North Pacific Research Board Final Report

NPRB project 1618: Original title: Genetic population structure of red seaweeds *Palmaria* and *Pyropia* in Alaska

Modified title: Phylogeography of the kelps *Saccharina latissima*, *Hedophyllum nigripes*, and *Alaria* spp. in Alaska and implications for resource managment

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Date: August 2019

Index

Abstract: Alaskans are eager to partake in the 6-billion-USD global seaweed industry. A first step in developing this industry is to resolve patterns of genetic variability among populations. Three molecular markers, mitochondrial DNA, chloroplast DNA and microsatellite DNA revealed strong genetic differences between populations but low levels of genetic diversity within populations of sugar kelp (*Saccharina latissima*), split kelp (*Hedophylum nigripes*), and winged-kelp (*Alaria* spp.). Significant differences between populations appeared on a range of spatial scales extending from several hundred kilimeters to only a few kilometers. Each of these species exhibited a genetic mosaic pattern of structure without isolation by distance and without a hierarchical pattern of regional differentiation. This chaotic structure is likely due to isolation in ice-age refugia around the Gulf of Alaska and post-glacial dispersals and is an example of 'isolation by colonization'. The relatively higher levels of genetic diversity in populations around the Gulf of Alaska relative to related populations to the south, in the Arctic and in the North Atlantic further supports the hypothesis of Alaskan ice-age refugia. Isolation in refugia and post-glacial colonizations have produced shallow, but significant differences between populations of sugar and slpit kelp, but five deeply separated lineages appeared in winged kelp that do not entirely correspond to species described on the basis of morphological variability. The low levels of genetic diversity in these ecologically success species indicate that epigenetic dynamics and not stores of standing genetic diversity may underpin adaptive responses to increasing temperature and ocean acidification.

Key Words: *Saccharina latissima*, *Hedophyllum nigripes*, *Alaria* spp., Mitochondrial DNA, Chloroplast DNA, Microsatellite DNA, Gulf of Alaska, Arctic Ocean, Phylogeography, Aquaculture

Citation: Grant WS. 2019. Phylogeography of the kelps *Saccharina latissima*, *Hedophyllum nigripes*, and *Alaria* spp. in Alaska and implications for resource managment. NPRB Project 1618. 325 p.

Chronology:

1. The PI of this project was a co-PI on the NPRB Project 1526 "Phylogeography and Management of Golden King Crab populations in Alaska"

2. The focus of the project was redirected toward kelps, when it became obvious that the greatest commercial interest was in kelps and not red seaweeds.

3. A no-cost extension of the project for one year was granted in June 2018.

Chapter 1: **General Introduction**

Alaska has a long coastline extending from temperate latitudes in southeastern Alaska to polar latitudes along the Arctic coast. The shores around the Gulf of Alaska are influenced by the subarctic Northeastern Pacific gyre and the Alaska Coastal Current. West Wind Drift in the North Pacific Current spits into northward and southward currents at it approaches North America (Figure 1.1). The warm northward current feeds the Alaska Coastal Current (ACC) and produces a warmer marine environment than exists along the coasts of the Northwestern Pacific at the same latitude.

The ACC is a boundary current with complex eddies and meanders (Figure 1.2). The ACC moves along the coast of the Gulf of Alaska and narrows to about 50 km as it reaches Kodiak Island and becomes the Alaska Stream (AS) with currents speeds of 50 cm per second or greater (1.8 km per hour) (Stabeno *et al.* 2004). The AS continues westward along the Aleutian Island Archipelago with arms of the current flowing northward into the Bering Sea. A large cyclonic gyre in the Bering Sea leads to a southwestern cold current along the eastern shores of the Kamtchatka Peninsula with an arm flowing into the Okhotsk Sea to form a large cyclonic gyre with complex eddies and meanders (Verkhunov 1997).

The focus of this project is on kelps, which are brown algae in the Class Phaeophyceae with a heteromorphic life-history cycle, consisting of a large sporophytic stage and a microscopic gametophytic stage (Figure 1.3). Sporophytes have a 2N complement of chromosomes and produce biflagellated 1N spores through meiosis. Spores settle rapidly on the bottom and grow into minute, dioeious, filamentous plants (gametophytes) that produce gametes through mitosis. Male gametes are released into the water and fertilize eggs produced by the female gametophyte. The fertilized egg remains attached to the filamentous female gametophyte and develops into a macroscopic sporophyte. Gametophytes of both sexes are in close proximity to one another so that mating between related plants and self-fertizilation between male and female gametophytes from the same sporophytic plant may be common (Reed *et al*. 2004). The dispersals of an enormous number of meiotic spores and male gametes are leptokurtic so that most move only a small distance from the parental plant (Reed *et al*. 1988). Only a few propagules survive to produce macroscopic sporophytes (Schiel & Foster 2006). However, long-distance dispersals can occur from the drift of detached mature plants in ocean currents (Nikula *et al*. 2010). Sporophytes grow on hard substrates and at tide heights and depths that are specific to a particular species. The intensity of wave action greatly influences the local distributions of kelps so that some species are found only on wave-swepte headlands and other only in wave-protected coves.

Interactions between light and temperature produce an age structure in kelps that leads to the production of sporophytes during the warmer parts of the year. Dispersal is limited by the longevity of spores and gametes, which have little starch or lipid reserves. Hence, spores and male gametes have a life expectancy of no more than two days. Gametophytes can survive unfavorable conditions and produce sporophytes at a later time. Under laboratory culture, gametophytes have been observed tro survive over 30 years (Klimova & Klochkova 2017). Unfertilized eggs can develop pathenogeneticaly in several kelps. For examaple, in *S. japonica*, sporophytes arising parthenogenetically from female gametophytes produce zoospores that develop into female gametophytes (Lewis *et al*. 1993). In *Alaria angusta* the maturation of oogonia and antheridia are asynchronous, so that some eggs may germinate parthenogenetically (Klimova & Klochkova 2017).

Sugar kelp (*Saccharina latissima*) are widely distributed in the North Pacific, Arctic and North Atlantic oceans, where they inhabit wave- and current-protected bays and coves. This species alternates between a large bladed sporophyte plants, anchored to small rocks with branched haptera, and a microscopic, filamentious gametophyte phase. Plants tend to be perennials in the North Atlantic, but largely annuals in Alaska (Bartsch *et al*. 2008).

The split kelp, *Hedophyllum nigripes*, inhabits shallow subtidal and low intertidal rocky shores with moderate to high wave action from central California to Alaska, the Aleutian Archipelago, Bering Sea and Russia in the North Pacific (Lindeberg and Lindstrom 2010). It also occurs in Arctic waters in Hudson's Bay, the Canadian Arctic and as far south as New Brunswick and the Gulf of Maine (Sears 2002; Longtin and Saunders 2016). The wide distribution in the North Pacific, Arctic, NW and NE Atlantic provides an opportunity investigate the timing of dispersals across the Arctic into the N Atlantic.

Biological surveys in the 1800s found a rich seaweed flora in Alaskan waters (Figure 1.4). The collections of seaweeds during a Russian expedition in the 1820 by Feodor Litke led to the formal description of several species of kelps found in Alaska. A catalogue of marine algae in Alaska was compiled in the 1970s (Lindstrom 1977) and an illustrated guide to seaweeds in Alaska appeared recently (Lindeberg & Lindstrom 2010).

The North Pacific Ocean is likely the area of origins of the world's kelps (Bolton 2010). However, the phylogeographical structures of Alaska'a algae are largely unknown. Studies of Alaskan seaweeds with molecular tools have been largely limited to taxonomic treatments (Lane *et al*. 2007; Bringloe & Saunders 2019) with limited attention to population diversity (Lindstrom *et al*. 1997; Lindstrom 2001, 2009; Lindstrom & Fredericq 2003; Lane *et al*. 2007; Coyer *et al.* 2011; Johanesson *et al*. 2015; Bringloe *et al*. 2017). The results of these studies depict a diverse and complex algal floral in Alaska that has not been fully described.

Genetic patterns among populations of marine organisms often show two levels of organization that reflect different isolating mechanisms (Grant & Bowen 1998). One level of structure among populations results from the contemporary interplay between random drift, which tends to produce allele-frequency differences between populations, and gene flow (‘migration’) between populations, which counters the diversifying effects of drift. The approach to drift-migration equlibrium may take hundreds or thousands of years depending on population size and the extent of gene flow between populations. Random drift is greater in small populations than in large populations, but the homogenizing effects of gene flow from dispersal are greater in small populations (Crow & Kimura 1970). In a region experiencing climatic variability, drift-migration equilibrium may not be achieved in contemporary populations because of frequent population turnovers.

A second component of genetic population structure arises from the legacy of historical isolations, population bottlenecks in size, extinctions, and founder events on longer time scales. These events can lead to the loss of genetic diversity when bottlenecks are severe, when only a few individuals found a population, or when founding individuals come from genetically depauperate source populations (Hewitt 1996). Genetic diversity is regained only after mutation or immigration introduces new genetic variation into a population.

The choice of a molecular marker to study population structure influences the temporal perspective of a study. Markers based on genes with a small mutation rates may be suitable for detecting ancient population events, but may not resolve contemporary population structure because of the lack of polymorphisms to track the effects of drift and gene flow. On the other hand, highly polymorphic markers with large mutation rates, such as microsatellite DNA, may resolve contemporary population structure, but may not be able to resolve deep population structure because mutations erase genetic imprints of historical events. In this study, mitochondrial (mt) and chloroplast (c) DNA with moderate mutations rates were used to provide a window onto historical events and several microsatellite loci with large mutation rates were used to resolve contemporary population structure. The mtDNA cytochrome oxidase I (*COI*) gene we used in this study is generally more polymorphic than the large subunit of the cDNA ribulose bisphosphate carboxylase (*rbcL*) gene (Grant 2016). Since both genes are commonly used to study genetic variability in seaweeds, their use here allows comparisons with population studies of other kelps with similar life histories.

Additional attributes of molecular markers influence what aspects of population history and structure can be resolved. Adaptation to local habitats through natural selection can lead to divergence between populations in addtion to random drift. In this study, we assumed that the variability within and among populations that was resolved with the two organellar DNA and microsatellite markers was not constrained by natural selection or was linked to regions of DNA influenced by selection. The assumption of neutrality allows us to estimate numerous population parameters from classic models based on genetically effective population size (N_e) and migration (m). In population genetic formulations, N_e is the number of individuals in a theoretical population that produces the same level of random drift as that found in a natural population, and m is the proportion of successfully reproducing individuals coming from another population.

Original objectives:

1. Estimate the genetic population structure of the red foliose seaweeds *Palmaria mollis-Palmaria hecatensis* in Alaska: 1. Delineate population production units with molecular markers, 2. Estimate dispersal rates between populations and patterns of genetic variability, 3. Estimate levels of genetic diversity and genetic effective population sizes.

2. Estimate the genetic population structure of the red foliose seaweed complex *Pyropia spp.* in Alaska: 1. Delineate population production units with molecular markers, 2. Estimate dispersal rates between populations and patterns of genetic variability, 3. Estimate levels of genetic diversity and genetic effective population sizes.

3. Use the population genetic information from this and other studies to formulate recommendations for the management of seaweed resources in Alaska.

The original focus of this project was on two red (rhodophyta) seaweeds. However, it became apparent from the numbers of applications submitted to the State of Alaska to operate culture facilities that kelps in the order Laminariales (Ochrophyta) were of greater interest to seaweed farmers. Hence, we shifted the focus of the studies to sugar kelp, *Saccharina latissima*, and ribbon (winged) kelp, *Alaria marginata*. Kelps are Stramenopiles that acquired chloroplasts through endosymbiosis from other eukaryotes. The overall objective to resolve the genetic population structure and to understand the origins of this structure remained the same. However, it became obvious that the genetic population structures of the three kelps in this study could not be understood by events and processes solely in Alaskan waters. Hence, each of the kelps were placed in a broader phylogeographic context by including data when available from British Columbia, Northwestern Pacific Ocean, Arctic Ocean and the North Atlantic. Along with this historical perspective, evolutionary principles should be used for formulation resource management guidelines (Hendry *et al*. 2010). Among these principles is the conservation of ‘evolutionary potential’, which is especially at risk in the waters of Alaska where climate warming is already having a substantial influence on marine organisms (Royer & Grosch 2006; Wing 2006; Mackas *et al*. 2007; Fabry *et al*. 2009).

Outline of report

The laboratory and statistical methods for the various sections of the report were collated into a single section to save repetition. A single section at the end of the report contains all of the literature that was cited to save space. Chapters 3–7 will form the basis for publications in the appropriate peer-reviewed journals. Following this Introduction, Section 2 details the biologies of the kelps included in the study, sample collection methods, DNA extraction methods and polymerase chain reaction protocols for two organellar genes and 8 microsatellite genes, and the statistical approaches.

Chapter 3 focuses on the phylogeographic history of the sugar kelp, *Saccharina latissima*, in a region of the North Pacific Ocean that has been greatly influenced by Milankovitch ice-age cycles and stadial-interstadial climate shifts between glacial maxima over the past 100 thousand years. The use of two classes of genetic markers with different modes of inheritance provides considerable resolution of genetic structure of sugar kelp populations in the Gulf of Alaska. This structure reflects not only contemporary restrictions on gene flow, but also divergences in isolation during the Pleistocene ice ages.

Chapter 4 explores the phylogeographic history of sugar kelp in the North Pacific and North Atlantic. The addition of *COI* sequences from Genbank showed that the common North Pacific lineage is present in Hudson's Bay and as far as the shores of western Greenland. Two previous studies of high latitude populations of sugar kelp proposed biogeographic hypotheses that are not supported by the larger collection of sequences. Unfortunately, a reconstruction of biogeographic pathways and dispersal timing, other than the fact of dispersal from the North Pacific, through the Arctic and into the North Atlantic, is still unresolved by the analysis of this single marker.

Chapter 5 focuses on split kelp, *Hedophyllum nigripes*, a rocky intertidal kelp that is morphologically similar to sugar kelp, especially during early growth stages. Our data, together with Genbank sequences, provide insights into the glacial refugia and post-glacial dispersals across the Arctic and into the Northwestern Atlantic Ocean. Genetic markers show a close relationship between populations in Alaska and those along the shores of the Canadian Maritime Provinces and New England States. The results of this study once again show that not only swimming fish and benthic crabs, but also seaweeds, show DNA imprints of recent dispersals through the Arctic.

Chapter 6 tells the story of ribbon kelps in the genus *Alaria* in the Northeastern Pacific, which has had a troubled taxonomic history. The results of our study uncovered five deeply separated lineages that were unanticipated and not geographically structured. These lineages are likely the result of repeated divergences during Pleistocene episodes of continental glaciation. The lineages hybridize at locations where they are in contact and appear to prevent full divergence into new species.

Chapter 7 continues to examine the species of *Alaria*. Available *COI* sequences from Genbank were combined with the sequences generated in this study. The results show that taxa in the Northeastern Pacific are less diverged from one another than are species in the Asian waters of the Northwestern Pacific. The combined dataset also shows that the nominal species of *Alaria* in the Northeastern Pacific do not fall consistently into the five lineages.

Chapter 8 is a comment article publication in the *ICES Journal of Marine Science* point out that available software using coalescence methods based on the Fisher-Wright model of evolutionary change is not appropriate for most marine species that produce often millions of larvae, but with only few surviving from a limited number of families (reproductive skew). The use of the Fisher-Wright model to interpret data for marine species can lead to erroneous conclusions.

Chapter 9 is an article in the *Proceedings of the National Academy of Sciences*, USA comparing phylogeographic boundaries based on molecular markers with biogeographic realms defined by traditional methods of species diversity and endemism. While intraspecific phylogeographic boundaries largely coincide with biogeographic boundaries, the biogeographic histories of high-latitude species contrast sharply with low-latitude species because of the greater environmental disturbances at high latitudes during the Pleistocene ice ages.

Chapter 10 is published review in *Reviews in Fish Biology and Fisheries* on the genetic dynamics of stock restorations, sea ranching, and stock enhancements of marine species. Several genetic principles should be considered when wild stocks are manipulated to increase production.

Chapter 11 considers the management implications of these studies to achieve sustainable commercial develop of seaweed resources in Alaska. The results show the broad regional outlines of genetic population structure in these three species of kelps, but hint at finer-scale population structures in each species. Further sampling is required to fill out the details necessary to formulate and implement a sound management strategy to guide the development of seaweed aquaculture in Alaska. In the interim, the results of this project provide the basis for at least some general guidelines for the culture and production of kelps in Alaska. The effects of ocean warming and acidification will greatly influence the abundances and distributions of kelps in Alaskan waters, and harvest and farming guidelines should anticipate these environmental changes.

Chapter 12 summarizes the study and outlines possible future directions for understanding macroalgal species in the Northeastern Pacific. The finely subdivided nature of population structure for kelps requires a further sampling effort. Since selectively neutral genetic markers were used, it is uncertain the extent that the genetically different populations are also locally adapted. Genomic studies will be important to better understand the extent of adaptation to local habitats.

References

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J. 2008. The genus *Laminaria sensu lato*: recent insights and developments. *European Journal of Phycology*, **43**, 1–86.

Bolton JJ. 2010. The biogeography of kelps (Laminariales, Phaeophyceae): a global analysis with new insights from recent advances in molecular phylogenetics. *Helgoland Marine Research* 64: 264–279.

Bringloe TT & Saunders GW. 2018. Mitochondrial DNA sequence data reveal the origins of postglacial marine macroalgal flora in the Northwest Atlantic. *Marine Ecology Progress Series* 589: 45–58.

Bringloe TT & Saunders GW. 2019. DNA barcoding of the marine macroalgae from Nome, Alaska (Northern Bering Sea) reveals many trans-Arctic species. *Polar Biology* 42: 851–864.

Bringloe TT, Dunton KH & Saunders GW. 2017. Updates to the marine algal flora of the Boulder Patch in the Beaufort Sea off Northern Alaska as revealed by DNA Barcoding. *Arctic* 70: 343–348.

Coyer JA, Hoarau G, Van Schaik J, Luijckx P & Olsen JL. 2011. Trans-Pacific and trans-Arctic pathways of the intertidal macroalga *Fucus distichus* L. reveal multiple glacial refugia and colonizations from the North Pacific to the North Atlantic. *Journal of Biogeography* 38: 756–771.

Crow, J.F. and Kimura, M., 1970. An introduction to population genetics theory. Harper & Row, New York.

Fabry VJ, McClintock JB, Mathis JT & Grebmeier JM. 2009. Ocean acidification at high latitudes: the bellwether. *Oceanography* 22: 160–171.

Grant WS. 2016. Paradigm shifts in the phylogeographic analysis of seaweeds. In: *Seaweed Phylogeography*, Z-M Hu, F Ceridwen (eds). Springer, Dordrecht.

Grant WS & Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* 89: 415–426.

Hendry AP, Lohmann LG, Conti E, Cracraft J, Crandall KA, Faith DP, Häuser C, Joly CA, Kogure K, Larigauderie A & Magallón S. 2010. Evolutionary biology in biodiversity science, conservation, and policy: a call to action. Evolution 64: 1517-28.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological journal of the Linnean Society* 58: 247–276.

Klimova AV & Klochkova TA. 2017. Peculiarities of development in the marine brown alga *Alaria angusta* Kjellman, 1889 (Alariaceae: Ochrophyta) under laboratory-controlled conditions. *Russian Journal of Marine Biology* 43: 42–48.

Johansson ML, Alberto F, Reed DC, Raimondi PT, Coelho NC, Young MA, Drake PT, Edwards CA, Cavanaugh K, Assis J, Ladah LB, Bell TW, Coyer JA, Siegel DA & Serrão EA. 2015. Seascape drivers of *Macrocystis pyrifera* population genetic structure in the northeast Pacific. *Molecular Ecology* 24: 4866–4885.

Lane CE, Lindstrom SC & Saunders GW. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Molecular Phylogenetics and Evolution* 44: 634–648.

Lewis R, Jiang B, Neushul M, 1993. Haploid parthenogenetic sporophytes of *Laminaria japonica* (Phaeophyceae). *Journal of Phycology* 29: 363–369.

Lindeberg MR & Lindstrom SC. 2010. *Field Guide to Seaweeds of Alaska*. Sea Grant College Program, University of Alaska Fairbanks.

Lindstrom SC. 1977. An annotated bibliography of the marine algae of Alaska. Alaska Department of Fish and Game. Technical Data Report No. 31, 172 pp.

Lindstrom SC. 2001. The Bering Strait connection: dispersal and speciation in boreal macroalgae. *Journal of Biogeography* 28: 243–251.

Lindstrom SC. 2009. The biogeography of seaweeds in Southeast Alaska. *Journal of Biogeography* 36: 401–409.

Lindstrom SC. 2009. The biogeography of seaweeds in southeast Alaska. *Journal of Biogeography* 36: 401–409.

Lindstrom SC & Fredericq S. 2003. *rbc*L gene sequences reveal relationships among north-east Pacific species of *Porphyra* (Bangiales, Rhodophyta) and a new species, *P. aestivalis*. *Phycological Research* 51: 211–211.

Lindstrom SC, Olsen JL & Stam WT. 1997. Postglacial recolonization and the biogeography of *Palmaria mollis* (Rhodophyta) along the Northeast Pacific coast. *Canadian journal of botany* 75: 1887–1896.

Longtin CM & Saunders GW 2015. On the untility of mucilage ducts as a taxonomic character in *Larminaria* and *saccharina* (Phaeophyceae)–the conundrum of *S. groenlandica*. *Phycologia* 54: 440–450. [This publication changed the name of *Saccharina groenlandica* to *S. nigripes*; Starko et al. 2019 has further changed the name to *Hedophyllum nigripes*.]

Mackas DL, Batten S & Trudel M. 2007. Effects on zooplankton of a warmer ocean: recent evidence from the Northeast Pacific. *Progress in Oceanography* 75: 223–252.

Nikula R, Fraser CI, Spencer HG & Waters JM. 2010. Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. *Marine Ecology Progress Series* 405: 221–230.

Redmond S, Green L, Yarish C, Kim J & Neefus C. 2014. *New England Seaweed Culture Handbook-Nursery Systems*. Connecticut Sea Grant CTSG-14-01. 92 pp.

Reed RK & Schumacher JD. 1986. Physical oceanography. In: DW Hood, ST Zimmerman (eds). *The Gulf of Alaska: Physical environment and biological resources.* Ocean Assessment Division, NOAA, USA. pp. 57–75.

Reed DC, Laur DR & Ebeling AW. 1988. Variation in algal dispersal and recruitment: the importance of episodic events. *Ecological Monographs* 58: 321–335.

Royer TC & Grosch CE. 2006. Ocean warming and freshening in the northern Gulf of Alaska. *Geophysical Research Letters* 33: e6.

Schiel DR & Foster MS. 2006. The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. *Annual Reviews in Ecology Evolution and Systematics* 37: 343–72.

Sears JR. (Ed.). 2002. *NEAS keys to benthic marine algae of the northeastern coast of North America from Long Island Sound to the Strait of Belle Isle*. 2nd edition. Express Printing, Fall River, MA. NEAS Contribution Number 2.

Stabeno PJ, Bond NA, Hermann AJ, Kachel NB, Mordy CW & Overland JE. 2004. Meteorology and oceanography of the Northern Gulf of Alaska. *Continental Shelf Research* 24: 859–897.

Verkhunov AV. 1997. The development of knowledge of the large-scale circulation in the Sea of Okhotsk. In:"Complex Studies of the Okhotsk Sea Ecosystem". VNIRO, Moscow. pp. 8–19 (in Russian) [cited in https://www.nodc.noaa.gov/OC5/okhotsk/ok_doc.html]

Wing BL. 2006. Unusual invertebrates and fish observed in the Gulf of Alaska, 2004–2005. *Pices Press* 14: 26–28.

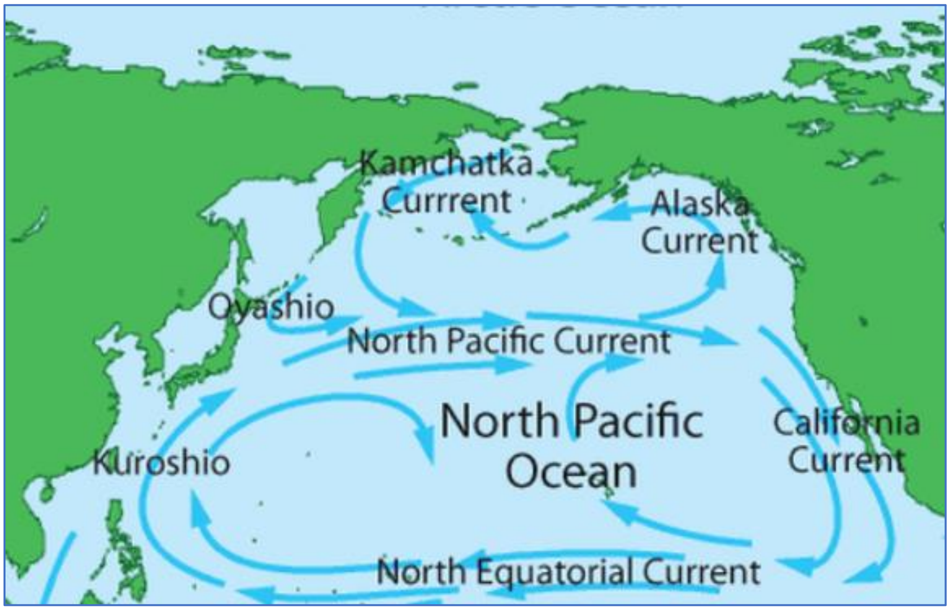

Figure 1.1 General current patterns in the North Pacific Ocean and marginal seas. Chart from https://www.oceanblueproject.org/ocean-current-maps.html

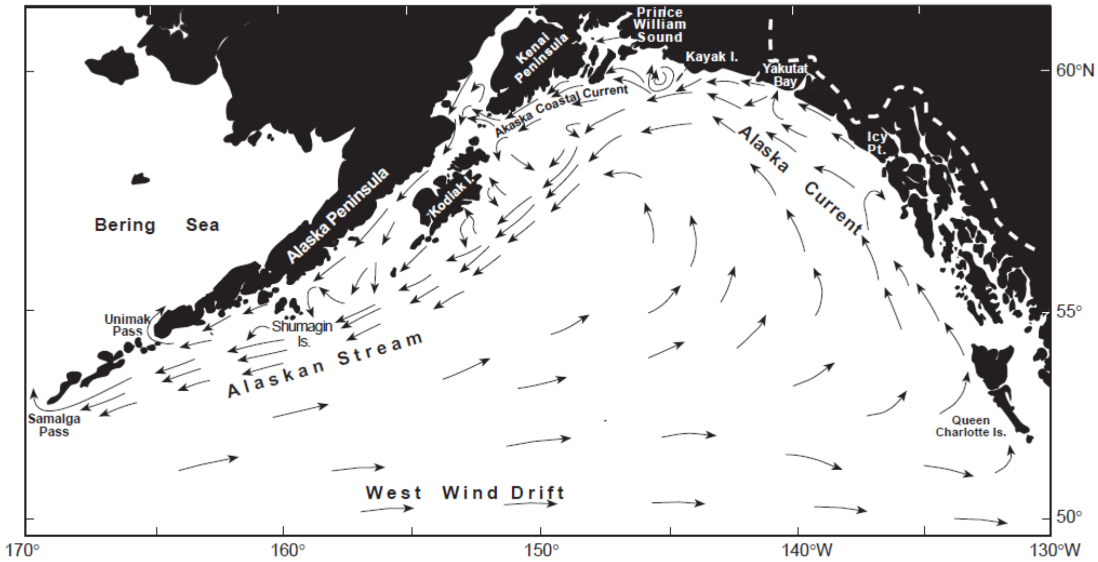

Figure 1.2 Currents in the Gulf of Alaska. From Stabeno *et al*. (2004) after Reed and Schumacher (1986).

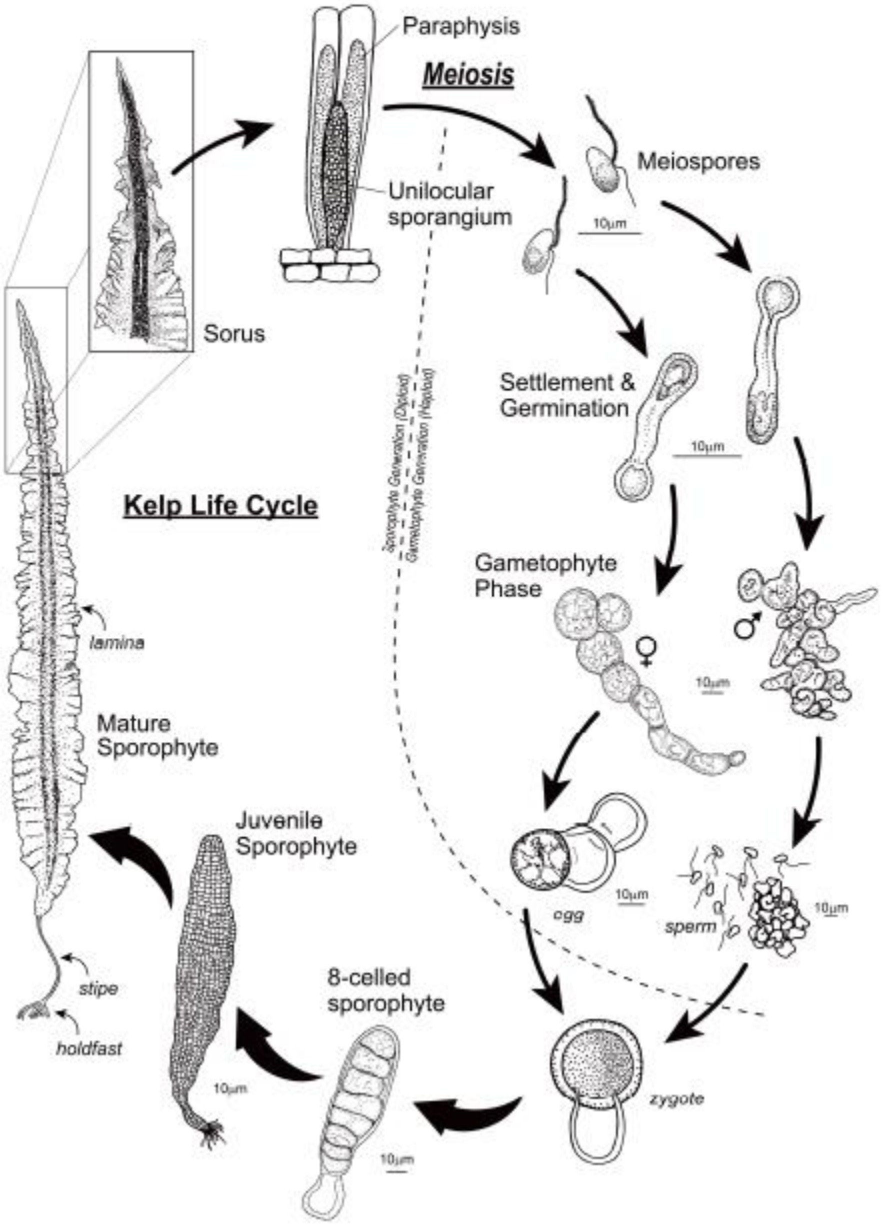

Figure 1.3 Generalized life-history cycle of kelps. From Redman *et al*. (2014)

Figure 1.4 *Algarum Vegitatio* drawn in 1827 by Aleksandr Filippovich Postals who explored sea life along Alaska's coast during an Russian expedition led by Feodor Litke. Several species of algae bear Postals' name as the describing author. The scene depicted above may have been near Sitka, given the combination of seaweed species and the rocky shore habitat. Note that the sea surface depicted in the drawing is remarkably flat. While features of intertidal and subtidal zonation and habitat assemblage may not be entirely correct, the drawing details several recognizable seaweeds. Judging from the discoid holdfast, the kelp being pulled from the water is likely *Laminaria yezoensis*. *Fucus distichus* clings to boulders just above the water line. *Nereocystis luetkeana* (Postels et Ruprecht) floats on the surface of the water in the center of the illustration. Bunched plants of *Hedophyllum sessilis* appear in the lower left corner of the picture. This species generally occurs in heavy surf. Behind and to the right of these plants are most likely *Hedophyllum nigripes*, one of the species appearing in our genetic study. Behind these plants, ribbed *Pleurophycus gardneri* flutter in the current. To the right is *Macrocystis pyrifera*, and to the right again is the sieve kelp, possibly *Agarum turneri* (Postels et Ruprecht), with holes in its frond. Several large plants of *Alaria marginata* (Postels et Ruprecht), with spore-bearing fronds near the bottom of the plant, appear in the center of the drawing. This species is the focus of seaweed farming and a center piece in our genetic study. To the right is *Lessoniopsis littoralis* with strap-like blades growing from a short robust stipe. *Cymathaere triplicata* (Postels et Ruprecht) would be expected in this habitat, but it is difficult to identify in the drawing. Also missing is *Saccharina latissima*, which grows in wave-protected coves and is another focus of our genetic study. Several species of red and green algae are found in this setting, but they are difficult to identify in the monochrome drawing. A further narrative of the history of early seaweed surveys in Alaska appears in Lindstrom (2009). Drawing from *Illustrationes Algarum* by Postels and Ruprecht, housed in the Beinecke Library at Yale University.

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Chapter 2: **Materials and Methods**

Abstract: We used two classes molecular markers to create a baseline of genetic diversity in sugar kelp, ribbon kelp and split kelp with the aim of developing management guidelines for the development of seaweed culture in Alaska. An understanding of patterns of population structure due to both contemporary processes and ancient evolutionary events are needed to devise resource management guidelines that will lead to the preservation of genetic diversity and to the sustainable use of kelps in Alaska. The use of both bi- and uni-parentally inherited markers provides different views of the same population events. Contrasts in mutation rates between marker types allows inferences of the relative timings of historical events. We used the *COI* species-'barcode' gene found in mitochondria and the gene, *rbcL,* coding for an important photosynthetic enzyme in chloroplasts. After DNA extraction, PCR was used to amplify regions of the two genes for sequencing. We also used microsatellite DNA, which has a high mutation rate, producing an abundance of polymorphic alleles with a high power to discriminate among populations. The PI and volunteer collectors around the state provided a wealth of samples for analyses. We used standard statistical software for our analyses of the organellar DNA and microsatellite DNA data. The use of relatively small samples in these studies can be justified from probability considerations and from large frequency differences among populations.

2.1 Marker selection

Two classes of markers were selected for this study, organellar genes and nuclear genes. The use of both kinds of makers provides complementary insights into historical and contemporary processes influencing genetic population structure of the species of kelps examined in this study. The results of our study bear this out. The analysis of both mitochondrial (mt) and chloroplast (cp) DNA provides independent views of the same population histories. Microsatellite markers, on the other hand, were used to resolve contemporary population processes, because the larger mutation rates in microsatellite DNA can potentially resolve events occurring on short time scales.

2.1.1 Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L, Rubisco)

Rubisco is a multifunctional enzyme in chloroplasts, catalyzing the fixation of CO_2 during photosynthesis and the release of O_2 during photorespiration. Once the rubisco gene has been expressed, two subunits undergo co- and post-translational modifications by interactions with structurally molecular-modifying enzymes. Rubisco's role in photosynthetic CO_2 assimilation depends on conformation modifications by another enzyme called Rubisco activase. Rubisco is also influenced by environmental variables, such as light intensity, resulting in its degradation and molecular turnover in the cell. The bio-engineering of this enzyme has been viewed as a strategy for improving plant productivity because the fixation of CO_2 by this enzyme is a rate-limiting step in photosynthesis. Hence, the genetic modification of this gene in kelps to increase CO_2 uptake may enhance their use as carbon sinks to help to mitigate increased concentrations of CO_2 in sea water.

Surveys of sequence variability in DNA encoding the large subunit of the enzyme (*rbc*L) has been used in phylogenetic studies of lamininarian kelps (Lane *et al*. 2007). Chloroplast DNA is maternally, or uniparentally in plants with undifferentiated life-history types, inherited in plants and lacks recombination during replication as in nuclear DNA (Li *et al*. 2016). These two traits allow reconstructions of gene genealogies and together with geography and provide insights into the ancient dispersals, colonizations, and historical demography. This gene was included in our study to provide a view of phylogeographic patterns independent of mitochondrial DNA and to capture possible deep structure steming from historical population events. Genetic imprints of ancient events in DNA are less likely to be obliterated the small mutation rates of *rbc*L.

2.1.2 Cytochrome oxidase subunit I (*COI*, *COX1*)

Three subunits of the heme-copper enzyme cytochrome c oxidase are encoded in mitochondrial DNA. Cytochrome oxidase I (*COI*) is the main subunit of the cytochrome c oxidase complex, but the functional cytochrome oxidase complex also contains components encoded by nuclear genes. Cytochrome c oxidase is attached to the inner membrane of the mitochondria. This enzyme is the third and final step of the electron transport chain of mitochondrial oxidative phosphorylation. Cytochrome c is a key enzyme in aerobic metabolism and has an amino acid structure that is highly conserved among eukaryotes.

Nevertheless, neutral mutations at third position nucleotide sites can provide insights into deep and shallow population structure. DNA sequences of *COI* have been used extensively in phylogeographic studies because of its moderate levels of polymorphism in third codon positions—the ‘wobble’ position. Nucleotide substitutions at this position do not change the amino acid encoded by a codon and hence are considered to be neutral to natural selection. A 658 base-pair segment of the 5’ end of *COI* is used as a molecular tag to identify species of animals (barcoding), and hence a large archive of sequences is available for analyses. In some plant groups, *COI* may evolve too slowly to be of value for barcoding, so the *rbc*L gene is used instead. In algae, however, levels of *rbc*L polymorphism appear to be about the same or less than those in *COI* (Grant 2015). Mitochondrial DNA follows uniparentally inheritance from generation to generation and lacks recombination during replication as in nuclear DNA in most species. These two traits allow the reconstruction of gene genealogies. Additionally, frequencies of haplotypes among samples can be used to test hypotheses of population structure. The use of *COI* DNA sequences in the present study facilitates comparisons with other related species.

2.1.3 Microsatellite DNA

Microsatellites are segments of DNA with repetitive motifs of 1 to 6 or more nucleotides in length that are repeated as many as 50 times. The repeat units are generally di-, tri- tetra- or pentanucleotides. Tri- or tetra-nucleotide repeats are most useful because di-nucleotide repeats often produce a strongly stuttered phenotype that is difficult to score. These repetitive segments belong to a class of genes called variable number of tandem repeats (VNTRs) and are also referred to as short tandem repeats (STRs) or as simple sequence repeats (SSRs) in the plant literature. Short repetitive sequences occur in hundreds of places throughout the genome and have a larger mutation rates than do other regions of nuclear DNA and of mitochondrial and chloroplast DNA.

Sequences in the locus-specific flanking regions on either side of a microsatellite are used to anchor polymerase chain reaction (PCR) forward and reverse primers to amplify the repeating seqment. The size of the PCR product depends on the number of repeats. Alleles are defined by the number of repeats and can be separated by size in an electric field. Alleles are co-dominant so that homozygous genotypes can be distinguished from heterozygous gentoypes. Marine organisms tend to have large numbers of microsatellite alleles, as many as 100 or more, and this high level of polymorphism provides considerable statistical power to detect fine-scale population structure or to infer family structure. Because of the large mutation rate, microsatellites are ideally suited to addressing some questions in population genetics, such as relatedness among individuals (Blouin 2003), or the effects of contemporary isolations and gene flow among populations (Michalakis & Excoffier 1996; Pritchard et al. 2000; Selkoe & Toonen. 2006).

Microsatellites have some drawbacks. They are not suited to resolving relationships among species or to detecting genetic imprints of ancient population events because the large mutation rate over-prints the genetic signatures of long-past events. A major problem is the convergence to the same allelic length from historically different allelic states. The same allelic states among individuals may not be identical by descent, but may have different genealogical histories (technically called homoplasies). Even though the number of DNA motif repeats may be constrained by natural selection, most microsatellite genotpes are thought to be influenced chiefly by gene flow and random genetic drift. Comparisons of results with other studies are often not possible because of the use of different microsatellite loci in the various studies. The chief use of microsatellites in population studies is to estimate levels of genetic diversity for comparison among samples and to test hypotheses of population structure with allele-frequency spectra among samples.

2.2 Sample collection

Numerous volunteers help to collect samples at locations around the Gulf of Alaska (Figures 2.1, 2.2). Several collectors, including the PI of this project, mistook split kelp (*Hedophyllum nigripes*) for sugar kelp in some collections because of the morphlogical similarity between the species, especially of younger plants. After closer inspection, the two species of kelp could be identified by morphology and habitat. Sugar kelp grows in sheltered coves and is attached to small stones or pebbles, whereas split kelp is attached to large boulders or bedrock at more wave- or current-exposed sites. Sugar kelp has a fragile frond with the consistancy of parchment paper. Split kelp, on the other hand, is leathery and slippery to the touch because of copious mucinilaginous cells on the frond (Longtin & Saunders 2015).

Common names and Latin bionomials followed Lindberg & Lindstrom (2010) except for a recent revision of the latin binomial for the spit kelp, which is now called *Hedophyllum nigripes* (ex *Saccharina groenlandica* , ex *Saccharina nigripes*) (Starko *et al*. 2019). The common name for *Saccharina latissima* is sugar kelp, reflecting the high carbohydrate content of this kelp. *Alaria marginata* is known in Alaska as ribbon kelp (Lindeberg & Lindstrom 2010), but many research articles refer to this species as winged kelp (e.g. Kusumo & Druehl 2000).

2.3 Laboratory methods

We used standard laboratory methods to produce sequences of the 5' end of mitochondrial *COI* and chloroplast *rbcL*. Needless to say, kelp tissues behave differently from fish tissues and it took some trial and error to routinely extract DNA to amplify the selected genes with the PCR for sequencing or microsatellite size analysis.

2.4 Statistical methods

Because of the limits of time and manpower, we chose to focus on the detection of major regional groups in the Gulf of Alaska. Future studies will tease apart fine-scale variability in the broader genetic seascape within the regions. Our samples were smaller than is customary in fishery population genetics, where greater precision is required, but are more than adequate for detecting genetic breaks among regions. Previous studies show the existence of boundaries between crabs (Grant & Cheng 2012) and fish (Matala *et al*. 2004; Palof *et al*. 2011). Studies of kelps show strong, often fixed differences between populations, even populations in geographical proximity (Zhang *et al*. 2015). Under these conditions, the samples sizes of 25–40 plants provided considerable power to detect large-scale frequency differences if they existed (Table 2.2). For example, the sample size needed to find a signficant allele-frequency differences of 0.2 (P_1 = 0.25 in population 1 and P_2 = 0.55) with 90% certainty is only 30 individuals (Sokal & Rohlf 1995). The microsatellite loci surveyed in this study were polymorphic for 4 to 15 alleles, which provided additional statistical power above the two-allele values in Table 2.1.

Another concern for statistical power is the ability to detect divergences between populations estimated with F_{ST} that occurred on long time scales through random drift and natuaral selection. Our selection of 12 microsatellite loci provides considerable power for detecting small levels of divergence between populations (Table 2.2) (POWSIM: Ryman & Palm 2006). For example, the use of 10 polymorphic microsatellite loci, even with sample sizes as small as 25 individuals, provides enough statistical power to detect an F_{ST} of 0.01 with 100% certainty.

2.5 References

Blouin MS. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology & Evolution* 18: 503–511.

Grant WS, Cheng W. 2012. Incorporating deep and shallow components of genetic structure into the management of Alaskan red king crab. *Evolutionary Applications* 5: 820–837.

Kusumo HT & Druehl LD. 2000. Variability over space and time in the genetic structure of the winged kelp *Alaria marginata*. *Marine Biology* 136: 397–409.

Lane CE, Lindstrom SC & Saunders GW. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Molecular Phylogenetics and Evolution* 44: 634–648.

Lindeberg MR & Lindstrom SC. 2010. *Field Guide to Seaweeds of Alaska*. Sea Grant College Program, University of Alaska Fairbanks.

Longtin CM & Saunders GW 2015. On the untility of mucilage ducts as a taxonomic character in *Larminaria* and *saccharina* (Phaeophyceae)–the conundrum of *S. groenlandica*. *Phycologia* 54: 440–450. [This publication changed the name of *Saccharina groenlandica* to *S. nigripes*; Starko et al. 2019 has further changed the name to *Hedophyllum nigripes*.]

Matala AP, Gray AK, Heifetz J & Gharrett AJ. 2004. Population structure of Alaskan shortraker rockfish, *Sebastes borealis*, inferred from microsatellite variation. *Environmental Biology of Fishes* 69: 201–210.

Michalakis Y & Excoffier L 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 142: 1061–1064.

Palof KJ, Heifetz J & Gharrett AJ. 2011. Geographic structure in Alaskan Pacific ocean perch (*Sebastes alutus*) indicates limited lifetime dispersal. *Marine Biology* 158: 779–792.

Pritchard JK, Stephens M & Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Ryman N & Palm S. 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* 6: 600-602.

Selkoe KA & Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* 9: 615–629.

Sokal RR & Rohlf FJ. 1995. Biometry. 3d ed., Freeman, New York.

Starko S, Gomez MS, Darby H, Demes KW, Kawai H, Yotsukura N, Lindstrom SC, Keeling PJ, Graham SW & Martone PT. 2019. A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem. *Molecular Phylogenetics and Evolution* 136: 138–150.

Zhang J, Yao JT, Sun ZM, Fu G, Galanin DA, Nagasato C, Motomura T, Hu ZM & Duan DL. 2015. Phylogeographic data revealed shallow genetic structure in the kelp *Saccharina japonica* (Laminariales, Phaeophyta). *BMC Evolutionary Biology* 15: 237.

Table 2.1 Sample sizes needed to detect a given allele-frequency difference between populations at a given level of statistical power ($1 - \beta$). Values generated from eq ... of (1969). Type I error (α), the probability of rejecting a null hypothesis of no allele-frequency difference, when it is true, was set at $\alpha = 0.05$. Type II error (β) is the opposite: it is the rejection of a null hypothesis that is true. Hence, ($1 - \beta$) is the probability of detecting a true difference, if it exists. Values to the right of the step line provide conderable power for the allele-frequency differences expected between populations of kelp. Samples sizes were generated using the equations in Box 17.13 of Sokal & Rohlf (1995).

		Allele frequency in sample P_1												
P_2	$1 - \beta$	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70
0.05	0.9	310	103	57	37	27	21	17	14	11	10	8	7	6
	0.8	237	80	44	29	21	17	13	11	9	8	7	6	5
	0.6	156	54	30	20	15	12	10	8	7	6	5	5	4
0.10	0.9	-	479	143	73	46	32	24	19	15	12	10	9	8
	0.8	-	363	109	56	36	25	19	15	12	10	8	7	6
	0.6	-	236	73	38	24	17	13	11	9	7	6	5	5
0.15	0.9	-	-	626	177	87	53	36	27	21	16	13	11	9
	0.8	-	-	473	135	67	41	28	21	16	13	11	9	7
	0.6	-	-	305	89	45	28	19	15	11	9	8	6	6
0.20	0.9	-	-	-	752	206	99	59	40	29	22	17	14	11
	0.8	-	-	-	567	157	76	46	31	23	17	14	11	9
	0.6	-	-	-	364	102	50	31	21	16	12	10	8	7
0.25	0.9	-	-	-	-	857	230	108	64	42	30	23	17	14
	0.8	-	-	-	-	645	174	83	49	33	24	18	14	11
	0.6	-	-	-	-	414	113	55	33	22	16	12	10	8
0.30	0.9	-	-	-	-	-	941	248	115	67	44	31	23	18
	0.8	-	-	-	-	-	708	188	88	51	34	24	18	14
	0.6	-	-	-	-	-	453	122	58	34	23	17	13	10
0.35	0.9	-	-	-	-	-	-	1004	261	120	69	45	31	23
	0.8	-	-	-	-	-	-	755	198	91	53	35	24	18
	0.6	-	-	-	-	-	-	483	128	60	35	23	17	13
0.40	0.9	-	-	-	-	-	-	-	1046	269	122	70	45	31
	0.8	-	-	-	-	-	-	-	787	204	93	53	35	24
	0.6	-	-	-	-	-	-	-	503	132	61	36	36	23
0.45	0.9	-	-	-	-	-	-	-	-	1067	272	122	69	44
	0.8	-	-	-	-	-	-	-	-	802	206	93	53	34
	0.6	-	-	-	-	-	-	-	-	513	133	61	35	23
0.50	0.9	-	-	-	-	-	-	-	-	-	1067	269	120	67
	0.8	-	-	-	-	-	-	-	-	-	802	203	91	51
	0.6	-	-	-	-	-	-	-	-	-	513	132	60	34

Table 2.3 Probability of detecting a significant value of F_{ST} for a given level of divergence between three populations, samples sizes and number of polymorphic loci. Loci consisted of 8 alleles with a frequency distribution of 0.25, 0.25, 0.125, 0.125, 0.0625, 0.0625, 0.0625, 0.0625. N_e was set to 2000 to generate a value of F_{ST} with 1000 repetitions.

		Number of loci									
		1		5		10		20		40	
F_{ST}	*n*	X^2	Fisher	X^2	Fisher	X^2	Fisher	X^2	Fisher	X^2	Fisher
0.001	25	0.053	0.067	0.067	0.069	0.095	0.096	0.140	0.137	0.232	0.212
	50	0.080	0.080	0.140	0.141	0.219	0.210	0.308	0.286	0.476	0.449
	100	0.149	0.143	0.299	0.291	0.469	0.461	0.732	0.705	0.923	0.910
	200	0.224	0.237	0.635	0.614	0.880	0.885	0.990	0.983	1.000	0.999
0.005	25	0.170	0.181	0.380	0.379	0.600	0.582	0.819	0.809	0.980	0.972
	50	0.313	0.319	0.771	0.755	0.953	0.944	0.996	0.995	1.000	1.000
	100	0.608	0.606	0.992	0.993	1.000	1.000	1.000	1.000	1.000	1.000
	200	0.898	0.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.01	25	0.287	0.315	0.749	0.744	1.000	1.000	1.000	1.000	1.000	1.000
	50	0.596	0.606	0.995	0.992	1.000	1.000	1.000	1.000	1.000	1.000
	100	0.896	0.896	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	200	0.991	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.02	25	0.579	0.607	0.989	0.992	1.000	1.000	1.000	1.000	1.000	1.000
	50	0.906	0.912	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	100	0.989	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	200	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.04	25	0.887	0.895	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	50	0.990	0.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	200	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.08	25	0.989	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	50	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	200	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.16	25	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	50	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	200	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Homer Spit, Kachemak Bay

Kyak Point, Kachemak Bay

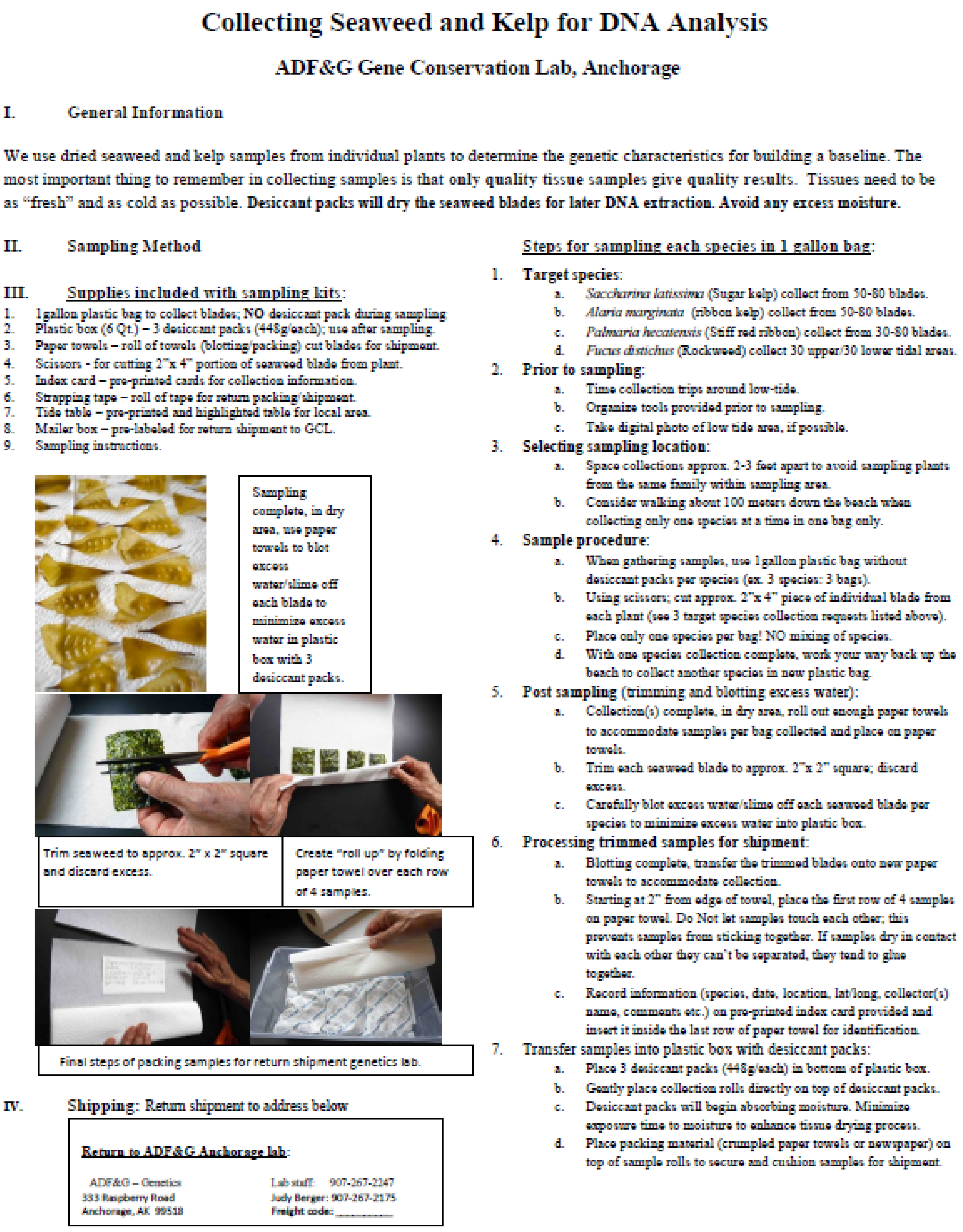
Harris Island, Sitka

Lowell Point, Resurrection Bay

Whittier, Prince William Sound

Kasitsna Lab Beach, Kachemak Bay

Figure 2.1 Photographs of seaweed collecting sites



Collecting Seaweed and Kelp for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

I. General Information

We use dried seaweed and kelp samples from individual plants to determine the genetic characteristics for building a baseline. The most important thing to remember in collecting samples is that **only quality** tissue samples give **quality** results. Tissues need to be as "fresh" and as cold as possible. **Desiccant packs will dry the seaweed blades for later DNA extraction. Avoid any excess moisture.**

II. Sampling Method

III. Supplies included with sampling kits:

1. 1gallon plastic bag to collect blades; NO desiccant pack during sampling
2. Plastic box (6 Qt.) – 3 desiccant packs (448g/each); use after sampling.
3. Paper towels – roll of towels (blotting/packing) cut blades for shipment.
4. Scissors - for cutting 2"x 4" portion of seaweed blade from plant.
5. Index card – pre-printed cards for collection information.
6. Strapping tape – roll of tape for return packing/shipment.
7. Tide table – pre-printed and highlighted table for local area.
8. Mailer box – pre-labeled for return shipment to GCL.
9. Sampling instructions.

Sampling complete, in dry area, use paper towels to blot excess water/slime off each blade to minimize excess water in plastic box with 3 desiccant packs.

Trim seaweed to approx. 2" x 2" square and discard excess.

Create "roll up" by folding paper towel over each row of 4 samples.

Final steps of packing samples for return shipment genetics lab.

IV. Shipping: Return shipment to address below

Return to ADF&G Anchorage lab:

ADF&G – Genetics
333 Raspberry Road
Anchorage, AK 99518

Lab staff: 907-267-2247
Judy Berger: 907-267-2175
Freight code: __________

Steps for sampling each species in 1 gallon bag:

1. **Target species:**
   a. *Saccharina latissima* (Sugar kelp) collect from 50-80 blades.
   b. *Alaria marginata* (ribbon kelp) collect from 50-80 blades.
   c. *Palmaria hecatensis* (Stiff red ribbon) collect from 30-80 blades.
   d. *Fucus distichus* (Rockweed) collect 30 upper/30 lower tidal areas.
2. **Prior to sampling:**
   a. Time collection trips around low-tide.
   b. Organize tools provided prior to sampling.
   c. Take digital photo of low tide area, if possible.
3. **Selecting sampling location:**
   a. Space collections approx. 2-3 feet apart to avoid sampling plants from the same family within sampling area.
   b. Consider walking about 100 meters down the beach when collecting only one species at a time in one bag only.
4. **Sample procedure:**
   a. When gathering samples, use 1gallon plastic bag without desiccant packs per species (ex. 3 species: 3 bags).
   b. Using scissors; cut approx. 2"x 4" piece of individual blade from each plant (see 3 target species collection requests listed above).
   c. Place only one species per bag! NO mixing of species.
   d. With one species collection complete, work your way back up the beach to collect another species in new plastic bag.
5. **Post sampling** (trimming and blotting excess water):
   a. Collection(s) complete, in dry area, roll out enough paper towels to accommodate samples per bag collected and place on paper towels.
   b. Trim each seaweed blade to approx. 2"x 2" square; discard excess.
   c. Carefully blot excess water/slime off each seaweed blade per species to minimize excess water into plastic box.
6. **Processing trimmed samples for shipment:**
   a. Blotting complete, transfer the trimmed blades onto new paper towels to accommodate collection.
   b. Starting at 2" from edge of towel, place the first row of 4 samples on paper towel. Do Not let samples touch each other; this prevents samples from sticking together. If samples dry in contact with each other they can't be separated, they tend to glue together.
   c. Record information (species, date, location, lat/long, collector(s) name, comments etc.) on pre-printed index card provided and insert it inside the last row of paper towel for identification.
7. Transfer samples into plastic box with desiccant packs:
   a. Place 3 desiccant packs (448g/each) in bottom of plastic box.
   b. Gently place collection rolls directly on top of desiccant packs.
   c. Desiccant packs will begin absorbing moisture. Minimize exposure time to moisture to enhance tissue drying process.
   d. Place packing material (crumpled paper towels or newspaper) on top of sample rolls to secure and cushion samples for shipment.

Figure 2.2 Sampling guidelines design and photographs by Judy Berger

Chapter 3

Molecular Ecology: Research article

Isolations in northern ice-age refugia shaped phylogeography of Sugar kelp *Saccharina latissima* in the Gulf of Alaska

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Abstract

Identifying the biotic and abiotic forces shaping genetic variability among wild populations remains a continuing challenge in evolutionary biology. Here, we used three genetic markers with different modes of inheritance to resolve patterns of genetic variability in sugar kelp (*Saccharina latissima*) populations around the rim of the Gulf of Alaska in Northeastern Pacific Ocean, an area that was heavily impacted by Pleistocene glaciations. Gene genealogies reconstructed from mitochondrial DNA *COI*-5P and chloroplast DNA *rbc*L sequences resolved several lineages that were discontinuously distributed around the Gulf of Alaska. Nine of the 14 populations were fixed, or nearly fixed, for a single haplotype (average h = 0.188 among populations). Heterozygosites of 12 microsatellite loci were larger (average H_O = 0.348 among populations), but were small compared to those in other kelps. The results of a PCA of microsatellite genotypes showed that sugar-kelp plants in the same organellar lineage clustered together, regardless of geographic origins. These findings indicate a recent expansion of the organellar lineages with corresponding microsatelite DNA variability. The distributions of the organellar DNA lineages were chaotic and were only weakly isolated by distance. Divergences between organellar lineages likely resulted from isolation in northern glacial refugia during the last Milankovitch 100 kyr cycle. The lack of microsatellite divergence between widely separated populations indicates post-glacial dispersals.

Keywords: chloroplast DNA, ice-age refugia, kelp, microsatellite DNA, mitochondrial DNA, Northeastern Pacific, paleoclimate, phylogeography, *Saccharina latissima*

Introduction

A continuing challenge is to understand the forces shaping genetic population structure in marine organsisms. Genetic patterns among populations can be molded by contemporary levels of gene flow and genetic drift, and by historical events, including divergences in isolation, dispersals and founder effects. In this study, we used genetic markers to resolve patterns of genetic variability in sugar kelp (*Saccharina latissima*) populations around the Gulf of Alaska in the Northeastern Pacific Ocean, an area that was heavily impacted by Pleistocene glaciations.

Marine ecosystems in the Northeastern Pacific periodically experienced profound changes during the Pleistocene as the margins of terrestrial glaciers blanketed coastal areas. Over the last 100 thousand years (kyrs), lobes of ice advanced and retreated several times during stadial-interstadial changes in temperature (Rasmussen *et al*. 2014; Li & Bor 2019) and produced a patchwork of suitable habitats for coastal marine species (Kaufman & Manley 2004; Marko *et al*. 2010). A gradual drop in global temperatures led to ever larger glaciers which reached their greatest extent about 22.5–18 kyrs ago during the Last Glacial Maximum (LGM). As glaciers grew, global sea levels drop 50 m below present-day levels for about 80% of the time over the last Milankovitch climate cycle. This drop in sea level resculpted shorelines along the Northeastern Pacific Ocean and influenced patterns of gene flow between populations.

A widely held view proposes that populations in northern seas were displaced to southern ice-free shorelines (Hewitt 1996, 2000), located as far south as the Washington-Oregon outer coasts (Thorson 1980). In this view, populations expanded northward and colonized the shore of Northern British Columbia and Alaska after glaciers recededed from the coast about 15 thousand years (ky) ago in Alaskan waters and about 12 krys ago from Puget Sound and the Salish Sea. As populations dispersed northward they would lose genetic diversity through founder effects so larger amounts of genetic diversity would be expected in southern populations. Stepwise dispersals and colonizations by only a few individuals at each advance would lead to a steeper gradient in genetic diversity than rapid ‘phalanx’ dispersals (Hewitt 1996).

Alternatively, ice-age populations may have survived in northern refugia, which has been postulated for North Atlantic seaweeds (Maggs *et al*. 2007) and Atlantic cod (Bigg *et al*. 2007), and for Pacific cod (Canino et al. 2010; Bigg 2014) and intertidal invertebrates in the Northeastern Pacific (Marko *et al*. 2010). While numerous genetic studies have been made of kelp populations along the coasts of Washington, Oregon and California (*eg* Alberto *et al*. 2010, 2011), few have been made of kelps at higher latitudes in the Northeastern Pacific. In one of the few studies, Lindstrom (2009) proposed that northern refugia accounted for breaks in some species' distributions in Southeastern Alaska.

Here, we focus on populations of sugar kelp (*Saccharina latissima*) that inhabit wave- and current-protected bays and coves from Central California to the shores of the Arctic coast of Alaska, and beyond. Populations of sugar kelp also occur in the Northwestern Pacific along the coasts the Kamchatka Peninsula and as far south as Japan under the names *S. coriacea* and *S. cichoriodes* (Balakirev *et al*. 2012). Divergent mitochondrial DNA lineages of sugar kelp also inhabit the Northwestern and Northeastern Atlantic (Luttikhuizen *et al*. 2018; Neiva *et al*. 2018), but are thought to have originated in the North Pacific. This species alternates between a large bladed sporophyte plants, anchored to rocks with branched haptera, and a microscopic, filamentious gametophyte phase (Lindeberg & Lindstrom 2010). Gametophytes sprout from meiospores and hence are haploid (*n* chromosome complement). Separate male and female plants and produce spermatozoa and oogonia, respectively. Zygotes (2*n*) grow in place into large sporophytic plants, which release meiospores at maturity that settle to the bottom and germinate into gametophytes, completing the life-history cycle. Plants tend to be perennials in the North Atlantic, but largely annuals in Alaska (Bartsch *et al*. 2008).

We used three genetic markers with different modes of inheritance to resolve patterns of genetic variability in sugar kelp in the Gulf of Alaska and eatern Aleutian Islands. Two organellar genes, one encoded in mitochrondrial DNA and the other in chloroplast DNA, were used to reconstruct gene genealogies that are independent of each other. The use of genetic markers with different modes of inheritance potentially provides greater resolution of population structure than the use of only one class of markers. We also used previously described microsatellite DNA markers to estimate contemporary processes influencing genetic variability (Paulino *et al*. 2016). The use of multiple molecular markers with different mutation rates and modes of inheritance provides a window into events on shallow and deep time scales.

Materials and Methods

Sampling

A 2 cm^2 piece of from near the basal meristem was excised at low tide in spring and summer from sporophytes growing in wave-protected waters, were damp dried with paper towels and dessicated for storage with silica beads soon after collection. Plants at least 1 m apart were sampled to avoid sampling related plants. Bone dry samples of kelp were transferred to 2 X 2 inch zip-loc bags, together with dried silica beads, and labeled for archiving.

DNA extration

DNA was extracted from about 10–20 mg of dried tissue with the NucleoSpin® 96 Plant II (Macherey-Nagel Inc., Düren, Gernabt) kit. The standard extraction kit protocol was followed, except dried subsamples were homogenized at room temperature by crushing or chopping on weighing paper with a scalpel. Samples were then funneled into their microtubes on a 96-well plate using a custom jig with manually pipetted PL1 lysis buffer (cetyltrimethylammonium bromide (CTAB) method). The homogenates were incubated in PL1 lysis buffer at 65°C overnight. Mucus on the surface of a frond fragment sometimes interfered with extraction, and we found that ample amounts of DNA for PCR could be obtained by diluting the extraction solution.

Organellar DNA amplificaton

A segment of *COI* at the 5P end of the gene was amplified with PCR using the forward primer *GazF2* (5’ CCAACCAYAAAGATATWGGTAC 3’) and reverse primer *GazR2* (5’ GGATGACCAAARAACCAAAA 3’) (Lane *et al*. 2007). A segment of *rbc*L was amplified with PCR using the forward primer *rbc*L-*543F* (5’ CCWAAATTAGGTCTTTCWGGWAAAAA 3’) (Bittner *et al*. 2008; Silberfeld *et al*. 2010) and reverse primer *rbc*L-*1381R* (5’ ATATCTTTCCATARRTCTAAWGC 3’) (Burrowes *et al*. 2003; Silberfeld *et al*. 2010). DNA was diluted 100-fold in deionized water before amplification by PCR. A PCR cocktail consisted of a 50 µL mixture of 2.0 µL diluted template DNA in 1x Colorless GoTaq Flexi buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1µM of forward and reverse primers, and 2.5U GoTaq Flexi DNA polymerase. The cocktails were thermo-cycled in a Gene Amp PCR System 9700 (Applied Biosystems, Inc., Foster City, CA) with an initial denaturation at 94^{o} C for 3 min, followed by 35 amplification cycles of 45 s at 94^{o} C, 1 min at primer annealing temperature 50^{o} C for *COI* and 52^{o} C for *rbc*L, and 1 min 30 s at 72^{o} C, with a final 5 min at 72^{o} C.

PCR amplified DNAs were sequenced in the forward and reverse directions by Genewiz Inc. (South Plainfield, NJ), or by the University of Arizona Genetics Core. Forward and reverse-complement sequences were aligned and edited with MEGA 7.0.20 (Kumar *et al*. 2016) and chromatograms viewed with Finch TV 1.4.0 (Geospiza Inc.). After editing, a 645 bp fragment of *COI* and a 735 bp fragment of *rbcL* were used for population analyses. Quality control consisted of re-extracting and re-sequencies unique haplotypes from each of the 96-well plates.

Microsatellite genotyping

We used a suite of 12 microsatellite loci previously developed for North Atlantic *Saccharina latissima*, including *SLN32*, *SLN320*, *SLN34*, *SLN35*, *SLN36*, *SLN54*; *SLN58*, *SLN62, SLN314*, *SLN319*, *SLN510* and *SLN511* (Paulino *et al*. 2016). The polymerase chain reaction (PCR) was used to amplify microsatellite alleles with a Gene Amp PCR System 9700 (Applied Biosystems, Inc., Foster City, CA). Each 10 µL reaction cocktail consisted of 2 µL template DNA diluted 4-fold in deionized water mixed with (~0.1µg/µL) 1x Colorless GoTaq Flexi Buffer (Promega Inc.

Madison, WI), 1.5–3.0 mM $MgCl_2$ (Promega Inc. Madison, WI), 0.20 mM of each nucleotide (Applied Biogsytems, Inc.), 0.05–0.25 µM of forward and reverse primers, 0.1 mg/mL of BSA (Sigma Inc. St. Louis, MO), 0.05 U GoTaq Flexi DNA polymerase (Promega Inc. Madison, WI), and deionized water. Optimal thermal cycling profiles varied among loci (Table S2). Microsatellites were fractionated by size using electrophoresis in an Applied Biosystems 3730 capillary DNA sequencer. Genotypes were scored with GeneMapper 5.0 (Applied Biosystems) independently by two technicians. A subset of 8% of the samples was re-extracted and re-genotyped by a third technician for quality control.

Statistical analyses

We used ARLEQUIN 3.5.2.2 (Excoffier & Lischer 2010) to estimate the number of polymorphic nucleotide sites, N_{poly}, the number of observed, N_{H}, and expected, N_{EH}, number of haplotypes under neutrality. ARLEQUIN was also used to estimate gene diversity, *h* (standard deviation), and nucleotide diversity, θ_π (standard deviation). Divergence between populations was estimated with *F* statistics (Weir & Cockerham 1984) and with Φ_{ST} with an appropriate mutation models in ARLEQUIN. Appropriate mutation models for the various datasets were determined with MEGA 7 (Kumar *et al*. 2016). IBD 1.52 (Bohonak 2002) was used to test for isolation-by-distance with Mantel's test between difference matrices of pairwise genetic distsances with correction for diversity $[\Phi_{\mathrm{ST}}/(1\text{-}\Phi_{\mathrm{ST}})]$ in organellar DNA or $[F_{\mathrm{ST}}/(1 - F_{\mathrm{ST}})$ for microsatellites and approximate shoreline distances between samples. Tests were made with geographic distances either with or without a log transformation and coefficients were esimated with 1000 randomizations.

We estimated migration between populations with the island model of migration to estimate the number of migrants between populations with $F_{\mathrm{ST}} = 1/(4Nm + 1)$, or $Nm = 0.25[(1/F_{\mathrm{ST}}) - 1]$ by assuming drift-migration equilibrium, no mutation, and a large number of populations exchanging individuals (Slatkin & Voelm 1991). We used Analysis of MOlecular VAriation (AMOVA) in ARLEQUIN to explore geographical structure in the distributions of haplotype frequencies using multiple groupings of samples based on geography. We recognized these tests will be confounded by the scattering of distinct genealogical lineages (ancestral polymorphisms) among the samples that arose on long time scale.

We made an initial analysis of the microsatellite genotype data with GENEPOP to search for null alleles. When these results indicated the presence of null alleles, we used MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to further confirm the presence of null alleles, and to determine whether allele stuttering or large-allele dropout had affected a dataset. Some loci failed to amplify with PCR in some individuals, even though amplifications were successful at other loci. These PCR failures were assumed to be homozygous genotypes of null alleles.

GENEPOP 4.6 (Rousset 2008) was used to test for deviations from Hardy-Weinberg genotypic proportions, using Markov-chain-Monte-Carlo chains 10,000 steps in 100 batches. Because of the repeated tests aong loci we used a Bonferroni correction (Rice 1989) of $P = 0.05/12 = 0.004$ to control type I error at $\alpha = 0.05$. We used GENETIC DIVERSITY ANALYSIS to estimate observed (H_O) and exptected (H_E) heterozyzygosity averaged over loci, to count the number of alleles at each locus and to estimate the inbreeding coeficient, F_{IS}. FSTAT (Goudet 1995) was used to estimate allelic richness based on the smallest sample size to be able to compare levels of diversity among samples of different sizes.

Microsatellite divergence between populations was estimated with F_{ST} and R_{ST} using GENEALEX 5.03 (Peakall & Smouse 2006, 2012) with 9999 permutations to establish departures from 0.0. F_{ST} is based on allele frequencies and R_{ST} uses allelic sizes in addition to allele frequencies. The number of migrants between pairs of populations per generation was estimated from F_{ST} with Wright's island model of migration $FST = 1/(4Nm + 1)$. GENEALAX was also use to define principal components (PCA) of allele-frequency variability among samples with standardized covariances and to search for genetic differences among samples using AMOVA. The model for AMOVA was framed by the geography of the samples and was the same model used for the analyses of organellar DNA. Missing genotypes for some loci in some plants were estimated with sample averaging for both the PCA and AMOVA. Results for each locus were summed across loci under the assumption of linkage equilibrium and independence among loci. To establish signficcance of AMOVA structure, we used 9999 permutations to shuffle individuals among samples and regions.

GENEPOP 4.6 (Rousset 2008) was used to test for deviations from Hardy-Weinberg genotypic proportions, using Markov-chain-Monte-Carlo chains 10,000 steps in 100 batches. Because of the repeated tests among loci we used a Bonferroni correction (Rice 1989) of $P = 0.05/12 = 0.004$ to control type I error at $\alpha = 0.05$. We used GENETIC DIVERSITY ANALYSIS to estimate observed (H_O) and exptected (H_E) heterozyzygosity averaged over loci, to count the number of alleles at each locus and to estimate the inbreeding coeficient, F_{IS}. FSTAT (Goudet 1995) was used to estimate allelic richness based on the smallest sample size to be able to compare levels of diversity among samples of different sizes.

Results

The analyses of cytochrome oxidase I-5P (*COI*) and Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) were made separately to facilitate comparisons with results of other studies. The sequences for the two genes concatenated for additional analysis. Sample sizes for the concatenated sequences were slightly smaller than for the individual genes, because both genes in some plants could not be successfully sequenced.

Concatenated COI *and* rbc*L*

The sequences of *COI* and *rbc*L were concatenated to combine the information in both genes. Sample sizes ranged from 6 to 80 plants and averaged 31.9 plants (Table S11). Polymorphisms at 13 nucleotide sites defined 16 haplotypes distributed among samples (Table 3, Figure 1e). The number of haplotypes expected under neutrality was 14.77. The *rbc*L lineage 2 (‘B’, blue) was resolved into two geographically separated sub-lineages (‘B2’, light blue), one occurring at Port Moller (sample 2) and the other in Kachemak Bay (samples 5 & 6) (Fig. 1f). Gene diversity (*h*) ranged from 0.0 in 5 samples to 0.574 in sample 6 and was 0.781 (SD = 0.012) overall in a pooled sample. Nucleotide diversity (θ_π) ranged from 0.0 to 0.00047 and was 0.0009 (SD = 0.0006) overall. Tajima’s *D* was -0.868 and was not signficant ($P = 0.208$).

We found 12 private haplotypes among 7 of the samples (Table 1). Genetic divergences (Φ_{ST}) between populations ranged from 0.0 between populations fixed for the same haplotype to 0.968 between populations 8 (Cordova) and 11 (Auke Bay), which did not share haplotypes (Table 2). Nearly all of the samples showed significant haplotype-frequences differences from one another, except for samples that were fixed or nearly fixed for the same haplotype. No significant isolation-by-distance appeared among the samples between genetic [$\Phi_{ST}/(1-\Phi_{ST})$] and geographic shore-line distance with distances either as km (Mantel’s $r = 0.138$, $P = 0.162$) or as log(km) ($r = 0.150$, $P = 0.118$) (Figure 2).

Microsatellites

The 12 microsatellite loci had 4–17 alleles and averaged 10.7 alleles (Tables S12, S13). Allelic richness ranged from 1.00 to 9.39 and averaged 5.06. Values of observed heterzygosity (H_O) ranged from 0.032 to 0.602 and averaged 0.347. Expected heterozygosity (H_E) ranged from 0.054 to 0.615 and average 0.559. H_E exceeded H_O for all of the loci, which produced positive values of the inbreeding coefficient for each locus, ranging from -0.086 to 0.372 and averaging 0.070 among loci.

Average population sample sizes over loci ranged from 25.7 to 85.8 and averaged 32.3 (Table 3). The number of observed alleles ranged from 1.67 to 4.50 among samples and averaged 3.59 alleles. Allelic richness ranged from 1.52 in the western most sample (1, Unalaska) to 4.16 in nearby sample 2 (Port Moller) and average 3.31. The number of private alleles ranged from 0 to 16 and average 3.7 per sample. Observed heterozygosities (H_O) ranged from 0.125 in sample 1 to 0.445 in sample 2 and averaged 0.348. Expected heterozygosities (H_E) were marginally larger in most samples, ranging from 0.0132 to 0.460 and averaging 0.360. This produced small but positive inbreeding coefficients (F_{IS}) in most samples. F_{IS} ranged from -0.052 (heterozygte excess) to 0.246 (heterzogote deficit) and averaged 0.046. The genotypes were examined further without modification, as GENEPOP indicated only a few null alleles were present among samples.

Variability among populations was examined in several ways. F_{ST} between populations varied from 0.010 between samples 5 (Homer) and 6 (Humpy Creek) from Kachemak Bay to

0.648 between samples 1 (Nateen Bay) and 9 (Cordova) (Table S15, lower triangle). All of the pairwise comparisons were significantly greater than 0.0 ($P < 0.0001$), except between samples 5 and 6 from Kachemak Bay ($P = 0.054$). Overall, $F_{ST} = 0.360$ among samples. AMOVA indicated that 36% of the variability was due to allele-frequency differences between populations, 2% was due to differences between plants within populations, and 62% was due to variability within plants (Table S16).

We tested for isolation by distance among populations comparing F_{ST}'s and shoreline distances between samples. Mantel's correlation was $r = 0.410$ ($P < 0.001$) and was consistent with a model of moderate step-wise isolation between populations (Figure 2b). The number of migrants between populations (*Nm*) was estimated from the observed values of F_{ST} and the island model of migration and was generally less than one (*Nm* = 0.190 to 1.14), except between (Table S15, upper triangle), except between populations 5 and 6 (*Nm* = 25.56) and between populations 12 and 13 (*Nm* = 2.33).

Estimates of divergence between populations with R_{ST} incorporate mutational distances between alleles as well as allele-frequency differences between populations, by assuming step-wise changes in allelic size. As expected, R_{ST} was larger for a given comparison than F_{ST}, ranging from 0.041 between samples 5 and 6 to 0.648 between samples 1 and 8 (Table S17). AMOVA of R_{ST} indicated that 41% of the total variability was due to allele-frequency and allelic divergences among populations, 7% was due to differences among plants within populations on average, and 52% was due to within plant diversity on average (Table S18).

Finally, a principal components analysis of genotypic variability among plants was made to search for geographic patterns in the distribution of microsatellite alleles among samples and lineages. Axes 1 and 2 accounted for 25.1% of the variability among plants in the 13 samples (Figure 3). Axis 3 accounted for only an additional 6.6% of the variability and was ignored in our presentation. The PCA showed several clusters (Figure 3a), from which we extracted the *COI-rbc*L haplotype lineages and placed them in separate graphs for clarity. Colours of closed circles representing individual plants in Figure 3 correspond to lineage colours in Figure 1.

Individuals in lineage A (red) fell into three groups (Figure 3b): two groups correspond to Yakutat and Tokeen Bay, respectively, but a third group contained plants from widely separated locations in Resolution Bay, Malina Bay Kaguk Bay and Harris Island. Lineage B1 plants from Port Moller (sample 2) form a single PCA cluster (Figure 3c), and lineage B2 (light blue) included plants from Homer Spit and Humpy Creek (Figure 3d). Lineage C (yellow) contained three micdrosatellite groups. One small group encompassed plants from Malina Bay, which were in the same PCA space as Malina Bay plants in lineage A. Lineage-C includes plants from Homer Spit and Humpy Creek, which are not in the same microsatellite PCA space as lineage B2 plants from these two localities. A third cluster included plants from Nateen Bay (sample 1) and distant Kaguk Bay (sample 14) in Southeastern Alaska. Lineage D1 and D2 plants clustered into two groups: one included plants from Cordova and a second included lineage D1 and D2 plants from Kuiuk Bay (Figure 3e). Lineage E included plants from Auke Bay and formed a unique microsatellite PCA group.

Log-likelihood assignments of individuals to populations showed 100% assignments to the population of origin for 4 of the 13 populations (Table S19). Mis-assignments of up to 3 plants occurred for 7 populations and large mis-assignments occurred between populations 5 and 6, which are both located in Kachemak Bay about 20 km from each other.

Discussion

The results of our multi-locus study show considerably more genetic variability among populations than was postulated previously in studies of *COI* variability based on a sample of 20 from British Columbia (Neiva *et al*. 2018). Populations of sugar kelp around the Gulf of Alaska show multiple levels of divergence that reflect both contemporary barriers to gene flow and divergences during ice-age isolations. Fixed organellar DNA haplotype differences between some populations produced a large overall level of divergence between populations ($\Phi_{ST} = 0.774$, $P < 0.0001$), but these populations did not show isolation by distance (IBD) ($r = 0.151$, $P = 0.132$). This haplotype variability most likely reflects ice-age isolations and and post-glacial founder effects. Microsatellite allele frequencies also varied strongly among populations ($R_{ST} = 0.411$, $P < 0.0001$) and produced a significant correlation between genetic and geographic distance between populations, supporting a model of contemporary IBD ($r = 0.410$, $P < 0.001$). Before discussing these contrasting signals of population structure in more detail, we offer cautions and strengths of our study.

Our study represents a snapshot of population structure in a species with a dynamic metapopulation structure similar to patterns of local extinctions and colonization in the North Atlantic populations of this species (Bekkby & Moy 2011; Moy & Christie 2012). Ecological studies and monitoring of genetic change are needed to gauge the rates of turnover of local populations in Alaskan waters. Since our goal was to discern regional patterns of diversity, we increased geographic coverage at the expense of using smaller sample sizes than is customary in fishery population genetics, where greater precision is required for estimating population parameters. Small sample sizes were justified because previous studies of kelps showed strong, often fixed differences between populations (Zhang *et al*. 2015), and our results bore this out. Under these conditions, the samples sizes of 25–40 plants are large enough to provide a sufficient amount of statistical power to detect large frequency differences between populations, if they exist. For microsatellite loci, statistical power is influenced by both sample size and the number of alleles at a locus. Again, the results show relatively fine-scale structure indicating that sample sizes and the number of loci examined were adequate to address the goals of the study.

Small sample sizes, however, cannot be used to capture some aspects of population structure and history. For example, our samples cannot be used to estimate allele-frequency spectra because of the inability to detect low-frequency alleles (Grant 2015). Comparisons of observed allele-frequency specta with theoretical models are commonly used to infer historical demographies, but the small sample sizes do not allow us to recontruct an accurate picture of some aspects of population history with several of the coalescent programs available (Grant *et al*. 2016).

We did not use programs such as BEAST (Drummond & Rambaut 2007) and IMa (Hey & Nielsen 2007) to estimate historical effective population sizes or migration because the assumptions in these models do not fit the type III ecologies of kelps. As with many marine organisms, kelps produce large numbers of offspring most of which do not survive to maturity, and this leads to a large variance in reproductive success called 'reproductive skew' (Grant *et al*. 2016). The Kingman 'n-coalescent' (Kingman 1982a, b, c) is a key theoretical tool used to estimate such parameters as effective population size and gene flow, but it is base on the Wright-Fisher forward model of evolution which is inappropriate for marine species with reproductive skew (Eldon & Wakeley 2006). The use of these programs can lead to serious mis-interpretations of patterns of genetic variability (Niwa *et al*. 2016; Matuszewski *et al*. 2018).

One strength of our study is the use of genetic markers with different modes of inheritance and mutation rates. Together, these markers provide insights into deep and shallow genetic population structure in sugar kelp and help to mitigate the large evolutionary variance among markers (Karl *et al*. 2012). Maternally inherited, non-recombining organellar genes can be used to reconstruct molecular genealogies that contain information about population history (Li *et al.* 2016). The use of bi-parentally inherited microsatellite DNA provides additional information about breeding structure and population connectivity. The *COI* and *rbc*L genes are inherited independently of each other and independently of microsatellite markers encoded in nuclear DNA, so together these markers provide a multifaceted view of population structure. Another advantage of using different marker types is that different mutation rates enable us to detect imprints of population events on different temporal scales. Microsatellites have larger mutation rates than organellar genes and hence can potentially resolve contemporary population dynamics. The smaller mutation rates of the two organellar genes can potentially resolve population events deeper in time.

COI-rbc*L Phylogeography*

For most macroalgal species, *COI* shows greater amounts of diversity than *rbc*L. However, in our study, 12 of the 14 locations we sampled were fixed or nearly fixed for the *COI*-A haplotype and had an overall haplotype diversity of only $h = 0.044$. In contrast, *rbc*L showed greater overall diversity, with six populations fixed or nearly fixed for haplotype *rbc*L-A, two populations fixed for haplotype *rbc*L-C and one population nearly fixed for *rbc*L-B, yielding an overall haplotype diversity of $h = 0.702$. This reversal in expectation may be due to the 5'-section of the *COI* gene that we used in our analyses. Balakirev *et al*. (2012) have shown that the

5' 'barcode' end of *COI* in *S. latissima* shows much less variability thant the 3' end of the gene. Their results showed average divergence between *S. latissima* and other species of *Saccharina* was $d = 0.038$ for the COI-5P end (~740 base pairs), but $d = 0.158$, nearly five times greater, for the COI-3P end (~862 bp). *COI*-5P is used in most population genetic studies for ease of comparison among studies and for use as a species barcode, but the *COI*-3P end may be more informative about phylogeographic structure in *Saccharina*.

The combined haplotypes of *COI* and *rbc*L showed strong frequency differences among locations ($\Phi_{ST} = 0.774$, $P < 0.0001$, Table S16, Figure 1f). This level of differentiation is much larger than that between populations of sugar kelp in the North Atlantic (Table 4). Ten of the 14 populations we sampled were fixed or nearly fixed for a single haplotype. Six of these populations had low-frequency private haplotypes that likely arose from recent mutations in the present-day populations. The remaining 4 populations had intermediate frequencies of two haplotypes separated by only one mutation. A question of interest is whether plants in these 4 populations are reproductively isolated from plants bearing other haplotype lineages at the same location. If the two lineages were reprodictively isolated, we would expect to find a strong deficits in the proportion of heterozygotes (Wahlund's effect) at the microsatellite loci for these populations, and this was not the case (Table 3). In the PCA of microsatellite variability, plants in different organellar lineages at the same collection sites (Kuiuk, Homer, Humpy & Kaguk) occupied the same areas, respectively, in the ordination of PCA axes 1 and 2 (Figure 3).

Plants at a few sites were fixed for a single 'private' allele not seen elsewhere, while other sites harboured divergent DNA haplotypes lineages. This mixing of lineages may compromised estimates of some population parameters. For example, estimates of diversity are artificially inflated and do not reflect the dynamics of a single population. The mixing of haplotypes may have origins in historical colonizations by the divergent lineages, or may result from contemporary gene flow from other populations.

Genetic connectivity between populations varies considerably. While adjacent populations tended to show similar haplotype frequencies, for example Homer-Humpy, Kodiak-Resurrection Bay, and Harris Island-Tokeen Bay, the correlation between genetic and geographic distance between samples was not significant ($r = 0.151$, $P = 0.123$, Figure 2a). Estimates of immigration (Nm) per generation from the island model of migration ranged from 0.0 between populations fixed for different haplotypes to infinity between populations that shared haplotypes, but were generally less than 1.0 between pairs of the other populations (Table 2). These estimates have to be interpreted cautiously, as it is uncertain that populations of sugar kelp in the Gulf of Alaska have reach drift-migration equilibrium after post-glacial colonizatons began about 12,000 years ago. Estimates for populations out of equilibrium tend to underestimate actual levels of gene flow (Slatkin 1993). Nevertheless, it is clear that dispersal is limited and that some populations are completely isolated from other populations.

The chaotic distribution of haplotypes cannot be easily explained. The distribution of haplotypes among localities may reflect historical isolations in ice-age refugia followed by post-glacial expansions and colonizations in newly available habitats. The question is whether the refugia were located in unglaciated patches in the north or along unglaciated shores off Washington, Oregon and California. The hypothesis of a southern refuge would be supported by finding shared haplotypes between Gulf of Alaska and southern populations. A step-wise post-glacial expansion into Alaska from a southern refuge might also have produced a gradient in gene diversity if frontier populations were small (Hewitt 2000). A limited amount of *COI* data indicates low diversities in southern populations, in which haplotype A predominates (Nieva *et al*. 2018). If further samples of shows that in fact southern populations are less diverse than northern populations, then northern glacial refugia are more likely. A better test of these hypothesis would be based on the more polymorphic *rbc*L marker, but no sequences are yet available for southern populations.

Microsatellites

The bi-parental inheritance of microsatellite loci encoded in the nucleus provides another view of genetic structure within and among Alaska's sugar kelp populations. Expected heterozygosities (H_E) exceeded observed heterozygosities (H_O) in 9 of the 13 samples produced inbreeding coefficients ranging from -0.052 to 0.245 and averaging 0.044. A single large F_{IS} (0.245) appeared in sample 9 (Cordova) and was likely due to missing genotypes. The lack of departures from Hardy-Weinberg proportions indicates that null alleles, if present, occurred at low frequencies and did not compromise the analyses. The high level of correct individual assignments back to originating populations also gives confidence in these microsatellite data (Table S18).

The use of the same microsatellite loci from other studies of *Saccharina latissima* provides an opportunity to compare levels of diversity and divergence among populations in different ocean basins (Table 5). The use of the same loci, or a large number of loci, averages out differences in mutations rates among loci and provides a rationale for making comparisons among studies. Of the 12 loci used in our study, all were used in Nieva *et al.* (2018) and 10 in the study of Breton *et al.* (2017). Other loci were used in Luttikhuizen *et al*. (2018) (n =10) and Guzinski *et al*. (2016) (n = 25). The overall average heterozygosity in Alaskan populations (H_O = 0.357) was similar to heterozygosities in populations of *S. latissima* in the Northwestern (H_O = 0.298, range 0.016–0.665) and Northeastern (H_O = 0.407, range 0.036–0.583) Atlantic. These comparisions show that the overall level of diversity was similar among the three regions, except for a few populations. These similarities among regions likely indicate similar responses to Pleistocene climate variability.

A range of values for the inbreeding coefficient F_{IS} was observed among populations but were similar among regions, except in the study of sugar kelp along the coast of Maine, which included a much smaller area than the other studies (Table 3). In addition to inbreeding within a population, apparent inbreeding can also be caused by the occurrence of null alleles that lead to produce heterozygotes that are mistaken for homozygotes. Since high frequencies of null alleles were not observed in these studies, the inbreeding coefficients can be interpreted in terms of reproductive biology. Values of F_{IS} tended to be larger than 0.0 in most populations indicating more genetic similarity between individuals in a population than would be expected with random mating and with equal contributions of offspring to the next generation. A large variance in the recuitment of offspring among plants generally occurs in kelp, because of the chaotic dispersals of spores and gametes into favorable micro-habitats and becauses of the effects environmental variability on the survivals of early life-history stages, typical of type III species.

Levels of divergence between populations varied considerably for populations around the Gulf of Alaska and showed a shallow, but significant, correlation between genetic and geographical distance (Figure 2b). The lack of divergence between adjacent populations can readily be explained by gene flow between them, as for example between populations 5 and 6 in Kachemak Bay, which appear to be parts of a single population in the bay. The association between genetic and geographical distance, however, weakens between populations separated by geographical distances greater than about 300 km, indicating that population history played a greater role than gene flow in shaping genetic divergences between populations.

Widely scattered populations bearing lineage-A haplotypes from several different sites around the Gulf of Alaska overlap along the 1st and 2nd axes of the PCA of microsatellite variability. The correspondence between *COI*-*rbc*L lineages and groups based on microsatellites (Figure 3) indicates that microsatellite divergences between populations are due to the same historical isolations that led to separations between the organellar DNA lineages and not to contemporary isolations. Populations of sugar kelp around the Gulf of Alaska appear to have not yet reached drift-migration equilibrium since the Last Glacial Maximum.

Phylogeographic, population genetic and ecological tools together are needed to further understand the origins of genetic variability among contemporary populations. Organellar genes allow us to reconstruct ancient population events because of their relatively slow mutation rates. The pattern of variability in the *COI*-*rbc*L lineages prompts the hypothesis of isolations in northern refugia during Global cooling in the last Milankovitch cycle of about 100,000 years. Most phylogeographic studies focus on the effects of the Last Glacial Maximum (LGM) 22.5–18 thousand years ago, but isolations also occurred during stadials over the last 100 thousand years. For about 80% of this time temperatures wer 5–6° C cooler, and global sea levels were at least 50 m below present-day sea levels. As terrestrial glaciers waxed and waned, lobes of ice intermitently reached the coast at several locations. A key feature of this hypothesis posits that these glacial lobes isolated sugar kelp populations along the coast for tens of thousands of years.

The results for populations of *S. latissima* in the North Atlantic Ocean are of particular interest because these populations also experienced a Pleistocene climate history of coastal glaciations. A close association between organellar DNA lineages (*COI*) and population groups, based on microsatellite allele-frequency variability, also appeared on both sides of the North Atlantic (Figure 4 in Luttikhuizen *et al*. 2018; Figure 4 in Neiva *et al*. 2018), indicating the importance of historical isolations among populations in producing contemporary patterns of diversity among populations. Hence, large values of F_{ST} cannot be interpreted entirely in terms of contemproary levels of gene flow. These patterns in the North Pacific and North Atlantic oceans reflect similar responses to climate variability by populations of plants with the same reproductive biologies and dispersal capabilities.

Synthesis

A major challenge is to explain how the patterns of haplotype variability within and among populations arose in the Gulf of Alaska. The genealogies of *COI* and *rbc*L separately and *COI-rbc*L combined are shallow with only 3 mutations at most separating haplotypes. These shallow genealogies are characterstic of evolutionarily young populations (Grant & Bowen 1998) and reflect a turbulent history of coastal glaciations in the late Pleistocene (Manley & Kaufman 2002). The low levels of genetic diversity may be due to the isolation of multiple small regufial populations during glaciations, or to founder effects during post-glacial colonizations. Both events are not mutually exclusive and both can potentially lead to the loss of genetic diversity and a mosaic population structure.

The juxtoposition of the COI-*rbc*L lineages with microsatellite variability in the PCA showed that opulations within a particular *COI-rbc*L lineage tended to have similar microsatellite allele frequencies, regardless of location along the coast (Figure 3). The microsatellite allele-frequency similarity between these populations separated by several hundred kilometers in some cases is unlikely to be due to on-going gene flow, given the poor ability of kelp meiospores and gametes to disperse long distances (Anderson & North 1966; Gaylord *et al*. 2002, 2004). Spore dispersal generally follows a log-normal distribution, so that most spores settle within a few meters of the parent plant (Stein *et al*. 1995; Gaylord *et al*. 2006). The chaotic geographic distributions of the various lineages imply indepent dispersals for the lineages. Since mutation rates of organellar gene are smaller than microsatellite mutation rates, the genetic structure imprinted in organellar genes must predate the structure apparent in the microsatellite data. The microsatellite genetic homogeneity among geographically separated populations around the Gulf of Alaska implies a recent dispersal from a common ancestral population after the *COI-rbc*L lineages were established.

The genetic pattern we have resolved among populations of sugar kelp is transient. Large genetic distances from related species of *Saccharina* indicate that sugar kelps have existed as a separate lineage for about 7 million years (Starko *et al*. 2019), yet the population structures revealed by mitochondrial and chloroplast DNA are shallow, implying recent origins of only tens of thousands of years. Repeated episodes of disturbance during Pleistocene ice ages appear to lead to the periodic extirpations of some lineages and later geographical expansions of the surviving lineages. The temporal time framework of this turmoil appears to coincide with the stadials and interstadials of the last glaciation, which began about 110 thousand years ago following the last warm interglaical period 125,000 to 135,000 years ago (Rasmussen *et al*. 2014; Li & Bor 2019). As a result, genetic patterns among populations in the Gult of Alaska likely differ from one glacial cycle to the next.

Acknowledgements

Donna Aderhold, Robert Baer, Asia Breder, Jess Colthorp, Ben Daly, Lisa Fox, Heather Liller, Mandy Lindeberg, Mark Patterson, Sunny Rice, Philip Tscherisich, Scott Walker, Miranda Westphal, Carrier Worton, and Eric Wyatt helped to collect samples through out the State of Alaska. Judy Berger catalogued the samples and Eric Lardizabal maintain the database of sample information. Paul Kuriscak, Mariel Terry, Zach Pechacek, Nick Ellickson and Chase Jalbert extracted DNA from the samples. Special thanks to Pat Tester (NOAA, Beaufort, North Carolina) whose support was critical to the funding of this project. This project was funded by the North Pacific Research Board Project 1618 and in part by the general fund of the Alaska Department of Fish and Game.

References

Alberto F, Raimondi PT, Reed DC, Coelho NC, Leblois R, Whitmer A, Serrão EA (2010) Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. *Ecology*, **91**, 49–56.

Alberto F, Raimondi PT, Reed DC, Watson JR, Siegel DA, Mitarai S, Coelho N, Serrao EA (2011) Isolation by oceanographic distance explains genetic structure for *Macrocystis pyrifera* in the Santa Barbara Channel. *Molecular Ecology*, **20**, 2543–2554.

Anderson EK, North WJ (1966) January. In situ studies of spore production and dispersal in the giant kelp, Macrocystis. In *Proceedings of the Fifth International Seaweed Symposium, Halifax, August 25–28, 1965*, pp. 73–86. Pergamon Press.

Balakirev ES, Krupnova TN, Ayala FJ (2012) DNA variation in the phenotypically-diverse brown alga *Saccharina japonica*. *BMC Plant Biology*, **12**, e108.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J. 2008. The genus *Laminaria sensu lato*: recent insights and developments. *European Journal of Phycology*, **43**, 1–86.

Bekkby T, Moy FE (2011) Developing spatial models of sugar kelp (*Saccharina latissima*) potential distribution under natural conditions and areas of its disappearance in Skagerrak. *Estuarine*, *Coastal and Shelf Science*, **95**, 477–483.

Bigg GR (2014) Environmental confirmation of multiple ice age refugia for Pacific cod, *Gadus macrocephalus*. *Evolutionary Ecology*, **28**, 177–191.

Bigg GR, Cunningham CW, Ottersen G, Pogson GH, Wadley MR, Williamson P (2007) Ice-age survival of Atlantic cod: agreement between palaeoecology models and genetics. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 163–173.

Bittner L, Payri CE, Couloux A, Cruaud C, de Reviers B, Rousseau F (2008) Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **49**, 211–226.

Bohonak AJ (2002) IBD (Isolation By Distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93**, 153–154.

Breton TS, Nettleton JC, O'Connell B, Bertocci M (2018) Fine-scale population genetic structure of sugar kelp, *Saccharina latissima* (Laminariales, Phaeophyceae), in eastern Maine, USA. *Phycologia*, **57**, 32–40.

Burrowes R, Rousseau F, Müller DG, de Reviers B (2003) Taxonomic placement of *Microzonia* (Phaeophyceae) in the Syringodermatales based on the rbcL and 28S nrDNA sequences. *Cryptogamie. Algologie*, **24**, 63–73.

Canino MF, Spies IB, Cunningham KM, Hauser L, Grant WS (2010) Multiple ice-age refugia in Pacific cod, *Gadus macrocephalus*. *Molecular Ecology*, **19**, 4339–4351.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, e214.

Eldon B, Wakeley J. 2006. Coalescent processes when the distribution of offspring number among individuals is highly skewed. *Genetics*, **172**, 2621–2633.

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Gaylord B, Reed DC, Raimondi PT, Washburn L, McLean SR (2002) A physically based model of macroalgal spore dispersal in the wave and current-dominated nearshore. *Ecology*, **83**, 1239–1251.

Gaylord B, Reed DC, Washburn L, Raimondi PT (2004) Physical-biological coupling in spore dispersal of kelp forest macroalgae. *Journal of Marine Systems*, **49**, 19–39.

Gaylord B, Reed DC, Raimondi PT, Washburn L (2006) Macroalgal spore dispersal in coastal environments: mechanistic insights revealed by theory and experiment. *Ecological Monographs*, **76**, 481–502.

Goudet JF (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.

Grant WS **(**2015) Problems and cautions with sequence mismatch analysis and Bayesian skyline plots to infer historical demography. *Journal of Heredity*, **106**, 333–346.

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**, 415–426.

Grant WS, Árnason E, Eldon B (2016) New DNA coalescent models and old population genetics software. *ICES Journal of Marine Science*, **73**, 2178–2180.

Guzinski J, Mauger S, Cock JM, Valero M (2016) Characterization of newly developed expressed sequence tag-derived microsatellite markers revealed low genetic diversity within and low connectivity between European *Saccharina latissima* populations. *Journal of Applied Phycology*, **28**, 3057–3070.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hey J, Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences* USA, **104**, 2785–2790.

Karl SA, Toonen RJ, Grant WS, Bowen BW (2012) Common misconceptions in molecular ecology: echoes of the modern synthesis. *Molecular Ecology*, **21**, 4171–4189.

Kaufman DS, Manley WF (2004) Pleistocene maximum and Late Wisconsinan glacier extents across Alaska, USA. *Developments in Quaternary Sciences*, **2**, 9–27.

Kingman JFC (1982a) The coalescent. *Stochastic Processes and their Applications*, **13**, 235–248.

Kingman JFC (1982b) On the genealogy of large populations. *Journal of Applied Probability*, **19**, 27–43.

Kingman JFC (1982c) Exchangeability and the evolution of large populations. pp. 97–112. In: *Exchangeability in Probability and Statistics*, eds, G Koch & F Spizzichino. North Holland, Amsterdam.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870–1874.

Lane CE, Lindstrom SC, Saunders GW (2007) A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Molecular Phylogenetics and Evolution*, **44**, 634–648.

Li C, Bor A (2019) Coupled atmosphere-ice-ocean dynamics in Dansgaard-Oeschgerevents. *Quaternary Science Reviews*, **203**, 1–20.

Li Q, Wang XL, Zhang J, Yao JT, Duan D (2016) Maternal inheritance of organellar DNA demonstrated with DNA markers in crosses of *Saccharina japonica* (Laminariales, Phaeophyta). *Journal of Applied Phycology*, **28,** 2019–2026.

Lindeberg MR, Lindstrom SC (2010) *Field Guide to Seaweeds of Alaska*. Sea Grant College Program, University of Alaska Fairbanks.

Lindstrom SC (2009) The biogeography of seaweeds in southeast Alaska. *Journal of Biogeography*, **36**, 401–409.

Luttikhuizen PC, van den Heuvel FHM, Rebours C, Witte HJ, van Bleijswijk JDL, Timmermans K (2018) Strong population structure but no equilibrium yet: Genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). *Ecology and Evolution*, **8**, 4265–4277.

Maggs CA, Castilho R, Foltz DW, Henzler C, Jolly MT, Kelly J, Olsen JL, Perez KE, Stam WT, Väinölä R, Viard F, Wares JP. 2008. Evaluating signatures of glacial refugia for north Atlantic benithic marine taxa. *Ecology*, **89,** S108–S112.

Manley WF, Kaufman DS (2002) Alaska PaleoGlacier Atlas: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado, instaar.colorado.edu/QGISL/ak_paleoglacier_atlas, v. 1.

Marko PB, Hoffman JM, Emme SA, McGoverrn TM, Keever CC, Cox N (2010) The 'expansion-contraction' model of Pleistocene biogeography: rocky shores suffer a sea change? *Molecular Ecology*, **19**, 146–169.

Matuszewski S, Hildebrandt ME, Achaz G, Jensen JD (2018) Coalescent processes with skewed offspring distributions and nonequilibrium demography. *Genetics*, **208**, 323–338.

Moy FE, Christie H (2012) Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. *Marine Biology Research*, **8**, 357–369.

Neiva J, Paulino C, Nielsen MM, Krause-Jensen D, Saunders GW, Assis J, Bárbara I, Tamigneaux É, Gouveia L, Aires T, Marbà N (2018) Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. *Scientific Reports*, **8**, e1112.

Niwa HS, Nashida K, Yanagimoto T (2016) Reproductive skew in Japanese sardine inferred from DNA sequences. *ICES Journal of Marine Science*, **73**, 2181–2189.

Paulino C, Neiva J, Coelho NC, Aires T, Marbà N, Krause-Jensen D, Serrão EA (2016) Characterization of 12 polymorphic microsatellite markers in the sugar kelp *Saccharina latissima*. *Journal of Applied Phycology*, **28**, 3071–3074.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6,** 288–295.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetics software for teaching and research–an update. *Bioinformatics*, **28**, 2537–2539.

Rasmussen SO, Bigler M, Blockley SP, Blunier T, Buchardt SL, Clausen HB, Cvijanovic I, Dahl-Jensen D, Johnsen SJ, Fischer H, Gkinis V, Guillevic M, Hoek WZ, Lowe JJ, Pedro JB, Popp T, Seierstad IK, Steffensen JP, Svensson AM, Vallelonga P, Vinther BM, Walker MJC, Wheatley JJ, Winstrup M (2014) A stratigraphic framework for abrupt climatic changes during the LastGlacial period based on three synchronized Greenland ice-corerecords: refining and extending the INTIMATE event stratigraphy. *Quaternary Science Reviews*, **106**, 14–28.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* **8,** 103–106.

Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, De Reviers B, Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the "brown algal crown radiation". *Molecular Phylogenetics and Evolution*, **56**, 659–674.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Slatkin M, Voelm L (1991) F_{ST} in a hierarchical island model. *Genetics*, **127**, 627–629.

Starko S, Gomez MS, Darby H, Demes KW, Kawai H, Yotsukura N, Lindstrom SC, Keeling PJ, Graham SW, Martone PT (2019) A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem. *Molecular Phylogenetics and Evolution*, **136**, 138–150.

Thorson RM (1980) Ice-Sheet Glaciation of the Puget Lowland, Washington, during the Vashon Stade (Late Pleistocene) 1. *Quaternary Research*, **13**, 303–321.

Stein F, Sjøtun K, Lein TE, Rueness J (1995) Spore dispersal in *Laminaria hyperborea* (Laminariales, Phaeophyceae). *Sarsia*, **80**, 47–53.

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. E*volution*, **38**, 1358–1370.

Zhang J, Yao JT, Sun ZM, Fu G, Galanin DA, Nagasato C, Motomura T, Hu ZM, Duan DL (2015) Phylogeographic data revealed shallow genetic structure in the kelp *Saccharina japonica* (Laminariales, Phaeophyta). *BMC Evolutionary Biology*, **15**, e237.

All authors contributed to this study. W.S.G. conceived the project, wrote the resarch proposal, designed a sampling scheme, made the statistical analyses and wrote the paper. E.C. commented on the manuscript. W.C., E.C and Z.G. conducted the molecular analyses.

Data accessibility

DNA sequences: GenBank *COI* Accession nos 000000–000000. *rbc*L Accession nos 000000–000000.

Microsatellite genotypes available from the North Pacific Research Board . . .

Supporting information

Addtional supporting information may be found in the online version of this article.

Table 1 *Saccharina latissima*: Estimates of genetic parameters based on contatenated fragments of mitochondrial DNA cytochrome oxidase I (*COI*) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1359 base pairs combined) in samples from the Gulf of Alaska and southeastern Bering Sea. Location number, sample size (N), number of polymorphic nucleotide sites (N_{poly}), number of haplotypes (N_H), expected number of haplotypes under neutrality (N_{EH}), haplotype diversity (h, SD: standard deviation), nucleotide diversity (θ_π, SD: standard deviation) and Tajima's D (P: probability of null hypothesis of neutrality)

Location	N	N_{poly}	N_H	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
1	37	3	2	1.18	1	0.054	0.051	0.012	0.019	-1.722	0.012
2	80	5	4	1.49	4	0.121	0.049	0.026	0.029	-1.423	0.046
3	30	1	2	3.48	1	0.515	0.027	0.038	0.036	1.621	0.976
4	28	0	1	1.00	0	0.0	–	0.0	–	–	–
5	30	1	2	3.06	0	0.460	0.061	0.034	0.034	1.280	0.919
6	31	3	4	4.06	2	0.574	0.048	0.047	0.042	-0.342	0.402
7	27	3	4	1.72	2	0.214	0.103	0.016	0.022	-1.734	0.014
8	6	0	1	1.00	0	0.0	–	0.0	–	–	–
9	22	2	3	1.55	1	0.178	0.106	0.013	0.020	-1.515	0.041
10	29	0	1	1.00	0	0.0	–	0.0	–	–	–
11	26	1	1	1.23	1	0.077	0.070	0.006	0.001	-1.156	0.139
12	23	0	1	1.00	0	0.0	–	0.0	–	–	–
13	31	0	1	1.00	0	0.0	–	0.0	–	–	–
14	46	1	2	3.12	0	0.433	0.055	0.032	0.032	1.239	0.908
Mean	31.9	1.4	2.1	1.85	0.9	0.188	–	0.016	–	–	–
Pooled	446	13	16	14.77	12	0.781	0.012	0.088	0.063	-0.868	0.208

Table 2 *Saccharina latissima*: Lower triangle: genetic distances (Φ_{ST}) (lower triangle) between samples from the Gulf of Alaska and southeastern Bering Sea based on contatenated fragments of mitochondrial DNA cytochrome oxidase I (*COI*) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1359 base pairs) with the Tamura & Nei (1992) model of mutation. Overall, $\Phi_{ST} = 0.774$ among samples. Italics $0.05 > P > 0.01$; Bold $P < 0.01$. Upper triangle: estimates of migration per generation (*Nm*) from the island model of migration.

1	–	0.090	0.075	0.024	0.143	0.170	0.050	0.038	0.045	0.024	0.022	0.026	0.023	0.092
2	0.847	**–**	0.174	0.143	0.535	0.936	0.187	inf	0.189	0.141	0.078	0.150	0.139	0.436
3	**0.870**	**0.742**	–	0.110	0.249	0.303	0.161	0.187	0.905	0.108	0.073	0.121	0.105	0.234
4	**0.954**	**0.778**	**0.819**	–	0.155	0.227	374.824	0.0	0.042	inf	0.020	inf	inf	1.594
5	**0.778**	**0.483**	**0.668**	**0.763**	–	inf	0.241	0.484	0.242	0.152	0.083	0.170	0.147	0.490
6	**0.747**	**0.348**	**0.622**	**0.688**	0.0	–	0.324	1.107	0.335	0.223	0.115	0.250	0.215	0.659
7	**0.909**	**0.727**	**0.757**	0.001	**0.675**	**0.607**	–	0.102	0.111	187.883	0.082	inf	6.217	2.789
8	**0.930**	0.0	**0.728**	**1.000**	*0.508*	0.311	**0.830**	–	0.082	0.0	0.017	0.0	0.0	0.435
9	**0.917**	**0.725**	**0.356**	**0.923**	**0.674**	**0.599**	**0.818**	**0.859**	–	0.041	0.033	0.047	0.039	0.245
10	**0.955**	**0.779**	**0.822**	0.0	**0.767**	**0.692**	0.003	**1.000**	**0.925**	–	0.020	inf	inf	1.567
11	**0.957**	**0.864**	**0.872**	**0.961**	**0.857**	**0.813**	**0.859**	**0.968**	**0.938**	**0.962**	–	0.022	0.019	0.152
12	**0.950**	**0.769**	**0.805**	0.0	**0.746**	**0.667**	0.0	**1.000**	**0.915**	0.0	**0.958**	–	inf	1.748
13	**0.956**	**0.783**	**0.827**	0.0	**0.773**	**0.699**	0.005	**1.000**	**0.927**	0.0	**0.964**	0.0	–	1.517
14	**0.844**	**0.534**	**0.681**	*0.239*	**0.505**	**0.431**	*0.152*	*0.534*	**0.671**	*0.242*	**0.767**	*0.222*	*0.248*	–
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sample number														

Table 3 *Saccharina latissima*: Summary statistics for 12 microsatellite loci in 13 samples from the Gulf of Alaska. N = mean sample size over loci. N_{A} = mean number of alleles. N_{E} = mean number of alleles expected under neutrality. N_{AR} = mean allelic richness. H_{O} = observed heterozygosity. H_{E} = expected heterozygosity. F_{IS} = inbreeding coefficient. *Pri* = number of private alleles. Total estimates for pooled sample. N_{AR} for the pooled sample was estimated with resamples of n = 344.

Sample	N	$\mathrm{N_A}$	$\mathrm{N_{AR}}$	*Pri*	H_O	H_E	F_{IS}
1	36.5	1.67	1.52	1	0.125	0.132	0.059
2	85.8	6.17	4.16	16	0.445	0.460	0.033
3	27.6	2.50	3.39	0	0.346	0.347	0.003
4	31.8	3.58	3.41	4	0.419	0.400	-0.050
5	30.1	4.42	3.96	2	0.368	0.376	0.096
7	23.3	3.75	3.67	0	0.365	0.467	0.031
8	20.4	4.00	3.82	3	0.458	0.358	0.019
9	16.7	4.17	4.01	4	0.272	0.265	0.245
10	25.7	2.58	2.33	2	0.261	0.405	0.017
11	31.3	2.83	2.75	4	0.425	0.356	-0.052
12	27.0	4.50	4.10	8	0.399	0.436	0.085
13	26.2	3.25	3.00	2	0.342	0.355	0.038
14	37.7	3.25	2.94	2	0.298	0.321	0.071
Mean	32.3	3.59	3.31	3.7	0.348	0.360	0.044
Pooled	419.9	10.67	123.57	48	0.357	0.558	0.039

Table 4 Organellar DNA (mitochondrial & chloroplast) variability in populations of *Saccharina latissima* and *S. japonica*

Region	No. Pops	No. haplotypes	*h* (range)	θ_π (range)	F_{ST} (range)	Reference
S. latissima						
NE Pacific	14	14	0.781	0.0009	0.774	This study
			0.0–0.574	0.0–0.005	0.0–1.0	
NE Atlantic	7	7	–	–	0.318	Luttikhuizen *et al*. 2018
			0.0–0.325	0.0–0.0005	0.0–1.000	
S. japonica						
Japan	11	35	0.725	0.0008	–	Zhang *et al*. 2015
			0.177–0.762	0.0001–0.0016		
Russia	11	25	0.585	0.0004	–	Zhang *et al*. 2015
			0.0–0.600	0.0–0.0006		
Korea	1	5	0.033	0.0003	–	Zhang *et al*. 2015
China	3	4	0.553	0.0005	–	Zhang *et al*. 2015
			0.0–0.722	0.0–0.0007		

Table 5 Microsatellite variability in *Saccharina latissima* and *S. japonica*. Values of H_O, F_{IS} and F_{ST} are based on a pooled sample and represent overall values. The range of values, when available, is given below.

Region	No. pops	No. loci	H_O (range)	F_{IS} (range)	F_{ST} (range)	Reference
Saccharina latissima						
NE Pacific	13	12	0.357	0.377	0.360	This study
			0.125–0.458	-0.052–0.246	0.010–0.648	
	2	12	0.312–0.326	0.040-0.140	–	Nieva *et al*. 2018
NW Atlantic	6	10	0.298	0.035	0.016	Breton *et al*. 2017
			0.016–0.665	-0.072–0.220	0.0–0.032	
	14	12	0.146–0.466	-0.050-0.519	–	Nieva *et al*. 2018
NE Atlantic	7	10	0.407	0.324	0.268	Luttikhuizen *et al*. 2018
			0.036–0.387	-0.010–0.173	0.050–0.469	
	7	12	0.296-0.583	-0.047-0.146		Nieva *et al*. 2018
	6	25	0.204-0.330	–	0.077-0.562	Guzinski *et al*. 2016
Saccharina japonica						
NW Pacific	15	11	–	–	0.0–0.762	Shan *et al*. 2017

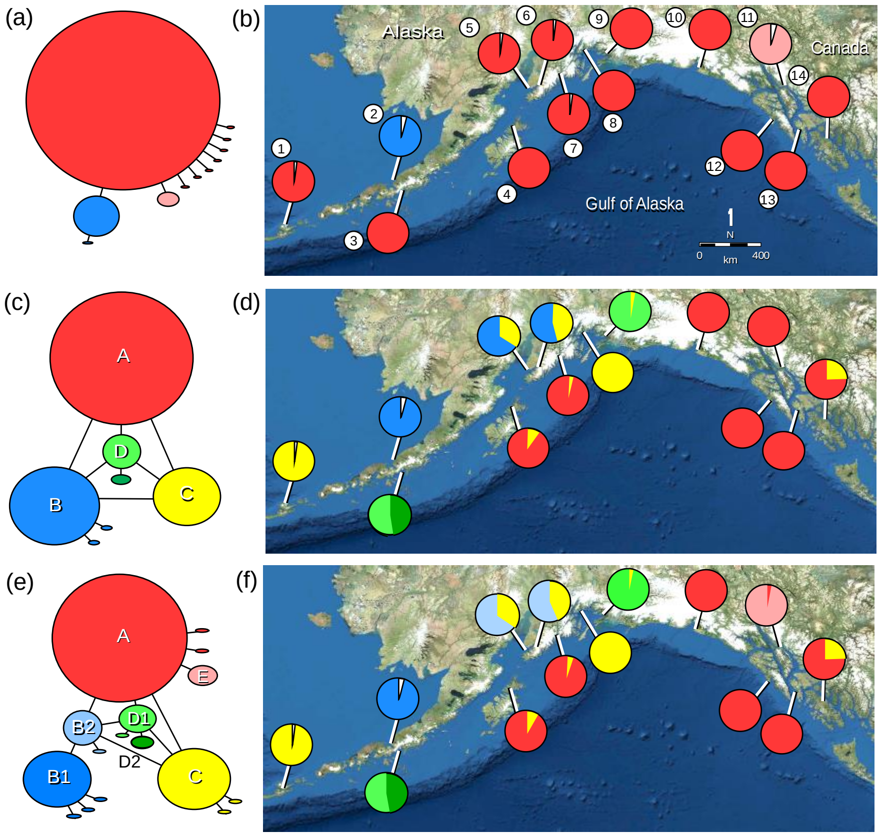

Figure 1 Haplotype networks and haplotype frequency distributions among samples of *Saccharina latissima*. (a, b) Cytochrome oxidase I (*COI*) (624 base pairs). (c, d) ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base pairs). (e, f) concatenated *COI* and *rbc*L sequences (1359 base pairs).

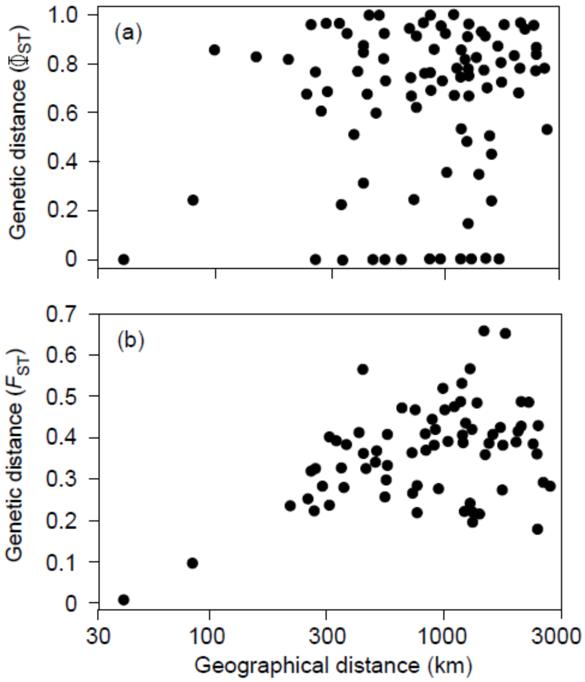

Figure 2 *Saccharina latissima*: Isolation by distance among populations in the NE Pacific with geographic distance transformed onto a log scale. (a) Concatenated sequences of *COI* and *rbc*L: Mantel's test of difference matrices of $\Phi_{ST}/(1 - \Phi_{ST})$ and geographical distance, $r = 0.151$, $P = 0.132$. (b) Microsatellite DNA: Mantel's test of difference matrices of $F_{ST}/(1 – F_{ST})$ between samples and geographical distance, $r = 0.410$, $P = 0.001$.

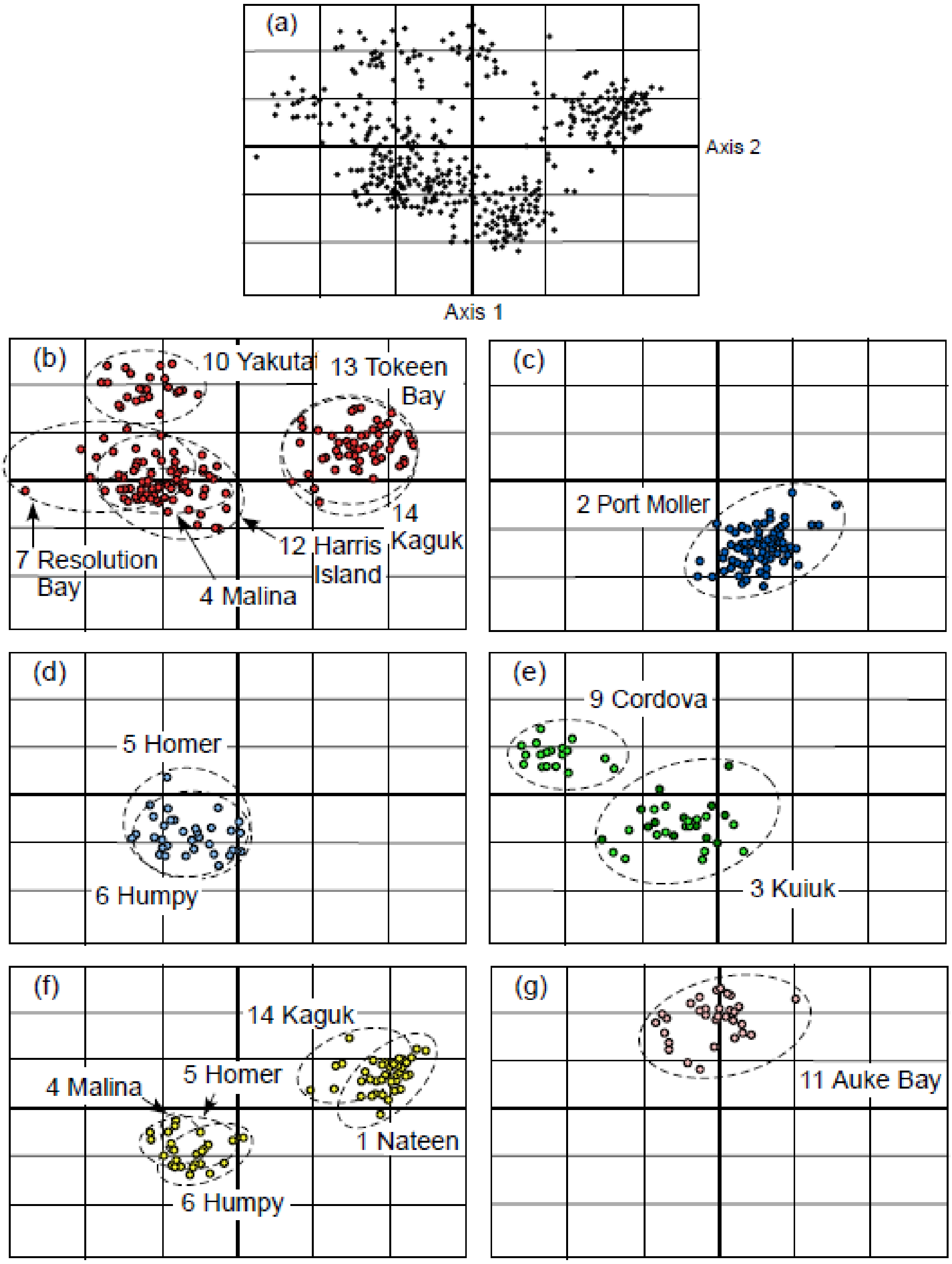

Figure 3 Principal components analysis (PCA) of microsatellite DNA (12 loci) alleles frequencies. (a) Total PCA of samples from 13 localities in the Gulf of Alaska. (b–g) PCAs of Plants carrying particular *COI-rbc*L haplotypes as in Figure 2: (b) Lineage A (red); (c) Lineage C (yellow); (d) Lineage B1 (dark blue); (e) Lineages D1 (green) & D2 (dark green); (f) Lineage B2 (light blue); (g) Lineage E (pink).

Supplemental information: Grant, Grauvogel, Chenoweth & Cheng. Isolations in northern ice-age refugia shaped phylogeography of Sugar kelp *Saccharina latissima* in the Gulf of Alaska

Sample collection

Table S1 *Saccharina latissima*: Locations and collection dates of samples of sugar kelp from the Gulf of Alaska and southeastern Bering Sea

Sample	Location in Alaska	N Latitude	W Longitude	Date
1	Nateen Bay, Unalaska	53.883	166.634	June 2018
2	Port Moller, Alaska Peninsula	55.989	135.278	June 2018
3	Kuiuk Bay, Alaska Peninsula	56.179	158.520	May 2016
4	Malina Bay, Kodiak Island	58.176	152.995	June 2018
5	Homer Spit, Kachemak Bay	59.604	151.418	June 2016
6	Humpy Creek, Kachemak Bay	59.668	151.135	June 2016
7	Lowell Point, Resurrection Bay	60.032	149.437	June 2016
8	Whittier, Prince William Sound	60.787	148.634	July 2016
9	Cordova, Prince William Sound	60.545	145.768	July 2016
10	Boat Harbor, Yakutat	59,563	139.743	June 2018
11	Auke Bay, Juneau	58.376	134.702	June 2018
12	Harris Island, Sitka	57.036	135.278	June 2018
13	Tokeen Bay, Scott Island	55.893	133.383	April 2017
14	Kaguk Bay, Prince of Wales Island	55.745	133.288	June 2018

Laboratory methods

Table S2 PCR thermal profiles of 12 microsatellite loci amplified in sugar kelp *Saccharina latissima*

Locus Name	PCR thermal profile
SLN314; *SLN510*	95 °C/4 min; 33 cycles of (95 °C/40 sec + 58 °C/40 sec + 72 °C/40 sec); 72 °C 20 min
SLN319; *SLN320*; *SLN34*; *SLN35*	95 °C/4 min; 33 cycles of (95 °C/40 sec + 56 °C/40 sec + 72 °C/40 sec); 72 °C 20 min
SLN32; *SLN36*	95 °C/4 min; 33 cycles of (95 °C/40 sec + 57 °C/40 sec + 72 °C/40 sec); 72 °C 20 min
SLN511	95 °C/4 min; 35 cycles of (95 °C/40 sec + 54 °C/40 sec + 72 °C/40 sec); 72 °C 20 min
SLN54	95 °C/4 min; 34 cycles of (95 °C/40 sec + 54 °C/40 sec + 72 °C/40 sec); 72 °C 20 min
SLN58; *SLN62*	95 °C/4 min; 32 cycles of (95 °C/40 sec + 54 °C/40 sec + 72 °C/40 sec); 72 °C 20 min

Cytochrome oxidase (*COI*)

Fourteen locations were sampled with sample sizes ranging from 6 to 90 and averaging 33 plants (Tables S1, S3). Ten nucleotide polymorphisms in a 624 bp fragment of the 5' end of *COI* defined 11 haplotypes, but only three haplotypes were abundant. A central abundant haplotype (1) was connected to 9 peripheral haplotypes by one mutation (Fig. 1a). Twelve of the 14 samples were fixed or nearly fixed for the most common haplotype (1, red). The sample from Port Moller (sample 2) was nearly fixed for a haplotype (3) that was one mutation removed from the central haplotype, and the sample from Auke Bay (sample 11) was nearly fixed for another haplotype (3) also one mutation removed from the central haplotype (Fig. 1b). Six of the samples had one or two private haplotypes that were one mutation removed from haplotype 1.

Haplotype diversity (*h*) ranged from 0.0 in 8 samples to 0.147 in sample 7 and was 0.044 (SD = 0.024) in a pooled sample (n = 463) (Table S4). Nucleotide diversity (θ_π) ranged from 0.0 in several samples to 0.0003 in sample 9, and was 0.0008 in the pooled sample. Tajima's D was marginally significant at three locations and in the pooled sample (D = -1.446, P = 0.040). Generally the expected number of haplotypes under neutrality was smaller than the numbr of observed haplotypes. Overall, the number of observed haplotypes was 11, when only 3.93 were expected under neutrality. A total of 10 private haplotypes appeared in six of the samples.

Genetic divergence (Φ_{ST}) between populations ranged from 0.0 between pairs fixed for the same haplotype to 0.972 between populations that were fixed or nearly fixed for different haplotypes and was 0.909 overall among populations (Table S5). 90.9% of the overall diversity was due to differences among populations and 9.1% was contained within populations as different haplotypes among plants (Table S6).

Table S3 *Saccharina latissima*: Haplotype frequencies of mitochondrial DNA *cytochrome oxidase I* (624 base pair fragment) haplotypes in samples from the Gulf of Alaska and southeastern Bering Sea

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1 ‘A’	29	.	31	32	31	29	25	6	21	31	1	26	32	42	336
2 ‘B’	.	87	.	.	.	.	.	.	.	.	.	.	.	.	87
3 ‘E’	.	.	.	.	.	.	.	.	.	.	30	.	.	.	30
4	.	3	.	.	.	.	.	.	.	.	.	.	.	.	3
5	.	.	.	.	.	.	.	.	1	.	.	.	.	.	1
6	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
7	.	.	.	.	.	.	.	.	1	.	.	.	.	.	1
8	.	.	.	.	.	.	1	.	.	.	.	.	.	.	1
9	.	.	.	.	.	.	1	.	.	.	.	.	.	.	1
10	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1
11	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1
Total	30	90	31	32	31	31	27	6	23	31	31	26	32	42	463

Table S4 Estimates of genetic parameters for mitochondrial DNA cytochrome oxidase I (624 base-pair fragment) in samples from the Gulf of Alaska and southeastern Bering Sea. Location number, sample size (N), number of polymorphic nucleotide sites (N_{poly}), number of haplotypes (N_{H}), expected nmber of haplotypes under neutrality (N_{EH}), haplotype diversity (h, standard deviation), nucleotide diversity (θ_π, standard deviation) and Tajima’s D (P: probability of null hypothesis of neutrality)

Location	N	N_{poly}	N_{H}	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
1	30	1	2	1.21	1	0.067	0.061	0.011	0.025	-1.147	0.133
2	90	1	2	1.26	1	0.065	0.035	0.010	0.024	-0.784	0.211
3	31	0	1	1	0	0.0	–	0.0	–	–	–
4	32	0	1	1	0	0.0	–	0.0	–	–	–
5	31	0	1	1	0	0.0	–	0.0	–	–	–
6	31	2	3	1.42	2	0.127	0.080	0.021	0.036	-1.506	0.042
7	27	2	3	1.47	2	0.145	0.090	0.024	0.039	-1.512	0.041
8	6	0	1	1	0	0.0	–	0.0	–	–	–
9	23	2	3	1.53	2	0.170	0.103	0.028	0.043	-1.515	0.040
10	31	0	1	1	0	0.0	–	0.0	–	–	–
11	31	1	2	1.20	1	0.070	0.059	0.010	0.025	-1.145	0.134
12	26	0	1	1	0	0.0	–	0.0	–	–	–
13	32	0	1	1	0	0.0	–	0.0	–	–	–
14	42	0	1	1	0	0.0	–	0.0	–	–	–
Mean	33.1	0.6	1.6	1.15	0.6	0.046	–	0.007	–	–	–
Pooled	463	10	11	3.93	10	0.044	0.024	0.077	0.074	-1.446	0.040

Table S5 *Saccharina latissima*: Genetic distances (Φ_{ST}; Tamura & Nei 1992) based on mitochondrial DNA cytochrome oxidase I (624 base-pair fragment) between samples from the Gulf of Alaska and southeastern Bering Sea. Overall $\Phi_{ST} = 0.909$ samples. Bold inidicate distance are significantly larger than 0.0 ($P < 0.00001$).

2	**0.939**												
3	0.001	**0.954**											
4	0.002	**0.954**	0.0										
5	0.001	**0.954**	0.0	0.0									
6	0.0	**0.925**	0.0	0.0	0.0								
7	0.001	**0.923**	0.005	0.006	0.005	0.001							
8	0.0	**0.942**	0.0	0.0	0.0	0.0	0.0						
9	0.005	**0.921**	0.013	0.015	0.013	0.002	0.001	0.0					
10	0.001	**0.954**	0.0	0.0	0.0	0.0	0.005	0.0	0.013				
11	**0.935**	**0.968**	**0.967**	**0.967**	**0.967**	**0.906**	**0.901**	**0.944**	**0.894**	**0.967**			
12	0.0	**0.952**	0.0	0.0	0.0	0.0	0.0	0.0	0.005	0.0	**0.964**		
13	0.002	**0.954**	0.0	0.0	0.0	0.001	0.006	0.0	0.015	0.0	**0.967**	0.0	
14	0.012	**0.957**	0.0	0.0	0.0	0.010	0.017	0.0	0.028	0.0	**0.972**	0.0	0.0
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Sample number												

Table S6 *Saccharina latissima*: Analysis of molecular variance (AMOVA) of mitochondrial DNA *cytochrome oxidase I* (624 base-pair fragment) sequence variability among samples from the Gulf of Alaska and southeastern Bering Sea.

Partition	d.f.	Variance component	% of variance	*P*
Among populations	13	0.238	90.9	<0.00001
Within populations	449	0.023	9.1	
Total	462	0.261	100.0	

Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L)

Sample sizes for this gene ranged from 6 to 81 and averaged 32.1 plants (Table S7). Four polymorphic nucleotide sites in a 735 bp fragment of *rbc*L defined 7 haplotypes (Table S8, Figure 1c). The four most abundant haplotypes were separated from one another by substitutions at a single nucleotide site. Mutations between the four haplotypes involved both transitions (haplotypes 1-3 C↔T; 2-4 G↔A) and transversions (1-2 C↔G; 1-4 C↔A; 2-3 G↔T; 3-4 T↔A). The most abundant haplotype, 1, (‘A’, red) appeared in 7 samples, was fixed in four samples and at a high frequency in three samples (Figure 1d). This haplotype was most abundant in the Central (samples 4 & 7) and eastern Gulf of Alaska (samples 10–14). The second most abundant haplotype, 2, (‘B’, blue) appeared in three samples located in Port Moller (sample 2) and Kachemak Bay (samples 5 & 6). Haplotype 3 (‘C’, yellow) appeared in samples from Dutch Harbor (sample 1), Kachemak Bay (samples 5& 6) and Prince William Sound (sample 8). Haplotype 4 (‘D’, green) appeared in a samples from Sand Point (sample 3) and Cordova (sample 9).

Four *rbc*L haplotypes appeared overall, but samples consisted of only 1 to 2 haplotypes (Table S8). The overall number of haplotypes expected under neutrality was 10.5. Haplotype diversity (*h*) ranged from 0.0 in 6 samples to 0.515 in sample 3 and was 0.702 in the pooled sample. Nucleotide diversity (θ_π) ranged from 0.0 in 6 samples and was 0.0011 in the pooled sample. Tajima’s *D* was not significant in any of the sample, nor in the pooled sample (D = 0.453, P = 0.719).

Genetic divergences (Φ_{ST}) between populations ranged from 0.0 between populations fixed for the same haplotype to 0.965 (Table S8). Overall, Φ_{ST} = 0.788 among samples (Table S9). AMOVA indicated that 78.8% of the total variation was due to differences among populations and 21.2% was due to differences among plants within populations (Table S10).

Table S7 *Saccharina latissima*: Haplotype frequencies of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base-pair fragment) haplotypes in samples from the Gulf of Alaska and southeastern Bering Sea

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1 ‘A’	.	.	.	28	.	.	27	.	.	29	27	23	31	32	197
2 ‘B’	.	78	.	.	20	17	.	.	.	.	.	.	.	.	115
3 ‘C’	37	.	.	4	10	15	1	6	1	.	.	.	.	10	84
4 ‘D1’	.	.	16	.	.	.	.	.	21	.	.	.	.	.	37
5 ‘D2’	.	.	14	.	.	.	.	.	.	.	.	.	.	.	14
6	.	2	.	.	.	.	.	.	.	.	.	.	.	.	2
7	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
Total	37	81	30	32	30	32	28	6	22	29	27	23	31	42	450

Table S8 *Saccharina latissima*: Estimates of genetic parameters for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base pairs) in samples from the Gulf of Alaska and southeastern Bering Sea. Location number, sample size (N), number of polymorphic nucleotide sites (N_{poly}), number of haplotypes (N_{H}), expected nmber of haplotypes under neutrality (N_{EH}), haplotype diversity (h, SD: standard deviation), nucleotide diversity (θ_{π}, SD: standard deviation) and Tajima’s D (P: probability of null hypothesis of neutrality)

Location	N	N_{poly}	N_{H}	N_{EH}	N_{PH}	h	SD	θ_{π} (%)	SD	D	P
1	37	0	1	1.00	0	0.0	–	0.0	–	–	–
2	81	2	3	1.29	2	0.073	0.040	0.010	0.022	-1.310	0.051
3	30	1	2	3.49	1	0.515	0.027	0.070	0.028	1.621	0.973
4	32	1	2	1.81	0	0.226	0.088	0.031	0.041	-0.138	0.328
5	30	1	2	3.06	0	0.460	0.061	0.063	0.063	1.280	0.918
6	32	1	2	3.53	0	0.514	0.025	0.070	0.067	1.634	0.972
7	28	1	2	1.22	0	0.071	0.065	0.001	0.020	-1.151	0.134
8	6	0	1	1.00	0	0.0	–	0.0	–	–	–
9	22	1	2	1.26	0	0.091	0.081	0.012	0.026	-1.162	0.149
10	29	0	1	1.00	0	0.0	–	0.0	–	–	–
11	27	0	1	1.00	0	0.0	–	0.0	–	–	–
12	23	0	1	1.00	0	0.0	–	0.0	–	–	–
13	31	0	1	1.00	0	0.0	–	0.0	–	–	–
14	42	1	2	2.66	0	0.372	0.070	0.051	0.055	0.844	0.866
Mean	32.1	0.6	1.6	1.74	0.2	0.165	–	0.022	–	–	–
Pooled	450	4	7	10.50	3	0.702	0.013	0.105	0.085	0.453	0.719

Table S9 *Saccharina latissima*: Genetic distances (Φ_{ST}) between samples from the Gulf of Alaska and southeastern Bering Sea based on seqence variability in ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base-pair fragment) with Tamura (1992) model of mutation. Overall, Φ_{ST} = 0.788 among samples. Italics 0.05 > *P* > 0.01; Bold *P* < 0.01.

2	**0.952**												
3	**0.841**	**0.864**											
4	**0.880**	**0.885**	**0.750**										
5	**0.681**	**0.380**	**0.668**	**0.645**									
6	**0.536**	**0.524**	**0.649**	**0.607**	0.005								
7	**0.968**	**0.930**	**0.796**	0.018	**0.726**	**0.69**1							
8	**0.000**	**0.935**	**0.728**	**0.793**	*0.508*	*0.350*	**0.939**						
9	**0.965**	**0.925**	**0.380**	**0.830**	**0.700**	**0.662**	**0.920**	**0.925**					
10	**1.000**	**0.948**	**0.822**	0.090	**0.767**	**0.733**	0.001	**1.000**	**0.991**				
11	**1.000**	**0.947**	**0.817**	0.085	**0.760**	**0.726**	0.0	**1.000**	**0.959**	0.0			
12	**1.000**	**0.945**	**0.805**	0.075	**0.746**	**0.711**	0.0	**1.000**	**0.956**	0.0	0.0		
13	**1.000**	**0.949**	**0.827**	0.095	**0.773**	**0.740**	0.004	**1.000**	**0.962**	0.0	0.0	0.0	
14	**0.744**	**0.824**	**0.704**	0.553	**0.553**	**0.507**	*0.117*	**0.624**	**0.732**	*0.185*	*0.180*	*0.167*	*0.191*
	1	2	3	4	5	6	7	8	9	10	11	12	13
							Sample number						

Table S10 *Saccharina latissima*: Analysis of molecular variance (AMOVA) of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base pairs) sequence variability among samples from the Gulf of Alaska and southeastern Bering Sea with the Tamura (1992) model of mutation.

Partition	d.f.	Variance component	% of variance	*P*
Among populations	13	0.325	78.8	<0.00001
Within populations	436	0.087	21.2	
Total	449	0.412	100.0	

Table S11 *Saccharina latissima*: Haplotype frequencies of contatenated fragments of contatenated seqences of mitochondrial DNA cytochrome oxidase I (*COI*) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1359 base pairs) in samples from the Gulf of Alaska and southeastern Bering Sea

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1 ‘A’	.	.	.	28	.	.	24	.	.	29	1	23	31	32	168
2 ‘C’	36	.	.	.	10	13	1	6	1	.	.	.	.	14	81
3 ‘B1’	.	75	.	.	.	.	.	.	.	.	.	.	.	.	75
4 ‘D1’	.	.	16	.	.	.	.	.	20	.	.	.	.	.	36
5 ‘B2’	.	.	.	.	20	16	.	.	.	.	.	.	.	.	36
6 ‘E’	.	.	.	.	.	.	.	.	.	.	25	.	.	.	25
7 ‘D2’	.	.	14	.	.	.	.	.	.	.	.	.	.	.	14
8	.	3	.	.	.	.	.	.	.	.	.	.	.	.	3
9	.	.	.	.	.	.	.	.	1	.	.	.	.	.	1
10	.	.	.	.	.	.	1	.	.	.	.	.	.	.	1
11	.	.	.	.	.	.	1	.	.	.	.	.	.	.	1
12	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1
13	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1
14	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
15	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
16	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Total	37	80	30	28	30	31	27	6	22	29	26	23	31	46	446

Table S12 *Saccharina latissima*: Analysis of molecular variance (AMOVA) of contatenated fragments of mitochondrial DNA cytochrome oxidase I (*COI*) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1359 base pairs) among samples from the Gulf of Alaska and southeastern Bering Sea with the Tamura & Nei (1992) model of mutation.

Partition	d.f.	Variance component	% of variance	*P*
Among populations	13	0.446	77.4	<0.00001
Within populations	432	0.131	22.6	
Total	445	0.577	100.0	

Microsatellites

Table S13 *Saccharina latissima*: Microsatellite DNA allele frequencies

Locus	Sample												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1 SLN32													
Allele N	36	90	30	32	31	24	20	16	30	32	27	27	40
217	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000
220	0.000	0.011	0.000	0.078	0.065	0.083	0.800	0.000	0.000	0.000	0.000	0.019	0.000
223	1.000	0.972	1.000	0.922	0.548	0.667	0.125	1.000	1.000	1.000	1.000	0.722	0.988
226	0.000	0.017	0.000	0.000	0.016	0.000	0.075	0.000	0.000	0.000	0.000	0.222	0.013
229	0.000	0.000	0.000	0.000	0.355	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000
232	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2 SLN34													
Allele N	37	83	27	31	31	24	20	14	23	31	26	27	39
171	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
186	0.000	0.042	0.019	0.048	0.258	0.292	0.150	0.107	0.000	0.694	0.000	0.000	0.154
189	0.959	0.259	0.093	0.419	0.645	0.500	0.700	0.786	1.000	0.290	0.750	0.611	0.731
192	0.014	0.608	0.889	0.532	0.065	0.146	0.150	0.071	0.000	0.016	0.192	0.000	0.064
195	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.000	0.000
198	0.000	0.018	0.000	0.000	0.016	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000
201	0.014	0.006	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.051
207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.352	0.000
210	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000
219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000
225	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3 SLN35													
Allele N	31	84	4	32	20	14	21	19	23	31	26	21	18
348	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
351	1.000	0.982	1.000	1.000	1.000	0.929	0.833	0.842	1.000	1.000	0.981	1.000	1.000
354	0.000	0.006	0.000	0.000	0.000	0.071	0.143	0.079	0.000	0.000	0.019	0.000	0.000
357	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.079	0.000	0.000	0.000	0.000	0.000
4 SLN36													
Allele N	38	90	30	32	31	24	20	16	29	32	31	27	40
252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.129	0.000	0.000
265	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
267	0.000	0.061	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063
271	0.171	0.217	0.367	0.000	0.048	0.000	0.075	0.000	0.017	0.000	0.081	0.333	0.050
274	0.829	0.639	0.100	0.172	0.548	0.625	0.525	0.000	0.000	0.438	0.194	0.556	0.400

277	0.000	0.056	0.533	0.016	0.194	0.208	0.100	0.031	0.000	0.000	0.081	0.056	0.338
280	0.000	0.022	0.000	0.000	0.048	0.063	0.000	0.031	0.000	0.000	0.177	0.037	0.125
283	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.031	0.052	0.000	0.065	0.000	0.025
286	0.000	0.000	0.000	0.359	0.048	0.063	0.000	0.031	0.328	0.000	0.032	0.000	0.000
289	0.000	0.000	0.000	0.156	0.032	0.000	0.125	0.281	0.017	0.031	0.113	0.000	0.000
292	0.000	0.000	0.000	0.063	0.016	0.042	0.050	0.313	0.569	0.188	0.065	0.019	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.156	0.017	0.047	0.065	0.000	0.000
298	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.063	0.000	0.000	0.000	0.000	0.000
301	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.297	0.000	0.000	0.000
304	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000
307	0.000	0.000	0.000	0.156	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000
310	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

5 SLN54

Allele N	37	80	30	32	31	24	17	15	24	32	26	25	39
302	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.115	0.000	0.000
308	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
314	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.313	0.000	0.000	0.000
317	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000
320	0.311	0.000	0.000	0.000	0.000	0.000	0.029	0.033	0.000	0.078	0.000	0.000	0.000
323	0.689	0.556	0.850	1.000	1.000	1.000	0.912	0.933	0.604	0.578	0.808	0.000	0.000
326	0.000	0.194	0.150	0.000	0.000	0.000	0.029	0.033	0.000	0.000	0.038	0.020	0.000
328	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
329	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.396	0.000	0.038	0.960	0.974
332	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
335	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.026
338	0.000	0.138	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
341	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
350	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

6 SLN58

Allele N	38	87	30	32	31	25	22	15	24	32	26	28	39
166	0.566	0.011	0.000	0.000	0.032	0.020	0.000	0.033	0.375	0.969	0.000	0.036	0.000
171	0.434	0.649	1.000	1.000	0.968	0.980	1.000	0.933	0.625	0.031	0.904	0.964	1.000
176	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.096	0.000	0.000
181	0.000	0.293	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
186	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

7 SLN62

Allele N	38	86	30	32	31	25	23	15	24	32	26	28	39
154	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.000	0.000	0.000	0.000	0.000	0.000
166	1.000	1.000	0.583	0.484	0.823	0.920	0.239	0.033	0.438	0.391	0.519	0.804	0.987
172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
200	0.000	0.000	0.000	0.297	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000
206	0.000	0.000	0.033	0.219	0.177	0.080	0.674	0.933	0.063	0.578	0.404	0.196	0.000

212	0.000	0.000	0.383	0.000	0.000	0.000	0.000	0.033	0.000	0.031	0.077	0.000	0.000

8 SLN314

Allele N	37	90	30	32	31	23	19	17	30	32	26	25	40
225	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.059	0.000	0.000	0.000	0.000	0.013
228	0.000	0.028	0.683	0.125	0.274	0.087	0.211	0.794	0.917	0.781	0.750	0.220	0.163
231	0.000	0.317	0.000	0.063	0.258	0.478	0.105	0.088	0.033	0.000	0.192	0.360	0.600
234	0.865	0.517	0.000	0.156	0.129	0.130	0.368	0.059	0.050	0.219	0.058	0.400	0.188
237	0.135	0.117	0.000	0.094	0.306	0.217	0.289	0.000	0.000	0.000	0.000	0.020	0.038
240	0.000	0.011	0.317	0.000	0.000	0.087	0.026	0.000	0.000	0.000	0.000	0.000	0.000
243	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
246	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
258	0.000	0.000	0.000	0.531	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
261	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

9 SLN319

Allele N	37	84	30	32	31	24	22	23	24	29	31	27	39
366	0.000	0.000	0.000	0.453	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000
369	0.000	0.470	0.000	0.547	0.000	0.042	0.364	0.587	0.000	0.241	0.694	0.056	0.179
372	1.000	0.131	0.350	0.000	0.065	0.083	0.545	0.000	1.000	0.759	0.113	0.926	0.821
375	0.000	0.244	0.650	0.000	0.839	0.833	0.000	0.000	0.000	0.000	0.000	0.019	0.000
377	0.000	0.143	0.000	0.000	0.097	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000
380	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
408	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000
411	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000
414	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.370	0.000	0.000	0.000	0.000	0.000
417	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000
420	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
428	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000
443	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
451	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000
454	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000

10 SLN320

Allele N	37	83	30	31	31	24	21	16	24	31	25	27	40
208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.306	0.000	0.000	0.000
214	0.000	0.000	0.000	0.000	0.000	0.000	0.119	0.000	0.021	0.000	0.000	0.000	0.000
217	0.000	0.018	0.317	0.790	0.710	0.833	0.762	1.000	0.979	0.016	0.200	0.019	0.000
220	0.000	0.066	0.017	0.161	0.016	0.000	0.095	0.000	0.000	0.177	0.000	0.204	0.125
223	0.973	0.187	0.667	0.048	0.145	0.104	0.024	0.000	0.000	0.419	0.380	0.741	0.625
226	0.027	0.602	0.000	0.000	0.081	0.063	0.000	0.000	0.000	0.081	0.420	0.037	0.250
229	0.000	0.108	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
232	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
235	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

11 SLN510

Allele N	37	91	30	32	31	25	19	17	30	32	28	26	40
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000
216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000
237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.583	0.000	0.000	0.000	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.013
247	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.096	0.050
252	0.000	0.044	0.000	0.031	0.097	0.040	0.000	0.000	0.000	0.000	0.036	0.000	0.000
257	1.000	0.643	0.567	0.766	0.774	0.820	0.079	0.000	0.017	0.000	0.661	0.712	0.413
262	0.000	0.115	0.000	0.109	0.065	0.100	0.368	0.324	0.317	0.438	0.179	0.058	0.338
267	0.000	0.016	0.133	0.047	0.000	0.000	0.000	0.176	0.083	0.563	0.018	0.135	0.188
272	0.000	0.132	0.017	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000
277	0.000	0.022	0.283	0.000	0.048	0.040	0.526	0.235	0.000	0.000	0.000	0.000	0.000
282	0.000	0.016	0.000	0.000	0.016	0.000	0.026	0.176	0.000	0.000	0.000	0.000	0.000
287	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.000	0.000	0.000	0.000
292	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
297	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

12 SLN511

Allele N	35	81	30	32	31	24	21	17	23	29	26	26	39
370	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000
375	0.000	0.012	0.000	0.000	0.016	0.104	0.000	0.000	0.000	0.000	0.019	0.000	0.000
380	0.000	0.037	0.000	0.000	0.097	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000
385	0.000	0.432	0.000	0.188	0.016	0.021	0.000	0.000	0.022	0.000	0.288	0.000	0.000
390	0.000	0.228	0.000	0.000	0.226	0.333	0.000	0.029	0.826	0.362	0.442	0.000	0.013
395	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.029	0.109	0.000	0.058	0.058	0.090
400	0.000	0.019	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000
405	0.000	0.000	0.000	0.031	0.000	0.083	0.167	0.176	0.000	0.121	0.135	0.000	0.026
410	1.000	0.068	0.117	0.438	0.065	0.042	0.024	0.471	0.022	0.466	0.038	0.596	0.705
415	0.000	0.105	0.417	0.094	0.113	0.042	0.143	0.176	0.000	0.052	0.000	0.308	0.090
420	0.000	0.037	0.017	0.031	0.081	0.063	0.262	0.029	0.000	0.000	0.000	0.000	0.000
425	0.000	0.012	0.417	0.203	0.258	0.146	0.310	0.059	0.000	0.000	0.000	0.038	0.000
430	0.000	0.012	0.033	0.000	0.097	0.104	0.024	0.029	0.000	0.000	0.000	0.000	0.077
435	0.000	0.000	0.000	0.000	0.016	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000
440	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table S14 *Saccharina latissima*: Summary statistics of variability at 12 microsatellite loci pooled over 13 samples. Number of alleles (N_A), allelic richness (N_{AR}) based on resampling 28 genes, observed heterozygosity (H_O), expected heterozygosity assume Hardy-Weinberg proportions (H_E), population inbreeding coefficient (F_{IS}), differentiation among populations (F_{ST}) and probability F_{ST} is greater than 0.0. Totals obtained by bootstraping over loci.

Locus	N_A	N_{AR}	H_O	H_E	F_{IS}	F_{ST}
SLN32	6	3.06	0.126	0.222	0.068	0.413
SLN34	12	4.45	0.378	0.615	0.105	0.335
SLN35	4	1.00[1]	0.032	0.054	0.372	0.067
SLN36	17	8.52	0.602	0.769	0.034	0.205
SLN54	14	5.20	0.296	0.544	0.005	0.477
SLN58	5	3.20	0.184	0.378	0.095	0.487
SLN62	6	3.56	0.296	0.442	-0.086	0.418
SLN314	10	5.48	0.479	0.756	0.055	0.328
SLN319	15	5.26	0.322	0.711	0.035	0.468
SLN320	9	5.15	0.379	0.726	0.012	0.440
SLN510	15	6.50	0.484	0.663	0.088	0.284
SLN511	15	9.39	0.589	0.831	0.051	0.271
Mean	10.7	5.06	0.347	0.559	0.070	0.349

[1] Base on resampling of 8 genes

Table S15 *Saccharina latissima*: Microsatellite DNA divergences between populations. Lower triangle: genetic distances (F_{ST}) between populations in the Gulf of Alaska based on 12 microsatellite loci. All values of F_{ST} were significantly larger than 0.0 ($P < 0.001$), except the value between samples 5 and 6 ($P = 0.0536$). Upper triangle: estimates of pairwise migration (Nm) per generation based on the island model of migration.

1	–	0.442	0.190	0229.	0.257	0220.	0.199	0.139	0.136	0.262	0.269	0.399	0.326
2	0.362	–	0.673	0.791	0.856	0.913	0.497	0.389	0.329	0.438	1.140	0.610	0.621
3	0.569	0.271	–	0.581	0.698	0.607	0.417	0.369	0.261	0.335	0.658	0.386	0.348
4	0.522	0.240	0.301	–	0.844	0.808	0.647	0.700	0.340	0.348	0.997	0.383	0.362
5	0.493	0.226	0.264	0.229	–	25.564	0.743	0.493	0.348	0.360	0.871	0.440	0.400
6	0.532	0.215	0.292	0.236	0.010	–	0.637	0.418	0.309	0.324	0.761	0.398	0.383
7	0.557	0.335	0.375	0.279	0.252	0.282	–	0.775	0.359	0.401	0.647	0.393	0.334
8	0.642	0.391	0.404	0.263	0.336	0.374	0.244	–	0.396	0.419	0.871	0.276	0.271
9	0.648	0.432	0.490	0.424	0.418	0.447	0.411	0.387	–	0.380	0.472	0.278	0.282
10	0.489	0.363	0.427	0.418	0.435	0.435	0.384	0.374	0.400	–	0.528	0.371	0.356
11	0.482	0.180	0.275	0.200	0.223	0.247	0.279	0.223	0.346	0.321	–	0.515	0.517
12	0.385	0.291	0.393	0.395	0.362	0.386	0.389	0.475	0.474	0.402	0.327	–	2.326
13	0.434	0.287	0.418	0.408	0.385	0.395	0.480	0.470	0.470	0.413	0.326	0.097	–
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Sample number												

Table S16 *Saccharina latissima*: Analysis of molecular variance (AMOVA) of microsatellite allele-frequency variability among samples using F_{ST}. Overall, $F_{ST} = 0.360$ ($P < 0.0001$)

Partition	d.f.	Variance component	% of variance	*P*
Among populations	12	1.256	36	<0.00001
Among plants within populations	448	0.079	2	
Within plants	461	2.149	62	
Total	921	3.483	100	

Table S17 *Saccharina latissima*: Genetic distances (R_{ST}) between populations in the Gulf of Alaska based on 12 microsatellite loci (lower triangle) and probability that R_{ST} is significantly greater than 0.0 (upper triangle)

1	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2	0.423	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3	0.449	0.568	–	0.001	0.001	0.001	0.008	0.001	0.001	0.001	0.001	0.001	0.001
4	0.491	0.548	0.276	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
5	0.121	0.325	0.162	0.270	–	0.026	0.001	0.001	0.001	0.001	0.001	0.002	0.001
6	0.196	0.192	0.316	0.310	0.041	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001
7	0.566	0.638	0.062	0.241	0.271	0.387	–	0.001	0.001	0.001	0.001	0.001	0.001
8	0.781	0.759	0.407	0.429	0.524	0.589	0.231	–	0.001	0.001	0.001	0.001	0.001
9	0.700	0.558	0.527	0.371	0.409	0.388	0.513	0.555	–	0.001	0.001	0.001	0.001
10	0.585	0.565	0.274	0.221	0.290	0.335	0.204	0.296	0.285	–	0.001	0.001	0.001
11	0.388	0.318	0.296	0.284	0.198	0.167	0.314	0.372	0.112	0.167	–	0.001	0.001
12	0.297	0.385	0.196	0.341	0.084	0.178	0.344	0.606	0.513	0.405	0.265	–	0.001
13	0.337	0.371	0.407	0.483	0.136	0.207	0.526	0.747	0.662	0.555	0.392	0.181	–
	1	2	3	4	5	6	7	8	9	10	11	12	13

Sample number

Table S18 *Saccharina latissima*: Analysis of molecular variance (AMOVA) of microsatellite allele-frequency variability among samples using R_{ST}. Overall, $R_{ST} = 0.411$ ($P < 0.001$).

Partition	d.f.	Variance component	% of variance	*P*
Among populations	12	386.479	41	<0.00001
Among plants within populations	448	617.308	7	
Within plants	461	491.634	52	
Total	921	940.950	100	

Table S19 *Saccharina latissima*: Log likelihood assignments individuals to populations. Assignments are from populations on the X-axis to populations on the Y-axis. Assignments back to orginal populations are in the diagonal.

1	38	1	–	–	–	–	–	1	1	–	2	–	–
2	–	89	–	–	–	–	–	–	–	–	–	–	–
3	–	–	30	–	–	–	2	–	–	–	–	–	–
4	–	–	–	32	–	–	–	–	–	–	–	–	–
5	–		–	–	20	8	–	–	–	–	–	–	–
6	–	1	–	–	11	17	–	–	–	–	–	–	–
7	–	–	–	–	–	–	22	–	–	–	–	–	–
9	–	–	–	–	–	–	1	24	–	–	–	–	–
10	–	–	–	–	–	–	–	–	30	–	–	–	–
11	–	–	–	–	–	–	–	–	–	32	–	–	–
12	–	–	–	–	–	–	–	–	–	–	29	–	–
13	–	1	–	–	–	–	–	–	–	–	–	25	1
14	–	–	–	–	–	–	–	–	–	–	–	3	39
	1	2	3	4	5	6	7	9	10	11	12	13	14
							Sample number						

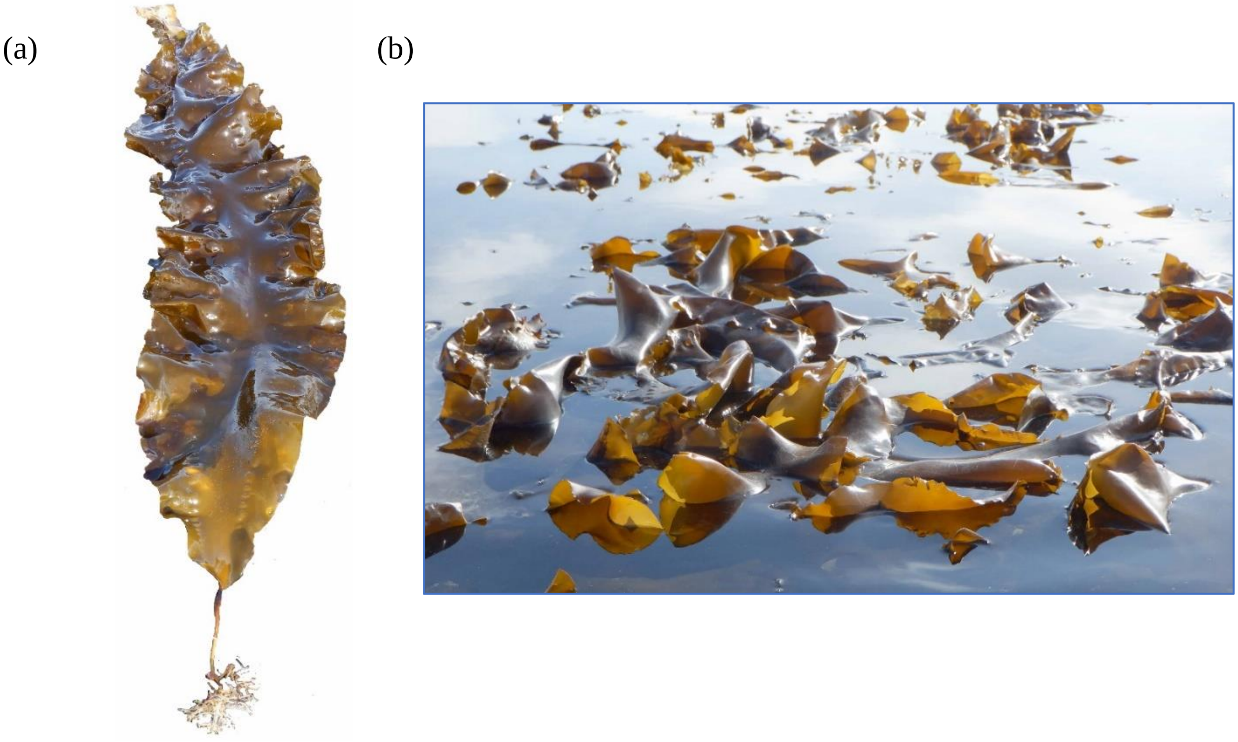

Figure S1 Photographs of *Saccharina latissima*. (a) entire plant 1.3 m in length representing growth in one season. (b) plants at low tide.

Chapter 4

Journal of Biogoeography: Research article

Parsimony can be misleading: Phylogeography of sugar kelp in the North Pacific, Arctic and North Atlantic oceans

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ABSTRACT

Aim Surveys of mitochondrial DNA sequence variability have provided insights into the transarctic dispersal and colonization histories of species inhabiting the North Pacific and North Atlantic oceans. Here, we explore contradictory phylogeographic models of dispersals of sugar kelp (*Saccharina latissima*) across the Arctic Ocean that were based on parsimonious reconstructions of a mitochondrial cytochrome oxidase I genealogy.

Location North Pacific, Arctic, North Atlantic oceans

Methods A total of 857 cytochrome oxidase I (*COI*) published sequences (586 bp) were re-examined to test alternative hypotheses of transarctic dispersal. A total of 36 polymorphic nucleotide sites defined 36 haplotypes in kelps from North Pacific and North Atlantic oceans that defined three major groups segregated by geography in the Northeastern Pacific, Northwestern Atlantic and Northeastern Atlantic oceans.

Results Results indicate that incomplete taxon sampling and use of a limited number of genetic markers hamper tests of sugar kelp phylogeogaphic hypotheses. Moreover, molecular clock estimates, which have wide confidence intervals, do not distinguish among oceanographic reconstructions of the opening of Bering Strait over the last Milankovitch climate cycle. Previous studies of northern Hemisphere populations of sugar kelp were basesd on haplotype networks that had been pruned using parsimony, but the unpruned network of the large dataset showed two mutation pathways, differing by one mutation, that do not definitively resolve dispersal pathways across the Arctic.

Main conclusion The use of strict parsimony can be misleading when alternative pathways in a geneology are similar, but have been pruned with the sheers of parsimony, or when convergences produce spurious similarities.

INTRODUCTION

Phylogeographic methods rest on the assumption that gene geneologies reconstructed from mitochondrial DNA (mtDNA) track population events. Reconstructions of population history together with paleo-climatic and paleo-ecological have can provided insights into the processes shaping genetic diversity and ultimately species diversity. The application of Occam's razor through parsimony is a cornerstone of phylogeographic analysis and limits the number of ad-hoc interpretations of the patterns in a dataset. However, various tree-generating algorthms can generate alterative genealogies and these genealogies can be interpreted in different ways depending on the hypothesis of interest. The use of strict parsimony can be misleading when alternative pathways in a geneology are similar or when convergences produce spurious similarities.

Genealogies of non-recombinant and uniparentally inherited DNA markers have been used extensively to chart phylogeographic relationships among populations of seaweeds (Hu *et al.*, 2010; Guillemin *et al*., 2016). These studies invariably show that paleo-climatic volatility over the Pleistocene has played an important role in shaping the genetic population structure by influencing extinction, dispersal and colonization (Assis *et al*., 2004, 2016; Hoarau *et al*., 2007). In the northern hemisphere, particular effort has been directed toward understanding the role the Arctic Ocean and climate variability during the Pleistocene have played in gating dispersals between the North Atlantic and North Pacific (Dunton, 1992; van Oppen *et al*., 1995; Lindstrom, 2001; Coyer *et al*., 2006, 2010, among many other studies). The North Atlantic and North Pacific oceans have been perodically connected through the Bering Strait and Arctic Ocean over the past 5.5 million years throughout the Pliocene and Pleistocene Epochs (Gladenkov et al., 2002; Marincovich *et al*., 2001). Inter-ocean dispersals are gated by sea level across Bering Srait and are blocked when sea level drops at least 50 m below present-day levels to expose the sill across Bering Strait (Fig. 1). Over the Pleistocene, the Strait has been open for only about 20% of the time (Bintanja *et al*., 2005; Jouzel *et al*., 2007).

Related species or populations in the North Pacific and North Atlantic oceans indicate dispersals across the Arctic time scales. Several levels of divergence are apparent, ranging from conspecific populations (e.g. snow crabs, Vulstek *et al*., 2013) to sister species (e.g. herring, Grant, 1986; cod, Grant & Ståhl, 1988). Some North Pacific species appear to have dispersed into the North Atlantic after the last glacial maximum (LGM), including Alaska pollock, Pacific herring, Pacific cod. While sister taxa in the two northern oceans demonstrate that dispersals have taken place, the details of the dispersals and their timings are poorly known especially for ancient dispersals predating the LGM.

Sugar kelp, *Saccharina latissima*, inhabits shallow subtidal and low intertidal shores along wave-protected shores. The greatest abundances of sugar kelp are in cold temperate waters, but this species shows physiological tolerances that allow it to survives in Arctic waters (Dunton, 1985). Previous studies show a close relationship between North Pacific and North Atlantic populations, which are considered to be conspecific. Previous surveys of mtDNA variability resolved four mtDNA lineages (Luttikhuizen *et al*., 2018; Neiva *et al*., 2018). Lineage **B** occurs in European waters from France to the high Arctic in Norway. Lineage **C** occurs in waters along North America from New England, through the maritime Canadian Provinces and into the Canadian Arctic, and lineage third, **A** inhabits the waters of the Northeastern Pacific Ocean, but is also found in Arctic waters. A fourth lineage **D** occurs in Asian waters off Japan and Russia.

Here we explore the reasons for phylogeographic contradictions between a model based on a genealogy reconstructed with parsimonious and a model based on geographic and post-LGM dispersal into the Arctic. We used mitochondrial DNA cytochrome oxidase C subunit I (*COI*) from Grant *et al*. (submitted) and from Genbank to infer phylogeographic relationships between populations of sugar kelp. Our focus was on the timings and dispersal pathways of multiple sugar-kelp migrations across the Arctic Ocean.

METHODS

Available sequences of mtDNA *COI*-5P of sugar kelp from Genbank (Table S1) were combined with sequences of populations around the Gulf of Alaska (Grant *et al*., in preparation). Sequences from the NW Pacific were labeled as *S. cichaera* and *S. cichoriodes*, but appear to be conspecific with *S. latissima* (Balkirev *et al*. 2012). The combined dataset included sequences from the Northwestern (NW) Pacific (n = 21), Northeastern (NE) Pacific (n = 488), eastern Arctic (n = 82), Northeastern Atlantic (n = 214). Samples were pooled into 5 groups—NW Pacific, NE Pacific, Arctic, NW Atlantic, NE Atlantic to provide a regional depiction of deep geneological structure without regard to the genetic population structure within a region (Fig. 2). This can be rationalized in that each region consists of a unique haplotype lineage. A small sample of *S. japonica* from the Northwestern Pacific was included as an outgroup. No plants of S. Latissima were found offshore from Nome, Alaska (Bringloe & Saunders, 2019), even though it is found in the Arctic (Bringloe *et al*., 2017). A detailed phylogeographic analysis of this species in Asian waters can be found in Zhang *et al*. (2015).

Genbank sequences of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) for *S. latissima* included sequences for plants in the ‘Boulder Patch’ in Arctic Alaska (n = 1) and in New England (n = 4), and for *S. cichorioides* from Asia (n = 8) (Table S2). These sequences were combined with those from Alaska (n = 450) (Grant *et al*., submitted) and were trimmed to 521 bp.

Summary statistics for the various samples were generated with ARLEQUIN 3.5.2.2 (Excoffier & Lischer 2010), including the number of polymorphic sites and haplotypes, expected number of haplotypes assuming a neutral model of evolution, expected genetic diversity, h, and nucleotide diversity, θ_π. The latter statistic accounts not only for haplotype frequencies in a group, but also sequence divergences between haplotypes. Sequence divergences between the four regional populations/lineages were estimated with Φ_{ST} and the Tamura & Nei (1993) of DNA mutation with a gamma = 0.05 in ARLEQUIN. We were forced to use the TN93 model (BIC = 17035.37) because the best-fit model, HKY+G (BIC = 17030.78), as determined with MEGA 7, was unavailable in ARLEQUIN.

The program TCS was used to produce a parsimonious haplotype network that contained a large closed loop. Closed loops are most often pruned with parsimony to eliminate ambiguities with steps are outlined in Templeton *et al.* (1987). In particular, the path with a shorter mutational distance between haplotypes is preferred over a longer path. The haplotype network produced by the combined sequences was not complex, but contained a large closed loop. We retained the entire network to for our analyses.

RESULTS

A total of 36 polymorphic nucleotide sites defined 36 haplotypes along a 586 base-pair fragment of COI in 836 plants of *S. latissima* from the North Pacific, Arctic and North Atlantic oceans (Fig. 3). These samples were subdivided into four regional groups to estimate haplotpe frequencies (Table 1) and population parameters (Table 2). Haplotype diversity (*h*) ranged from 0.392 to 0.650 and averaged 0.533. Overall $h = 0.756$. Nucleotide diversity (θ_π%) ranged from 0.080 to 0.600, and averaged 0.302. Overall $\theta_\pi\% = 0.596$.

The average number of nucleotide differences between haplotpes within regions ranged from 0.47 in the NE Pacific to 3.54 in the NW Pacific. After accounting for this within group variability, the adjusted number of differences ranged from 0.90 between the NE Pacific and Arctic to 6.06 between the NE Pacific and NW Atlantic (Table 3). The average number of adjusted differences between the five regional sugar kelp groups and *S. japonica* ranged from 26.98 to 31.51. Tamura & Nei (1993) genetic distances between 5 regional groups of sugar kelp varied from 0.279 between the NW Pacific and Arctic groups to 0.945 between the NE Pacific and the NW Atlantic (Table 4). Values of F_{ST} between regions ranged from 0.116 the NE Pacific and Arctic to 0.585 between the NE Pacific and NW Atlantic. All of these distance measures show greater amonts of differention between the NE Pacific and NW Atlantic than between the NE Pacific and the NE Atlantic.

An important feature of the haplotype network in Figure 3 is a closed loop between lineages **A**, **B**, and **C**. The central haplotype of lineage **B** is removed from the central haplotype of Lineage **A** by five mutation traversing four unobserved haplotypes. Lineage **C**, on the other hand, is removed from lineage **A** by six mutations along a path passing through four unobserved haplotypes and one singleton haplotype. Five mutational steps separate the central haplotypes of lineages **B** and **C**. As an outgroup, the Asian kelp *Saccharina japonica* was included and was removed from the central haplotype of lineage **A** by 28 mutational steps.

The *rbc*L sequences (521bp) produced four haplotypes that were present in samples from Alaska (Fig. 4). These haplotypes were 1–2 mutations removed from one another and were defned by transitions and transversions at a single nucleotide position. Haplotype 1 included plants from Arctic Alaska, New England and *S. cichodorioides* from Asia. Sequences for *S. angustissima* were one step removed from haplotype 4 and haplotypes for *S. Japonica* were removed by 5–6 mutational steps from haplotype 4. This haplotype network provides no further insights into sugar kelp phylogeography than is apparent for *COI*.

DISCUSSION

We used published *COI* sequences from several studies of sugar kelps in the NE Pacific, E Arctic, NW Atlantic and NE Atlantic oceans to reconstruct a gene genealogy to test models of historical dispersals and colonizations. The assembled dataset, however, did not completely resolve sugar kelp phylogeography because of the undersampling of populations in the NW Pacific and the western Arctic. The use of a single gene marker is also provided only one among many possible gene genealogies in these populations. Importantly, the genealogies previously constructed with parsimoney were inconsistent proposed models of dispersal, so that dispersal pathways and relative timings of dispersal remained unresolved.

Several features of the composite *COI* haplotype network for sugar kelp (Fig. 3) bear on understanding the phylogeographic history of the major lineages in the North Pacific and North Atlantic oceans. Four major lineages, **A**, **B**, **C**, and **D**, correspond to those described by Nieva *et al*. (2018) and Luttikhuizen *et al*. (2018). Lineage **A** was distributed among plants in the North Pacific but also appeared in Arctic waters in samples collected from the northern coast of Alaska and along the shores of Hudson's Bay, Manitoba. Plants bearing **A-**lineage haplotypes appeared as far east as as western Greenland. Lineage **B** haplotypes were distributed among plants in the Northeastern Atlantic European waters. Lineage **C** was most abundant in the Northwestern Atlantic but also appeard in samples from Hudson's Bay. Lineage **D** included plants from the Northwestern Pacific that were assigned to *S. coriacea* (n = 8) and *S. cichoriodes* (n = 12). These two **D** sub-lineages were separated through the central **A**-lineage haplotype by six mutations.

Evolutionary histories in the Pleistocene

The genetic patterns in the mtDNA *COI* of sugar kelp were imprinted by events that occurred on different time scales. On 'contemporary' time scales low-frequency variants have likely appeared since the edge of the last glacier receded from the coast 11–13 thousand years ago and sea level reached present-day levels about 10 thousand years ago (Miller *et al*. 2005). All of the mutations producing the low-frequency haplotypes anchored to the abundant central haplotype likely occurred in the past 10 thousand years. Two populations were nearly fixed for alternative haplotypes (3 & 6) (Grant *et al*., in preparation) indicating founder effect by colonizations of plants with mutant haplotypes. Sugar kelp in the NE Pacific showed an overall significant departure from neutality (D = -1.55, $P = 0.024$) and low levels of nucleotide diversity ($\theta_\pi = 0.0008$) relative to other regions. Together, these results reflect post-glacial population expansions as colonists followed the retreat of the ice.

Similar histories of coastal glaciation also influenced populations of sugar kelp in the North Atlantic. The coastlines of both the Northwestern Atlantic Ocean were covered in ice and were largely unavailable to sugar kelp until 11–12 thousand years ago. The star like genealogies also appear around the central haplotypes of **C** lineage in the Northwestern Atlantic and can also be interpreted to indicate an expanding post-glacial population. The regional population shows a small value of nucleotide diversity ($\theta_\pi = 0.0009$) reflecting the presence of only new mutations and a significant departure from neutality ($D = -2.194$, $P = 0.001$).

The genetic imprints on the **B** lineage in the Northeastern Atlantic populations are different in some respects. As in the two other regions, some populaitons are fixed for haplotypes that are only one or two mutational steps from an abundant central haplotype. Haplotypes at three locations in France (haplotype 5), the Netherlands (8) and Norway (7) are fixed, or nearly fixed, for locally distinctive haplotypes. However, nucleotide diversity is moderately large ($\theta_\pi = 0.0016$) and no departure from neutrality was detected ($D = -0.637$, $P = 0.308$). These populations may have retreated to southern refuges during glaciations without suffering large losses of genetic diversity and moved northward after glaciers retreated a few thousands years ago.

Alternatively, the diversities and distributions of sugar kelp haplotypes in the North Atlantic and North Pacific may reflect survivals in northern refugia. Northern refugia above the southern limit of coastal glaciation have been suggested for coastal organisms, including the Hurd Deep off in the western English Channel, link the Northeastern Atlantic, Iceland in the central North Atlantic, locations along Canadian maritimes in the Northwestern Atlantic, and coastal island around the Gulf of Alaska. While the conjecture of ice-age northern refugia may have been prompted by paleoenvironmental evidence, the identification of refugial locations of particular taxa is not always certain (Dahlgren *et al*., 2000; Wares & Cunningham, 2001; Young *et al*., 2002; Shaw *et al*., 2006; Ingólfsson, 2009; Maggs *et al*., 2008; Kelly *et al*., 2009; Canino *et al*., 2010; Olsen *et al*., 2010).

Timings of dispersals

In addition to the geographical connectivity, a haplotype network contains a temporal component. However, precise estimates of dispersals are difficult to extract from a molecular genalogy for several reasons. A genealogy based on a single locus is affected by evolutionary variance and by finite sample sizes of individuals and genes, so that it is difficult to distinguish between most competing hypotheses. Nevertheless, the timings of trans-Arctic dispersals can be constrained by paleo-oceanographic evidence of global sea levels and the submergence of the Bering Strait sill (Fig. 1). Dispersals were possible not only during the major warm interglacials periods about every 100 ky at Marine Isotope Stages (MIS) 5e (maximum 123 kya), MIS 7e (230 kya), MIS 8 (330 kya), MIS 9 (410 kya) and so on for about the past 800 ky (Milankovitch cycles), but also during interstadials at MIS 5e (82 kya), 5c (96 kya), 7a (190 kya) and 7c (210 kya) (Lisiecki & Raymo 2005). Most recently, dispersals across the Arctic Ocean were possible after 10 kya (PALE Beringian Work Group, 1999). Seasonally ice-free periods in some regions occurred from 6–10 kya ago when solar insolation in the Arctic was strongest (Polyak *et al*., 2010; Müller *et al*., 2012). Perennial sea-ice cover did not fully develop until about 5000 years ago (Darby *et al*., 2006). Opportunities for dispersal since the last glaical maximum have been driven by Arctic warming during Dansgaard-Oeschger events (Li & Bor 2019).

It is tempting to use genetic distances between North Pacific and North Atlantic lineages and a molecular-clock calibration to assign dispersals to a particular period. However, point estimates of genetic distance have large confidence intervals that include several opportunities for dispersal. The largest source of error for molecular clock estimates are mutation rates used to calibration a molecular clock. Several studies show that the use of phylogenetic calibrations greater than about one million years tend to greatly underestimate mutation rates (Ho *et al*., 2005). This source of molecular-clock calibration error is especially important for estimating population-level events. Errors in temporal estimates also arise from the use of a finite numbers of genetic markers and individuals to estimate genetic distance, and from evolutionary randomness shaping a particular gene genealogy (Karl *et al*., 2012). Another source of error comes from the mismatch between the reproductive biology of kelps and assumptions of software algorthims producing temporal estimates (Grant *et al*., 2016).

Transarctic Dispersals

The geography of the four *COI* lineages prompts four biogeogaphical models to explain the geographical distributions of and levels of divergence between the three COI lineages. In the first, dispersal from and ancestral European population (lineage **B**) to the the Northwestern Atlantic and to the North Pacific led to a circumpolar distribution (McDevit & Saunders, 2010). Northern hemisphere cooling shifted the distributions of the three lineages to the south, and the lineages diverged genetically from one another in isolation. Luttikhuizen *et al*. (2018) concluded that the relationships among the lineages depicted in their edited haplotype network corroborated historical dispersals from Europe to North America.

Recent dispersals into the Arctic from North Pacific and Northwestern Atlantic populations have resulted in hybridizations, as indicated by nuclear ITS genotypes (McDevit & Saunders, 2010). Because of the lack of recombination in organellar DNA, COI haplotypes have remained distinct revealing the lineage of origin in areas of overlap in the Arctic. Hybrid individuals have been detected as far as Newfoundland, and plants with North Pacific **A**-lineage COI haplotypes have been detected as far as Baffin Island and the west Coast of Greenland (Neiva *et al*., 2018). These hybridizations between genetically distinctive populations may lead to populatons that are more fit in postglacial habitats than are parental types and may create evolutionary novelty (Riginos & Cunningham, 2007).

In a second scenario, populations intially dispersed in the other direction from the North Pacific through Bering Strait into the Northeastern Atlantic. This scenario was inferred from the *COI* haplotype networks in Luttikhuizen *et al*. (2018) and Neiva *et al*. (2018). In both networks, lineage **B** in the NE Atlantic lies between lineages **A** in the N Pacific and **C** in the NW Atlantic. Hence, **C** show a closer affinity to **B** than to **A** even though post-LGM gene flow is occurring between **A** and **C**. In our unedited network both **B** and **C** show essentially the same amount of divergence from **A**. Luttikhuizen *et al*. (2018) postulated two separate dispersals from the Pacific through the Arctic into the North Atlantic, an earlier one into European waters and a later one into the Northwestern Atlantic. However, the topology of their haplotype networks does not support this conclusion. Instead, the network implies that **C**-lineage populations in the Northwestern Atlantic arose from dispersal of colonists from the Northeastern Atlantic.

An alternative scenario postulates dispersal from the North Pacific into the Northwestern Atlantic and subsequent, but almost simultaneous dispersal and colonization of Europe from the Northwestern Atlantic. In this model, a sufficient amount of time has elapsed to produce reciprocal monophyly between **C** and **B** by lineage sorting. The lack of shared haplotypes between the two groups argues for an ancient dispersal and divergence, but limits inferences about dispersal pathways. Shared haplotypes between North Pacific, Arctic and NW Atlantic populations reveals post-glacial dispersals from the North Pacific. These contemporary dispersals confirm the feasibility of historical dispersals through the Canadian Arctic.

A fourth scenario invokes two dispersal routes through Arctic waters: one through the Canadian Arctic into the NW Atlantic, as above, and another along western Arctic shores to the NE Atlantic. A second dispersal route through the western Arctic is supported by the ‘near’ parsimonious connection between the **A** and **B** lineages. A western Arctic dispersal route was discounted by Luttikhuizen *et al*. (2018), who conjectured that the freshwater discharges of rivers along the Siberian coast acted as a barrier to dispersal. However, the large discharges of the Kuskokwim and Yukon rivers into the Bering Sea and the Mckensie River into the Arctic

Ocean have not prevented post-LGM dispersals across the eastern Arctic. Kelp beds have been observed off the Siberian coast, but have not yet been sampled for genetic analyses (Filbee-Dexter *et al*., 2019). While rocky substrates are not typical of western Arctic shores, even occasional hard substrates can act as dispersal stepping stones (Dunton, 1992). This fourth scenario cannot presently be tested because of the lack of samples from the Russian Arctic. Unsampled diversity can lead to erroneous conclusions (Joly *et al*., 2007). In this model of separate dispersals, the similarity between the **B** and **C** lineages can be explained by convergence and does not imply historical connectivity.

None of these biogeographic models is fully supported by the haplotype networks appearing in Luttikhuizen *et al*. (2018) and Neiva *et al*. (2018), and the relationships among lineages in the network in Figure 2 are ambigous and also cannot distinguish among the four models. Nevertheless, we can safely postulate 1) pre-LGM dispersals and isolations of the three lineages from one another, 2) post-glacial population expansions into previously glaciated shorelines inferred from genetic signatures and from paleoclimate histories of the northern oceans The resolution of historical dispersals and colonizations of sugar kelp can be improved by including additoinal genetic markers and by sampling under- or un-sampled areas in the Northwestern Pacific and Russian Arctic.

Strict parsimony can be misleading

Parsimony is used to produce networks and minimal-spanning trees to show mutational relationships among haplotypes. However, we show here that strict parsimony leads to erroneous conclusions about dispersals of sugar kelp from the Pacific Ocean into the North Alantic Ocean. The haplotype network for the combined dataset contrasts with the networks in Luttikhuizen *et al*. (2018) and Neiva *et al*. (2018), who edited out the least parsimonious connections between the **A** lineage in the North Pacific and the two Atlantic lineages, **B** in the Northeastern Atlantic and **C** in the Northwestern Atlantic (Fig. 5). In our analysis the difference between these two links was only one mutation, a small difference that could have arisen by sampling error or by random mutational and population events that shaped the network. This small edit of the network, however, leads to different phylogeographic models that imply different origins, dispersal routes and colonizations of lineages **B** and **C**. Haplotype networks are important tools for constructing hypotheses of historical biogeography and how to edit, often complex, haplotype networks is a universal problem among phylogeographers (Templeton *et al*., 1987; Cassens *et al*., 2005). Mis-interpretations of a haplotype network can arise with the use of software that automatically produces an edited version of a network (e.g. Teacher & Griffiths, 2011).

CONCLUSIONS

Three important points arise from this study. The first is that incomplete taxon sampling and the use of a limited number of genetic markers hamper tests of sugar kelp phylogeogaphic hypotheses. While the nuclear gene ITS examined by Luttikhuizen *et al*. (2018) indicated that lineage A and C plants were hybridizing, it was not informative about phylogeographic relationships among the COI lineages. Microsatellite data are valuable for resolving contemporary population structure, but because of a large mutation rate, are not suitable for resolving events in deep time. Genomic panels are likely needed to resolve the phylogeographic structure of North Atlantic and North Pacific sugar kelp.

A second point is that temporal estimates of divergence have a large error associated with them that arises chiefly from time calibrations. Luttikhuizen *et al*. (2018) used a phylogenetic calibration and concluded that the star-shaped genealogies in North Atlantic populations indicated population expansions that pre-dated the LGM. The harsh environmental conditions in the northern seas during the LGM were clearly not conducive to population growth.

Third, this study shows that pruned haplotype networks can potentially lead to erroneous biogeographic scenarios. Large mutational distances between haplotypes in a network lead to unambiguous conclusions, but nearly equal distances between haplotype may not resolve some aspects of phylogeographical history. Parsimony to trim a haplotype networks has to be used judiciously.

ACKNOWLEDGEMENTS

Funding for this project was provided by the North Pacific Research Board Project 1618 and in part by the general funds of the Alaska Department of Fish and Game, Anchorage, Alaska.

REFERENCES

Assis, J., Serrão, E.A., Claro, B., Perrin, C. & Pearson, G.A. (2014) Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. *Molecular Ecology*, **23**, 2797–2810.

Bintanja, R., van de Wal, R.S. & Oerlemans, J. (2005) Modelled atmospheric temperatures and global sea levels over the past million years. *Nature*, **437**, 125–128.

Bringloe, T.T. & Saunders, G.W. (2019) DNA barcoding of the marine macroalgae from Nome, Alaska (Northern Bering Sea) reveals many trans-Arctic species. *Polar Biology*, **42**, 851–864.

Bringloe, T.T., Dunton, K.H. & Saunders, G.W. (2017) Updates to the marine algal flora of the Boulder Patch in the Beaufort Sea off Northern Alaska as revealed by DNA Barcoding. *Arctic*, **70**, 343–348.

Canino, M.F., Spies, I.B., Cunningham, K.M., Hauser, L. & Grant, W.S. (2010) Multiple ice-age refugia in Pacific cod, *Gadus macrocephalus*. *Molecular Ecology*, **19**, 4339–4351.

Cassens, I., Mardulyn, P. & Milinkovitch, M.C. (2005) Evaluating intraspecific "Network" construction methods using simulated sequence data: do existing algorithms outperform the global maximum parsimony approach? *Systematic Biology*, **54**, 363–372.

Coyer, J.A., Hoarau, G., Oudot-Le Secq, M.-P., Stam, W.T. & Olsen, J.L. (2006) A mtDNA-based phylogeny of the brown algal genus *Fucus* (Heterokontophyta; Phaeophyta). *Molecular Phylogenetics and Evolution*, **39**, 209–222.

Coyer, J.A., Hoarau, G., Van Schaik, J., Luijckx, P. & Olsen, J.L. (2011) Trans-Pacific and trans-Arctic pathways of the intertidal macroalga *Fucus distichus* L. reveal multiple glacial refugia and colonizations from the North Pacific to the North Atlantic. *Journal of Biogeography*, **38**, 756–771.

Dahlgren, T.G., Weinber, J.R. & Halanych, K.M. (2000) Phylogeography of the ocean quahog (*Arctica islandica*): influences of paleoclimate on genetic diversity and species range. *Marine Biology*, **137**, 487–495.

Darby, D.A., Polyak, L. & Bauch, N.A. (2006) Past glacial and interglacial conditions in the Arctic Ocean and marginal seas - a review. *Progress in Oceanography*, **71**: 129–144.

Dunton, K. (1992) Arctic biogeography: the paradox of the marine benthic fauna and flora. *Trends in Ecology & Evolution*, **7**, 183–189.

Dunton, K.H. (1985) Growth of dark-exposed *Laminaria saccharina* (L.) Lamour. and *Laminaria solidungula* J. Ag. (laminariales: phaeophyta) in the Alaskan Beaufort Sea. *Journal of Experimental Marine Biology and Ecology*, **94**, 181–189.

Filbee-Dexter, K., Wernberg, T., Fredriksen, S., Norderhaug, K.M. & Pedersen, M.F. (2019) Arctic kelp forests: Diversity, resilience and future. *Global and Planetary Change*, **172**, 1–14.

Gladenkov, A.Y., Oleinik, A.E., Marincovich, Jr L. & Barinov, K.B. (2002) A refined age for the earliest opening of Bering Strait. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **183**, 321–328.

Grant, W.S. (1986) Biochemical genetic divergence between Atlantic, *Clupea harengus*, and Pacific, *C. pallasi*, herring. *Copeia*, **1986**, 714–719.

Grant, W.S., & Ståhl, G. (1988) Evolution of Atlantic and Pacific cod: loss of genetic variation and gene expression in Pacific cod. *Evolution*, **42**, 138–146.

Grant, W.S., Árnason, E. & Eldon, B. (2016) New DNA coalescent models and old population genetics software. *ICES Journal of Marine Science*, **73**, 2178–2180.

Grant, W.S., Grauvogel, Z., Chenoweth, E., Cheng, W. (in preparation). Isolations in northern ice-age refugia shaped phylogeography of Sugar kelp *Saccharina latissima* in the Gulf of Alaska. To be submitted to *Molecular Ecology*.

Guillemin, M.L., Valero, M., Tellier, F., Macaya, E.C., Destombe, C. & Faugeron, S. (2016) Phylogeography of seaweeds in the South East Pacific: complex evolutionary processes along a latitudinal gradient. pp. 251–277, In *Seaweed phylogeography* (eds. ZM Hu & C Fraser). Springer, Dordrecht.

Ho, S.Y.W., Phillips, M.J., Cooper, A. & Drummond, A.J. (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution*, **22**, 1561–1568.

Hoarau, G., Coyer, J.A., Veldsink, J.H., Stam, W.T. & Olsen, J.L. (2007) Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. *Molecular Ecology*, **16**, 3606–3616.

Hu, Z., Guiry, M.D., Critchley, A.T. & Duan, D. (2010) Phylogeographic patterns indicate transatlantic migration from Europe to North America in the red seaweed *Chondrus crispus* (Gigartinales, Rhodophyta). *Journal of Phycology*, **46**, 889–900.

Ingólfsson, A. (2009) A marine refugium in Iceland during the last glacial maximum: fact or fiction? *Zoological Scripta*, **38**, 633–665.

Joly, S., Stevens, M.I. & van Vuuren, B.J. (2007) Haplotype networks can be misleading in the presence of missing data. *Systematic Biology*, **56**, 857–862.

Jouzel, J., Masson-Delmotte, V., Cattani, O., Dreyfus, G., Falourd, S., Hoffman, G., Minster, B., Nouet, J., Barnola, J.M., Chapellaz, J., Fischer, H., Gallet, J.C., Johnsen, S., Leuenberger, M., Loulergue, L., Luethi, D., Oerter, H., Parrenin, F., Raisbeck, G., Raynaud, D., Schilt, A., Schwander, J., Selmo, E., Souchez, R., Spanhi, R., Stauffer, B., Steffensen, J.P., Stenni, B., Stocker, T.F., Tison, J.L., Werner, M. & Wolff, E.W. (2007) Orbital and millennial Antarctic climate variability over the past 800,000 years. *Science*, **317**, 793–796.

Karl, S.A., Toonen, R.J., Grant, W.S. & Bowen, B.W. (2012) Common misconceptions in molecular ecology: echoes of the modern synthesis. *Molecular Ecology*, **21**, 4171–4189.

Lisiecki, L.E. & Raymo, M.E. (2005) A Pliocene-Pleistocene stack of 57 globally distributed benthic δ18 O records. *Paleoceanography*, **20**, PA1003.

Li, C. & Bor, A. (2019) Coupled atmosphere-ice-ocean dynamics in Dansgaard-Oeschger events. *Quaternary Science Reviews*, **203**, 1–20.

Lindstrom, S.C. (2001) The Bering Strait connection: dispersal and speciation in boreal macroalgae. *Journal of Biogeography*, **28**, 243–251.

Luttikhuizen, P.C., van den Heuvel, F.H.M., Rebours, C., Witte, H.J., van Bleijswijk, J.D.L., Timmermans, K. (2018) Strong population structure but no equilibrium yet: Genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). *Ecology and Evolution*, **8**, 4265–4277.

Maggs, C.A., Castilho, R., Foltz, D.W., Henzler, C., Jolly, M.T., Kelly, J., Olsen, J.L., Perez, K.E., Stam, W.T., Väinölä, R., Viard, F. & Wares, J.P. (2008) Evaluating signatures of glacial refugia for north Atlantic benithic marine taxa. *Ecology*, **89**, S108–S112.

Marincovich, L., Barinov, K.B. & Oleinik, A.E. (2002) The Astarte (Bivalvia: Astartidae) that document the earliest opening of Bering Strait. *Journal of Paleontology*, **76**, 239–245.

McDevit, D.C. & Saunders, G.W. (2010) A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. *Phycologia*, **49**, 235–248.

Miller, K.G., Kominz, M.A., Browning, J.V., Wright, J.D., Mountain, G.S., Katz, M.E., Sugarman, P.J., Cramer, B.S., Christie-Black, N. & Pekar, S.F. (2005) The Phanerozoic record of global sea-level change. *Science,* **310**, 1293–1298.

Müller, J., Werner, K., Stein, R., Fahl, K., Moros, M. & Jansen, E. (2012) Holocene cooling culminates in sea ice oscillations in Fram Strait. *Quaternary Science Reviews*, **47**, 1–14.

Neiva, J., Paulino, C., Nielsen, M.M., Krause-Jensen D., Saunders, G.W., Assis, J., Bárbara, I., Tamigneaux, É., Gouveia, L., Aires, T. & Marbà, N. (2018) Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. *Scientific Reports*, **8**, e1112.

Olsen, J.L., Zechman, F.W., Hoarau, G., Coyer, J.A., Stam, W.T., Valero, M. & Åberg, P. (2010) The phylogeographic architecture of the fucoid seaweed *Ascophyllum nodosum*: an intertial 'marine tree' and survivor of more than one glacial-interglacial cycle. *Journal of Biogeography*, **37**, 842–856.

PALE Beringian Working Group. (1999) Paleoenvironmental Atlas of Beringia presented in electronic form. *Quaternary Research*, **52**, 270–271.

Polyak, L., Alley, R.B., Andrews, J.T., Brigham-Grette, J., Cronin, T.M., Darby, D.A., Dyke, A.S., Fitzpatrick, J.J., Funder, S., Holland, M. & Jennings, A.E. 2010. History of sea ice in the Arctic. *Quaternary Science Reviews*, **29**, 1757–1778.

Riginos, C. & Cunningham, C.W. (2007) Hybridization in postglacial marine habitats. *Molecular Ecology*, **16,** 3971–3972.

Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Teacher, A.G.F. & Griffiths, D.J. (2011) HapStar: automated haplotype network layout and visualization. *Molecular Ecology Resources*, **11**, 151–153.

Templeton, A.R., Boerwinkle, E. & Sing, C.F. (1987) A cladistic analysis of phenotypic associations with haplotypes inferred from restrictions endonuclease mapping. I. Basic theory and analysis of alcohol dehydrogenase activity in *Drosophila*. *Genetics*, **117**, 343–351.

van Oppen, M.J.H., Draisma, S.G.A., Olsen, J.L. & Stam, W.T. (1995) Multiple trans-Arctic passages in the red alga *Phycodrys rubens*: evidence from nuclear rDNA ITS sequences. *Marine Biology*, **123**, 179–188.

Vulstek, S.C., Linderoth, T.P., Guyon, J.R. & Tallmon, D.A. (2013) Spatio-temporal population genetic structure and mating system of red king crab (*Paralithodes camtschaticus*) in Alaska. *Journal of Crustacean Biology*, **33**, 691–701.

Wares, J.P. & Cunningham, C.W. (2001) Phylogeogaphy and historical ecology of the North Atlantic intertidal. *Evolution*, **55**, 2455–2469.

Zhang, J., Yao, J.-T., Sun, Z.-M., Fu, G., Galanin, D.A., Nagasato, C., Motomura, T., Hu, Z.-M. & De-Lin Duan, D.-L. (2015) Phylogeographic data revealed shallow genetic structure in the kelp *Saccharina japonica* (Laminariales, Phaeophyta). *BMC Evolutionary Biology*, **15**, e237.

Table 1 Haplotype frequences pooled by region of cytochrome oxidaxe I in samples of sugar kelps *Saccharina latissima*. The NW Pacific forms of sugar kelp, *S. cicorea*, and *S. cichoriodes* (group 1), were also included. A small sample of the genetically well-studied populations of *S. japonica* from the NW Pacific were included as an outgroup.

	Group						
	1	2	3	4	5	6	
Haplotype	NW Pacific	NE Pacific	Arctic	NW Atlantic	NE Atlantic	*S. japonica*	Total
1	.	359	17	.	.	.	376
2	.	.	.	.	117	.	117
3	.	87	1	.	.	.	88
4	.	.	7	63	.	.	70
5	.	.	.	1	33	.	34
6	.	30	.	.	.	.	30
7	.	.	.	.	30	.	30
8	.	.	.	.	25	.	25
9	.	.	.	.	.	15	15
10	.	.	.	12	.	.	12
11	12	.	.	.	.	.	12
12	8	.	.	.	.	.	8
13	.	.	4	.	.	.	4
14	.	3	.	.	.	.	3
15	.	.	.	.	3	.	3
16	.	.	.	.	3	.	3
17	.	2	.	.	.	.	2
18	.	.	.	.	.	2	2
19	.	1	.	.	.	.	1
20	.	1	.	.	.	.	1
21	.	1	.	.	.	.	1
22	.	1	.	.	.	.	1
23	.	1	.	.	.	.	1
24	.	1	.	.	.	.	1
25	.	1	.	.	.	.	1
26	.	.	.	.	1	.	1
27	.	.	.	.	1	.	1
28	.	.	.	.	1	.	1
29	.	.	.	1	.	.	1
30	.	.	.	1	.	.	1
31	.	.	.	1	.	.	1
32	.	.	1	.	.	.	1
33	.	.	.	1	.	.	1
34	.	.	.	.	.	.	1
35	.	.	.	1	.	.	1
36	.	.	.	1	.	.	1
37	.	.	1	.	.	.	1
38	1	.	.	.	.	.	1
39	.	.	.	.	.	1	1
Total	21	70	39	82	214	18	749

Table 2 Summary statistics for sugar kelp *Saccharina latissima, S. coriacea, and S. cichoriodes* for the 5' end of cytochrome oxidase I (*COI*) (586 base pairs), including sample size (*N*), number of polymorphic nucleotide sites (N_{poly}), number of haplotypes (N_H), number of haplotypes expected under neutrality (N_{EH}), haplotype diversity (*h*) and standard deviation (*SD*), nucleotide diversity (θ_π) given as a percentage, and Tajima's D and the the one-sided probability that the observed value is less than 0.0.

Group	N	N_{poly}	N_H	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
1	21	11	3	3.49	3	0.552	0.066	0.600	0.354	0.512	0.937
2	488	11	12	4.35	10	0.424	0.024	0.080	0.077	-1.549	0.024
3	31	9	6	4.94	4	0.650	0.075	0.576	0.337	1.535	0.943
4	82	13	9	3.11	7	0.392	0.063	0.093	0.086	-2.194	0.001
5	214	8	9	7.54	7	0.647	0.029	0.163	0.124	-0.637	0.308
Mean	167.2	10.4	7.8	4.69	6.2	0.533	–	0.302	–	-0.467	–
Pooled	537	36	36	14.62	34	0.756	0.013	0.596	0.336	-0.752	0.256

Table 3 Average number of nucleotide differences between five groups of sugar kelp (*Saccharina latissima*, *Saccharina coriacea*, *Saccharina cichoriodes*) and *Saccharina japonica* (above diagonal), average number of differences within samples (diagonal), and corrected number of differences between groups, based on the 5’ end of cytochrome oxidase I (*COI*) sequences (586 base pairs). Group names by number appear Table 1.

1	3.54	3.62	4.98	7.16	6.29	30.96
2	1.62	0.47	2.85	6.57	5.92	28.65
3	1.50	0.90	3.43	4.23	5.76	28.86
4	5.12	6.06	2.24	0.55	5.51	29.65
5	4.05	5.21	3.57	4.76	0.96	32.15
6	29.03	28.26	26.98	29.21	31.51	0.32
	1	2	3	4	5	6
Group number						

Table 4 Tamura & Nei (1993) genetic distances based on the 5’ end of cytochrome oxidase I (*COI*) sequences (586 base pairs) between regional groups of *Saccharina latissima*, *Saccharina coriacea*, *Saccharina cichoriodes*, and *Saccharina japonica* (below diagonal). Convential F_{ST} (above diagonal). Group names by number appear Table 1.

1	–	0.545	0.395	0.556	0.384	0.564
2	0.729	–	0.116	0.585	0.486	0.600
3	0.279	0.665	–	0.400	0.352	0.496
4	0.850	0.945	0.641	–	0.453	0.632
5	0.802	0.909	0.744	0.874	–	0.453
6	0.974	0.994	0.961	0.993	0.991	–
	1	2	3	4	5	6
Group number						

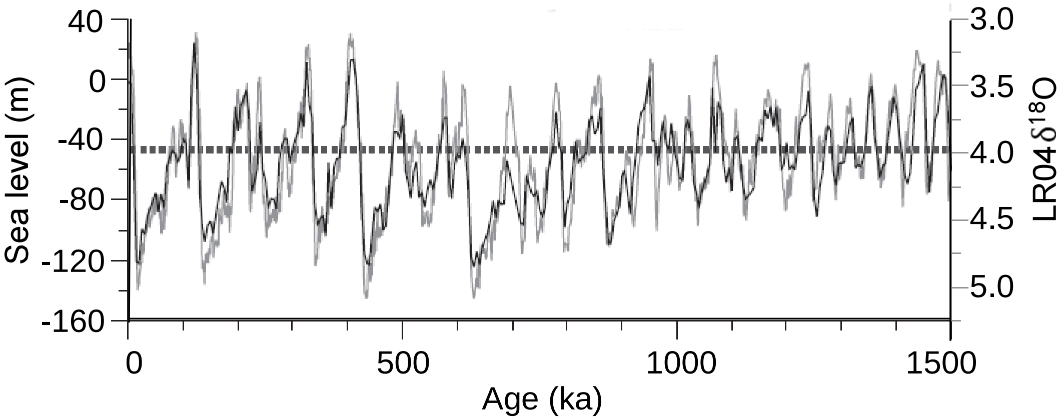

Figure 1 Global sea levels through the late Pleistocene Epoch. Dashed line shows the depth of the Bering Strait sill relative to present-day sea level. $\delta^{18}O$ is a proxy for temperature. Sea levels after Miller et al. (2005) and $\delta^{18}O$ after Lisiecki and Raymo, (2005). Redrawn from Polyack *et al*. (2013) Figure 6.

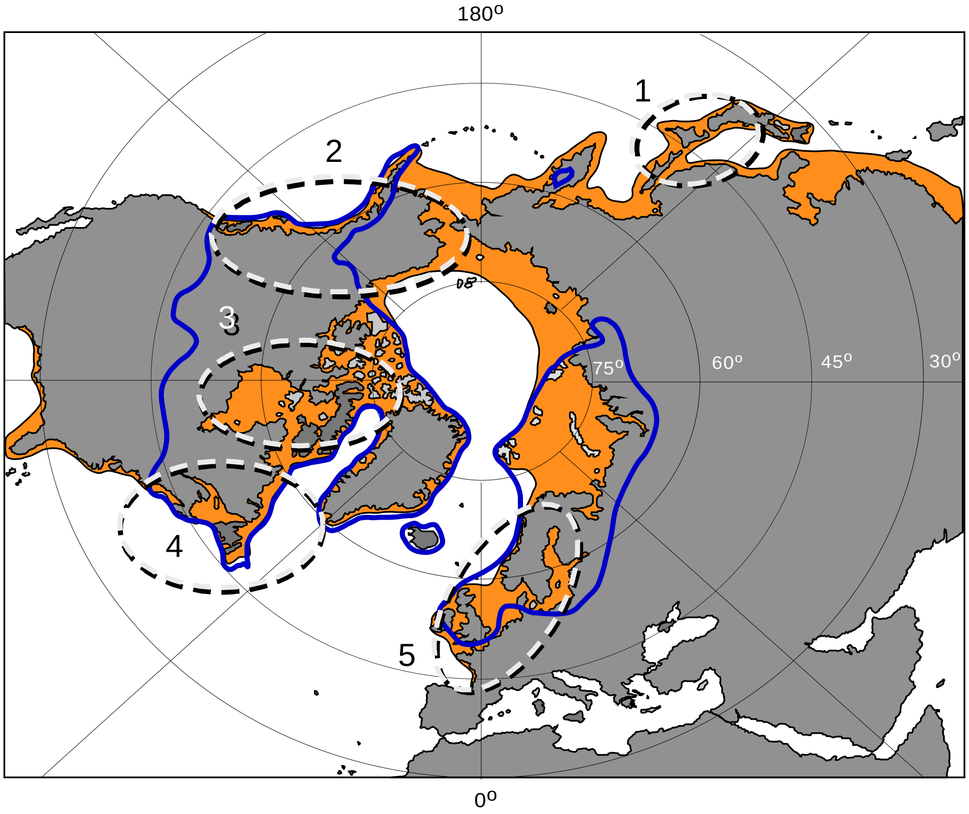

Figure 2 Polar-view map of the North Pacific, Arctic and North Atlantic oceans. Dashed elipses indicate locations of five groups of samples of *Saccharina latissima*, *S. coriacea*, and *S. cichoriodes* examined in the present study. Blue enclosure indicate the extention of glacial ice during the last glacial maximum.

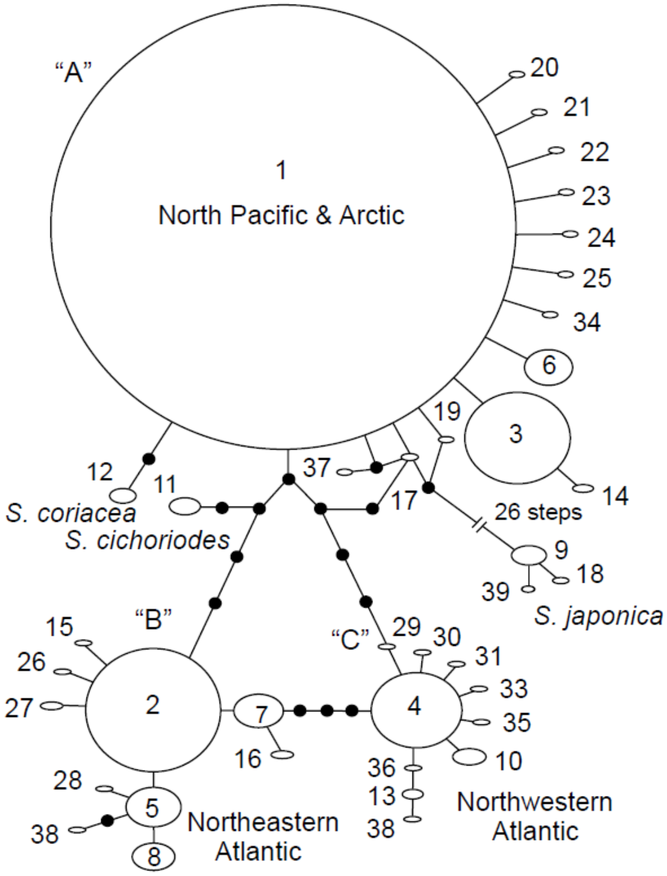

Figure 3 Mutatonal network of the 5' end of cytochrome oxidase I (586 base pairs) in samples of *Saccharina latissima*, *S. coriacea*, *S. cichoriodes*, and *S. japonica* from the North Pacific and North Atlantic oceans. Haplotype numbers and sample sizes appear in Table 1. Closed circles represent hypothetical, unobserved haplotypes needed to show the most parsimonious mutational paths between haplotypes.

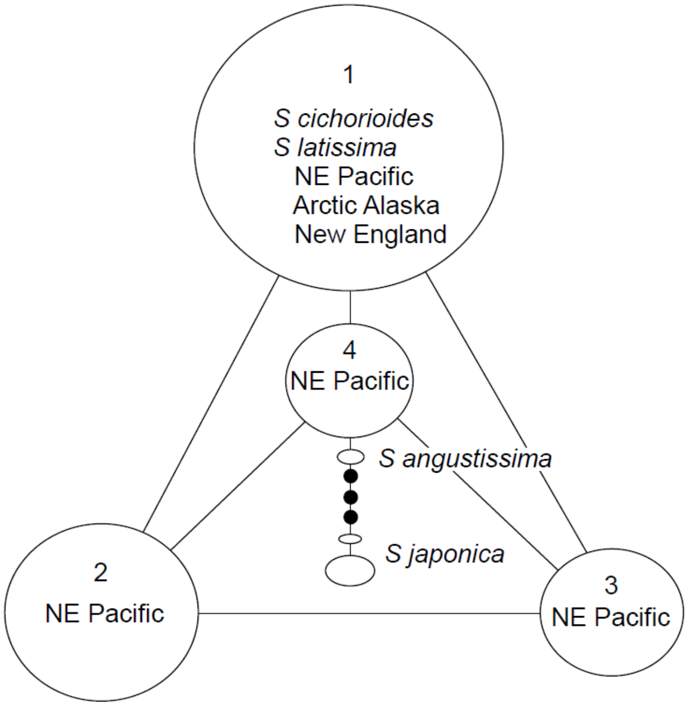

Figure 4 Mutatonal network of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit *rbc*L (521 base pairs) in samples of *Saccharina latissima*, *S. cichoriodes*, *S. angustissima* and *S. japonica* from the North Pacific and North Atlantic oceans. Haplotype numbers and sample sizes appear in Table 1. Closed circles represent hypothetical, unobserved haplotypes needed to show the most parsimonious mutational paths between haplotypes.

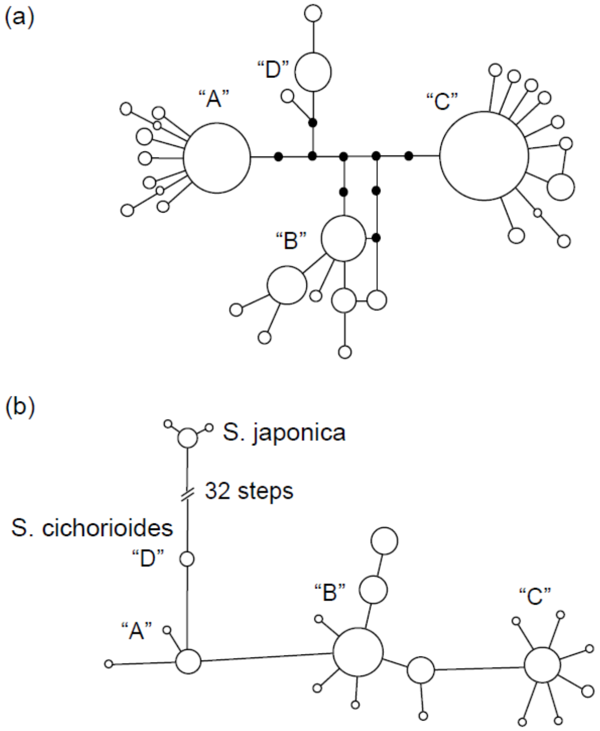

Figure 5 *Saccharina latissima, S. cichorioides, S. japonica*: Haplotype networks of cytochrome oxidase I haplotypes. a) redrawn from Figure 1 of Neiva *et al*. (2018). b) redrawn from Figure 3 of Luttikhuizen *et al*. (2018). Closed circles represent hypothetical, unobserved haplotypes needed to show the most parsimonious mutational paths between haplotypes. Lineage designations, **A**, **B**, **C** & **D**, follow Neiva *et al.* (2018).

Supplemental information:

WS Grant and J Neiva: Parsimony can be misleading: Phylogeography of sugar kelp in the North Pacific, Arctic and North Atlantic oceans

Supplemental Table S1 List of haplotypes of cytochrome oxidase C subunit I (*COI*) Genbank sequences from samples of sugar kelp *Saccharina latissima* from the North Pacific, Arctic and North Atlantic oceans

Genbank accession No.	Haplotype No., Lineage[1]	Location	North latitude	Longitude	Reference
Northeastern Atlantic Ocean					
EU681420	5, B	France	–	–	Silberfeld et al. unpublished
MF447855	15, B, (A)	Grenå, Denmark	56.3978	10.9206	Luttikhuzen et al. 2018
MF447856	27, B, (B)	Grenå, Denmark	56.3978	10.9206	Luttikhuzen et al. 2018
MF447857	8, B, (C)	Marsdiep, Netherlands	53.00194	4.78722	Luttikhuzen et al. 2018
MF447858	26, B, (D)	Galway, Ireland	53.2400	-9.30972	Luttikhuzen et al. 2018
MF447859	2, B, (E)	Numerous European localities	–	–	Luttikhuzen et al. 2018
MF447860	5, B, (F)	Brittany, France	48.70833	-3.82306	Luttikhuzen et al. 2018
MF447861	7, B, (G)	Helsem, Norway	62.25583	6.95389	Luttikhuzen et al. 2018
GU097786	2, B, SC1	Oranmore, Ireland	53.27	-8.93	McDevit & Saunders 2010
GU097750	16, B	Dratturin, Denmark	61.533	6.817	McDevit & Saunders 2010
GU097751	16, B	Dratturin, Denmark	61.533	6.817	McDevit & Saunders 2010
GU097752	2, B	Kaldbacksbornur, Denmark	62.067	6.817	McDevit & Saunders 2010
GU097753	16, B	Sinabour, Denmark	62.1	6.817	McDevit & Saunders 2010
KY572466	2, B	Hakonsund, Norway	60.1762,	5.1113	Bringloe & Saunders 2019
KY572199	2, B	Kleppesjoen, Norway	60.1847	5.1494	Bringloe & Saunders 2019
KY572296	2, B	Kleppesjoen, Norway	60.1847	5.1494	Bringloe & Saunders 2019
KY572340	2, B	Kleppesjoen, Norway	60.1847	5.1494	Bringloe & Saunders 2019
KY572385	2, B	Kleppesjoen, Norway	60.1847	5.1494	Bringloe & Saunders 2019
KY572797	2, B	Kleppesjoen, Norway	60.1847	5.1494	Bringloe & Saunders 2019

KY572608	2, B	Bergen, Norway	60.2412	5.2021	Bringloe & Saunders 2019
KY572465	2, B	Hordaland, Norway	60.242	5.24	Bringloe & Saunders 2019
KY572514	2, B	Espegend, Norway	60.2692	5.2212	Bringloe & Saunders 2019
KY572600	2, B	Stora Lauvoyna, Norway	60.4425	5.0646	Bringloe & Saunders 2019
KY572700	2, B	Hellesoy, Norway	60.6627	4.7875	Bringloe & Saunders 2019
KY572709	2, B	Hellesoy, Norway	60.6627	4.7875	Bringloe & Saunders 2019
KY572759	2, B	Hellesoy, Norway	66.6627	4.7875	Bringloe & Saunders 2019
KY572425	2, B	Hellesoy, Norway	60.6627	4.7875	Bringloe & Saunders 2019
MF288586	28, C, EV-3-B01	–	–	–	Neiva *et al*. 2018
GU097830	4, C	Narragansett, Rhode Island	41.42255	-71.45460	McDevit & Saunders 2010
GU097829	4, C	Narragansett, Rhode Island	41.42255	-71.45460	McDevit & Saunders 2010
GU097826	4, C	Fort Wetherill, Rhode Island	41.47910	-71.36066	McDevit & Saunders 2010
GU097831	4, C	Fort Wetherill, Rhode Island	41.47910,	-71.36066	McDevit & Saunders 2010
MF156535	4, C, SA21	NW Atlantic	–	–	Augyte *et al*. 2018
MF156536	4, C, SA22	NW Atlantic	–	–	Augyte *et al*. 2018
MF156537	4, C, SL6	NW Atlantic	–	–	Augyte *et al*. 2018
GU097828	30, C	Cape Neddick, Maine	43.16658	-70.5924	McDevit & Saunders 2010
GU097764	36, C	Cape Neddick, Maine	43.1658,	-70.5924	McDevit & Saunders 2010
GU097766	4, C	Cape Neddick, Maine	43.1658,	-70.5924	McDevit & Saunders 2010
GU097798	4, C	Cape Neddick, Maine	43.1658,	-70.5924	McDevit & Saunders 2010
KY319059	4, C	Harpswell, Maine	–	–	Brimley *et al*. unpublished
GU097769	4, C	South Portland, Maine	43.626	-70.214	McDevit & Saunders 2010
GU097773	4, C	South Portland, Maine	43.6256	-70.2138	McDevit & Saunders 2010
GU097777	4, C	South Portland, Maine	43.6256	-70.2138	McDevit & Saunders 2010
GU097783	4, C	South Portland, Maine	43.6256	-70.2138	McDevit & Saunders 2010
GU097782	4, C	Digby, Nova Scotia	44.3951	-66.2042	McDevit & Saunders 2010
GU097784	4, C	Digby, Nova Scotia	44.3951	-66.2042	McDevit & Saunders 2010
GU097780	4, C	Digby, Nova Scotia	44.3951	-66.2042	McDevit & Saunders 2010
GU097770	4, C	Digby, Nova Scotia	44.3951	-66.2042	McDevit & Saunders 2010
GU097781	4, C	Brier Island, Nova Scotia	44.2876	-66.3422	McDevit & Saunders 2010
GU097778	4, C	Brier Island, Nova Scotia	44.2876	-66.3422	McDevit & Saunders 2010
GU097776	4, C	Brier Island, Nova Scotia	44.2876	-66.3422	McDevit & Saunders 2010
GU097774	4, C	Brier Island, Nova Scotia	44.2876	-66.3422	McDevit & Saunders 2010

GU097772	4, C	Brier Island, Nova Scotia	44.2876	-66.3422	McDevit & Saunders 2010
GU097771	4, C	Brier Island, Nova Scotia	44.2876	-66.3422	McDevit & Saunders 2010
GU097768	4, C	Brier Island, Nova Scotia	44.2876	-66.3422	McDevit & Saunders 2010
KY572303	4, C	Manone Bay, Nova Scotia	44.508	-64.126	Bringloe & Saunders 2019
KY572261	4, C	St Mararets Bay, Nova Scotia	44.568	-64.036	Bringloe & Saunders 2019
GU097801	4, C	Meadow Cove, Nova Scotia	45.0381	-66.8913	McDevit & Saunders 2010
KY572467	4, C	Crow Island, Nova Scotia	45.0425	-66.873	Bringloe & Saunders 2019
GU097827	4, C	Pointe Sapin, Nova Scotia	46.96	-64.83	McDevit & Saunders 2010
GU097763	4, C	Pointe Sapin, New Brunswick	46.96	-64.83	McDevit & Saunders 2010
GU097823	31, C	Letete, New Brunswick	45.0382	-66.8912	McDevit & Saunders 2010
GU097767	4, C	Beaver Harbour, Nova Scotia	45.0717	-66.7372	McDevit & Saunders 2010
GU097822	4, C	Beaver Harbour, New Brunswick	45.0563	-66.7358	McDevit & Saunders 2010
GU097821	4, C	Beaver Harbour, New Brunswick	45.0563	-66.7358	McDevit & Saunders 2010
GU097799	4, C	Beaver Harbour, New Brunswick	45.0563	-66.7358	McDevit & Saunders 2010
GU097804	4, C	Lepreau, New Brunswick	45.0722	-66.4690	McDevit & Saunders 2010
KY572705	4, C	Lepreau, New Brunswick	45.0722	-66.4690	Bringloe & Saunders unpublished
EF218847	5, C	Lepreau, New Brunswick	45.0722	-66.4690	Lane *et al.* 2007
KY572442	4, C	Canso Causeway, New Brunswick	45.648	-61.415	Bringloe & Saunders unpublished
KY572692	10, C	New Brunswick	47.831	-66.083	Bringloe & Saunders unpublished
KY572147	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572213	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572241	10, C	B ay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572266	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572380	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572428	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572507	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572617	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572631	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572652	10, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
KY572788	29, C	Bay of Fundy, Nova Scotia	45.173	-64.757	Bringloe & Saunders unpublished
GU097814	33, C	Bay of Fundy, Nova Scotia	45.173	-64.757	McDevit & Saunders 2010
KY572271	10, C	Nova Scotia	44.508	-64.126	Bringloe & Saunders unpublished
GU097809	4, C	White Point, Nova Scotia	46.88226	-60.35077	McDevit & Saunders 2010

GU097811	4, C	Escoumins, Quebec	48.35062	-69.39722	McDevit & Saunders 2010
GU097813	4, C	Escoumins, Quebec	48.35062	-69.39722	McDevit & Saunders 2010
GU097815	4, C	Escoumins, Quebec	48.35062	-69.39722	McDevit & Saunders 2010
GU097807	4, C	Escoumins, Quebec	48.35062	-69.39722	McDevit & Saunders 2010
KY572766	4, C	Escoumins, Quebec	48.35062	-69.39722	Bringloe & Saunders 2018
KY572674	4, C	Quebec	50.2019	-63.5578	Bringloe & Saunders 2018
KY572824	4, C	Quebec	50.2267	-63.5425	Bringloe & Saunders 2018
MH310052	4, C	Cable Head, Prince Edward Island	46.4773	-62.6295	Bringloe & Saunders unpublished
MH309409	4, C	Naufrage-deep, Prince Edward Island	46.4744	-62.5477	Bringloe & Saunders unpublished
MH309137	4, C	Naufrage-middle, Prince Edward Island	46.4863	-62.5469	Bringloe & Saunders unpublished
MH309134	4, C	Naufrage-deep, Prince Edward Island	46.4744	-62.5477	Bringloe & Saunders unpublished
MH308764	4, C	Naufrage-deep, Prince Edward Island	46.4744	-62.5477	Bringloe & Saunders unpublished
GU097824	4, C	English Harbour, Newfoundland	47.63192	-54.88630	McDevit & Saunders 2010
GU097825	4, C	English Harbour, Newfoundland	47.6331	-54.87	McDevit & Saunders 2010
GU097832	4, C	English Harbour, Newfoundland	47.633	-54.87	McDevit & Saunders 2010
GU097794	4, C	English Harbour, Newfoundland	47.63192	-54.88630	McDevit & Saunders 2010
GU097775	4, C	Grand Barrachois, Newfoundland	47.0065,	-56.3598	McDevit & Saunders 2010
GU097808	4, C	Eastport, Newfoundland	48.65521	-53.75191	McDevit & Saunders 2010
GU097820	4, C	Bottle Cove, Newfoundland	49.1142	-58.4136	McDevit & Saunders 2010
KY572765	4, C	Bonne Bay, Newfoundland	49.4778	-57.9014	Bringloe & Saunders unpublished
GU097779	35, C	Bonne Bay, Newfoundland	49.52826	-57.82495	McDevit & Saunders 2010
GU097765	4, C	Bonne Bay, Newfoundland	49.68198	-57.96275	McDevit & Saunders 2010
GU097764	36, C	Bonne Bay, Newfoundland	49.5253	-57.8256	McDevit & Saunders 2010
KY572144	4, C	Turnagain Island, Labrador	56.766	-61.311	Bringloe & Saunders unpublished
KY572669	4, C	Evans Bight, Torngat, Labrador	59.429	-63.715	Bringloe & Saunders unpublished
KY572575	4, C	Duck Islands, Torngat, Labrador	60.234	-64.341	Bringloe & Saunders unpublished
KY572356	1, A	Broughton Chan, Nunavut	67.522	-64.062	Bringloe & Saunders unpublished
LT546291	1, A, BI063	Cape Hatt, Baffin Island	73.49056	-79.80972	Küpper *et al*. 2016
GU097754	37, A	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097755	1, A	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097756	1, A	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097796	13, C	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097806	4, C	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010

GU097810	4, C	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097817	4, C	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097818	32, A	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097819	1, A	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097802	4, C	Churchill, Manitoba	58.7678	-93.8897	McDevit & Saunders 2010
GU097792	1, A	Churchill, Manitoba	58.7787	-94.1582	McDevit & Saunders 2010
GU097797	1, A	Churchill, Manitoba	58.7787	-94.1582	McDevit & Saunders 2010
GU097805	13, C	Churchill, Manitoba	58.7787	-94.1582	McDevit & Saunders 2010
GU097785	4, C	Churchill, Manitoba	58.78057	-94.27670	McDevit & Saunders 2010
GU097812	3, A	Churchill, Manitoba	58.78057	-94.27670	McDevit & Saunders 2010
GU097816	1, A	Churchill, Manitoba	58.78057	-94.27670	McDevit & Saunders 2010
GU097787	1, A	Churchill, Manitoba	58.78671	-94.21583	McDevit & Saunders 2010
GU097788	4, C	Churchill, Manitoba	58.78671	-94.21583	McDevit & Saunders 2010
GU097757	1, A	Fort Prince Wales, Manitoba	58.7979	-94.2068	McDevit & Saunders 2010
GU097758	1, A	Fort Prince Wales, Manitoba	58.7979	-94.2068	McDevit & Saunders 2010
GU097759	1, A	Fort Prince Wales, Manitoba	48.7979	-94.2068	McDevit & Saunders 2010
GU097760	1, A	Fort Prince Wales, Manitoba	58.7979	-94.2068	McDevit & Saunders 2010
FJ409199	1, A	Churchill, Manitoba	58.8020	-94.2078	McDevit & Saunders 2010
GU097795	13, C	Churchill, Manitoba	58.8115	-94.2197	McDevit & Saunders 2009
GU097761	4, C	Churchill, Manitoba	58.81154	-94.21970	McDevit & Saunders 2010
GU097762	1, A	Churchill, Manitoba	58.8115	-94.2197	McDevit & Saunders 2010
GU097793	1, A	Churchill, Manitoba	58.8115	-94.2197	McDevit & Saunders 2010
GU097791	13, C	Churchill, Manitoba	58.8020,	-94.2078	McDevit & Saunders 2010
GU097790	1, A	Churchill, Manitoba	58.81154	-94.21970	McDevit & Saunders 2010
KY683294	1, A	Boulder Patch, Alaska	70.322	-147.579	Bringloe *et al*. 2018
KY683299	1, A	Boulder Patch, Alaska	70.322	-147.579	Bringloe *et al*. 2018
KY683301	1, A	Boulder Patch, Alaska	70.322	-147.579	Bringloe *et al*. 2018
KY683304	1, A	Boulder Patch, Alaska	70.322	147.579	Bringloe *et al*. 2018
KY683349	1, A	Boulder Patch, Alaska	70.322	147.579	Bringloe *et al*. 2018
KY683358	1, A	Boulder Patch, Alaska	70.322	147.579	Bringloe *et al*. 2018
KY683376	1, A	Boulder Patch, Alaska	70.322	147.579	Bringloe *et al*. 2018
MH327958	1, A	Boulder Patch, Alaska	–	–	Starko *et al.* 2018
GU097789	34, A	Prince Rupert, BC British Columbia	54.3150	-130.3208	McDevit & Saunders 2010

FJ409200	1, A	Palmerston, British Columbia	49.84	- 124.701	McDevit & Saunders 2010
FJ409204	1, A	Port Renfrew, British Columbia	48.362	-123.805	McDevit & Saunders 2010
FJ409203	1, A	Otter Point, British Columbia	48.423,	-123.42	McDevit & Saunders 2010
FJ409202	1, A	Saxe Point, British Columbia	48.4227	-123.4195	McDevit & Saunders 2010
FJ409201	1, A	Saxe Point, British Columbia	48.815	-123.61	McDevit & Saunders 2010
KM675818	38, B, ye-C14	'B' lineage from Northeastern Atlantic	–	–	Wang *et al*. unpublished
Saccharina coriacea					
AP011499	12, SCOR1	Japan, *n* = 8	–	–	Yotsukura *et al*. unpublished
Saccharina cichoriodes			–	–	
JN873240	11, SHA-2	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873243	11, CHE-1	Cape Khitrovo, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873246	11, CHE-4	Cape Khitrovo, Sea of Japan	–	–	Balakirev *et al*. 2012
JQ792007	11, CIC-1	Risovaya Bay, Sea of Japan	41.6317	131.1311	Balakirev *et al*. 2012
JQ792008	11, CIC-2	Risovaya Bay, Sea of Japan	41.6317	131.1311	Balakirev *et al*. 2012
JQ792009	11, CIC-3	Risovaya Bay, Sea of Japan	41.6317	131.1311	Balakirev *et al*. 2012
JQ792010	11, CIC-4	Risovaya Bay, Sea of Japan	41.6317	131.1311	Balakirev *et al*. 2012
Saccharina japonica					
JN873222	9, TYP-1	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873223	9, TYP-2	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873224	9, TYP-3	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873225	9, TYP-4	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873226	9, TYP-07	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873227	18, TYPF-1	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873228	18, TYPF-2	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873229	9, TYPF-3	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873230	9, TYPF-4	Cape Dal'niy, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873231	9, TYPA-1	Cape Khitrovo, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873232	9, TYPA-2	Cape Khitrovo, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873233	9, TYPA-3	Cape Khitrovo, Sea of Japan	–	–	Balakirev *et al*. 2012

JN873234	9, TYPA-4	Cape Khitrovo, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873235	9, TYPW	Cape Khitrovo, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873236	9, LON-1	Cape Zolotoi, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873237	39, LON-2	Cape Zolotoi, Sea of Japan	–	–	Balakirev *et al*. 2012
JN873238	9, LON-07	Cape Zolotoi, Sea of Japan	–	–	Balakirev *et al*. 2012
KC491236	9	Northwestern Pacific	–	–	Zhao unpublished

[1] Haplotype number in present study; Major lineage designation follows Nieva et al. (2018); haplotype letter by Luttikhuizen et al. (2017) in parentheses

Supplemental Table S2 List of haplotypes of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) Genbank sequences in samples of *Saccharina latissima*, *S. cichorioides*, *S. angustissima*, and *S. japonica* from the North Pacific, Arctic and North Atlantic oceans. Haplotype numbers as in Table 2 of Grant *et al*. (in preparation)

Genbank accession No.	Haplotype No.	Location	North latitude	Longitude	Reference
***S. latissima*: Arctic & NW Atlantic oceans**					
KY683521	1	Alaskan Arctic, ‘Boulder Patch’ *n* = 1	70.322	-147.579	Bringloe *et al*. 2017
MF156510	1	Land’s End, Harpswell, Maine	43.7172	-70.0047	Augyte *et al*. 2018
MF156511	1	Land’s End, Harpswell, Maine	43.7172	-70.0047	Augyte *et al*. 2018
MF156512	1	Land’s End, Harpswell, Maine	43.7172	-70.0047	Augyte *et al*. 2018
MF156513	1	Land’s End, Harpswell, Maine	43.7172	-70.0047	Augyte *et al*. 2018
***S. cichorioides*: NW Sea of Japan**					
JN873289	1, SHA-1	Cape Dal’niy, Primorye Region, Sea of Japan			Balakirev *et al*. 2012
JN873290	1, SHA-2	Cape Dal’niy, Primorye Region, Sea of Japan			Balakirev *et al*. 2012
JN873291	1, SHA-3	Cape Dal’niy, Primorye Region, Sea of Japan			Balakirev *et al*. 2012
JN873292	1, SHA-4	Cape Dal’niy, Primorye Region, Sea of Japan			Balakirev *et al*. 2012
JN873293	1, CHE-1	Chernoruch’e Bay, Sea of Japan			Balakirev *et al*. 2012
JN873294	1, CHE-2	Chernoruch’e Bay, Sea of Japan			Balakirev *et al*. 2012
JN873295	1, CHE-3	Chernoruch’e Bay, Sea of Japan			Balakirev *et al*. 2012
JN873296	1, CHE-4	Chernoruch’e Bay, Sea of Japan			Balakirev *et al*. 2012
***S. angustissima*: NW Atlantic Ocean**					
MF1565		Giant’s Staircase, Harpswell, Maine	43.7231	-69.9942	Augyte *et al*. 2018
MF1565		Giant’s Staircase, Harpswell, Maine	43.7231	-69.9942	Augyte *et al*. 2018
MF1565		Giant’s Staircase, Harpswell, Maine	43.7231	-69.9942	Augyte *et al*. 2018
MF1565		Giant’s Staircase, Harpswell, Maine	43.7231	-69.9942	Augyte *et al*. 2018
MF1565		Giant’s Staircase, Harpswell, Maine	43.7231	-69.9942	Augyte *et al*. 2018
***S. japonica*: NW Sea of Japan**					
JN873272	TYP-1	Cape Dal’niy, Primorye Region, Sea of Japan			Balakirev *et al*. 2012
JN873273	TYP-2	Cape Dal’niy, Primorye Region, Sea of Japan			Balakirev *et al*. 2012

JN873274	TYP-3	Cape Dal’niy, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873275	TYP-4	Cape Dal’niy, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873276	TYP-07	Cape Dal’niy, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873277	TYPF-1	Cape Dal’niy, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873278	TYPF-2	Cape Dal’niy, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873279	TYPF-3	Cape Dal’niy, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873280	TYPF-4	Cape Dal’niy, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873281	TYPA-1	Chernoruch’e Bay, Cape Khitrovo, Sea of Japan	Balakirev *et al*. 2012
JN873282	TYPA-2	Chernoruch’e Bay, Cape Khitrovo, Sea of Japan	Balakirev *et al*. 2012
JN873283	TYPA-3	Chernoruch’e Bay, Cape Khitrovo, Sea of Japan	Balakirev *et al*. 2012
JN873284	TYPA-4	Chernoruch’e Bay, Cape Khitrovo, Sea of Japan	Balakirev *et al*. 2012
JN873285	LON-1	Cape Zoloto, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873286	LON-1	Cape Zoloto, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873287	LON-1	Cape Zoloto, Primorye Region, Sea of Japan	Balakirev *et al*. 2012
JN873288	TYPW	Chernoruch’e Bay, Cape Khitrovo, Sea of Japan	Balakirev *et al*. 2012

References

Augyte, S., Lewis, L., Lin, S., Neefus, C.D. & Yarish, C. (2018) Speciation in the exposed intertidal zone: the case of *Saccharina angustissima* comb. nov. & stat. nov. (Laminariales, Phaeophyceae). *Phycologia*, **57**, 100–112.

Balakirev, E.S., Krupnova, T.N. & Ayala, F.J. (2012) DNA variation in the phenotypically-diverse brown alga *Saccharina japonica*. *BMC Plant Biology*, **12**, e108.

Bringloe, T.T. & Saunders, G.W. (2018) Mitochondrial DNA sequence data reveal the origins of postglacial marine macroalgal flora in the Northwest Atlantic. *Marine Ecology Progress Series*, **589**, 45–58.

Bringloe, T.T., Dunton, K.H. & Saunders, G.W. (2017) Updates to the marine algal flora of the Boulder Patch in the Beaufort Sea off Northern Alaska as revealed by DNA Barcoding. *Arctic*, **70**, 343–348.

Luttikhuizen, P.C., van den Heuvel, F.H.M., Rebours, C., Witte, H.J., van Bleijswijk, J.D.L. & Timmermans, K. (2018) Strong population structure but no equilibrium yet: Genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). *Ecology and Evolution*, **8**, 4265–4277.

McDevit, D.C. & Saunders, G.W. (2009) On the utility of DNA barcoding for species differentiation among brown macroalgae (Phaeophyceae) including a novel extraction protocol. *Phycological Research*, **57**, 131–141.

McDevit, D.C. & Saunders, G.W. (2010) A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. *Phycologia*, **49**, 235–248.

Neiva, J., Paulino, C., Nielsen, M.M., Krause-Jensen, D., Saunders, G.W., Assis, J., Bárbara, I., Tamigneaux, É., Gouveia, L., Aires, T. & Marbà, N. (2018) Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. *Scientific Reports*, **8**, e1112.

Chapter 5

Polar Biology: Research article

Phylogeography of Split kelp *Hedophyllum nigripes*: transArctic dispersals and northern ice-age refugia

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Abstract

Genetic variability within and among populations arises from historical isolations that create partitions between genealogical lineages and from contemporary processes that affect population size and dispersal. Periodic coastal glaciations in the Northeastern (NE) Pacific Ocean through the Pleistocene Epoch have led to the displacement or local extinctions of populations of shallow water and littoral invertebrates and seaweeds. The present-day distributions of these species around the Gulf of Alaska reflect population expansions from several possible refugia. In the present study, we surveyed genetic variability in the mitochondrial DNA 5' end of cytochrome oxidase I (*COI*-5P) and plastid ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) in the kelp *Hedophyllum nigripes* and found a moderate amount of haplotype diversity (*COI*-5P: $h = 0.652$; *rbc*L: $h = 0.578$), but low levels of nucleotide diversity (*COI*-5P: $\theta_\pi = 0.0015$; *rbc*L: $\theta_\pi = 0.0001$). These diversities reflect often strong haplotype frequency differences between populations, but shallow divergences between haplotypes. The general lack of southern haplotypes and higher levels of genetic diversity in the Gulf of Alaska points to northern ice-age refugia. The addition of Genbank sequences to the new *COI*-5P sequences shows a separation between Arctic-NW Atlantic populations and NE Pacific poulations of only one or two mutations. Unexpectedly, the Arctic and NE Atlantic populations were more closely related to southern populations in British Columbia than they were to northern populations in Alaska. The phylogeographic pattern revealed in this study indicates a complex history of persistence in glacial refugia, most certainly in a northern refuge in the Gulf of Alaska, but possibly also in the eastern Arctic or North Atlantic.

Keywords *Hedophyllum nigripes* · Kelp · Phylogeography · Transarctic dispersal · Mitochondrial DNA · Chloroplast DNA

Introduction

The mechanisms producing species diversity in a lineage are still poorly understood. Reproductive isolation, whether by geographical separation, ecological divergence or chromosomal change, is thought to be vital to the origins of new species. A first-step in understanding mechanisms promoting species diversity is a description of pattern of genetic divergence among populations and the relationship of this pattern to historical and contemporary environmental variability. Dispersal pathways can be inferred by the distributions of a genealogical lineages across a land- or seascape (Avise 2000) and dispersal timing can be estimated by the amount of divergence between lineages in the genealogy. Population size, metapopulation dynamics and gene flow between populations shape contemporary patterns of genetic diversity. Large population size and population longevity promote high levels of genetic diversity, and gene flow between populations tend to limit genetic divergence between populations (Grant and Bowen 1998).

The shores of the Northeastern (NE) Pacific Ocean have had a turbulent history over the Pleistocene Epoch. The edges of large continental glaciers periodically covered shorelines along western North America and regularly subdivided, displaced or extirpated coastal populations of invertebrates and seaweeds. Pesent-day species in these previously glaciated areas are expected to bear genetic imprints typical of young populations by having low levels of genetic diversity and by showing a lack of mutation-drift equilibrium. Of particular interest are the phylogeographic origins of contemporary populations, which can be inferred by the geographical distributions of genealogical lineages of uniparentally inherited genes. Did populations persist during the ice ages in refugia scattered between glacial lobes or were populations displaced to unglaciated shores to the south, to the southern edges of the Bering Land Bridge, or to the Northwestern Pacific, which was largely unglaciated?

The identification of the biogeographical origins of Arctic populations is also of interest in understanding the influences of dispersal and isolation on within-species diversity. The opening and closing of Bering Strait in concert with glacially driven sea-level changes has regulated the dispersals of marine species into the Arctic and often into the North Atlantic Ocean. The timings of possible dispersals across the Arctic can be inferred by well documented historical records of global sea levels associated with Pleistocene temperature excursions in the Northern Hemisphere (Bintanja et al. 2005; Jouzel et al. 2007).

The focus of the present study is on the shallow subtidal and low intertidal kelp, *Hedophyllum nigripes* (Rosenvige) Starko, S.C. Lindstrom & Martone, that is distributed on rocky shores with moderate to high wave action from central California to Alaska, the Aleutian Archipelago, Bering Sea and Russia in the North Pacific (Hansen 1997; Lindeberg and Lindstrom 2010; Klinkingberg 2018) (Figure 4.1). It also occurs in Arctic waters in Hudson's Bay, the Canadian Arctic and as far south as New Brunswick and the Gulf of Maine (Sears 2002; Longtin and Saunders 2016). The wide distribution in the North Pacific, Arctic, NW and NE Atlantic provides opportunity investigate the timing of dispersals across the Arctic into the North Atlantic and identify potential glacial refugia. In particular, populations rising from glacial refugia are expected to have higher levels of genetic diversity than recently colonized populations (Hewitt 2004). We surveyed genetic variability in the mitochondrial 5' end of the cytochrome *c* oxidase subunit I gene (*COI*-5P) and plastid ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) in samples collected from Alaskan waters to understand population structure and added published sequences of *COI*-5P from Arctic and NW Atlantic localities to test hypotheses of glacial refugia, dispersal and colonization.

Materials and methods

Samples of sporophytes were collected at numerous rocky intertidal habitats along the coast of Alaska (Fig. 1). A 2x2 cm piece of frond near the basal meristem was damp dried then dried on silica beads soon after collection. DNA was extracted with the NucleoSpin® 96 Plant II (Macherey-Nagel Inc., Bethlehem, PA) kit. A 576 bp segment of *COI*-5P was amplified with PCR using the forward primer *GazF2* (5' CCAACCAYAAAGATATWGGTAC 3') and reverse primer *GazR2* (5' GGATGACCAAARAACCAAAA 3') (Lane et al. 2007). A 735 bp segment of *rbc*L was amplified with PCR using the forward primer *rbcL-543F* (5' CCWAAATTAGGTCTTTCWGGWAAAAA 3') (Bittner *et al*. 2008; Silberfeld et al. 2010) and reverse primer *rbc*L*-1381R* (5' ATATCTTTCCATARRTCTAAWGC 3') (Burrowes et al. 2003; Silberfeld et al. 2010). The PCR cocktail consisted of a 50 μL mixture of 2.0 μL template DNA in 1x Colorless GoTaq Flexi buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1μM of forward and reverse primers, and 2.5U GoTaq Flexi DNA polymerase. PCR amplifications were conducted in ABI 9700 thermocyclers with initial denaturation at 94^{o} C for 3 min, followed by 35 amplification cycles of 45 s at 94^{o} C, 1 min at primer annealing temperature 50^{o} C for *COI*-5P and 52^{o} C for *rbc*L, and 1 min 30 s at 72^{o} C, and a final 5 min at 72^{o}C. PCR amplifications were sequenced in the forward and reverse directions by Genewiz Inc. (South Plainfield, NJ) or by the University of Arizona Genetics Core.

Forward and reverse-complement sequences were aligned and edited with MEGA 7.0.20 (Kumar et al. 2016) and chromatograms viewed with Finch TV 1.4.0 (Geospiza Inc.). Plants with unique haplotypes were selected from each of the 96-well plates for re-extraction and re-sequencing for quality control. ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010) was used to estimate the number of polymorphic nucleotide sites, N_{poly}, the number of observed, N_{H}, and expected, N_{EH}, number of haplotypes under neutrality. ARLEQUIN was also used to estimate gene diversity, *h* (standard deviation), and nucleotide diversity, θ_π (standard deviation). Divergence between populations was estimated with *F* statistics (Weir & Cockerham 1984) and with Φ_{ST} with an appropriate mutation models in ARLEQUIN. Appropriate mutation models for the various datasets were determined with MEGA 7 (Kumar et al. 2016). Departures from neutrality were tested with Tajima’s *D* (Tajima 1989).

Results

This study of genetic variability in *H. nigripes* presents new sequences from Alaska and adds to published sequences available in Genbank for populations in British Columbia, Manitoba and eastern Canada. The entire dataset places Alaskan populations in a broader biogeographical context. Both mitochondrial *COI*-5P and chloroplast *rbc*L sequences are available for Alaskan samples, but only *COI*-5P sequences are available for samples from the various localities in Canada.

Gulf of Alaska

*Cytochrome oxidase I-5P (COI-*5P*)*

We sampled at 6 sites in Alaskan waters, including 3 sequences from Genbank (Table 1). Eleven polymorphic nucleotides sites along a 576 fragment of *COI*-5P defined 11 haplotypes among 57 plants (Table S2; Figure 1a). Nine haplotypes were unique to a particular site. Haplotype diversity (*h*) ranged from 0.0 to 1.0 and was 0.652 (SD 0.043) overall. Nucleotide diversity (θ_π) ranged from 0.0 to 0.0046 and was 0.0015 (SD = 0.0012) overall. Tajima’s test showed an overall significant departure from neutrality overall in a pooled sample (D = -1.67, P = 0.021) that was due to an excess of low-frequency haplotypes. A total of 6.24 haplotypes were expected, but 11 were observed.

Twenty additional *COI*-5P sequences from northern and southern British Columbia were added to the collection of Alaskan samples (Figure 2). No sequence variability was observed among plants from northern British Columbia (n = 15; h = 0.0; θ_π = 0.0) and one variant haplotype appeared among plants from southern British Columbia (n = 5; h = 0.400; θ_π = 0.0024). In a pooled sample, haplotype (h = 0.100, SD = 0.088) and nucleotide (θ_π = 0.0007, SD = 0.0009) diversities were small. A test for neutrality showed no significant departure (D = -1.164, P =0.135) in the pooled samples from British Columbia.

Haplotype frequencies varied strongly among locations in the NE Pacific (Figure 2b). Samples from British Columbia were nearly fixed for haplotype 3 (blue), which occurred at only low frequencies in Alaskan waters. Haplotype 1 (red) appeared in all of the samples from the Gulf of Alaska at various frequencies. Locations 6, 7 & 10 were fixed for this haplotype. The plants from Cordova at the entrance to Prince William Sound was nearly fixed for haplotype 8 (yellow). Genetic distances between populations ranged from Φ_{ST} = 0.0, between locations fixed for the same haplotype, to Φ_{ST} = 1.0, between locations fixed for alternative haplotypes. Locations in British Columbia differed significantly from locations in Alaska, except between locations 5 (western Gulf of Alaska) and 12 (southern British Columbia).

Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbc*L*)

Polymorphisms at 4 nucleotide sites in a 735 base-pair segment of *rbc*L defined 5 haplotypes among 54 plants collected at 5 localities in Alaska (Tables S5, S6; Figures 12c,d). One nucleotide site defining haplotypes 1 (red), 2 (green) and 3 (yellow) segregated for both transitions and transversions and formed a closed loop in the haplotype network. Haplotype diversity (h) ranged from 0.0 in three locations fixed for a single haplotype to 0.667 and was 0.578 (SD 0.047) overall. Nucleotide diversity (θ_π) ranged from 0.0 to 0.0009 and was 0.0001 (SD 0.0009) overall. A test for neutralilty overall was not significant (D = 0.343, P = 0.677).

Concatenated COI-*5P and* rbc*L sequences*

A total of 10 polymorphic nucleotide sites in 52 concatentated sequences (1492 bp) produced 12 haplotypes (Tables 2, 3; Figures 1e, f). Haplotype diversities (h) ranged from 0.0 in populations that were fixed for a single haplotype to 0.402 in plants from Halibut Point that had a dominant haplotype and 6 singleton haplotypes. Nucleotide diversity (θ_{ST}) in the samples was small ranging from 0.0 to 0.0004 and was 0.0009 overall. Tajima’s test of neutrality was not significant (D = -1.210, P = 0.114).

Haplotype frequencies varied strongly among locations (Figure 5.2d). Samples from four collecting sites (7–10) were fixed or nearly fixed for alternative *rbc*L haplotypes. Genetic distances (Φ_{ST}) between samples ranged from 0.342 to 1.0 and were significant for all of the pair-wise comparisions (Table 5.10).

Global phylogeography of *COI*-5P

The addition of 25 sequences of *COI*-5P from 4 localities in northern and eastern Canada provided an overview of genetic variability on a larger geographical scale. Only a single nucleotide site was polymorphic in these sequences producing two haplotypes, one of which occurred in a single plant (Tables S2, S3). Overall, haplotype (h = 0.08, SD = 0.072) and nucleotide (θ_π = 0.0001) diversity were small. Tajima's test for neutrality was not significant (D = -1.158, P = 0.782).

These 25 sequences were added to the *COI*-5P sequences examined above. Overall, 13 polymorphic sites produced 13 haplotypes in 102 plants (Tables S2, S3; Figure 2). Haplotype diversity (h) ranged from 0.0 in populations fixed for a single haplotype to 0.500, but was 0.746 (SD = 0.026) overall. Nucleotide diversity (θ_π) ranged from 0.0 to 0.0046 among the 12 locations and was 0.0021 overall. Tajima's test for neutality overall was not signficant (D = -1.380, P = 0.622). No haplotypes were shared between the NE Pacific and Arctic-eastern Canadian samples. Hence, all pairwise genetic distances between the northern and eastern Canadian samples and all other samples were significantly greater than 0.0 (Table 5.4).

Discussion

The results of this study provide an initial view of the phylogeography of a kelp that inhabits NE Pacific, Arctic and NW Atlantic waters. On regional spatial scales, the analyses of *COI*-5P and *rbcL* sequence variability shows that populations in the Gulf of Alaska are largely reproductively isolated from one another on scales of tens to hundreds of kilometers. The addition of *COI*-5P sequences from Genbank for kelps in British Columbia reveals a phylogeographic break betweeen Southeastern Alaska and southern populations. The lack of haplotype diversity among samples from British Columbia does not allow us to infer the nature of population structure in these populations. The high level of genetic diversity in the Gulf of Alaska populations relative to southen populations and the general lack of shared haplotypes between the Gulf of Alaska and British Columbia populations indicates the existence of northern ice-age refugia. On a larger geographical scale, populations in Arctic and eastern Canada showed reduced *COI*-5P variability of haplotypes that were more closely related to populations in British Columbia than populations in the Gulf of Alaska. The small genetic distances between Arctic and eastern Canadian populations may indicate dispersal from the North Pacific through Bering Strait after the last glacial maximum. However, dispersals before the Last Glacial Maximum (~21 ka) are possible and would imply eastern Arctic or North Atlantic refugia.

Before discussing these results in detail, we note some cautions regarding the interpretataion of patterns. First, sample sizes at some localities were small. Despite this, the strong frequency differences among populations in the NE Pacific are unlikely to change with the addition of more samples. Also the conclusion of reduced variability in Arctic and eastern Canadian populations relative to NE Pacific populations is unlikely to change with additional sampling, given the large geographical range of these locations relative to sampled populations in the Gulf of Alaska. We caution, however, that our conclusions are based on two genetic markers for inferences of population structure in the NE Pacific, but only a single marker for the global comparisons. We also expect the inclusion of nuclear genes in future studies will provide additional insights into the biogeography of *H. nigripes*, such as in the extent of hybridizations between organellar gene lineages.

Finally, we did not attempt to estimate a time of divergence between NE Pacific populations and those in the eastern Arctic and NE Atlantic oceans. Coalescent algorithms used in such programs as BEAST and IMa are based on the Wright-Fisher model of genealogical evolution, which does not accurately reflect the multiple-merger coalescences found in species with type III larval survival and massive numbers of offspring. Kelps are capable of producing millions of propagules, but few survive. As such, genealogical coalescences in these species are generally short, and the use of incorrect models leads to overestimates of divergence time. In addition, mutations rates cannot be calibrate accurately to apply to short time scales investigated here (Ho *et al*. 2005).

Northeastern Pacific Ocean

The combination of *COI*-5P and *rbc*L markers show that nearly all the populations sampled are genetically unique. Four of the populations (7–10) were fixed, or nearly fixed for one of the four combined haplotypes lineages. Haplotypes in these lineages, however, were separated by only 1 or 2 mutational steps indicating a recent origin in these populations, or alternatively strong metapopualtion structure with frequent local extinctions and recolonzations. Strong shifts in abundance have been observed for populations of *H. nigripes* in the Bay of Fundy (Longtin & Saunders 2016), for populations of a similar kelp, *Saccharina latissima*, in Europe (Moy & Christie 2012; Christie *et al*. 2019), and for *Macrocystis pyrifera* in California (Reed *et al*. 2006), shifts attributed to local biotic and abiotic variables. Variability in fecundity, in addition to dispersal capability, is an important driver of colonization in suitable but vacated habitat patches (Castorani *et al*. 2017). Little is known about the population dynamics of *H. nigripes* along NE Pacific shores. From a genetics point of view, metapopulation dynamics can act synergisticaly with fecundity and reproductive skew so that extirpated habitat patches are recolonized by genetically homogeneous propagules arising from only a few parents, or in extreme circumstances from a single plant. The fixed haplotype differences between populations are consistent with this model.

Metapopulation dynamics and reproductive biology may explain fixed differences between populations, but local extinctions and recolonizations tend to erode genetic diversity by reducing the overall effective sizes of populations (Gilpin 1991). The greater genetic diversity in the Gulf of Alaska relative to neighboring southern populations in British Columbia is indicative of northern refugia (Maggs *et al*. 2008). Divergences between isolated populations driven by drift and natural selection also add to larger diversity estimates among regional populations. Paleotemperatures recorded in marine sediments show that near-shore temperatures were not frigid even though Alaska's southern shores were covered by hundreds of meters of ice. Even though the eastward flowing North Pacific drift and the transition zone were pushed farther south from their present position along central British Columbia (Sabin & Pisias 1996), average sea surface temperatures in the Gulf of Alaska dropped only by about 4°C during the last glacial maximum (Moore *et al*. 1980) to 5–6°C, which is well within physiological tolerances of *H. nigripes*.

Even if temperatures were not limiting, suitable rocky substrates are required for these plants to attach and grow. Several coastal ice-age refugia for large mammals and terrestrial plants have been postulated in the NE Pacific on the basis of semi-fossils and patterns of genetic variability (Peteet & Mann 1994; Heaton *et al*. 1996; Byun *et al*. 1997; Holder *et al.* 1999). The existence of these terrestrial refugia implies that some coastal areas were also free of ice. However, entirely ice-free shorelines may not have been required for kelp refugia. The well-study kelp assemblage on 'boulder patch' off Alaska's Arctic coast can be considered a contemporary analogue of a glacial refugial community. Kelps at this site persist on isolated boulders located on a vast expanse of muddy bottom despite nine months of darkness under snow-covered sea ice (Dunton 1985; Wilce & Dunton 2014). Similar kelp communities have been identified across the high Arctic (Bringloe & Saunders 2019; Küpper *et al*. 2016), with kelp growing as deep as 61 m along the coasts of Greenland (Krause-Jensen et al. 2019). The persistence of kelp communities in the high Arctic shows that algal refugia in the Northeastern Pacific during glacial maxima do not require ice-free shoreines, although seasonally ice-free open water would be required.

The traditionally held view suggests that shallow-water and marine species in the North Pacific and North Atlantic oceans were displaced into southern refugia (Hewitt 1996). However, the imprints of northern glacial refugia are convincingly demonstrated by numerous phylogeographic studies identify patterns of genetic diversity and phylogeographic signal of post-glacial dispersal (Maggs *et al*. 2008) and by ecosystem niche modeling that identify suitable ice-age habitats (Bigg 2014; Assis *et al*. 2014, 2018). The chief genetic evidence for the existence of northern refugia in the Northeastern Pacific is higher levels of genetic diversity (h = 0.652, $\theta_\pi = 0.0015$) relative to diversity in southern populations (h = 0.100, $\theta_\pi = 0.0002$). The general lack of shared haplotypes between the Gulf of Alaska and British Columbia is also consistent with a northern refugium (Figure 2b). The distinctive haplotype in British Columbian populations indicates a separate southern glacial refugium, with subsequent northward dispersal as far as Southeastern Alaska. The lack of samples from western Alaska and the Northwestern Pacific limits our ability to determine whether these northern refugia were located in the central and eastern Gulf of Alaska, or along unglaciated shorelines of the Bering Land Bridge, or additionally in the Northwestern Pacific. Ancestors of of the genetically distinctive Arctic and Atlantic populations may have originated these areas.

Arctic and Eastern Canada

A single dominant *COI*-5P haplotype occured in samples from the Arctic and eastern Canada that was one mutation removed from the dominant haplotype in samples from British Columbia. This disjunct geographical distribution of nearest-neighbor haplotype lineages is difficult to explain with the available data. One possibility is that the eastern Canadian haplotype is present along the Aleutian Islands, in the Bering Sea, or in the Northwestern Pacific, indicating migration across the Arctic following the LGM. This hypothesis is support by the low haplotype and nucleotide diversities estimated for these Arctic-Atlantic populations (Table 1). Further supporting this hypothesis, two haplotypes (6 & 7) from the southern lineage appeared in a small number of plants from the western Gulf of Alaska and may indicate that the southern lineage is common farther to the west. If true, the western Gulf of Alaska may represent the ancestral locaion giving rise to populations in both British Columbia and the Arctic-Atlantic oceans.

Alternatively, the Arctic-Atlantic haplotype may have survived in Atlantic refugia. Simplistic recolonization scenarios have similarly been invoked for the North Atlantic, with marine populations hypothesized to have survived in southern European refugia (Maggs et al. 2008). Genetic studies, however, have increasingly revealed genetic disjunctions between trans-Atlantic marine populations (Wares and Cunninghamn 2001; Bringloe and Saunders 2018; Neiva *et al.* 2018), indicating glacial refugia indeed occurred in the Northwest Atlantic. Unfortunately, the general lack of haplotype and nucleotide diversity in Arctic-Atlantic populations of *H. nigripes* hampers robust assessment of potential refugial locations, particularly whether these populations occurred in southlerly refugia or further north as inferred in Gulf of Alaska populations. Interestingly, the only other haplotype found in Arctic-Atlantic populations of *H. nigripes* was recovered in northern Labrador (location 3; Fig. 2), despite limited sampling for the area. If additional sampling were to reveal enhanced levels of genetic diversity in Arctic populations, the eastern Canadian Arctic could be inferred as a probable location for glacial refugia in marine populations. Though this possibility is not yet corroborated with genetic data, northern refugia have recently been inferred in Atlantic kelp populations based on distributional modelling during the LGM, particularly along the southern shorelines of Greenland and Baffin Island and the northern shorelines of Labrador (Assis et al. 2018). If *H. nigripes* indeed survived the LGM in the Arctic or Atlantic, we can infer that dispersal occurred during a warm interstadial period. Over the last Milankovitch climate cycle, the timings of trans-Arctic dispersals are constrained by the opening of Bering Strait at 140–115, 105–95, 85–75, 85–75 and 9.5–10.0 kyr ago when sea levels rose above the Bering Land Bridge (PALE Beringian Working Group 1999; Grant *et al*. 2012: Spratt & Lisiecki 2016). Dispersals, may also have occurred more recently in the mid Holocene during a millennial cycle of reduced sea-ice cover and increased marine production (Yamamoto *et al*. 2017).

Conclusions

Northern marine populations bear imprints of past glaciations, with long-held assumptions that boreal and Arctic populations are depauperate extensions of southern refugia. Genetic data are shifting the narrative by revealing population structure at odds with simplistic recolonization scenarios. Here, we demonstrated that the kelp species *Hedophyllum nigripes* displays greater genetic diversity in the Gulf of Alaska compared with southern populations. We also demonstrated that Arctic-Atlantic populations are genetically distinct and may have survived in locations previously thought to have been inhospitable to marine populations during glacial maxima. The dispersal and colonization hypotheses outlined here raise exciting new prospects for investigating northern marine biodiversity that warrant further sampling and analysis using additional molecular markers and genomic data.

Acknowledgements Heather Liller, Mandy Lindeberg, and Scott Walker helped to collect samples in Alaska. Judy Berger catalogued the samples and Eric Lardizabal maintain the database of sample information. Paul Kuriscak, Mariel Terry, Zach Pechacek, Nick Ellickson and Chase Jalbert extracted DNA from the samples and Wei Cheng oversaw the sequencing. This project was funded by the North Pacific Research Board Project 1618 and in part by the general fund of the Alaska Department of Fish and Game.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflictf of interest.

Suplemental information

Table S1 Locations of samples, Genbank Accession numbers of cytochrome *c* oxidase subunit I gene (*COI*-5P) & ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) sequences, with references

Table S2 Haplotype frequencies of mitochondrial cytochrome *c* oxidase subunit I gene (COI-5P) (576 base pairs) in samples of *Hedophyllum nigripes* from the northwestern Atlantic and northeastern Pacific oceans

Table S3 Genetic distances (Φ_{ST}) with the Tamura & Nei (1993) model of mutation between samples based on mitochondrial DNA cytochrome oxidase I (COI-5P) (576 base pairs) in *Hedophyllum nigripes*

Table S4 Summary statistics for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) (735 base pairs) in *Hedophyllum nigripes*

Table S5 Haplotype frequencies of choloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base pairs) in samples of *Hedophyllum nigripes* from the Gulf of Alaska

Table S5 Genetic distances (Φ_{ST}) with the Tamura & Nei (1993) model of mutation between samples of *Saccharina nigripes* based on ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base pairs) in *Hedophyllum nigripes*

References

Assis J, Serrão EA, Claro B, Perrin C & Pearson GA. 2014. Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. Mol Ecol 23:2797–2810.

Assis J, Araújo MB, Serrão EA. 2018. Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. Glob Change Biol 24:e55-e66.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Harvard university press.

Bigg GR. 2014. Environmental confirmation of multiple ice age refugia for Pacific cod, *Gadus macrocephalus*. Evol Ecol 28:177–191.

Bintanja R, van de Wal RS & Oerlemans J. 2005. Modelled atmospheric temperatures and global sea levels over the past million years. Nature 437:125–128.

Bittner L, Payri CE, Couloux A, Cruaud C, de Reviers B & Rousseau F. 2008. Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Mol Phylogen Evol* 49:211–226.

Bringloe TT & Saunders GW. 2018. Mitochondrial DNA sequence data reveal the origins of postglacial marine macroalgal flora in the Northwest Atlantic. Mar Ecol Prog Ser 589:45-58.

Bringloe TT & Saunders GW. 2019. DNA barcoding of the marine macroalgae from Nome, Alaska (Northern Bering Sea) reveals many trans-Arctic species. Pol Biol 42:851–864.

Burrowes R, Rousseau F, Müller DG and de Reviers B (2003) Taxonomic placement of *Microzonia* (Phaeophyceae) in the Syringodermatales based on the rbcL and 28S nrDNA sequences. Cryptogam Algol 24:63–73.

Byun S, Koop BF & Reimchen TE. 1997. North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. Evol 51:1647–1653.

Castorani MCN, Reed DC, Raimoni PT, Alberto F, Bell TW, Cavanaugh KC, Siegel DA & Simons RD. 2017. Fluctuations in population fecundity drive variation in demographic connectiviy and metapopulation dynamics. Proc Roy Soc *B* 284:e20162086.

Christie H, Andersen GS, Bekkby T, Fagerli CW, Gitmark JK, Gunderson H & Rinde E. 2019. Shifts between sugar kelp and turf algae in Norway: regime shifts or fluctuations between different opportunistic seaweed species? Font Mar Sc 6:e72.

Dunton KH. 1985. Growth of dark-exposed *Laminaria saccharina* (L.) Lamour. and *Laminaria solidungula* J. Ag. (laminariales: phaeophyta) in the Alaskan Beaufort Sea. J Exp Mar Biol Ecol 94:181–189.

Excoffier L and Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res 10:564–567.

Gilpin M. 1991. The genetic effective size of a metapopulation. Biol J Linn Soc 42:165–175.

Grant KM, Rohling EJ, Bar-Matthews M, Ayalon A, Medina-Elizalde M, Ramsey CB, Satow C & Roberts AP. 2012. Rapid coupling between ice volume and polar temperature over the past 150,000 years. *Nature* 491:744–747.

Grant WS. 2015. Problems and cautions with sequence mismatch analysis and Bayesian skyline plots to infer historical demography. J Hered 106:333–346.

Grant WS & Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J Hered 89:415–426.

Hansen GI. 1997. A revised checklist and preliminary assessment of microbenthic marine algae and seagrasses of Oregon. pp. 175–200. In: TN Kay, A Liston, RM Love, DL Luoma, RJ Meinke & MV Wilson (eds), Conservation and Management of Native Flora and Fungi. Native Plant Society of Oregon, Corvallis.

Heaton TH, Talbot SL & Shields GF. 1996. An ice age refugium for large mammals in the Alexander Archipelago, southeastern Alaska. Quat Res 46:86–192.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological journal of the Linnean Society* 58: 247–276.Hewitt GM. 2004. Genetic consequences of climatic oscillations in the Quaternary. Phil Trans Roy Soc London B 359:183–195.

Hewitt GM. 2004. Genetic consequences of climatic oscillations in the Quaternary. Phil Trans Roy Soc London B 359: 183–195.

Ho SYW, Phillips MJ, Cooper A & Drummond AJ. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Mol Biol Evol 22:1561–1568.

Holder K, Montgomerie R & Friesen VL. 1999. A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). Evolution 53:1936–1950.

Jouzel J, Masson-Delmotte V, Cattani O, Dreyfus G, Falourd S, Hoffman G, Minster B, Nouet J, Barnola JM, Chapellaz J, Fischer H, Gallet JC, Johnsen S, Leuenberger M, Loulergue L, Luethi D, Oerter H, Parrenin F, Raisbeck G, Raynaud D, Schilt A, Schwander J, Selmo E, Souchez R, Spanhi R, Stauffer B, Steffensen JP, Stenni B, Stocker TF, Tison JL, Werner M & Wolff EW. 2007. Orbital and millennial Antarctic climate variability over the past 800,000 years. Science 317:793–796.

Klinkenberg B (Editor). 2018. E-Flora BC: Electronic Atlas of the Flora of British Columbia. http://ibis.geog.ubc.ca/biodiversity/eflora/NorthPacificSeaweeds.html Accessed several times in 2019.

Krause-Jensen D, Serj MK, Bruhn A, Rasmussen MB, Christensen PB, Hansen JLS, Duarte CM, Bruntse G, Wegeberg S. 2019. Deep penetration of kelps offshore along the west coast of Greenland. Front Mar Sci 6:375.

Kumar S, Stecher G and Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Küpper FC, Peters AF, Shewring DM, Sayer MD, Mystikou A, Brown H, Azzopardi E, Dargent O, Strittmatter M, Brennan D, Asensi AO, van West P, Wilce RT. 2016. Arctic marine phytobenthos of northern Baffin Island. J Phycol 52:532–549.

Lindeberg MR & Lindstrom SC. 2010. *Field Guide to Seaweeds of Alaska*. Sea Grant College Program, University of Alaska Fairbanks.

Longtin CM & Saunders GW. 2016. The relative contribution of *Saccharina nigripes* (Phaeophyceae) to the Bay of Fundy Laminariaceae: spatial and temporal variability. Mar Ecol Prog Ser 543:153–162.

Maggs CA, Castilho R, Foltz DW, Henzler C, Jolly MT, Kelly J, Olsen JL, Perez KE, Stam WT, Väinölä R, Viard F & Wares JP. 2008. Evaluating signatures of glacial refugia for north Atlantic benithic marine taxa. Ecology 89:S108–S112.

Moore TC Jr, Burckle LH, Geitzenauer K, Luz B, Molina-Cruz A, Roberston JH, Sachs H, Sancetta C, Thiede J, Thompson P & Wenkam C. 1980. The reconstruction of sea surface temperatures in the Pacific Ocean of 18,000 B.P. Mar Micropal 5:215–247.

Moy FE & Christie H. 2012. Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. Mar Biol Res 8:357–369.

Neiva J, Paulino C, Nielsen MM, Krause-Jensen D, Saunders GW, Assis J, Bárbara I, Tamigneaux É, Gouveia L, Aires T & Marbà N. 2018. Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. Sci Rep 8:e1112.

PALE Beringian Working Group. 1999. Paleoenvironmental Atlas of Beringia presented in electronic form. Quat Res 52:270–271.

Peteet DM & Mann DH. 1994. Late-glacial vegetational, tephra, and climatic history of southwestern Kodiak Island, Alaska. Ecoscience 1:255–267.

Reed DC, Kinlan BP, Raimondi PT, Washburn L, Gaylord B & Drake PT. 2006. A metapopulation perspective on the patch dynamics of giant kelp in southern California. *Marine metapopulations*. Academic Press. Pp. 353–386.

Sabin AL & Pisias NG. 1996. Sea surface temperature changes in the Northeastern Pacific Ocean during the past 20,000 years and their relationship to climate change in Northwestern North America. Quat Res 46:48–61.

Sears JR. (Ed.). 2002. *NEAS keys to benthic marine algae of the northeastern coast of North America from Long Island Sound to the Strait of Belle Isle*. 2nd edition. Express Printing, Fall River, MA. NEAS Contribution Number 2.

Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, De Reviers B and Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the “brown algal crown radiation”. Mol Phylogen Evol 56:659–674.

Spratt RM & Lisiecki LE. 2016. A Late Pleistocene sea level stack. Clim Past 12:1079–1092.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Wares JP & Cunningham CW. 2001. Phylogeography and historical ecology of the North Atlantic intertidal. Evolution 55:2455-2469.

Weir BS and Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wilce RT & Dunton KH. 2014. The boulder Patch (North Alaska, Beaufort Sea) and its benthic algal flora. Arctic 67:43–56.

Table 1 Summary statistics for mitochondrial DNA cytochrome oxidase I (*COI*-5P) (576 base pairs). N = sample size, N_{poly} = number of polymorphic nucleotide sites, N_{H} = number of haplotypes, N_{EH} = expected number of haplotypes under neutrality, N_{PH} = number of private haplotypes, h = haplotype diversity (Standard Deviation), θ_π = nucleotide diversity (Standard Deviation), D = Tajima's test for neutralilty (Probability)

Location	N	N_{poly}	N_{H}	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
Arctic and NE Atlantic											
1	7	0	1	1.00	0	0.0	–	0.0	–	–	–
2	3	0	1	1.00	0	0.0	–	0.0	–	–	–
3	5	1	2	1.78	1	0.400	0.237	0.069	0.088	-816	0.305
4	10	0	1	1.00	0	0.0	–	0.0	–	–	–
Mean	6.25	0.25	1.3	1.20	0.25	0.100	–	0.017	–	–	–
Pooled	25	1	2	1.24	1	0.080	0.072	0.014	0.030	-1.158	0.782
NE Pacific, Alaska											
5	3	4	3	1.00	2	1.0	0.272	0.463	0.416	0.0	1.0
6	4	1	2	1.90	0	0.500	0.265	0.087	0.011	-0.612	0.389
7	7	0	1	1.00	0	0.0	–	0.0	–	–	–
8	9	1	2	1.52	1	0.222	0.166	0.039	0.057	-1.088	0.202
9	29	5	6	2.51	4	0.374	0.113	0.071	0.075	-1.868	0.009
10	5	0	1	1.00	0	0.0	–	0.0	–	–	–
Mean	9.5	1.8	2.5	1.49	1.2	0.349	–	0.110	–	–	–
Pooled	57	11	11	6.24	9	0.652	0.043	0.149	0.118	-1.673	0.021
NE Pacific, British Columbia											
11	15	0	1	1.00	0	0.0	–	0.0	–	–	–
12	5	1	2	1.00	1	0.400	0.237	0.069	0.088	-0.817	0.311
Mean	10	0.5	1.5	1.00	0.5	0.200	–	0.035	–	–	–
Pooled	20	1	2	1.28	1	0.100	0.088	0.017	0.034	-1.164	0.135
Overall	102	13	13	9.25	10	0.746	0.026	0.211	0.150	-1.380	0.622

Table 2 Summary statistics for concatenated sequences of cytochrome oxidase I (*COI*-5P) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) (1394 base pairs) for populations in the Gulf of Alaska. N = sample size, N_{poly} = number of polymorphic nucleotide sites, N_H = number of haplotypes, N_{EH} = expected number of haplotypes under neutrality, N_{PH} = number of private haplotypes, h = haplotype diversity (Standard Deviation), θ_π = nucleotide diversity (Standard Deviation), D = Tajima's test for neutralilty (Probability)

Location	N	N_{poly}	N_H	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
1	4	2	3	3.04	1	0.833	0.222	0.072	0.071	-0.710	0.285
2	7	0	1	1.00	0	0.0	–	0.0	–	–	–
3	9	1	2	1.52	2	0.222	0.166	0.016	0.023	-1.088	0.203
4	27	7	7	2.63	6	0.402	0.119	0.037	0.036	-2.166	0.002
5	5	0	1	1.00	1	0.0	–	0.0	–	–	–
Mean	10.4	2.0	2.8	1.84	2.0	0.291	–	0.025	–	–	–
Pooled	52	10	12	8.46	10	0.770	0.046	0.091	0.065	-1.210	0.114

Table 3 Haplotype frequencies of contatenated fragments of concatenated seqences of mitochondrial DNA cytochrome oxidase I (*COI*-5P) and chloroplast DNA ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1492 base pairs) for populaions in the Gulf of Alaska

Haplotype	1	2	3	4	5	Total
1	1	.	.	21	.	22
2	2	7	.	.	.	9
3	.	.	8	.	.	8
4	.	.	.	.	5	5
5	1	.	.	.	.	1
6	.	.	1	.	.	1
7	.	.	.	1	.	1
8	.	.	.	1	.	1
9	.	.	.	1	.	1
10	.	.	.	1	.	1
11	.	.	.	1	.	1
12	.	.	.	1	.	1
Total	4	7	9	27	5	52

Table 4 Genetic distances (Φ_{ST}) between populations in the Gulf of Alaska based on concatenated sequences of mitochondrial DNA cytochrome oxidase I (*COI*-5P) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) (1492 base pairs) with the Tamura (1992) model of mutation. Italics $0.05 > P > 0.01$; Bold $P < 0.01$.

2	0.1513			
3	**0.7852**	**0.9332**		
4	**0.4668**	**0.6805**	**0.7941**	
5	**0.7811**	*1.0*	**0.9492**	**0.6803**
	1	2	3	4
		Location		

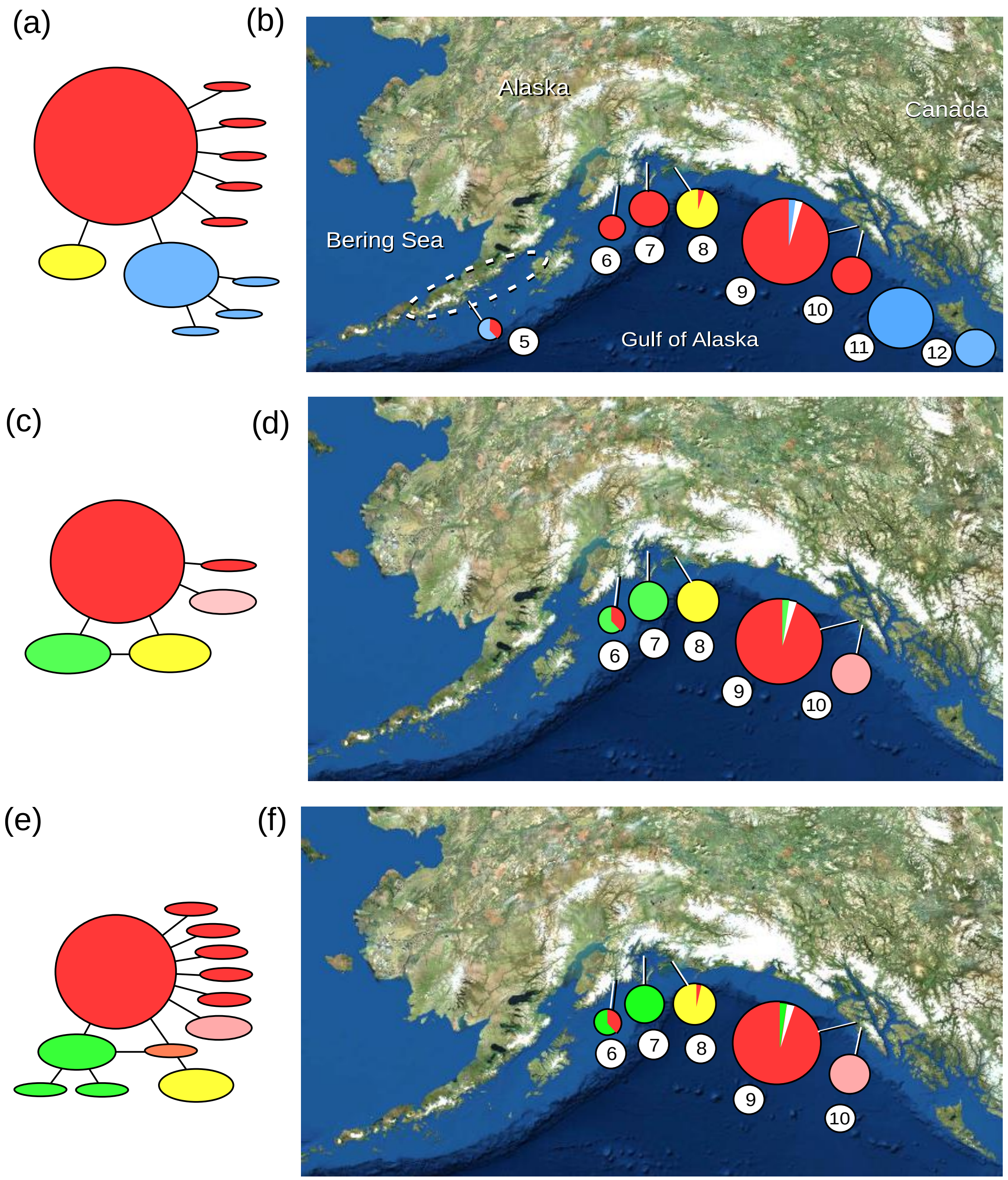

Figure 1 Geographical distributions of haplotypes in the NE Pacific Ocean for *Hedophyllum nigripes*. (a) Haplotype network of *COI*-5P sequences (576 bp). (b) Frequencies of mitochondrial *COI*-5P haplotypes among samples. (c) Haplotype network of chloroplast *rbc*L sequences (735 bp). (d) Frequencies of chloroplast *rbcL* among samples. (e) Haplotype networks of concatenated *COI*-5P and *rbc*L sequences (1492 bp). (f) Frequencies of *COI*-5P+*rbc*L haplotypes among samples.

Figure 2 Geographical distributions of cytochrome oxidase I haplotypes (576 base pairs) for *Hediphyllum nigripes* in North America

Table S1 Locations of samples, Genbank Accession numbers of cytochrome *c* oxidase subunit I gene (COI-5P) & ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) sequences, with references

Group, Location	North latitude	West longitude	COI Genbank Accession No.	rbcL Genbank Accession No.	Source
1. New Brunswick, Nova Scotia, Canada					
Grand Manan, New Brunswick	44.625	66.860	GU097741		McDevit & Saunders 2009
Wallace Cove, New Brunswick	45.039	66.808	MH309295		Bringloe & Saunders, in prep.
Bay of Fundy, New Brunswick	45.072	66.469	GU097746		McDevit & Saunders 2009
Bay of Fundy, Nova Scotia	45.173	64.757	MH309610		Bringloe & Saunders, in prep
Brier Island, Nova Scotia	44.288	66.342	GU097742		McDevit & Saunders 2009
Cap St. LeMoine, Nova Scotia	44.692	65.785	GU097744, GU097747		McDevit & Saunders 2009
2. New Foundland, Quebec, Canada					
Cape ray, Newfoundland	47.623	59.291	GU097749		McDevit & Saunders 2009
English Harbor, Newfoundland	47.633	54.87	GU097748		McDevit & Saunders 2009
Escoumins, Quebec	48.318	69.414	HM891053		
3. Labrador, Canada					
Hogg Island	59.429	63.715	MH309630		Bringloe & Saunders, in prep
Evans Bight	59.429	63.524	MH309211, MH309500		Bringloe & Saunders, in prep
Duck Islands	60.234	64.341	MH310020, MH309117,		Bringloe & Saunders, in prep
4. Manitoba, Canada					
NW of Eskimo Island, Churchill	58.8115	94.2197	HM891022, HM891028, HM891045, HM981058, GU097736, GU097737, GU097743		McDevit & Saunders 2009
Bluff A, Churchil	57.77	93.847	GU097739		McDevit & Saunders 2009
Wreck of Ithaca, Churchill	58.768	93.89	GU097745, HM891064		McDevit & Saunders 2009

Alaska					
5. Western Gulf of Alaska			MH327950, MH327952, MH327953		Starko *et al*. 2018
6. Resurrection Bay	60.0643	149.4427	n = 4	n = 3	This study
7. Northwest Bay, Prince William Sound	60.5147	147.5947	n = 7	n = 8	This study
8. Cordova, Prince William Sound	60.5425	145.7686	n = 9	n = 10	This study
9. Halibut Point, Sitka	57.0796	135.3769	n = 29	n = 28	This study
10. Harris Island, Sitka	57.0366	135.2789	n = 5	n = 5	This study
11. Northern British Columbia					
Prince Rupert	54.301	130.251	FJ409194, FJ409195		McDevit & Saunders 2009
Prince Rupert	54.221	130.329	FJ409196, FJ409196		McDevit & Saunders 2009
Mazarredo Island, Haida Gwaii	54.091	132.551	HM890930		Biodiversity Institute, U of Guelph
North Beach, Haida Gwaii	54.033	132.053	HQ990659		Biodiversity Institute, U of Guelph
Cowley Islands, Haida Gwaii	53.692	132.368	HQ990676		Biodiversity Institute, U of Guelph
Kwuna Island, Haida Gwaii	53.217	131.986	HQ990664		Biodiversity Institute, U of Guelph
Ramsey Island, Gwaii Haanas	52.586	131.372	GU097734, GU097735		McDevit & Saunders 2009
Ramsey Island, Gwaii Haanas	52.569	131.403	GU097733		52.569McDevit & Saunders 2009
Murchison Island Lagoon, Gwaii Haanas	52.604	131.45	GU097732		McDevit & Saunders 2009
Burnaby Island, Gwaii Haanas	52.449	131.283	HM890938		Biodiversity Institute, U of Guelph
Alder Island, Gwaii Haanas	52.442	131.319	HQ990681		Biodiversity Institute, U of Guelph
Burnaby Narrows, Gwaii Haanas	52.360	131.352	GU097731		McDevit & Saunders 2009
12. Southern British Columbia					
Point Holmes, Comox, Vancouver Island	49.6903	124.87	GU097738		McDevit & Saunders 2009
Botany Bay, Port Renfrew	48.53	124.454	FJ409198		McDevit & Saunders 2009
Spring Bay, Vancouver Island	48.456	123.269	MH310014, HM891339, HM891341		Bringloe & Saunders, in prep.

Table S2 Haplotype frequencies of mitochondrial cytochrome *c* oxidase subunit I gene (*COI*-5P) (576 base pairs) in samples of *Hedophyllum nigripes* from the northwestern Atlantic and northeastern Pacific oceans. Sample numbers as in Table 1.

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	Total
1	.	.	.	.	1	3	7	1	23	5	.	.	41
2	7	3	4	10	.	.	.	.	.	.	.	.	25
3	.	.	.	.	.	.	.	.	2	.	15	4	21
4	.	.	.	.	.	.	.	8	.	.	.	.	8
5	.	.	1	.	.	.	.	.	.	.	.	.	1
6	.	.	.	.	1	.	.	.	.	.	.	.	1
7	.	.	.	.	1	.	.	.	.	.	.	.	1
8	.	.	.	.	.	1	.	.	.	.	.	.	1
9	.	.	.	.	.	.	.	.	1	.	.	.	1
10	.	.	.	.	.	.	.	.	1	.	.	.	1
11	.	.	.	.	.	.	.	.	1	.	.	.	1
12	.	.	.	.	.	.	.	.	1	.	.	.	1
13	.	.	.	.	.	.	.	.	.	.	.	1	1
Total	7	3	5	10	3	4	7	9	29	5	15	5	104

Table S3 Genetic distances (Φ_{ST}) with the Tamura & Nei (1993) model of mutation between samples based on mitochondrial DNA cytochrome oxidase I (COI-5P) (576 base pairs) in *Hedophyllum nigripes*. Italics indicates signficance of 0.05 > *P* > 0.01; Bold numbers indicate significance at *P* < 0.01.

2	0.0										
3	0.073	0.0									
4	0.0	0.0	0.149								
5	**0.658**	0.428	*0.505*	**0.734**							
6	**0.924**	*0.868*	**0.819**	**0.942**	0.245						
7	**1.0**	**1.0**	**0.926**	**1.0**	0.482	0.152					
8	**0.956**	**0.939**	**0.909**	**0.964**	**0.665**	**0.729**	**0.859**				
9	**0.846**	**0.825**	**0.820**	**0.857**	**0.515**	0.029	0.0	**0.678**			
10	**1.0**	*1.0*	**0.909**	**1.0**	0.378	0.062	0.0	**0.838**	0.0		
11	**1.0**	**1.0**	**0.921**	**1.0**	**0.530**	**0.923**	**1.0**	**0.957**	**0.759**	**1.0**	
12	**0.864**	*0.784*	**0.715**	**0.893**	0.131	**0.694**	**0.864**	**0.864**	**0.680**	**0.833**	0.240
	1	2	3	4	5	6	7	8	9	10	11
	Location										

Table S3 *Hedophyllum nigripes*: Summary statistics for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) (735 base pairs). N = sample size, N_{poly} = number of polymorphic nucleotide sites, N_{H} = number of haplotypes, N_{EH} = expected number of haplotypes under neutrality, N_{PH} = number of private haplotypes, h = haplotype diversity (Standard Deviation), θ_π = nucleotide diversity (Standard Deviation), D = Tajima's test for neutralilty (Probability)

Location	N	N_{poly}	N_{H}	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
1	3	1	2	2.03	0	0.667	0.314	0.091	0.113	0.0	1.0
2	8	0	1	1.00	0	0.0	–	0.0	–	–	–
3	10	0	1	1.00	1	0.0	–	0.0	–	–	–
4	28	3	3	1.45	1	0.140	0.087	0.029	0.040	-1.511	0.047
5	5	0	1	1.00	1	0.0	–	0.0	–	–	–
Mean	10.8	0.8	1.6	1.30	0.6	0.161	–	0.024	–	–	–
Pooled	54	4	5	6.22	3	0.578	0.047	0.011	0.089	0.343	0.677

Table S5 Haplotype frequencies of choloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base pairs) in samples of *Hedophyllum nigripes* from the Gulf of Alaska

Haplotype	1	2	3	4	5	Total
1	1	.	.	26	.	27
2	2	8	.	1	.	11
3	.	.	10	.	.	10
4	.	.	.	.	5	5
5	.	.	.	1	.	1
Total	3	8	10	28	5	54

Table S6 Genetic distances (Φ_{ST}) with the Tamura & Nei (1993) model of mutation between samples of *Saccharina nigripes* from the Gulf of Alaska based on ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (735 base pairs) in *Hedophyllum nigripes*. Italics indicates $0.05 > P > 0.01$. Bold indicates $P < 0.01$.

2	**0.342**			
3	**0.861**	**1.000**		
4	*0.672*	**0.890**	**0.900**	
5	*0.862*	**1.000**	**1.000**	**0.888**
	1	2	3	4
		Location		

Figure S1 *Hedophyllum nigripes* (a) young plants (photo Jenn Burt). (b) young plants at Whittier, Alaska

Chapter 6

Molecular Ecology Research Article

Chaotic geographical structure of five cryptic lineages of ribbon kelp *Alaria* in Alaska reflects post-glacial isolation-by-colonization

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Abstract

Population structures of marine species can be shaped by three mechanisms: isolation by distance (IBD), isolation by adaptation (IBA) and isolation by colonization (IBC). Mitochondrial *COI*-5P, chloroplast *rbc*L and 8 microsatellite were surveyed in 16 populations of the rocky intertidal kelp, *Alaria*, along a 2700 km transect around the Gulf of Alaska in the Northeastern Pacific Ocean. The two organellar genes resolved 5 genetically well-separated lineages (d = 0.0101 to 0.0398) with several populations fixed, or nearly fixed, for a single haplotype lineage. Microsatellite allele frequencies tended to cluster samples by organellar lineage rather than by geography, likely indicating a recent expansion of the lineages. Microsatellite markers also indicated that plants in separate lineages hybridized where they co-occurred at three localities. Both organellar and nuclear genes failed to show IBD, indicating that migration is limited or that populations have not reach drift-migration equilibrium after post-glacial colonizations. IBA may be responsible for some genetic differences between populations located along wave-energry and salinity gradients. However, the mosaic pattern of variability among populations on geographical scales of hundreds of kilometers appears to be due to IBC and incumbency, which limits the successful immigration of genetically divergent plants from other localities.

Keywords: *COI*-5P, chloroplast DNA, incumbency, isolation-by-colonization, mitochondrial DNA, Pleistocene glacial refugia, Northeastern Pacific Ocean, phylogeography, *rbc*L

Introduction

Genetic differentiation among populations of marine organisms can arise from several mechanisms that limit gene flow. Understanding how gene flow is restricted between populations is a continuing challenge and is the focus of most population genetic studies. Most marine species have large dispersal potentials enabled by planktonic gametes or propagules, but the appearance of genetic differences between populations provides evidence that these dispersal potentials are not fully realized. Several mechanisms can limit gene flow. While dispersal potential in marine species is large, it is not infinite so that some populations may be isolated from one another by distance; more widely separated populations are expected to show greater genetic divergence (Bohonak 1999). Surveys of molecular markers show that genetic variability in numerous species can be explained by isolation by distance (IBD) (Raimondi *et al.* 2004; Krueger-Hadfield 2013; Wright *et al*. 2015). Correlations between geographic isolation and genetic distance are greatest for a given dispersal ability when populations are in drift-migration equilibrium (Slatkin 1993).

Other mechanisms, however, may also restrict gene flow between populations. Adaptive responses to habitat variables can be another source of genetic heterogeneity among populations (Wang & Bradburd 2014; Drinan *et al*. 2015). While adaptation may be an important source of variability among populations, the use of neutral markers in the present study can detect only the indirect effects of selection as shifts in allele frequencies among populations that may be difficult to distinguish from random drift. Several examples of local adaptation can be found among seaweeds (Nielsen *et al*. 2009; Nielsen 2016a, b). Although individuals may disperse into a population, they may not pass on as many offspring as locally adapted individuals do, and hence do not contribute significantly to gene flow. The alignment of phylogeographic boundaries with biogeographic boundaries may reflect isolation by adaptation (IBA) (Orsini *et al*. 2013; Bowen *et al.* 2015). To distinguish IBA from IBD, genetic distances between populations, estimated with neutral markers, are compared with ecological or adaptive distances. The two correlations may or may not be consistent with each other (*e.g*. Calegario *et al*. 2019), or may be confounded when both distance and adaptation are isolating populations from one another (Orsini *et al*. 2013; Sexton *et al*. 2014).

Yet other mechanisms can shape patterns of genetic diversity. Post-glacial colonizations from refugia, gene surfing, high-density blocking and competetive exclusion are all underpinned by ‘founder-takes-all’ exclusions of immigrants (Waters *et al*. 2013). The chief evidences for adaptive incumbency or high-density blocking are sharp genetic boundaries between populations that cannot be explained by environmental discontinuity (Neiva *et al*. 2012) and on long time scales the replacment of one group of species with fundamentally different species after mass extinctions (Jablonski & Sepkoski 1996). Classic examples of incumbency include the diversification of mammals after the end-Cretaceous extinctions of dinosaurs (Valentine 1980) and the radiation of telost bone-ray fishes in the Paleocene (Siebert & Norris 2015; Alfaro *et al.*

2018). In both cases long-standing minor components of a biota diversified after the demise of incumbent competitors.

Opportunities to diversify also arise with the appearance of new colonizable habitats, such as on newly created islands or along recently deglaciated shorelines. It is uncertain, however, whether incumbency shapes diversity on ecological time scales (Jalonski & Sepkoski 1996). Waters *et al*. (2013) argued that density-dependent exclusions by incumbents are common and have been overlooked by phylogeographers. In the marine realm, Neiva *et al*. (2012) concluded that sharp genetic discontinuities between populations of an estuarine seaweed arose from seconary contact and density-dependent exclusion of genetically divergent lineages by the initial founders, despite the potential for gene flow. On larger geographical and temporal scales, temperate and subpolar marine habitats were profoundly affected by periodic ice ages that led to isolations and extinctions, and to post-glacial dispersals and colonizations (Hoarau *et al.* 2007). Genetic variability among populations of some marine species can be traced to these ice-age influences (Grant & Utter 1984; Marko *et al.* 2010; McGovern *et al*. 2010).

Several approaches can be used to distinguish among these mechanisms that shape contemporary patterns of genetic diversity within and among populations. Restrictions on gene flow can be assessed with hierarchical tests of allele-frequency differences among populations and with a test for isolations by distance (IBD). Allele-frequency differences, however, can appear not only as a direct result of contemporary barriers to dispersal, but also as relict divergences from historical isolations. IBD is expected to appear among populations that have limited amounts of dispersal so that gene flow is a function of geographical distance. The results of these tests help to identify more or less homogeneous metapopulations with demographies that are independent of other metapopulations.

On larger spatial and temporal scales, climate change can lead to adaptive shifts, or drive populations to extinction if they cannot adapt. The shores of the Northeastern Pacific Ocean have periodically been buried under hundreds of meters of ice at some localities. Species may have survived these ice ages in scattered habitats between glacial lobes. Surviving populations should bear genetic signatures of these event that would be evident in estimates of genetic diversity and genetic distances between populations or lineages. The depth of divergence is assumed to be proportional to time, and several coalescence methods have been used measure time from a common ancestor. While this approach has considerable appeal, the assumptions in the Kingman *n*-coalescent used in these methods is inappropriate to model the genetics of species, such as seaweeds, that have strong reproductive skew (Eldon & Wakely 2006). These analyses cannot clearly distinguish imprints of historical population growth from the effects of reproductive skew (Niwa *et al*. 2016; Matuszewski *et al*. 2018). The approach used in the present study is to use the molecular clock hypothesis to make rough estimates of times of divergence between lineages.

In the present study, we show that the rocky-intertidal kelp, *Alaria*, has a highly subdivided population structure but lacks an IBD signal among populations. *Alaria* has two intertidal phases, a sporophyte with large blades up to 2–3 meters in length and a microscopic phase of filamentous male and female gametophytes. These small plants produce gametes with limited dispersal that fuse to produce a new generation of macroscopic sporophytes. Seasonal environmental influences on either or both phases can shape the demography of a local population. *Alaria* tends have an annual life cycle in southern localities but a perennial at high latitudes (McConnico & Foster 2005). Twelve species of *Alaria* are currently recognized: one in the North Atlantic and 11 in the North Pacific Ocean and Bering Sea (Widdowson 1971; Lane *et al*. 2007); four of the 11 Pacific species, *A. marginata*, *A. nana*, *A. tenuifolia* and *A. taeniata*, inhabit the shores of Northeastern Pacific and variously extend from Central California to the eastern Aleutian Islands.

The present study builds on the previous work of Lane *et al*. (2007), who examined sequence variability in the nuclear internal transcribed spacer (*ITS*), the chloroplast Rubisco operon spacer (*rbc*Sp) and the mitochondrial cytochrome oxidase I (*COI*-5P) in samples largely from the Northeastern Pacific. Their study included Northeastern Pacific taxa, *A. marginata*, *A. nana*, *A. taeniata*, *A. tenuifolia*, and *Eularia fisulosa*, Northwestern Pacific *A. praelonga* and *A crassifolia*, and Bering Sea *A. crispa*. Except for *Eualaria. fistulosa*, the morphological traits used to identify Northeastern Pacific taxa did not correspond well partitions seen with the molecular markers. They concluded that Northeastern Pacific *Alaria* consisted of a ‘species complex’ of partially divergent, but reassociating and interbreeding taxa that had previously been isolated by glacial ice sheets.

The goal of the present study was to expand on these results with a survey of populations extending 2700 km from the western Aleutians to Southeastern Alaska with three marker types, including mitochondrial DNA, chloroplast DNA and microsatellites. We then used the results to distinguish among mechanims shaping the genetic structure of populations around the Gulf of Alaska. *COI*-5P resolved five deeply divided lineages that did not coincide with taxonomic categories based on morphology. These lineages are scattered across the Gulf of Alaska in a mosaic pattern that discounts IBD, but could be consistent with IBA on spatial scales of tens of meters to hundreds of kilometers, or in some cases the distance between wave-exposed and wave-protected sites along a shore. On larger scales, rapid post-glacial colonizations in the Northeastern Pacific may have established incumbencies that resisted the intrusion of localy less-adapted migrants from other populations. The contrast between shallow genetic distances between taxa in the Northeastern Pacific and deeply divergent species of *Alaria* in the Northwestern Pacific and Bering Sea likely reflect greater disturbances from repeated glaciations along Northeastern Pacific shores than along the Asian shores of the Northwestern Pacific.

Materials and Methods

Laboratory methods

Samples were collected at 16 rocky intertidal sites along the coast of Alaska (Fig. 1a, Table S1). About 2 cm^2 of frond, or sporophyll was damp dried and placed in a dessicator filled with silica beads soon after collection. DNA was extracted from tissues with the NucleoSpin® 96 Plant II (Macherey-Nagel Inc., Bethlehem, PA) kit.

A segment of *COI* at the 5' end of the gene was amplified with the polymerase chain reaction (PCR) using the forward primer *GazF2* (5' CCAACCAYAAAGATATWGGTAC 3') and reverse primer *GazR2* (5' GGATGACCAAARAACCAAAA 3') (Lane *et al*. 2007). A segment of *rbcL* was amplified with PCR using the forward primer *rbc*L-*543F* (5' CCWAAATTAGGTCTTTCWGGWAAAAA 3') (Bittner *et al*. 2008; Silberfeld *et al*. 2010) and reverse primer *rbc*L-*1381R* (5' ATATCTTTCCATARRTCTAAWGC 3') (Burrowes *et al*. 2003; Silberfeld *et al*. 2010). A PCR cocktail consisted of a 50 µL mixture of 2.0 µL template DNA in 1x Colorless GoTaq Flexi buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1µM of forward and reverse primers, and 2.5U GoTaq Flexi DNA polymerase. PCR amplifications were conducted in ABI 9700 thermocyclers with an initial denaturation at 94° C for 3 min, followed by 35 amplification cycles of 45 s at 94° C, 1 min at primer annealing temperature 50° C for *COI* and 52° C for *rbcL*, and 1 min 30 s at 72° C, with a final 5 min at 72°C. PCR amplifications were sequenced in the forward and reverse directions by Genewiz Inc. (South Plainfield, NJ) or by the University of Arizona Genetics Core. Forward and reverse-complement sequences were aligned and edited with MEGA 7.0.20 (Kumar *et al*. 2016) and chromatograms viewed with FINCH TV 1.4.0 (Geospiza Inc.). Unique haplotypes from each of the 96-well plates were re-extracted and re-sequenced for quality control.

Eight microsatellite loci were amplified with PCR primers *An21*, *An23*, *An26*, *An27*, *An30*, *An31*, *An38* and *An29* (Collens 2009) previously developed for *Alaria marginata* with Gene Amp PCR System 9700 (Applied Biosystems, Inc., Foster City, CA). Each 10 µL reaction cocktail consisted of 2 µL template DNA (~0.1µg/µL) in 1x Colorless GoTaq Flexi Buffer (Promega Inc. Madison, WI), 1.5–3.0 mM $MgCl_2$ (Promega Inc. Madison, WI), 0.20 mM of each nucleotide (Applied Biogsytems, Inc.), 0.05–0.25 µM of forward and reverse primers, 0.1 mg/mL of BSA (Sigma Inc. St. Louis, MO), 0.05 U GoTaq Flexi DNA polymerase (Promega Inc. Madison, WI), and deionized water. Optimal thermal cycling profiles varied among loci (Table S2). Microsatellites were fractionated by size with electrophoresis in an Applied Biosystems 3730 capillary DNA sequencer, and scored with GENEMAPPER 5.0 (Applied Biosystems) independently by two technicians. A subset of 8% of the samples was re-extracted and re-genotyped by a third technician for quality control.

Statistical methods

For the organellar DNA sequences , ARLEQUIN 3.5.2.2 (Excoffier & Lischer 2010) was used to estimate the number of polymorphic nucleotide sites, N_{poly}, the number of observed, N_{H}, and expected, N_{EH}, number of haplotypes under neutrality. ARLEQUIN was also used to estimate gene diversity, *h*, and nucleotide diversity, θ_{π}. Divergence between populations was estimated with *F* statistics (Weir & Cockerham 1984) and with Φ_{ST} with an appropriate mutation model determined with MEGA 7 (Kumar *et al*. 2016). IBD 1.52 (Bohonak 2002) was used to test for isolation-by-distance with Mantel's test between difference matrices of pairwise genetic distsances with correction for diversity [$\Phi_{\mathrm{ST}}/(1-\Phi_{\mathrm{ST}})$] in organellar DNA or [$F_{\mathrm{ST}}/(1 - F_{\mathrm{ST}})$ for microsatellites and approximate large-scale shoreline distances between samples. Tests were made with geographic distances, with or without a log transformation, and coefficients were esimated with 1000 randomizations. Analysis of molecular variation (AMOVA) in ARLEQUIN was used to describe geographical structure.

An initial analysis of microsatellite genotypic data was made with GENEPOP 4.6 (Rousset 2008) to search for null alleles. When these results indicated the presence of null alleles, we used MICRO-CHECKER 2.2.3 (Van Oosterhout *et al*. 2004) to further confirm the presence of null alleles, and to determine whether allele stuttering or large-allele dropout had affected a dataset. Some loci failed to amplify with PCR in some individuals, even though amplifications were successful at other loci. These PCR failures were assumed to be homozygous genotypes of null alleles.

GENEPOP was used to test for deviations from expected Hardy-Weinberg genotypic proportions, using Markov-chain-Monte-Carlo chains 10 000 steps in 100 batches. Because of the repeated tests aong loci we used a Bonferroni correction (Rice 1989) of $P = 0.05/12 = 0.004$ to control type I error at $\alpha = 0.05$. GENETIC DATA ANALYSIS (GDA, Lewis & Zaykin 2001) was used to estimate observed (H_{O}) and exptected (H_{E}) heterozyzygosity averaged over loci, to count the number of alleles at each locus and to estimate the inbreeding coeficient, F_{IS}. FSTAT (Goudet 1995) was used to estimate allelic richness based on the smallest sample size to be able to compare levels of diversity among samples of different sizes.

F_{ST} between populations was estimated with GENEALEX 5.03 (Peakall & Smouse 2012) with 9999 permutations to establish departures from 0.0. The number of migrants between pairs of populations per generation was estimated from F_{ST} with Wright's island model of migration $F_{\mathrm{ST}} = 1/(4Nm + 1)$. Principal components analysis (PCA) of allele-frequency variability among samples with standardized covariances was used to search for genetic differences among samples. The model for AMOVA was framed by the geography of the samples and was the same model used for the analyses of organellar DNA. To establish signficance of AMOVA structure, 9999 permutations was used to shuffle individuals among samples and regions. Missing genotypes for some loci in some plants were estimated with sample averaging for both the PCA and AMOVA. Results for each locus were summed across loci under the assumption of linkage equilibrium and independence among loci.

IBD 1.52 (Bohonak 2002) was used to test for isolation-by-distance with Mantel's test between difference matrices of linearized F_{ST} [F_{ST}/(1-F_{ST})] between paris of samples and approximate shoreline distances between samples, with and without a $\log_{10}$ transformations of distance. ARLEQIUN was used to estimate the number of migrants between populations with Nm = 0.25[(1/F_{ST}) – 1] under the island model of migration and by assuming drift-migration equilibrium, no mutation, and a large number of populations exchanging individuals (Slatkin 1991).

Results

rbcL

Four nucleotide polymorphisms in a 740 base pair segment of the chloroplast gene *rbc*L defined five haplotypes (Table S3, Fig. 1a). Most plants carried one of the two most abundant haplotypes, which were were separated from each other by two mutations. Haplotype diversity (*h*) ranged from 0.0 to 0.512 and averaged 0.127. Nucleotide diversity (θ_π) ranged from 0.0 to 0.0014 and average 0.0006 (Table S4). Tajima's D was not significant in any of the samples, or overall in the pooled sample. Mean sequence divergence (Φ_{ST}) between samples varied widely from 0.0 for populations fixed for the same haplotype to 1.0 between samples not sharing any haplotypes (Figure 1b, Table S5). Sequence divergence overall was 0.830. AMOVA indicated that 83% of sequences variability occurred among populations and 17% was due to variability on average among individuals within populations (Table S6).

COI-*5P*

The mitochondrial DNA *COI*-5P was much more polymorphic than *rbc*L. Polymorphisms at 36 nucleotide sites defined 27 haplotypes that fell into five lineages (A-E), each separated from other by at least 5 mutations (Fig. 1c; Tables S7, S8). Samples tended to be fixed or have a large frequency of one haplotype and low frequencies of related haplotypes (Fig. 1d. Twelve of the 16 samples had private haplotypes. Samples 1, 13, 14, had a mix of lineages producing the largest haplotype diversities (0.232–0.636) and nucleotide diversities (0.0023–0.0052). Tajima's *D* indicated that haplotype distributions in three populations (5, 12, 15) deviated from neutralilty, but not over all in the pooled sample. Average sequence divergences (Φ_{ST}) between samples ranged from 0.0, for populations fixed for the same haplotype, to 1.0, for populations that did not share haplotypes (Table S9). AMOVA indicated that 90.4% of the variability was due to differences among populations and 9.6% was due to diversity within populations, on average (Table S10). Overall, Φ_{ST} was 0.904.

Concatenated rbcL & COI-*5P*

We concatenated the *rbc*L and *COI*-5P sequences (1404 bp) for further analyses of a dataset containing 543 plants (Tables 1, S11). A total of 41 nucleotide polymorphisms defined 33 haplotypes (Fig. 1e; Table 1). Gene diversity (h) ranged from 0.0 to 0.636 and averaged 0.290 among samples. Overall gene diversity in the pooled sample was much larger ($h = 0.903$) because a large portion of the diversity was due to differences among populations. Nucelotide diversity ranged from 0.0 to 0.00264 and averaged 0.061. The number of private alleles ranged from 0 to 4 among samples and averaged 1.8 alleles. Tajima's D was significant in only sample 5 ($D = -2.206$, $P = 0.002$, Afognak Island) and was not significant overall in a pooled sample ($D = 0.776$, $P = 0.832$).

Sequence divergence between populations ranged from 0.0 between adajcent populations 9 (Cordova) and 11 (Yakutat), which were both fixed for haplotype A1 (Fig. 1f), to 1.0 between populations 8 and 9 and between 8 and 11, which were fixed for different haplotypes (Table S12). No IBD was detected with Mantel's test for a correlation between genetic distance [$F_{ST}/(1-F_{ST})$] and large-scale shoreline distance between samples ($r = 0.074$, $P = 0.737$) (Fig. 2a). AMOVA indicated that 98.6% of the variability was due to differences among populations and 10.4% was due to diversity among plants within populations on average (Table S13). Overall, Φ_{ST} was 0.896.

The genealogy reconstructed from the concatenated sequences resolved additional phylogeographic structure within lineages B, C and D. Lineage B consisted of geographically separated B1 in Kachemak Bay and B2 around Kodiak Island (3 & 4) and in Kachemak Bay (5) (Fig. 1f). Lineage C was resolved into C1 in Kachemak Bay (7) and Little Port Walter (15) and C2 around Magoun Island (13). C1 appeared in widely scattered populations 1, 7 & 15. Haplotype D1 appeared around Afognak Island (5) in the western Gulf of Alaska and at Watson Point (14) in Southeastern Alaska.

Microsatellites

Four loci had more than two alleles as expected for a diploid locus in samples 1, 2, 3 & 12 and were interpreted to represent chromosomal duplications (Table 2). Since the alleles at these loci were similar in size and did not appear to come from other populations, the number of alleles at these loci was reduced to 2 using random numbers to exclude excess alleles. Genotypes were added to samples 8, 9 & 10 for loci 4, 2 & 8 which showed complete PCR failures for these samples. The additions of invariant genotypes to missing data is rationalized in part by the general occurrence of fixed, or nearly, genotype frequences in several of the samples. Other missing genotypes were estimated in GENEALEX with the missing data function that uses averages of observed genotypes before commencing a particular analysis. These modifications may lead to underestimates of allelic diversity and heterozygosity, but should leave analyses based on allelic numbers, such at the relatively unaffected.

After these modifications, the number of alleles at a locus ranged from 16 to 87 and averaged 41.6 (Table S14). Observed heterozygosity (H_E) ranged from 0.057 to 0.548 and averaged 0.346. Expected heterozygosity (H_E) were larger for each locus, ranging from 0.287 to 0.695 and averaging 0.563. The inbreeding coefficients (F_{IS}) were positive and large, ranging from 0.120 to 0.748 and averaging 0.382. These large values may be due to the contrast between H_O and H_E , which may be due to null alleles, or to Wahlund's effect among well-differentiated populations. Large amounts of differentiation among populations (F_{ST} varied from 0.275 to 0.616 among loci) is in part also consistent with Wahlund's effect.

Sample sizes varied from 20.6 to 43.4 and average 28.0 over loci among the 16 samples (Table 3). The number of alleles ranged from 4.5 to 12.6 on average over samples. The number of private alleles ranged from 1 to 28 and averaged 9.1 over samples, with average frequencies ranging from 0.018 to 1.0. Observed heterozygosities (H) were less than expected heterozygosities (H_E) in all of the samples and this led to positive inbreeding coefficients (F_{IS}), ranging from 0.205 to 0.515.

Allele-frequency divergence between populations measured with F_{ST} ranged from 0.086 to 0.505 (Table S15) and overall was 0.457 ($P < 0.0001$). Gene flow per generation estimated from the pairwise values of F_{ST} and the island model of migration were small, ranging from 0.24 to 2.72 individuals between populations on average. An AMOVA showed that 46% of the variability was due to differences among popultaions and 54% was contained within populations (Table 4). No correlation between linearlized F_{ST} and shoreline distance appeared between samples, without ($r = 0.1341$, $P = 0.763$) and with a log transformation of geographical distance ($r = 0.016$, $P = 0.445$) (Fig. 2b). An assigment test showed individual mis-assignments for 8 of the 16 samples (Table 5). A PCA of allelic frequencies showed that some samples grouped by organellar lineage rather than by location. For example plants in the A lineage (red) clustered together (Fig. 3b) even though they were geographically widely separated (e.g. samples 1 and 12, 16). Another example are widely separated samples 5 and 15 in lineage D1 (green) which grouped together (Fig. 3e).

Discussion

The use of three classes of genetic markers, based on cellular compartmentilization and mode of inheritance, provide novel insights into the population structures and relationships among species of *Alaria* in the Northeastern Pacific. Mitochondrial and chloroplast DNA are presumably maternally inherited in *Alaria*, as in other kelps (Kato *et al*. 2006; Kraan & Guiry 2000; Li *et al*. 2016), so that maternal genealogies can be reconstructed. Mitochondrial and chloroplast DNAs evolve independently of each other and thus provide different perspectives of the same population history. The use of microsatellite markers, which are biparentaly inherited, provides yet another view of population events. These markers together reveal a complex population structure that appears to be a legacy of isolations during Pleistocene ice ages and post-glacial colonizations around the Gulf of Alaska.

The conclusions in this study are limited to some extent by several factors. First, sampling was necessarily coarse along the shores of the Gulf of Alaska becauses of the limited resources for travel and collecting at remote shorelines accessible only by air or boat. The collection of voucher specimens was not possible given the limitations of this study. Plants were collected by volunteer samplers generically as '*Alaria*', which is easy to identify because of presence of sporophylls, without an attempt to identify species. Hence, questions about taxonomy remain unaddressed and await further study of morphology in the light of the cryptic lineages identified in this study. Nevertheless, several conclusions about mechanisms leading to species are possible.

Even though we used three genetic marker classes, the amount of information provided by each marker was limited by the use of relatively short sequences relative to the sizes of the mitochondrial and chloroplastic genomes and by small sample sizes for the allele-frequency analysis of microsatellite DNA. Additionally, not all of the microsatellite markers readily amplified with PCR, so a few sample-locus combinations had missing genotypes. This was remedied in two ways. The software package GENEALEX estimated missing data by averaging over genotypes for some analyses. In three instances locus-sample data were completely missing, and a single genotype was substituted for all of the individuals. This can be rationalized in part by the occurrence of fixed allele-frequency differences between samples for several locus-sample combinations. Microsatellite loci were also affected by null alleles, so that estimates of diversity must be interpreted cautiously. Because of these weaknesses, the analyses of microsatellite data were limited to inferring geographical patterns among populations without assuming Hardy-Weinberg proportions. Despite these shortcomings, the results of this study resolved unexpected genetic structure among Alaska's populations.

Genetic population structure in the Gulf of Alaska

Populations of *Alaria* around the Gulf of Alaska show a complex, highly subdivided structure that cannot be explained by invoking isolation by distance. No IBD was found among the 16 populations for either the organellar or the microsatellite markers (Figs 2a & b), even though these genetic markers polymorphic and showed strong allele-frequency differences among populations (Figs 1, 3). Many populations were fixed, or nearly fixed, for a single *rbc*L-*COI*-haplotype, or for a single allele at some microsatellite loci, a pattern that can result only when gene flow is limited or absent. Most of the total variation was due to differences among populations: 89.6% for *rbc*L-*COI* and 46% for the 8 microsatellite markers on average.

Isolation between populations is further demonstrated by pervasive numbers of private alleles in each population, 1.8 haplotypes on average for *rbc*L-*COI* haplotypes and 9.1 alleles on average for 8 microsatellite loci (Tables 1, 3), that implies little gene flow between populations. Island model estimates of the number of migrants between populations. Island-model estimates of migration between populations per generation were small, generally less than 1.0 on average for *rbc*L-*COI* and less than 2.0 for microsatellites (Tables S12, S15).

Differences between populations may be due to IBA in which populations are isolated from one another by adapted to local environmental conditions. This may be the case on small geographical scales. For example, populations 10 to 15 km apart in Kachemak Bay were fixed for two divergent lineages C and B with out any mixing ((Figs 1c, 1e). A similar shift between lineages occurred over distances of tens of kilometres between Kodiak and Afognak islands and between populations in Sitka Sound. These genetic differences are most likely due to adaptation along a wave-action gradient. A larger collection of populations will likely uncover similar small-scale heterogeneity in other areas. Ecological and genomic research are needed to bring the details of local adaption into sharper focus.

The genetic mosaics among populations of *Alaria* are unlikely to be completely understood in terms of IBA. Lineage A haplotypes (red in figure 1f) were most abundant in inland populations from Prince William Sound in south central Alaska to Prince of Wales Island in southernmost Alaska. This cluster of populations may have arisen from a common ancestor surviving perhaps in the Alexander Archipelago of Southeastern Alsaka during glacial episodes (Byun *et al.* 1997). A-lineage plants, however, also occur in the western part of the Gulf of Alaska in the easternmost Aleutian Islands. Assuming an Alexander Archipelago origin, the Aleutian Island occurrence of a red lineage indicates long-distance dispersal in a direction enhanced by fast-moving westerly Alaska Coastal Current and the Alaska Stream.

B-lineage haplotypes are most abundant in the west central portion of the Gulf of Alaska, but are interspersed with C- and D-lineage haplotypes. Plants with C-lineage haplotypes appear on moderately wave-swept beaches in our small sample of only three sites. Ecological studies are needed to better understand the phenotypic and genotypic bases of adaptation to high-engergy habitats. The comparison of *COI*-5P sequendes to Genbank sequences from British Columbia indicates that the D lineage (green) plants represent the northern edge of the distribution of *A. marginata* (data not shown).

The geographical distributions of organellar haplotypes can be understood only in the light of displacements and refugial isolation during the Pleistocene glaciations and by post-glacial dispersals and colonizations. The shores of the Northeastern Pacific were periodically covered by the margins of large terrestrial glaciers during the nadir of Milankovitch climate cycles (Mann & Peteet 1994; Manley & Kaufman 2002; Kaufman & Manley 2004). High-amplitude, high-frequency climate variability has buffeted North Pacific subpolar areas for the past 1.26 million years not only during major glacial maxima but also during stadials and interstadials (Morley & Dworetzky 1991; Thunell & Mortyn 1995; Lisiecki & Raymo 2005).

These glacial advances and retreats led to a continual turnover of suitable intertidal habitats, which in turn led to repeated local extinctions, dispersals and recolonizations. A similar model was used to explain similar chaotic population structure of kelps in the North Atlantic (Neiva *et al.* 2018). Frequent local extirpations and colonizations in the Northeastern Pacific produced a mosaic in the geographical distributions of *COI*-5P and *rbc*L haplotypes among present-day populations. Highly heterogeneous, ephemeral populations have likely characterized *Alaria* in the Northeastern Pacific over the Pleistocene Epoch. Even short-term decadal and interdecadal environmental shifts can lead to major changes in kelp populations (Gedalof & Smith 2001; Bekkby & Moy 2011; Moy & Christie 2012; Pfister *et al*. 2018).

Despite the conjecture that environmental extirpations and colonizations are a prominent feature of the history of populations of Alaria around the Gulf of Alaska, average microsatellite heterozygosities among populations were not greatly reduced but ranged from H_E = 0.381 to 0.767 (mean = 0.563) (Table 3). These moderate levels of genetic diversity indicate that glacial refugia scattered around the Gulf of Alaska may have been large and numerous. A PCA of 8 microsatellite loci indicated that most plants clustered on the bases of organellar lineage and not by geographical location, indicating the lack of drift-migration equilibrium of *Alaria* populations in the Gulf of Alaska.

The results of the present study, confirm the conjecture of Lane *et al*. (2007) that North Pacific Alaria consists of a complex of weakly differentiated taxa. Thirteen of the 16 populations consisted of individuals with haplotypes from only one of the five lineages. This may be due to the effects of incumbency and the exclusion of immigrants. Mixing of more than one major lineage was found at only three sites. A comparison of groups ordered by microsatellite loci and the organellar haplotypes of plants in the groups indicated nuclear hybridizations between lineages when they occurred at the same location. Plants in lineage A and C in the eastern Aleutians (sample 1) and some plants in samples 13 (lineages A & C) and 14 (lineages A & D) appeared to have microsatellite frequencies typical of the location rather than of microsatellite frequencies typical of plants in the same lineage at other localities. This pattern can be explained by hybridization between lineages.

Conclusions

Alaria is one of the most abundant kelps in rocky intertidal habitats in the Northeastern Pacific. The results of this study uncovered unexpected molecular diversity that was not anticipated by taxonomies based on morphological traits. Both organellar and microsatellite population markers revealed a highly subdivided population structure that does not follow a model of isolation by distance. Some populations are likely isolated from other populations by adaptation to various levels of wave-expose on geographical scales on only a few kilometers. Detailed studies of environmental and genomic variability will likely show adaptations on a finer spatial scale along a shoreline. The patterns of genetic variability among populations unquestionably show imprints of ice-age isolations in refugia around the Gulf of Alaska, chaotic colonizations and incumbency. Repeated coastal disturbances from glacial advances and retreats appear produce divergences between lineages isolated in glacial refugia, but subsequent hybridizations between lineages limit complete reproductive isolation that would lead to new species. Two areas of research are of interest. Finer-scale surveys of populations with molecular markers with undoubtedly uncover additional genetic variability, and eco-genetic studies are needed to map the adaptive seascape of the evolutionary lineages in *Alaria*.

References

Alfaro ME, Faircloth BC, Harrington RC, Sorenson L, Friedman M, Thacker CE, Oliveros CH, Černý D, Near TJ (2018) Explosive diversification of marine fishes at the Cretaceous–Palaeogene boundary. *Nature Ecology & Evolution*, **2**, 688–696.

Bekkby T, Moy FE (2011) Developing spatial models of sugar kelp (*Saccharina latissima*) potential distribution under natural conditions and areas of its disappearance in Skagerrak. *Estuarine, Coastal and Shelf Science*, **95**, 477–483.

Bittner L, Payri CE, Couloux A, Cruaud C, de Reviers B, Rousseau F (2008) Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **49**, 211–226.

Bohonak AJ (1999) Dispersal, gene flow, and population structure. *The Quarterly Review of Biology*, **74**, 21–45.

Bohonak AJ (2002) IBD (Isolation By Distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93**, 153–154.

Bowen BW, Gaither MR, DiBattista JD, Iacchei M, Andrews KR, Grant WS, Toonen RJ, Briggs JC (2016) Comparative phylogeography of the ocean planet. *Proceedings of the National Academy of Sciences USA*, **113**, 7962–7969.

Burrowes R, Rousseau F, Müller DG, de Reviers B (2003) Taxonomic placement of *Microzonia* (Phaeophyceae) in the Syringodermatales based on the rbcL and 28S nrDNA sequences. *Cryptogamie. Algologie*, **24**, 63–73.

Byun S, Koop BF, Reimchen TE (1997) North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. *Evolution* 51: 1647–1653.

Calegario G, Freitas L, Santos E, Silva B, Oliveira L, Garcia G, Omachi C, Pereira R, Thompson C, Thompson F (2019) Environmental modulation of the proteomic profiles from closely phylogenetically related populations of the red seaweed *Plocamium brasiliense*. *PeerJ*, **7**: e6469.

Collens JD (2009) Isolation and description of eight polymorphic microsatellite loci for the winged-kelp *Alaria nana*. *Molecular Ecology Resources*, **9**, 1421–1423.

Drinan DP, Gruenthal KM, Canino MF, Lowry D, Fisher MC, Hauser L (2018) Population assignment and local adaptation along an isolation-by-distance gradient in Pacific cod (*Gadus macrocephalus*). *Evolutionary Applications*, **11**, 1448–1464.

Eldon B, Wakeley J (2006) Coalescent processes when the distribution of offspring number among individuals is highly skewed. *Genetics*, **172**, 2621–2633.

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Gedalof Z, Smith DJ (2001) Interdecadal climate variability and regime-scale shifts in Pacific North America. *Geophysical Research Letters*, **28**, 1515–1518.

Goudet JF (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of heredity* **86**, 485–486.

Grant WS, Utter FM (1984) Biochemical population genetics of Pacific herring (Clupea pallasi). *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 856–864.

Hoarau G, Coyer JA, Veldsink JH, Stam WT, Olsen JL (2007) Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. *Molecular Ecology*, **16**, 3606–3616.

Jablonski D, Sepokoski JJ Jr (1996) Paleobiology, community ecology, and scales of ecological pattern. *Ecology*, **77**, 1367–1378.

Kato Y, Kogame K, Nagasato C, Motomura T (2006) Inheritance of mitochondrial and chloroplast genomes in the isogamous brown alga *Scytosiphon lomentaria* (Phaeophyceae). *Phycological Research*, **54**, 65–71.

Kaufman DS, Manley WF (2004) Pleistocene maximum and Late Wisconsinan glacier extents across Alaska, USA. *Developments in Quaternary Sciences*, **2**, 9–27.

Kraan S, Guiry M (2000) Molecular and morphological character inheritance in hybrids of *Alaria esculenta* and *A. praelonga* (Alariaceae, Phaeophyceae). *Phycologia*, **39**, 554–559.

Krueger-Hadfield SA, Roze D, Mauger S, Valero M (2013) Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed *Chondrus crispus*. *Molecular Ecology*, **22**, 3242–3260.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870–1874.

Kusumo HT, Druehl LD. 2000. Variability over space and time in the genetic structure of the winged kelp *Alaria marginata*. *Marine Biology*, **136**, 397–409.

Lane CE, Lindstrom SC, Saunders GW (2007) A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Molecular Phylogenetics and Evolution*, **44**, 634–648.

Lewis PO, Zaykin D (2001) Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Available: http://lewis.eeb.uconn.edu/lewishome/software.html

Lisiecki LE, Raymo ME (2005) A Pliocene-Pleistocene stack of 57 globally distributed benthic δ18 O records. *Paleoceanography*, **20**, PA1003.

Li C, Bor A (2019) Coupled atmosphere-ice-ocean dynamics in Dansgaard-Oeschgerevents. *Quaternary Science Reviews*, **203**, 1–20.

Li Q, Wang XL, Zhang J, Yao JT, Duan D (2016) Maternal inheritance of organellar DNA demonstrated with DNA markers in crosses of *Saccharina japonica* (Laminariales, Phaeophyta). *Journal of Applied Phycology*, **28**, 2019–2026.

Manley WF, Kaufman DS (2002) Alaska PaleoGlacier Atlas: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado, instaar.colorado.edu/QGISL/ak_paleoglacier_atlas, v. 1.

Mann DH, Peteet DM (1994) Extent and timing of the Last Glacial Maximum in southwestern Alaska. *Quaternary Research*, **42**, 136–148.

Marko PB, Hoffman JM, Emme SA, McGoverrn TM, Keever CC, Cox N (2010) The ‘expansion-contraction’ model of Pleistocene biogeography: rocky shores suffer a sea change? *Molecular Ecology*, **19**, 146–169.

Matuszewski S, Hildebrandt ME, Achaz G, Jensen JD (2018) Coalescent processes with skewed offspring distributions and nonequilibrium demography. *Genetics*, **208**, 323–338.

McConnico LA, Foster MS (2005) Population biology of the intertidal kelp, *Alaria marginata* Postels and Ruprecht: a non-fugitive annual. *Journal of Experimental Marine Biology and Ecology*, **324**. 61–75.

McGovern TM, Keever CC, Saski CA, Hart MW, Marko PB (2010) Divergence genetics analysis reveals historical population genetic processes leading to contrasting phylogeographic patterns in co-distributed species. *Molecular Ecology*, **19**, 5043–5060.

Morley JJ, Dworetzky BA (1991) Evolving Pliocene-Pleistocene climate: A North Pacific perspective. *Quaternary Science Reviews*, **10**, 225–237.

Moy FE, Christie H (2012) Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. *Marine Biology Research*, **8,** 357–369.

Neiva J, Pearson GA, Valero M, Serrão (2012) Fine-scale genetic breaks driven by historical range dynamics and ongoing density-barrier effects in the estuarine seaweed *Fucus ceranoides* L. *BMC Evolutionary Biology*, **12**, e78.

Neiva J, Paulino C, Nielsen MM, Krause-Jensen D, Saunders GW, Assis J, Bárbara I, Tamigneaux É, Gouveia L, Aires T, Marbà N (2018) Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. *Scientific Reports*, **8**, e1112.

Nielsen EE, Hemmer Hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine fishes: identifying adaptive variation in space and time. *Molecular Ecology*, **18**, 3128–3150.

Nielsen MM, Paulino C, Neiva J, Krause-Jensen D, Bruhn A, Serrão EA (2016a) Genetic diversity of *Saccharina latissima* (Phaeophyceae) along a salinity gradient in the North Sea–Baltic Sea transition zone. *Journal of Phycology*, **52**, 523–531.

Nielsen MM, Mans D, D'Este M, Krause-Jensen D, Rasmussen MB, Larsen MM, Alvarado-Morales M, Angelidaki I, Bruhn A (2016b) Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters. *Algal Research*, **13**, 235–245.

Niwa HS, Nashida K, Yanagimoto T (2016) Reproductive skew in Japanese sardine inferred from DNA sequences. *ICES Journal of Marine Science*, **73**, 2181–2189.

Orsini L, Vanoverbeke J, Swillen I, Mergeay J, Meester L (2013) Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology*, **22**, 5983–5999.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetics software for teaching and research–an update. *Bioinformatics*, **28**, 2537–2539.

Peteet DM, Mann DH (1994) Late-glacial vegetational, tephra, and climatic history of southwestern Kodiak Island, Alaska. *Ecoscience*, **1**, 255–267.

Pfister CA, Berry HD & Mumford T. 2018. The dynamics of kelp forests in the Northeast Pacific Ocean and the relationship with environmental drivers. *Journal of Ecology* 106: 1520–1533.

Raimondi PT, Reed DC, Gaylord B, Washburn L (2004) Effects of self-fertilization in the giant kelp, *Macrocystis pyrifera*. *Ecology*, **85**, 3267–3276.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Sabin AL, Pisias NG (1996) Sea surface temperature changes in the Northeastern Pacific Ocean during the past 20,000 years and their relationship to climate change in Northwestern North America. *Quaternary Research*, **46**, 48–61.

Sexton JP, Hangartner SB, Hoffmann AA (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution*, **68**, 1–15.

Sibert EC, Norris RD (2015) New age of fishes initiated by the Cretaceous− Paleogene mass extinction. *Proceedings of the National Academy of Sciences* USA, **112**, 8537–8542.

Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, De Reviers B, Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the "brown algal crown radiation". *Molecular Phylogenetics and Evolution*, **56**, 659–674.

Slatkin M (1991) Inbreeding coefficients and coalescence times. *Genetical Research*, Cambridge, **58**, 167–175.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Thunell RC, Mortyn PG (1995) Glacial climate instability in the Northeast Pacific Ocean. *Nature*, **376**, 504–506.

Valentine JW (1980) Determinants of diversity in higher taxonomic categories. *Paleobiology*, **6**, 444–450.

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Wang IJ, Bradburd GS (2014) Isolation by environment. *Molecular Ecology*, **23**, 5649–5662.

Waters JM, Fraser CI, Hewitt GM (2012) Founder takes all: density-dependent processes structure biodiversity. *Trends in Ecology & Evolution*, **28**, 78–85.

Weir BS (1990) *Genetic data analysis. Methods for discrete population genetic data*. Sinauer Associates, Inc. Publishers.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. E*volution*, **38**, 1358–1370.

Widdowson TB (1971) A taxonomic revision of the genus *Alaria* Greville. *Syesis*, **4**, 11–49.

Wright D, Bishop JM, Matthee CA, von der Heyden S (2015) Genetic isolation by distance reveals restricted dispersal across a range of life histories: implications for biodiversity conservation planning across highly variable marine environments. *Diversity and Distributions*, **21**, 698–710.

Table 1 *Alaria*: Estimates of genetic parameters based on contatenated fragments of mitochondrial DNA cytochrome oxidase I (*COI*-5P) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1359 base pairs combined) in samples from the Gulf of Alaska and Aleutian Islands. Location number, sample size (N), number of polymorphic nucleotide sites (N_{poly}), number of haplotypes (N_{H}), expected number of haplotypes under neutrality (N_{EH}), haplotype diversity (h, SD: standard deviation), nucleotide diversity (θ_π, SD: standard deviation) and Tajima's D (P: probability of null hypothesis of neutrality)

Location	N	N_{poly}	N_{H}	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
1	32	9	3	1.84	2	0.234	0.095	0.137	0.089	-0.421	0.390
2	38	2	3	1.93	3	0.243	0.088	0.018	0.023	-0.902	0.200
3	28	2	3	1.97	2	0.265	0.105	0.020	0.024	-0.972	0.190
4	31	1	2	1.41	1	0.125	0.077	0.009	0.016	-0.774	0.219
5	32	18	5	4.28	4	0.593	0.056	0.112	0.076	-2.206	0.002
6	39	3	3	4.76	2	0.614	0.041	0.089	0.064	1.686	0.957
7	40	1	2	1.17	1	0.050	0.047	0.004	0.010	-1.124	0.126
8	48	0	1	–	0	0.0	–	0.0	–	–	–
9	34	0	1	–	0	0.0	–	0.0	–	–	–
10	47	2	3	2.41	2	0.324	0.076	0.024	0.027	-0.487	0.332
11	32	0	1	–	0	0.0	–	0.0	–	–	–
12	29	4	4	3.80	3	0.554	0.064	0.048	0.042	-0.874	0.221
13	41	7	5	4.02	4	0.545	0.072	0.173	0.0106	1.327	0.910
14	31	14	3	2.08	1	0.288	0.097	0.264	0.152	0.184	0.628
15	12	1	2	1.42	1	0.167	0.134	0.012	0.019	-1.141	0.175
16	29	2	3	4.67	2	0.636	0.056	0.061	0.049	1.414	0.916
Average	33.9	4.1	2.8	2.75	1.8	0.290	0.513	0.061	0.202	-0.330	–
Pooled	543	41	33	34.3	28	0.903	0.006	0.547	0.282	0.776	0.832

Table 2 *Alaria*: Apparent chromosomal polyploidy based on number of alleles at a microsatellite locus

	Locus											
	AN23			AN26			AN30			AN38		
Sample	2N	3N	4N	2N	3N	4N	2N	3N	4N	2N	3N	4N
1	6	12	11	–	–	–	22	5	1	–	–	–
2	–	–	–	–	–	–	–	–	–	32	1	–
3	5	12	13	–	–	–	29	3	–	–	–	–
12	10	8	12	29	1	1	–	–	–	–	–	–

Table 3 *Alaria*: Summary statistics for 8 microsatellite loci in 16 samples from the Gulf of Alaska. N = mean sample size over loci. N_A = mean number of alleles. N_E = mean number of alleles expected under neutrality. H_O = observed heterozygosity. H_E = unbiased expected heterozygosity. F_{IS} = inbreeding coefficient. N_{PR} = number of private alleles.

Sample	N	N_A	N_{PR}	f(PR)	H_O	H_E	F_{IS}
1	24.5	12.6	28	0.068	0.406	0.767	0.515
2	20.6	6.4	8	0.115	0.275	0.606	0.510
3	24.3	10.5	9	0.092	0.414	0.762	0.489
4	26.8	10.5	11	0.114	0.494	0.680	0.303
5	21.0	4.5	4	0.037	0.361	0.474	0.275
6	34.8	6.5	9	0.030	0.386	0.594	0.353
7	36.6	8.5	5	0.128	0.391	0.655	0.392
8	38.8	5.3	12	0.109	0.262	0.357	0.205
9	24.5	5.3	4	0.438	0.253	0.491	0.315
10	43.4	4.9	1	1.0	0.277	0.396	0.215
11	23.8	5.0	1	0.018	0.362	0.499	0.305
12	26.5	12.0	17	0.089	0.488	0.708	0.335
13	36.3	12.0	11	0.035	0.368	0.604	0.349
14	23.5	7.0	8	0.160	0.370	0.629	0.401
15	21.0	5.0	4	0.263	0.207	0.410	0.426
16	22.1	5.4	13	0.073	0.227	0.381	0.199
Mean	28.0	7.6	9.1	0.173	0.346	0.563	0.375
Pooled	530	333	–	–	0.375	0.882	0.601

Table 4 *Alaria*: Analysis of molecular variance (AMOVA) of microsatellite allele-frequency variability among samples using F_{ST}. Overall, $F_{ST} = 0.457$ ($P < 0.001$).

Partition	d.f.	Variance component	% of variance	*P*
Among populations	15	5.367	46	<0.00001
Within populations	570	6.370	54	
Total	585	11.373	100	

Table 5 *Alaria*: Log likelihood assignments of individuals to populations. Assignments are from populations on the X-axis to populations on the Y-axis. Assignments back to orginal populations are in the diagonal.

1	36	–	–	1	–	–	–	–	–	–	–	–	2	–	–	1
2	–	35	–	–	–	–	1	–	–	–	–	–	–	–	–	–
3	–	–	26	4	–	1	–	1	–	–	–	–	–	–	–	–
4	–	–	–	30	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	1	29	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	44	1	1	–	–	–	–	–	–	–	–
7	–	–	–	–	–	5	42	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	48	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–	34	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	–	45	–	–	–	–	–	–
11	–	–	–	–	–	–	–	–	–	–	31	–	–	–	–	1
12	–	–	1	1	–	–	–	–	–	–	–	29	–	–	–	–
13	–	–	–	–	1	–	–	–	–	–	–	–	42	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	4	26	–	–
15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	29	–
16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	32
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Sample number															

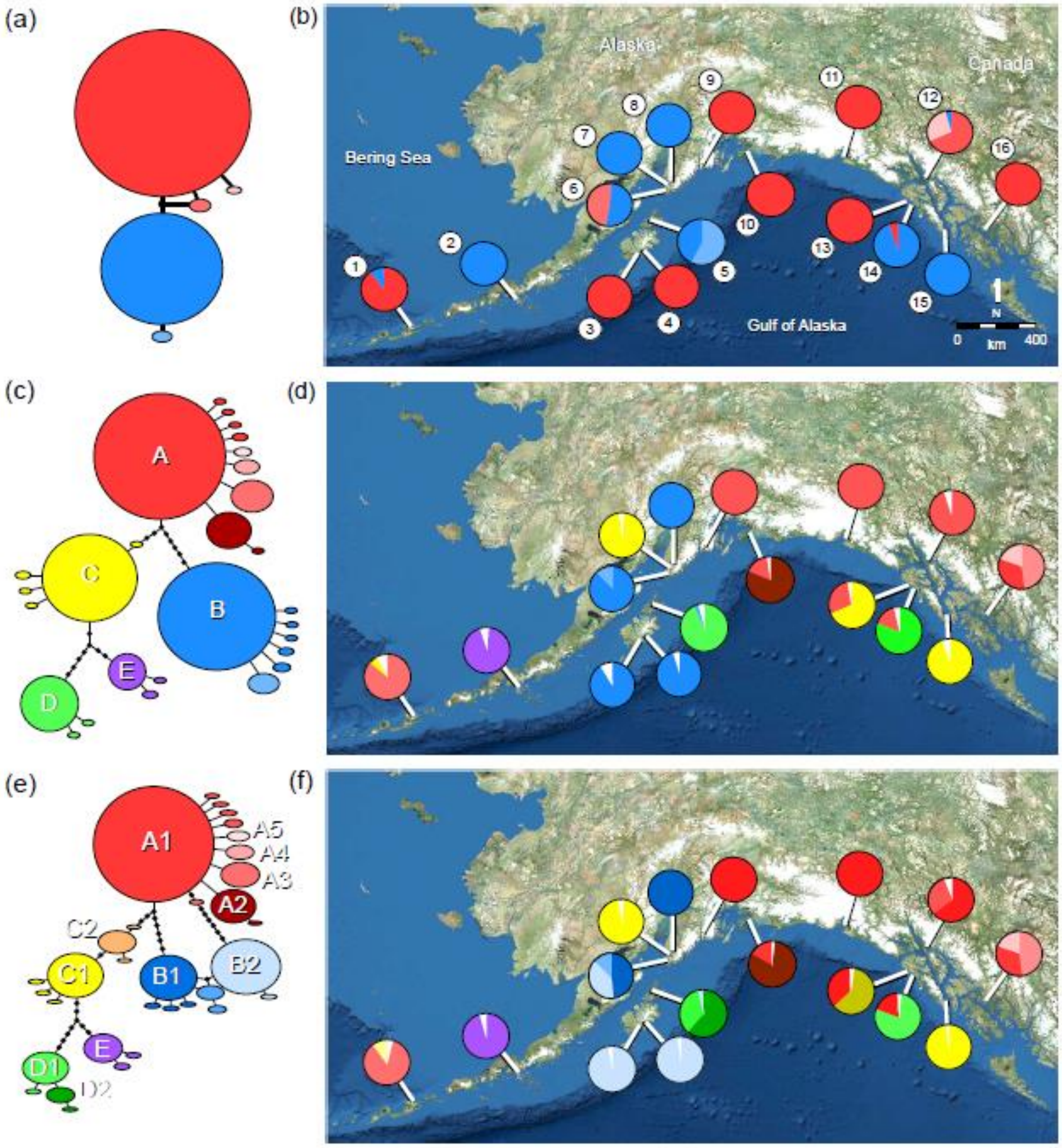


Fig. 1 *Alaria marginata*: Networks showing mutational relationships between haplotypes and pie diagrams for *rbc*L (a, b), *COI*-5P (c, d) and *rbc*L + *COI*-5P combined (e, f). White wedges indicate frequencies of local private alleles.

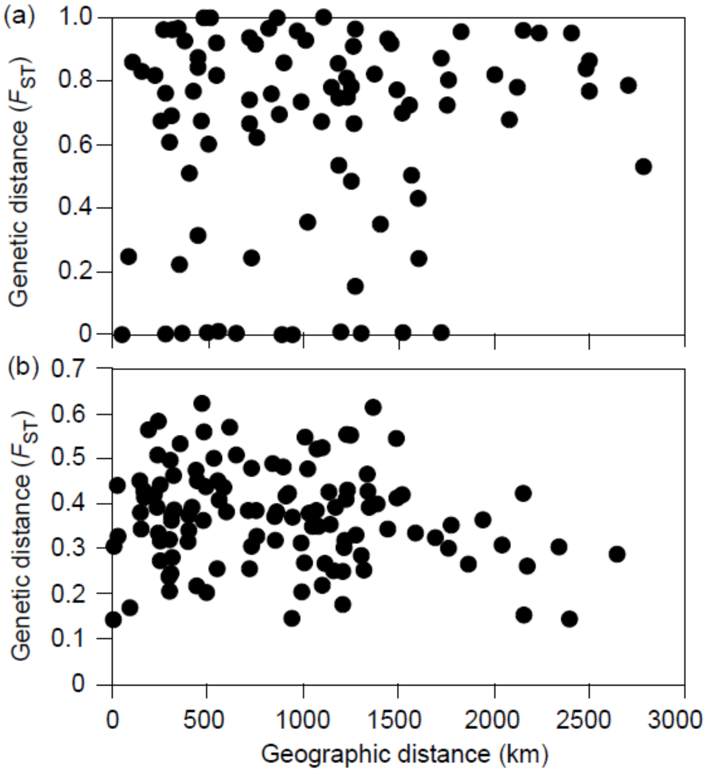


Fig. 2 *Alaria marginata*: Relationship between genetic distance (F_{ST}) and shoreline distance (kilometers). Mantel's test for isolation by distance were made between linearized F_{ST} and geographical distance and log(geographical distance). (a) Concatenated *rbc*L and *COI*-5P sequences: linear F_{ST} and geographical distance: $r = 0.074$, $P = 0.737$; linear F_{ST} and log(geographical distance): $r = 0.0009$, $P = 0.420$. (b) 8 microsatellite loci: linear F_{ST} and geographical distance: $r = 0.1341$, $P = 0.763$; linear F_{ST} and log(geographical distance): $r = 0.016$, $P = 0.445$.

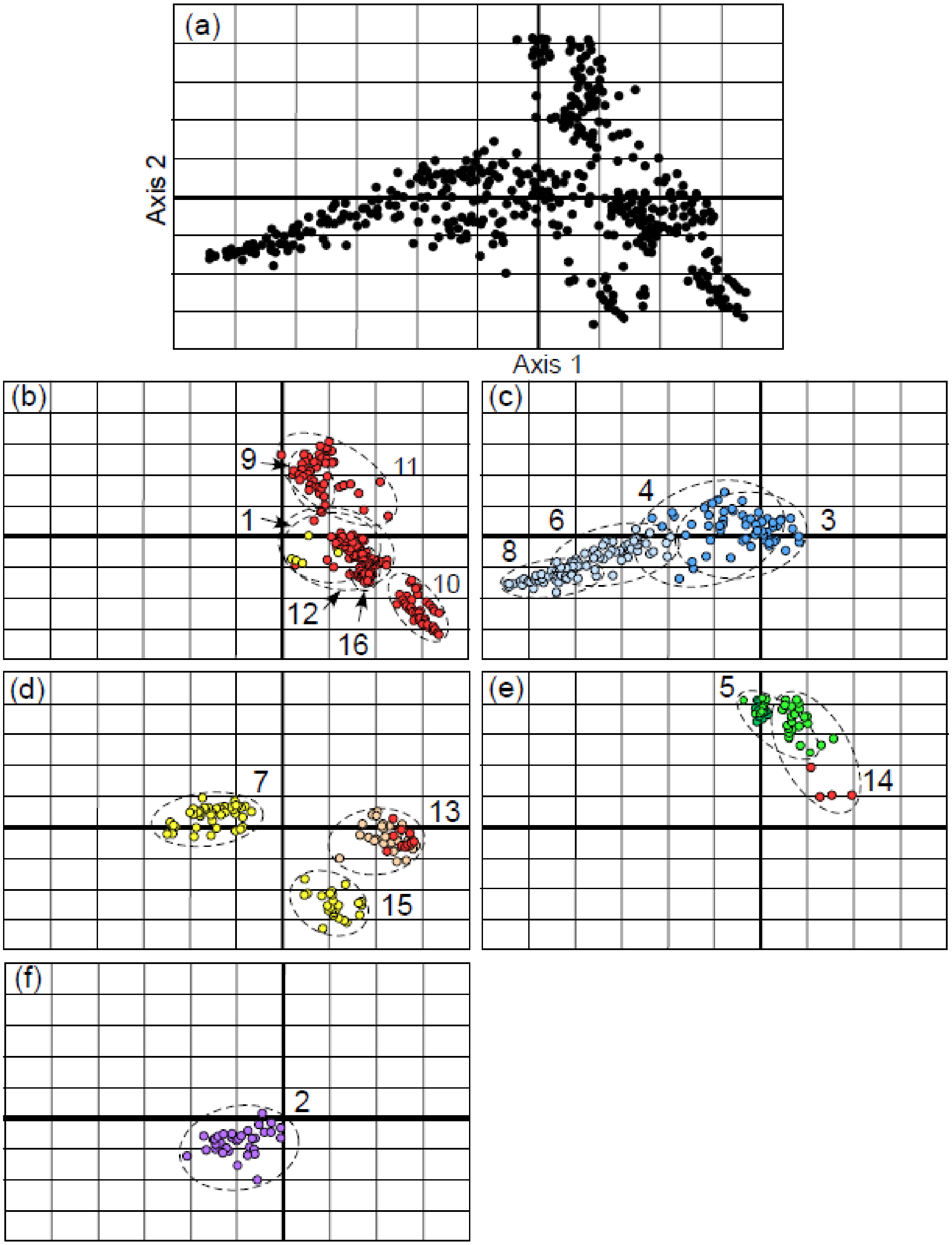


Fig. 3 *Alaria marginata*: Principle components analysis of allele-frequency variability at 8 microsatellite loci. Numbers indicate sample location, and colours correspond to organellar DNA lineages in Figure 2.

Supplemental Information

W.S. Grant & T.T. Bringloe: Phylogeography of ribbon kelp *Alaria* in Alaska

Table S1 *Alaria marginata*: Locations and collection dates of samples from the Gulf of Alaska and Aleutian Islands

Sample	Location	Habitat	N Latitude	W Longitude	Date
1	Morris Cove, Unalaska Island	Protected	53.919	166.438	June 2018
2	Sand Point, Popf Island, Alaska Peninsula	Exposed	55.309	160.513	June 2016
3	Table Island, Kodiak Island	Exposed	57.188	152.925	June 2018
4	Near Island, Kodiak Island	Protected	57.788	152.388	June 2018
5	Black Cape, Afognak Island	?	58.402	152.882	May 2016
6	Kasitsna Bay, Kachemak Bay	Protected	59.468	151.553	June 2015
7	Kayak Beach, Kachemak Bay	Semi-exposed	59.497	151.472	August 2016
8	Homer Spit, Kachemak Bay	Protected	59.604	151.418	August 2016
9	Lowell Point, Resurrection Bay	Semi-exposed	60.064	149.443	July 2016
10	Cordova, Prince William Sound	Protected	60.545	145.768	July 2016
11	Bridge Site, Yakutat	Current swept	60.056	149.443	May 2018
12	Auke Bay, North of Juneau	Protected	58.374	134.728	May 2018
13	Magoun Island, W Baranof Island	Protected	57.157	135.567	July 2016
14	Watson Point, W Baranof Island	Semi-Exposed	57.070	135.368	May 2018
15	Little Port Walter, SE Baranof Island	Protected	56.384	134.641	June 2018
16	Tokeen Bay, Prince of Wales Island	?	55.993	133.464	June 2018

Table S2 Microsatellite loci surveyed in winged kelp, *Alaria marginata*, and associated polymerase chain reactions thermal profiles

Locus Name	Thermal profile
An21	95 °C/4 min; 41 cycles of (95 °C/45 sec + 58 °C/45 sec + 72 °C/1 min); 72 °C 20 min
An23; *An27*	95 °C/4 min; 31 cycles of (95 °C/40 sec + 58 °C/40 sec + 72 °C/40 sec); 72 °C 20 min
An26; *An30*	95 °C/4 min; 32 cycles of (95 °C/40 sec + 58 °C/40 sec + 72 °C/40 sec); 72 °C 20 min
An31; *An39*	95 °C/4 min; 40 cycles of (95 °C/45 sec + 58 °C/45 sec + 72 °C/1 min); 72 °C 20 min
An38	95 °C/4 min; 39 cycles of (95 °C/45 sec + 56 °C/45 sec + 72 °C/1 min); 72 °C 20 min

Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L)

Four nucleotide polymorphisms in a 740 base pair segment of the chloroplast gene *rbc*L defined five haplotypes (Table S3, Fig. 1a). Most plants carried one of the two most abundant haplotypes, which were were separated from each other by two mutations. Mutations separating three of the five haplotypes occurred at two nucleotide sites with linked polymorphisms. Site 568 segregated for the transversion A↔C, and site 570 segregated for both transitions (C↔T) and transversions (A↔T, A↔C). Seven of the 16 samples were fixed for haplotype 1 and 4 samples were fixed for haplotype 2. Haplotype diversity (*h*) ranged from 0.0 to 0.512 and averaged 0.127. Nucleotide diversity (θ_π) ranged from 0.0 to 0.0014 and average 0.0006 (Table S4). Tajima's D was not significant in any of the samples, or overall in the pooled sample. Mean sequence divergence (Φ_{ST}) between samples varied widely from 0.0 for populations fixed for the same haplotype to 1.0 between samples not sharing any haplotypes (Fig. 1b, Table S5). Sequence divergence overall was 0.830. AMOVA indicated that 83% of sequences variability occurred among populations and 17% was due to variability on average among individuals within populations.

Table S3 *Alaria*: Haplotype frequencies of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (740 base-pair fragment) haplotypes in samples from the Gulf of Alaska and Aleutian Islands

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
1	28		28	31					34	48	32	18	41	5		32	297
2	4	38			14	23	46	48				1		26	13		213
3						22											22
4					18												18
5												10					10
Total	32	38	28	31	32	45	46	48	34	48	32	29	41	31	13	31	560

Table S4 *Alaria*: Estimates of genetic parameters for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (740 base pairs) in samples from the Gulf of Alaska and southeastern Bering Sea. Location number, sample size (N), number of polymorphic nucleotide sites (N_{poly}), number of haplotypes (N_{H}), expected nmber of haplotypes under neutrality (N_{EH}), haplotype diversity (h, SD: standard deviation), nucleotide diversity (θ_π, SD: standard deviation) and Tajima's D (P: probability of null hypothesis of neutrality)

Location	N	N_{poly}	N_{H}	N_{EH}	N_{PH}	h	SD	θ_π (%)	SD	D	P
1	32	2	2	1.81	0	0.226	0.088	0.61	0.062	-0.182	0.398
2	38	0	1	1.0	0	0.0	–	0.0	–	–	–
3	28	0	1	1.0	0	0.0	–	0.0	–	–	–
4	31	0	1	1.0	0	0.0	–	0.0	–	–	–
5	32	1	2	3.48	1	0.508	0.031	0.069	0.066	1.597	0.964
6	45	2	2	3.77	1	0.511	0.016	0.138	0.105	2.261	0.994
7	46	0	1	1.0	0	0.0	–	0.0	–	–	–
8	48	0	1	1.0	0	0.0	–	0.0	–	–	–
9	34	0	1	1.0	0	0.0	–	0.0	–	–	–
10	48	0	1	1.0	0	0.0	–	0.0	–	–	–
11	32	0	1	1.0	0	0.0	–	0.0	–	–	–
12	29	3	3	3.44	1	0.512	0.063	0.082	0.075	-0.491	0.348
13	41	0	2	1.0	0	0.0	–	0.0	–	–	–
14	31	2	1	2.04	0	0.280	0.090	0.076	0.071	0.237	0.068
15	13	0	1	1.0	0	0.0	–	0.0	–	–	–
16	32	0	1	1.0	0	0.0	–	0.0	–	–	–
Average	35.0	0.63	1.4	1.60	0.19	0.127	0.122	0.061	0.115	0.684	–
Pooled	560	4	5	6.92	3	0.572	0.012	0.150	0.109	1.437	0.916

Table S5 *Alaria*: Genetic distances (Φ_{ST}) between samples from the Gulf of Alaska and southeastern Bering Sea based on seqence variability in ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (740 base-pair fragment) and with the Tamura (1992) model of mutation. Overall Φ_{ST} = 0.830

2	0.881														
3	0.088	1.0													
4	0.095	1.0	0.0												
5	0.793	0.572	0.894	0.900											
6	0.473	0.456	0.610	0.621	0.489										
7	0.892	0.0	1.0	1.0	0.600	0.480									
8	0.895	0.0	1.0	1.0	0.606	0.486	0.0								
9	0.101	1.0	0.0	0.0	0.903	0.630	1.0	1.0							
10	0.129	1.0	0.0	0.0	0.920	0.669	1.0	1.0	0.0						
11	0.097	1.0	0.0	0.0	0.901	0.624	1.0	1.0	0.0	0.0					
12	0.184	0.883	0.263	0.276	0.805	0.543	0.895	0.897	0.288	0.337	0.280				
13	0.116	1.0	0.0	0.0	0.913	0.650	1.0	1.0	0.0	0.0	0.0	0.313			
14	0.665	0.151	0.826	0.833	0.397	0.245	0.171	0.175	0.840	0.865	0.836	0.706	0.854		
15	0.823	0.0	1.0	1.0	0.447	0.353	0.0	0.0	1.0	1.0	1.0	0.822	1.0	0.070	
16	0.097	1.0	0.0	0.0	0.901	0.624	1.0	1.0	0.0	0.0	0.0	0.280	0.0	0.836	1.0
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Sample number

Table S6 *Alaria*: Analysis of molecular variance (AMOVA) of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (740 base pairs) sequence variability among samples from the Gulf of Alaska and Aleutian Islands, and with the Tamura (1992) model of mutation.

Partition	d.f.	Variance component	% of variance	*P*
Among populations	15	0.489	83.0	<0.00001
Within populations	544	0.10	17.0	
Total	559	0.589	100.0	

Cytochrome oxidase I-5P (*COI*-5P)

The mitochondrial DNA *COI*-5P was much more polymorphic than *rbc*L. Polymorphisms at 36 nucleotide sites defined 27 haplotypes that fell into five lineages (A-E), each separated from other by at least 5 mutations (Fig. 1c; Tables S6, S7). Samples tended to be fixed or have a large frequency of one haplotype and low frequencies of related haplotypes. Twelve of the 16 samples had private haplotypes. Lineage A was widespread, but tended to be most abundant in the eastern Gulf of Alaska, whereas lineage B was confined to the central Gulf of Alaska. Lineages D and E occurred in the central and eastern Gulf of Alaska. Lineage E was confined to a single site in the western Gulf of Alaska (Fig. 1d). Three localities, (1, 13, 14) showed a mix of haplotypes from different major lineages, and two samples (10, 16) had a mix of closely related haplotypes in the same lineage. As expected, these populations had the largest haplotype diversities (0.232–0.636), but only the three populations with divergent lineages had the largest nucleotide diversities (0.0023–0.0052). Tajima's *D* indicated that haplotype distributions in three populations (5, 12, 15) deviated from neutralilty, but not over all in the pooled sample. Average sequence divergences (Φ_{ST}) between samples ranged from 0.0, for populations fixed for the same haplotype, to 1.0, for populations that did not share haplotypes (Table S8). Sequence divergences between pairs of populations were significantly greater than 0.0, except for four comparisons, samples 6 & 8 in Kachemak Bay and samples 9, 11 & 12 in the central and eastern Gulf of Alaska. AMOVA indicated that 90.4% of the variability was due to differences among populations and 9.6% was due to diversity within populations, on average (Table S9). Overall, Φ_{ST} was 0.904.

Table S7 *Alaria*: Haplotype frequencies of mitochondrial DNA cytochrome oxidase I-5P (664 base pair fragment) haplotypes in samples from the Gulf of Alaska and Aleutian Islands

	Sample number																
Haplotype/ Lineage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
1 A	.	.	.	.	.	.	.	.	34	8	32	29	11	4	.	8	126
2 B	.	.	27	29	.	36	.	48	.	.	.	.	.	.	.	.	140
3 C	2	.	.	.	.	.	40	.	.	.	.	.	30	.	29	.	101
4 D	.	.	.	.	29	.	.	.	.	.	.	.	.	26	.	.	55
5 A2	.	.	.	.	.	.	.	.	.	38	.	.	.	.	.	.	38
6 A3	35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	35
7 E	.	34	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34
8 A4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	15	15
9 A5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6	6
10 B	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	5
11 C	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	4
12 C	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
13 E	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
14 B	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	3
15 E	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
16 B	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	2
17 B	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	2
18 C	.	.	.	.	.	.	1	.	.	.	.	.	1	.	.	.	2
19 A3	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
20 C	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1
21 A	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1
22 A	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1
23 D	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
24 D	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
25 B	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
26 C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1
27 A	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1
Total	40	39	32	31	32	41	41	48	34	47	32	31	47	31	30	29	585

Table S8 *Alaria*: Estimates of genetic parameters for mitochondrial DNA cytochrome oxidase I-5P (664 base-pair fragment) in samples from the Gulf of Alaska and southeastern Bering Sea. Location number, sample size (N), number of polymorphic nucleotide sites (N_{poly}), number of haplotypes (N_{H}), expected nmber of haplotypes under neutrality (N_{EH}), haplotype diversity (h, standard deviation), nucleotide diversity (θ_{π}, standard deviation) and Tajima's D (P: probability of null hypothesis of neutrality)

Location	N	N_{poly}	N_{H}	N_{EH}	N_{PH}	h	SD	θ_{π} (%)	SD	D	P
1	40	7	3	1.89	2	0.232	0.085	0.231	0.158	-0.265	0.447
2	39	2	3	1.91	3	0.238	0.087	0.037	0.048	-0.914	0.193
3	32	2	3	2.07	2	0.284	0.098	0.045	0.054	-0.810	0.220
4	31	1	2	1.41	1	0.125	0.077	0.019	0.033	-0.774	0.217
5	32	17	4	1.63	3	0.182	0.090	0.016	0.012	-2.509	0.0001
6	41	1	2	1.84	1	0.195	0.077	0.034	0.046	-0.086	0.331
7	41	1	2	1.16	0	0.049	0.046	0.007	0.002	-1.122	0.126
8	48	0	1	–	0	0.0	–	0.0	–	–	–
9	34	0	1	–	1	0.0	–	0.0	–	–	–
10	47	2	3	2.41	2	0.324	0.076	0.050	0.057	-0.487	0.335
11	32	0	1	–	0	0.0	–	0.0	–	–	–
12	31	2	3	1.42	2	0.127	0.080	0.020	0.034	-1.506	0.042
13	47	7	5	4.10	2	0.541	0.068	0.370	0.226	1.439	0.928
14	31	17	3	2.08	1	0.288	0.097	0.522	0.306	-0.709	0.272
15	30	7	2	1.21	1	0.067	0.061	0.071	0.071	-2.174	0.002
16	29	2	3	4.67	2	0.636	0.056	0.130	0.105	1.414	0.910
Average	36.6	4.3	2.3	2.14	1.4	0.206	0.014	0.097	0.012	-0.944	–
Pooled	585	36	27	22.51	23	0.847	0.007	1.024	0.536	0.679	0.800

Table S9 *Alaria*: Genetic distances (Φ_{ST}) between samples from the Gulf of Alaska and Aleutian Islands based on mitochondrial DNA cytochrome oxidase I-5P (664 base-pair fragment) the Tamura & Nei (1993) model of mutation. Italics indicates $P < 0.05$ and bold indicates $P < 0.01$ of the null hypothesis of homogeneity between two populations.

2	0.911														
3	**0.888**	0.978													
4	**0.895**	**0.984**	*0.046*												
5	**0.891**	**0.930**	**0.953**	**0.959**											
6	**0.900**	**0.981**	**0.075**	*0.073*	**0.959**										
7	**0.862**	**0.981**	**0.987**	**0.995**	**0.945**	**0.989**									
8	**0.918**	**0.991**	**0.076**	0.056	**0.971**	0.111	**1.000**								
9	**0.504**	**0.986**	**0.980**	**0.992**	**0.956**	**0.983**	**1.000**	**1.000**							
10	**0.637**	**0.971**	**0.961**	**0.969**	**0.949**	**0.965**	**0.970**	**0.979**	**0.780**						
11	**0.497**	**0.985**	**0.979**	**0.991**	**0.954**	**0.983**	**1.000**	**1.000**	0.000	**0.776**					
12	**0.479**	**0.979**	**0.971**	**0.983**	**0.948**	**0.975**	**0.989**	**0.993**	0.038	**0.733**	0.035				
13	**0.529**	**0.804**	**0.836**	**0.840**	**0.802**	**0.851**	**0.273**	**0.870**	**0.619**	**0.685**	**0.612**	**0.602**			
14	**0.773**	**0.803**	**0.859**	**0.863**	*0.052*	**0.876**	**0.806**	**0.894**	**0.829**	**0.848**	**0.825**	**0.817**	**0.663**		
15	**0.811**	**0.943**	**0.963**	**0.971**	**0.909**	**0.968**	0.011	**0.982**	**0.958**	**0.936**	**0.956**	**0.944**	**0.216**	**0.749**	
16	**0.478**	**0.949**	**0.929**	**0.939**	**0.918**	**0.939**	**0.938**	**0.958**	**0.430**	**0.650**	**0.421**	**0.384**	**0.586**	**0.783**	**0.888**
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Sample number														

Table S10 *Alaria*: Analysis of molecular variance (AMOVA) of sequence variability among samples from the Gulf of Alaska and Aleutian Islands based on mitochondrial DNA *cytochrome oxidase I*-5P (664 base-pair fragment) with the Tamura & Nei (1992) model of mutation. Overall $\Phi_{ST} = 0.904$

Partition	d.f.	Variance component	% of variance	*P*
Among populations	13	3.283	90.4	0.00001
Within populations	512	0.348	9.6	
Total	525	3.631	100.0	

Contatenated cytochrome oxidase I-5P (*COI*-5P) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L)

Sequences of *COI*-5P and *rbc*L were concatenated for further analyses, producing 1404 bp sequences for 543 plants and 33 haplotypes in 16 populations (Table S11). About 90% of the overall variability was due to differences among populations, which included five major lineages, and only 10% was due to variability among plants within populations (Table S12). Genetic distances were generally large (Table S13) and this reflected divergences between the five lineages. Genetic distances between nearby populations or populations that shared the same lineage were smaller.

Table S11 *Alaria*: Haplotype frequencies of contatenated fragments of mitochondrial DNA cytochrome oxidase I-5P (*COI*-5P) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1404 base pairs) in samples from the Gulf of Alaska and Aleutian Islands

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
1	.	.	.	.	.	.	.	.	34	8	32	17	10	4	.	8	113
2	.	.	.	.	.	19	.	48	.	.	.	.	.	.	.	.	67
3	.	.	24	29	.	.	.	.	.	.	.	.	.	.	.	.	53
4	2	.	.	.	.	.	39	.	.	.	.	.	.	.	11	.	52
5	.	.	.	.	12	.	.	.	.	.	.	.	.	26	.	.	38
6	.	.	.	.	.	.	.	.	.	38	.	.	.	.	.	.	38
7	.	33	.	.	.	.	.	.	.	.	.	.	.	.	.	.	33
8	28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	28
9	.	.	.	.	.	.	.	.	.	.	.	.	26	.	.	.	26
10	.	.	.	.	17	.	.	.	.	.	.	.	.	.	.	.	17
11	.	.	.	.	.	15	.	.	.	.	.	.	.	.	.	.	15
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	15	15
13	.	.	.	.	.	.	.	.	.	.	.	10	.	.	.	.	10
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6	6
15	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	5
16	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	3
17	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
18	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
19	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	2
20	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
21	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	2
22	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	2
23	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1
24	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1
25	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
26	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
27	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
28	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
29	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1
30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1
31	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1
32	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1
33	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1
Total	32	38	28	31	32	39	40	48	34	47	32	29	41	31	12	29	543

Table S12 *Alaria*: Analysis of molecular variance (AMOVA) of samples from the Gulf of Alaska and Aleutian Islands based on contatenated fragments of mitochondrial DNA cytochrome oxidase I-5P (*COI*-5P) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1404 base pairs) with the Tamura (1992) model of mutation. Overall $\Phi_{ST} = 0.896$

Partition	d.f.	Variance component	% of variance	*P*
Among populations	15	3.676	89.6	<0.00001
Within populations	527	0.426	10.4	
Total	542		100.0	

Table S13 *Alaria*: Genetic distances (Φ_{ST}) between samples from the Gulf of Alaska and southeastern Bering Sea based on contatenated fragments of mitochondrial DNA cytochrome oxidase I-5P (*COI*-5P) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) (1404 base pairs) with the Tamura (1992) model of mutation (below diagonal). Island model estimates of migration between populations per generation (above diagonal).

1	–	0.489	0.076	0.068	0.072	0.094	0.073	0.042	0.552	0.317	0.571	0.711	0.495	0.161	0.121	0.623
2	0.911	–	0.009	0.007	0. 053	0.030	0.012	0.005	0.006	0.013	0.006	0.020	0.088	0.135	0.019	0.023
3	0.868	0.982	–	12.706	0. 031	0.429	0.006	0.025	0.009	0.020	0.009	0.034	0.098	0.081	0.010	0.039
4	0.880	0.986	0.038	–	0.028	0.384	0.003	0.012	0.004	0.016	0.004	0.027	0.091	0.075	0.006	0.033
5	0.874	0.904	0.941	0.947	–	0.050	0.046	0.023	0.030	0.031	0.031	0.045	0.105	2.634	0.076	0.048
6	0.842	0.943	0.538	0.566	0.910	–	0.031	0.530	0.042	0.043	0.043	0.063	0.104	0.100	0.048	0.065
7	0.873	0.976	0.988	0.993	0.916	0.942	–	0.001	0.002	0.013	0.002	0.022	0.253	0.136	10.898	0.027
8	0.922	0.991	0.952	0.976	0.956	0.485	0.998	–	0.0	0.008	0.0	0.014	0.056	0.063	0.002	0.017
9	0.475	0.988	0.983	0.992	0.944	0.923	0.996	1.000	–	0.141	∞	1.377	0.293	0.097	0.003	0.662
10	0.612	0.975	0.961	0.969	0.942	0.921	0.974	0.983	0.780	–	0.145	0.289	0.220	0.085	0.019	0.269
11	0.467	0.988	0.982	0.991	0.942	0.921	0.996	1.000	0.000	0.776	–	1.431	0.301	0.100	0.003	0.686
12	0.413	0.962	0.937	0.948	0.918	0.889	0.957	0.973	0.266	0.634	0.259	–	0.358	0.123	0.038	0.942
13	0.502	0.851	0.836	0.846	0.826	0.828	0.664	0.899	0.631	0.694	0.624	0.583	–	0.217	0.399	0.347
14	0.757	0.788	0.860	0.870	0.160	0.834	0.786	0.888	0.838	0.855	0.833	0.802	0.697	–	0.233	0.129
15	0.805	0.963	0.980	0.989	0.868	0.913	0.044	0.997	0.994	0.963	0.994	0.929	0.556	0.682	–	0.045
16	0.445	0.957	0.928	0.939	0.912	0.884	0.949	0.967	0.431	0.650	0.422	0.347	0.591	0.795	0.917	–
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sample number																

Microsatellite DNA

Table S14 *Alaria*: Summary statistics of variability at 8 microsatellite loci pooled over 16 samples. Number of alleles (N_A), observed heterozygosity (H_O), unbiased expected heterozygosity assuming Hardy-Weinberg proportions (H_E), population inbreeding coefficient (F_{IS}), differentiation among populations (F_{ST}) and probability F_{ST} is greater than 0.0. Totals obtained by bootstraping over loci.

Locus	N_A	H_O	H_E	F_{IS}	F_{ST}
AN21	50	0.167	0.695	0.748	0.278
*AN23**	43	0.353	0.456	0.210	0.508
*AN26**	87	0.548	0.678	0.178	0.275
AN27	16	0.057	0.287	0.793	0.616
AN30	29	0.431	0.618	0.276	0.334
AN31	28	0.444	0.512	0.120	0.416
*AN38**	43	0.409	0.683	0.391	0.268
AN39	37	0.361	0.576	0.340	0.419
Mean	41.6	0.346	0.563	0.382	0.389

Table S15 *Alaria*: Below diagonal: divergence (F_{ST}) between populations based on 8 microsatellite loci. Above diagonal: estimates of migration *Nm* from the island model of migration.

1	–	1.507	2.461	1.520	0.709	1.105	1.253	0.564	0.785	1.010	0.863	2.671	2.447	1.172	1.055	1.064
2	0.142	–	1.618	1.198	0.567	1.322	0.956	0.774	0.669	0.605	0.570	1.215	0.974	0.757	0.780	0.546
3	0.092	0.134	–	1.919	0.829	1.482	1.301	0.781	0.886	0.837	0.880	1.958	1.550	1.211	0.890	0.829
4	0.141	0.173	0.115	–	0.701	1.252	1.191	0.771	0.739	0.532	0.674	1.159	0.976	0.960	0.595	0.580
5	0.261	0.306	0.232	0.263	–	0.553	0.580	0.326	0.443	0.336	0.446	0.611	0.569	1.398	0.337	0.333
6	0.184	0.159	0.144	0.166	0.311	–	2.719	1.009	0.597	0.496	0.576	1.003	0.699	0.725	0.609	0.481
7	0.166	0.207	0.161	0.174	0.301	0.084	–	0.562	0.659	0.538	0.697	1.166	0.770	0.826	0.556	0.663
8	0.307	0.244	0.243	0.245	0.434	0.199	0.308	–	0.308	0.245	0.345	0.499	0.367	0.412	0.387	0.243
9	0.242	0.272	0.220	0.253	0.360	0.295	0.275	0.448	–	0.400	0.568	0.717	0.541	0.595	0.367	0.286
10	0.198	0.293	0.230	0.320	0.427	0.335	0.317	0.505	0.384	–	0.348	1.048	0.811	0.516	0.450	0.317
11	0.225	0.305	0.221	0.271	0.359	0.303	0.264	0.420	0.305	0.418	–	0.738	0.661	0.713	0.402	0.388
12	0.086	0.171	0.113	0.177	0.290	0.200	0.177	0.334	0.259	0.193	0.253	–	1.884	1.110	1.058	0.994
13	0.093	0.204	0.139	0.204	0.305	0.264	0.245	0.405	0.316	0.236	0.274	0.117	–	0.950	0.951	0.710
14	0.176	0.248	0.171	0.207	0.152	0.256	0.232	0.378	0.296	0.326	0.260	0.184	0.208	–	0.524	0.477
15	0.192	0.243	0.219	0.296	0.426	0.291	0.310	0.393	0.405	0.357	0.383	0.191	0.208	0.323	–	0.614
16	0.190	0.314	0.232	0.301	0.429	0.342	0.274	0.507	0.466	0.441	0.392	0.201	0.260	0.344	0.289	–
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
								Sample number								

Table S16 *Alaria*: Microsatellite allele frequencies in 16 samples from the Northeastern Pacific Ocean

	Population															
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AN21																
Allele/*N*	19	17	16	23	13	26	20	16	11	39	2	19	22	4	9	9
58	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111
64	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111
75	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
85	0.237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
88	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111
112	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
122	0.000	0.000	0.031	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
129	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
145	0.105	0.059	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.026	0.000	0.000	0.091	0.000	0.111	0.000
163	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
166	0.000	0.000	0.000	0.022	0.077	0.038	0.075	0.688	0.000	0.000	0.000	0.053	0.000	0.000	0.333	0.556
169	0.158	0.118	0.406	0.065	0.154	0.577	0.350	0.063	0.000	0.000	0.000	0.026	0.136	0.000	0.111	0.000
171	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.237	0.000	0.000	0.000	0.000
172	0.026	0.059	0.125	0.000	0.038	0.346	0.100	0.000	0.682	0.744	0.000	0.263	0.045	0.000	0.000	0.000
175	0.211	0.176	0.000	0.326	0.000	0.038	0.300	0.000	0.000	0.051	0.250	0.026	0.091	0.000	0.000	0.000
178	0.026	0.118	0.063	0.022	0.692	0.000	0.000	0.000	0.000	0.000	0.000	0.105	0.000	0.500	0.111	0.000
181	0.053	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.111
184	0.000	0.000	0.125	0.261	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.250	0.000	0.000
186	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.227	0.000	0.167	0.000
189	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.318	0.000	0.000	0.000	0.000	0.000	0.000	0.000
201	0.000	0.147	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.750	0.000	0.000	0.000	0.000	0.000
204	0.000	0.176	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.179	0.000	0.000	0.000	0.000	0.000	0.000
223	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
229	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
244	0.000	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000
256	0.105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000

322	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000
328	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000
361	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
367	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
373	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
376	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000
379	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000
386	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000
400	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000
415	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000
434	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
437	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
448	0.000	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
452	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000
458	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
472	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
475	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000
478	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000
517	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
520	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
535	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
538	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
541	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
597	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000

*AN23**

Allele/N	13	21	32	29	30	35	22	46	34	45	26	31	29	29	15	32
101	0.000	0.000	0.000	0.000	0.000	0.000	0.341	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
104	0.000	0.000	0.000	0.000	0.000	0.343	0.455	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
106	0.000	0.167	0.000	0.000	0.000	0.000	0.114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
107	0.077	0.024	0.000	0.017	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
109	0.000	0.048	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.989	0.000	0.000	0.000	0.000	0.000	0.000

110	0.077	0.000	0.047	0.207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
112	0.115	0.762	0.250	0.207	0.000	0.657	0.000	1.000	0.000	0.000	0.019	0.000	0.000	0.000	0.033	0.000
113	0.423	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.058	0.000	0.103	0.000	0.000	0.000
114	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.423	0.000	0.000	0.000	0.867	1.000
116	0.154	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.879	0.086	0.100	0.000
119	0.154	0.000	0.016	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.017	0.000	0.000
122	0.000	0.000	0.000	0.000	0.550	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.655	0.000	0.000
124	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.000
127	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.000	0.000
130	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000
133	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
135	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
136	0.000	0.000	0.078	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
138	0.000	0.000	0.000	0.000	0.217	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.000	0.000
139	0.000	0.000	0.094	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
141	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
142	0.000	0.000	0.047	0.017	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
145	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000
148	0.000	0.000	0.031	0.069	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
151	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
154	0.000	0.000	0.000	0.121	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
157	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000
160	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000
163	0.000	0.000	0.063	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.145	0.000	0.000	0.000	0.000
166	0.000	0.000	0.047	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.113	0.000	0.000	0.000	0.000
169	0.000	0.000	0.016	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.194	0.000	0.000	0.000	0.000
172	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.113	0.000	0.000	0.000	0.000
175	0.000	0.000	0.016	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000
178	0.000	0.000	0.016	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000
181	0.000	0.000	0.016	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.081	0.000	0.000	0.000	0.000
184	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000

187	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000
190	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
202	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
205	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
217	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*AN26**

Allele/N	37	23	32	28	18	46	47	48	30	44	30	31	41	24	27	24
154	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
157	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
163	0.162	0.000	0.000	0.018	0.028	0.000	0.213	0.000	0.000	0.000	0.050	0.016	0.000	0.000	0.000	0.000
166	0.081	0.761	0.078	0.179	0.000	0.707	0.085	0.990	0.000	0.000	0.000	0.065	0.037	0.000	0.852	0.000
169	0.000	0.000	0.016	0.018	0.000	0.054	0.426	0.010	0.000	0.000	0.000	0.016	0.000	0.000	0.019	0.000
172	0.000	0.000	0.000	0.000	0.000	0.022	0.011	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000
175	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.073	0.000	0.000	0.000
178	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
181	0.000	0.000	0.000	0.018	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
183	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.073	0.000	0.000	0.021
186	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.021	0.000	0.000
189	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
192	0.000	0.109	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000
198	0.027	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
201	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.012	0.000	0.000	0.000
210	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
213	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
215	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000
221	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000

224	0.054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.024	0.000	0.000	0.000
227	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.019	0.000
230	0.014	0.043	0.016	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.110	0.000	0.000	0.000
233	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.012	0.021	0.000	0.000
236	0.014	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.114	0.000	0.000	0.037	0.000	0.000	0.000
239	0.081	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.012	0.000	0.000	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.024	0.000	0.000	0.000
245	0.027	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.016	0.037	0.000	0.000	0.000
248	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
253	0.054	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.012	0.000	0.000	0.000
256	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.012	0.000	0.000	0.000
259	0.014	0.000	0.047	0.036	0.000	0.000	0.000	0.000	0.000	0.148	0.000	0.000	0.012	0.000	0.000	0.000
262	0.027	0.000	0.047	0.018	0.000	0.011	0.000	0.000	0.000	0.125	0.000	0.000	0.012	0.000	0.037	0.000
265	0.014	0.000	0.078	0.000	0.000	0.000	0.011	0.000	0.000	0.125	0.000	0.000	0.037	0.000	0.000	0.000
268	0.027	0.000	0.063	0.036	0.000	0.000	0.011	0.000	0.000	0.205	0.000	0.000	0.012	0.000	0.000	0.000
271	0.027	0.000	0.063	0.071	0.000	0.000	0.021	0.000	0.100	0.057	0.000	0.016	0.000	0.000	0.000	0.000
274	0.014	0.000	0.063	0.036	0.000	0.000	0.021	0.000	0.150	0.068	0.000	0.000	0.049	0.000	0.019	0.000
276	0.000	0.000	0.047	0.000	0.000	0.022	0.043	0.000	0.083	0.011	0.017	0.000	0.037	0.000	0.000	0.000
280	0.027	0.000	0.109	0.000	0.000	0.000	0.043	0.000	0.133	0.034	0.000	0.000	0.012	0.000	0.000	0.000
282	0.000	0.000	0.109	0.071	0.000	0.011	0.011	0.000	0.067	0.023	0.050	0.000	0.000	0.021	0.000	0.000
285	0.000	0.000	0.016	0.018	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.016	0.012	0.042	0.000	0.000
288	0.014	0.000	0.000	0.036	0.000	0.065	0.011	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
291	0.027	0.000	0.000	0.036	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
294	0.000	0.000	0.016	0.036	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
297	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.016	0.000	0.000	0.000	0.000
300	0.000	0.000	0.047	0.000	0.000	0.011	0.032	0.000	0.000	0.000	0.050	0.081	0.037	0.000	0.000	0.146
303	0.000	0.000	0.016	0.000	0.000	0.011	0.021	0.000	0.033	0.000	0.183	0.016	0.012	0.000	0.000	0.021
305	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.217	0.065	0.012	0.000	0.000	0.000
308	0.000	0.000	0.000	0.018	0.000	0.000	0.011	0.000	0.000	0.000	0.183	0.016	0.024	0.000	0.000	0.000
311	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.183	0.032	0.000	0.021	0.000	0.000
314	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.016	0.000	0.000	0.000	0.042

317	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.113	0.012	0.000	0.000	0.000
320	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.042
323	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
326	0.000	0.000	0.016	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.012	0.000	0.000	0.021
329	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.012	0.000	0.000	0.021
332	0.014	0.000	0.000	0.089	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.016	0.000	0.000	0.000	0.042
335	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.016	0.024	0.000	0.000	0.000
338	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.032	0.012	0.000	0.000	0.000
340	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000
343	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.012	0.021	0.000	0.000
346	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.016	0.000	0.000	0.000	0.000
349	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.048	0.000	0.000	0.000	0.042
352	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
355	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000
358	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.042
361	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063
364	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
367	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
372	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
375	0.014	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
378	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000
384	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000
387	0.000	0.000	0.000	0.000	0.944	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.813	0.000	0.063
398	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.083
401	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021
410	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021
416	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042
424	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063
430	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063
433	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042
436	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083
444	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021

AN27

Allele/N	17	4	6	21	15	26	47	48	6	45	30	31	41	16	19	17
214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.469	0.000	0.000
217	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.882	0.750	0.667	0.429	0.067	0.000	0.043	0.000	0.083	1.000	0.133	0.935	0.988	0.188	1.000	1.000
221	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
222	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
223	0.059	0.250	0.000	0.524	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.012	0.000	0.000	0.000
226	0.000	0.000	0.000	0.000	0.000	0.000	0.117	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
230	0.000	0.000	0.167	0.048	0.000	0.000	0.074	0.000	0.000	0.000	0.867	0.065	0.000	0.000	0.000	0.000
231	0.000	0.000	0.000	0.000	0.933	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000
233	0.000	0.000	0.000	0.000	0.000	0.692	0.713	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
236	0.000	0.000	0.000	0.000	0.000	0.192	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
242	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
253	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.313	0.000	0.000
285	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
425	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000
431	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*AN30**

Allele/N	34	31	32	28	2	39	35	25	31	43	28	31	34	27	23	29
224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000
227	0.000	0.032	0.000	0.000	0.000	0.000	0.214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
230	0.015	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
231	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000
233	0.029	0.048	0.188	0.518	0.500	0.321	0.086	0.880	0.000	0.000	0.161	0.000	0.015	0.000	0.022	0.000
234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000	0.000	0.000
235	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.256	0.643	0.323	0.206	0.333	0.022	0.000
236	0.029	0.065	0.125	0.125	0.250	0.308	0.129	0.020	0.032	0.000	0.000	0.000	0.029	0.000	0.000	0.000
237	0.412	0.032	0.109	0.036	0.000	0.000	0.014	0.000	0.000	0.000	0.036	0.097	0.456	0.074	0.000	0.983

238	0.029	0.726	0.031	0.071	0.000	0.231	0.486	0.000	0.694	0.128	0.000	0.000	0.000	0.000	0.022	0.000
240	0.132	0.016	0.047	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.221	0.019	0.000	0.000
241	0.000	0.065	0.016	0.036	0.000	0.103	0.043	0.000	0.065	0.593	0.000	0.000	0.000	0.000	0.109	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.226	0.000	0.019	0.000	0.000
243	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.161	0.000	0.125	0.000	0.029	0.000	0.022	0.017
244	0.000	0.016	0.078	0.054	0.000	0.000	0.000	0.040	0.000	0.023	0.000	0.000	0.000	0.000	0.348	0.000
245	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.145	0.000	0.556	0.000	0.000
246	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.044	0.000	0.000	0.000
247	0.000	0.000	0.156	0.036	0.250	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.283	0.000
248	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000
249	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
250	0.088	0.000	0.156	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.109	0.000
252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.048	0.000	0.000	0.000	0.000
253	0.000	0.000	0.047	0.036	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000
255	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
256	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
257	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
258	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
261	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

AN31

Allele/N	40	34	30	29	30	46	47	47	34	45	30	31	40	29	27	31
136	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
139	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
147	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
165	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
168	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.191	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
171	0.000	0.191	0.633	0.431	0.000	0.348	0.149	0.574	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
174	0.150	0.044	0.100	0.172	0.033	0.522	0.851	0.011	0.000	0.044	0.200	0.484	0.025	0.052	0.167	1.000
175	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.117	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000

177	0.125	0.044	0.217	0.397	0.000	0.000	0.000	0.000	0.941	0.000	0.000	0.032	0.063	0.000	0.056	0.000
180	0.075	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.544	0.050	0.387	0.700	0.069	0.704	0.000
181	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
183	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.050	0.190	0.074	0.000
186	0.038	0.000	0.017	0.000	0.183	0.000	0.000	0.000	0.000	0.000	0.383	0.000	0.025	0.621	0.000	0.000
189	0.075	0.000	0.000	0.000	0.750	0.000	0.000	0.000	0.000	0.100	0.183	0.016	0.038	0.034	0.000	0.000
190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
192	0.238	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.289	0.150	0.000	0.088	0.000	0.000	0.000
195	0.225	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.022	0.033	0.000	0.013	0.017	0.000	0.000
198	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
201	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
203	0.000	0.618	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
204	0.000	0.000	0.033	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
206	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
207	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
210	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
213	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
215	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*AN38**																
Allele/N	28	33	28	26	30	39	46	43	16	41	27	27	42	30	19	30
104	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.094	0.024	0.019	0.037	0.012	0.000	0.000	0.017
107	0.036	0.000	0.000	0.000	0.000	0.000	0.011	0.012	0.063	0.000	0.000	0.056	0.000	0.000	0.053	0.017
112	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000
121	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
147	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
163	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
173	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
174	0.000	0.015	0.000	0.000	0.000	0.013	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
177	0.018	0.015	0.000	0.327	0.000	0.654	0.533	0.523	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
179	0.000	0.000	0.036	0.000	0.000	0.000	0.011	0.000	0.094	0.000	0.000	0.148	0.167	0.250	0.158	0.000
182	0.000	0.182	0.000	0.173	0.700	0.013	0.000	0.000	0.156	0.000	0.000	0.000	0.476	0.500	0.000	0.033

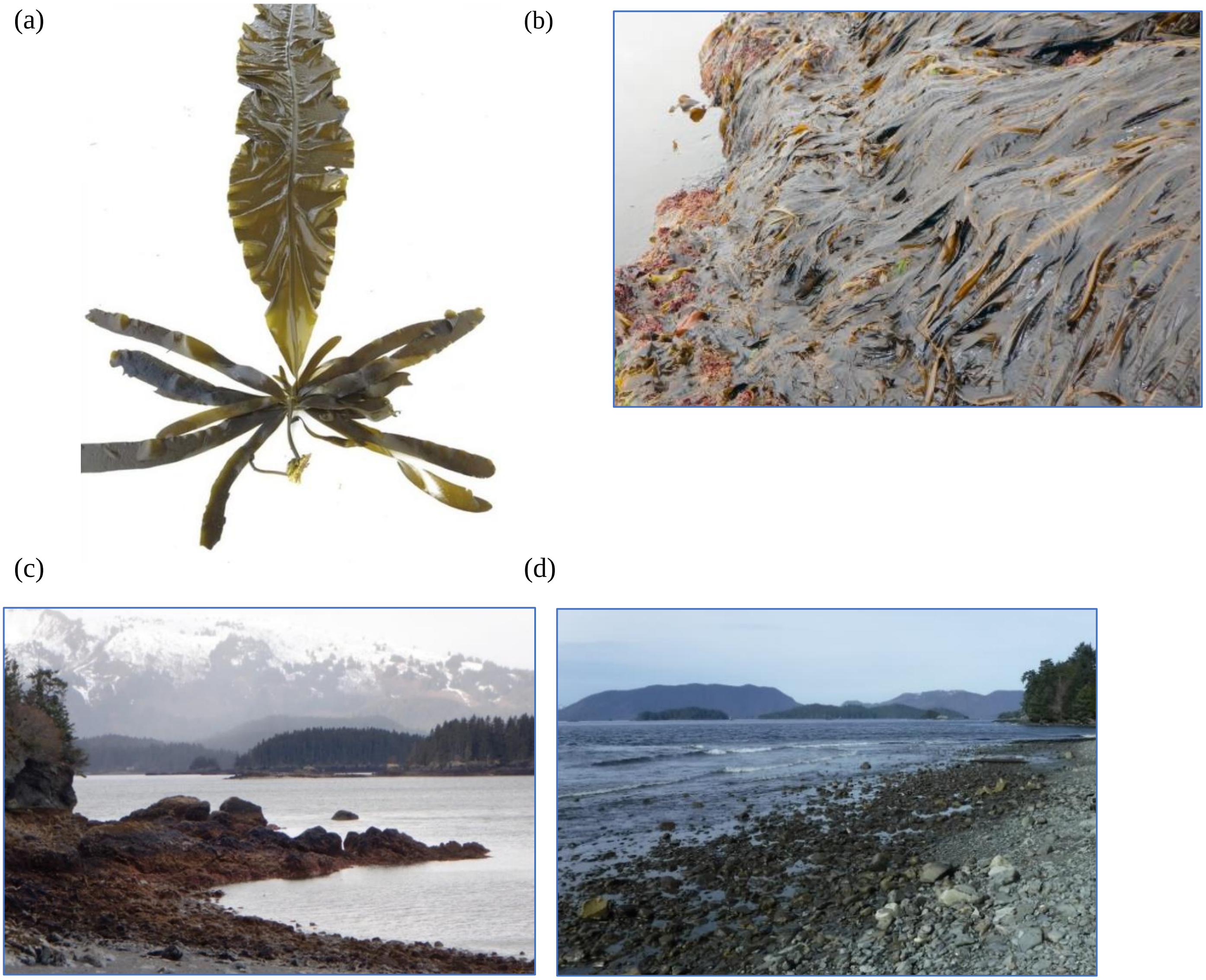
185	0.000	0.333	0.286	0.077	0.283	0.000	0.000	0.000	0.313	0.000	0.037	0.000	0.238	0.150	0.000	0.033
188	0.000	0.091	0.250	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.037	0.019	0.060	0.000	0.000	0.183
191	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
192	0.000	0.015	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
195	0.000	0.000	0.036	0.000	0.000	0.064	0.022	0.000	0.000	0.122	0.000	0.370	0.000	0.017	0.000	0.000
198	0.036	0.076	0.000	0.135	0.000	0.000	0.065	0.023	0.000	0.000	0.019	0.000	0.048	0.067	0.000	0.600
200	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
201	0.018	0.167	0.071	0.000	0.000	0.115	0.022	0.047	0.000	0.305	0.000	0.111	0.000	0.000	0.000	0.017
204	0.036	0.030	0.161	0.231	0.000	0.000	0.076	0.081	0.000	0.000	0.815	0.037	0.000	0.000	0.000	0.000
206	0.000	0.061	0.089	0.058	0.017	0.013	0.087	0.140	0.000	0.000	0.037	0.056	0.000	0.000	0.000	0.000
207	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.047	0.156	0.500	0.037	0.000	0.000	0.000	0.632	0.100
209	0.000	0.000	0.000	0.000	0.000	0.038	0.054	0.012	0.000	0.000	0.000	0.130	0.000	0.000	0.079	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
213	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000
218	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.000
221	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
223	0.000	0.000	0.000	0.000	0.000	0.051	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
224	0.054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
227	0.196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000
228	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
230	0.232	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
233	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
238	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
250	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
256	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
262	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
277	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
323	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
397	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
436	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

AN39

Allele/ N	8	2	18	30	30	21	29	37	34	45	17	11	41	29	29	5
239	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
249	0.000	0.500	0.000	0.017	0.450	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.400
250	0.000	0.000	0.000	0.967	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
254	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200
259	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138	0.000	0.000
264	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.088	0.000	0.012	0.017	0.000	0.100
269	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.122	0.034	0.000	0.000
274	0.000	0.000	0.056	0.000	0.167	0.000	0.000	0.000	0.176	0.000	0.000	0.000	0.049	0.138	0.034	0.000
275	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.966	0.000
276	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
279	0.000	0.000	0.556	0.017	0.250	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.073	0.172	0.000	0.300
284	0.000	0.000	0.111	0.000	0.083	0.000	0.000	0.000	0.765	0.000	0.912	0.045	0.012	0.052	0.000	0.000
289	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
293	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
294	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.073	0.000	0.000	0.000
299	0.000	0.000	0.167	0.000	0.000	0.000	0.069	0.000	0.000	0.000	0.000	0.000	0.341	0.121	0.000	0.000
304	0.125	0.000	0.000	0.000	0.017	0.262	0.241	0.000	0.000	0.000	0.000	0.000	0.244	0.052	0.000	0.000
309	0.000	0.250	0.000	0.000	0.000	0.190	0.052	0.270	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
314	0.000	0.000	0.000	0.000	0.000	0.048	0.155	0.216	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000
320	0.000	0.000	0.000	0.000	0.000	0.000	0.103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
324	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.682	0.000	0.000	0.000	0.000
329	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000
350	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
355	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
360	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
370	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
418	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000
422	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
427	0.000	0.000	0.000	0.000	0.000	0.095	0.034	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000

432	0.000	0.000	0.000	0.000	0.000	0.167	0.103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
437	0.000	0.250	0.000	0.000	0.000	0.143	0.069	0.284	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
442	0.000	0.000	0.000	0.000	0.000	0.071	0.121	0.230	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000
447	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
537	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000
542	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.034	0.000	0.000
547	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.034	0.000	0.000
552	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.086	0.000	0.000

*Locus with more than two alleles was diplodized for populations by randomly reducing the number of alleles to two



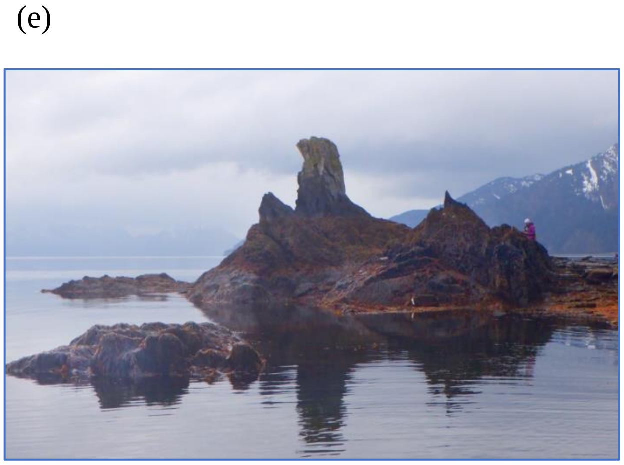


Figure S1. *Alaria*: a) photo of plant showing sporophylls and frond. b) a mat of *Alaria* at low tide. c) Kyak Beach, Kachemak Bay. d) Watson Beach, Sitka. e) Lowry Point, Seward.

References

Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+ C-content biases. *Molecular Biology and Evolution*, **9**, 678–687.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

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Chapter 7

Molecular Phylogenetics and Evolution Research Article

Pleistocene climate cycles produce and arrest divergences between populations of winged kelps in the genus *Alaria* in the Northeastern Pacific

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Abstract

In the Northeastern (NE) Pacific, kelps in the genus *Alaria* have had a tangled taxonomic history because convergent morphological plasticity among taxa has made it difficult to clearly delineate some species and to identify individual plants. In this study, we assembled available sequences (n = 658) of mitochondrial DNA cytochrome oxidase I-5P (*COI*-5P) sequences (634 bp) from 5 Northwestern (NW) Pacific and Bering Sea species and five major lineages in the NE Pacific to examine geographical patterns of divergence. Evolutionary units defined by *COI*-5P only partially corresponded to current taxonomies of *Alaria* in the NE Pacific. Sequence divergences between the five lineages in the NE Pacific (0.0101–0.040) were much smaller than divergences between species in the NW Pacific (0.0470–0.1861). The contrasting levels of genetic differentiation between taxa within the NW and NE Pacific is likely due a different climatic histories in the two basins. Shallow divergences between lineages of *Alaria* in the NE Pacific reflect a history of extinctions, repeated isolations in glacial refugia and secondary contact with hybridization. The deeper divergences between species in the NW Pacific, on the other hand, reflect longer-lived taxa in a region that did not experience extensive coastal glaciation during the Pleistocene Epoch.

Keywords: Alaria spp.; *COI*-5P; ice-ages; kelp; mitochondrial DNA; North Pacific Ocean; speciation

1. Introduction

The mechanisms producing new species in marine macroalgae are poorly understood. The description of patterns of genetic variability among populations is the first step in understanding the genetic events that lead to new species. The focus of the present study is on resolving patterns of differentiation of among populations, subspecies and species. Recent shifts in thinking have been prompted by the development of technologies that provide genomic views of differentiation. The focus, especialy in plants, has been on the roles of reinforcement (Butlin, 1987; Servedio and Noor, 2003; Hoskin et al., 2005), ecological divergence (Orr and Smith, 1998; Nosil, 2012), hybridization (Soltis and Soltis, 2009; Abbott et al., 2013), chromosomal rearrangements (Rieseberg, 2001) and polypoidy (Adams and Wendel, 2005). Another focus has been on patterns of reticulation brought about by hybridizations between partially isolated taxa (Linder and Rieseberg, 2004). Hypotheses of allopatric speciation have been the mainstay of evolutionary studies, but sympatric speciation may explain divergence, but only in a few cases (Coyne and Orr, 2004).

Species of kelps in the genus *Alaria* are often difficult to identify because environment variability influences morphology so that different species may show similar characters in some habitats (Widdowson, 1971a). The genus *Alaria* contains the second largest number of species in the kelp order Laminariales (Lane et al., 2006). The most widely distributed species, *A. esculenta*, has a range extending from France in the Northeastern (NE) Atlantic, to New England in the NW Atlantic and across the Arctic into the Bering Sea and Norwestern (NW) Pacific as far as northern Japan. As many as 10 additional species of *Alaria* are recognized in the North Pacific (Lane et al. 2007). The following geographical distributions were described in Widdowson (1971a). In the NW Pacific, *Alaria angusta* extends from Hokkaido, Japan to Bering Island. *Alaria crassifolia* has a restricted distribution extending from central Japan to the Kurile Islands. *Alaria paradisea* occurs in the Kurile Islands. *Alaria ochotensis* occurs from the Kamchatka Peninsula to Sakhalin, Russia. *Alaria crispa* occurs in the Bering Sea and along the Aleutian Island and appears to intergrade with *A. taeniata* along the southern shores of the Alaska Peninsula. *Alaria taeniata* extends from the Commander Island to British Columbia. *Alaria tenuifolia* extends from the western Aleutian Islands to Puget Sound, Washington. *Alaria praelonga* has a scattered distribution ranging from Japan to Southeastern Alaska. In the NE Pacific, *Alaria marginata* extends from the Gulf of Alaska to Point Conception, California (Widdowson, 1971a). *Alaria nana* extends from Yakutat, Alaska to Oregon.

Lane et al. (2007) used three molecular markers that produced conflicting results. The phylogenetic relationships detected with cytochrome oxidase 1 (*COI*-5P) showed 5 groups B–F that roughly corresponded to described species. Group B included *A. crispa* and *A. praelonga*. Group C encompassed populations of *A. esculenta*. Group D included only *A. taeniata*, Group E included *A. marginata*, *A. nana*, *A. taeniata* and *A. tenuifolia*. However these major lineages were not consistent with relationships among individual plants inferred from sequence variability of the nuclear internal transcribed spacer (ITS) of the ribosomal gene and the chloroplast Rubisco operon spacer (*rbc*Sp). They postulated that divergences between three groups, D, E and F, which may represent incipient species resolved with *COI*-5P did not correspond with relationships inferred with ITS and *rbc*Sp. The unrersolved relationships in a tree based on the nuclear gene, ITS, indicated hybridizations between organellar lineages and a breakdown of species' boundaries. In another study, nuclear microsatellite markers also showed hybridizations between divergent lineages of *COI*-5P and *rbc*L at sites where different lineages co-occurred (Grant and Bringloe, in preparation). Hybrids also appear between other pairs of species of *Alaria* (Kraan and Guiry, 2000).

The goal of this study was to provide further resolution of taxa of *Alaria* in the Gulf of Alaska relative to species in the NW Pacific and Bering Sea. We compiled available sequences of *COI*-5P for *Alaria* from Genbank and added them to sequences for the five NE Pacific lineages (Grant and Bringloe, in preparation). Genbank sequences were available for individuals identified as *A. marginata*, *A. nana, A. taeniata*, and *A. tenuifolia* from the NE Pacific, and as *A. esculenta*, *A. paradisea*, *A. crassifolia*, *A. praelonga* and *A. crispa* from the NW Pacific and Bering Sea. As in Lane et al. (2007), NE Pacific Genbank sequences did not sort by species, nor did they align consistently with the five well-define lineages. Shallow sequence divergences between taxa in the NE Pacific contrasted with the deeper divergences between species in the NW Pacific. This contrast appears to reflect the different climate histories in the two regions.

2. Methods

A total of 72 sequences of cytochrome oxidase I *COI*-5P (634 bp) from 9 species of *Alaria* were downloaded from Genbank and aligned with sequences (n = 586) from Grant and Bringloe (in preparation) (Table 1, 2). Several short sequences could not be used because the sequences did not encompass informative polymorphic nucleotide sites. C. Lane kindly provided chromatograms for sequences publilshed in Lane et al. (2007) to score an informative nucleotide site located three steps before the start of published sequences.

We used MEGA 7 (Kumar *et al*. 2016) to establish the best model of mutation and ARLEQUIN 3.5.2.2 (Excoffier & Lischer 2010) to estimate sequence divergences between taxa. Neighbor-joining trees based on sequence divergences between taxa were made with MEGA 7. We used TCS (Clements et al. 2000) to produce minimum spanning networks of haplotypes with parsimony.

3. Results

3.1 COI phylogeny

Average sequence divergence between the Alaskan samples ranged from 0.0 (samples 9 and 10) to 0.0394 (5 and 3, 5 and 6) (Table 3). Sequence divergences between putative species were generally larger, ranging from 0.0178 between *A. tenuifolia* and *A. paradisea* to 0.1853 between *A. crispa* and sample 5 (nominally *A. marginata*) (Table 3).

In a neighbor-joining tree of these sequence divergences, samples 1, 9, 10, 11, 12 and 16 clustered together and consituted a sister group to samples 3, 4, 6 and 8 and *A. taeniata* (Fig. 1a). Samples 5 and 14 were included in a group with *A. marginata* (southern distribution: Lane et al., 2007) and *A. nana* (wide spread). Sample 13 was a sister group to these two groups. Samples 7 and 15 (lineage C) were not associated with any of the species included in the tree. Sample 2 (lineage E, southern Alaska Peninsula) was also not associated with any of the species. *A. crispa* (Bering Sea), *A. crassifolia* (Northwestern Pacific) and *A. esculenta* (North Atlantic to Bering Sea) were distantly related to other taxa with sequence divergences ranging from 0.0470 to 0.1021 (Table 32).

Summaries of sequence divergences among plants by location, however, obscure complex relationships when samples include more than one of the major lineages. Sequences were summarized by lineage (Table 4). Average sequence divergences between Northeastern Pacific lineages ranged from 0.0101 between A and C to 0.0398 between B and D (Fig. 1b). The smallest divergence between recognized species and the lineages ranged from 0.0117 between C and *A. paradisea* to 0.1861 between D and *A. crispa*. *A. paradisea* (Kurile Islands) was closely related to the Northeastern Pacific lineages with sequence divergences ranging from 0.0117 to 0.0277. Relationships among the other species of *Alaria* remained the same as those in Fig. 1a.

In a parsimony network, the five lineages were separated from adjoining lineages by 5 to 10 mutational steps (Fig. 2). *A. paradisea* was separated from lineage C by only 6 steps and from lineage E by 8 steps. *A. praelonga* was separated from *A. crispa* by 6 steps. Haplotypes in lineage A included sequences of *A. marginata*, *A. tenuifolia*, *A. taeniata*, and *A. nana*. Lineage B included sequences from only *A. taeniata*. Lineage C included sequences from *A. taeniata*, *A. tenuifolia*, and *A. nana*. Lineage D included most of the sequences of *A. marginata* and two sequences of *A. nana* , and lineage E included none of the added sequences.

4. Discussion

We found a lack of correspondence between the five deep lineages in *Alaria* of the NE Pacific and nominal species described for this region. The mismatches may be due to the difficulty of identifying individual plants to species or to flawed species descriptions plagued by morphological plasticity among plants of *Alaria*. We also found greater sequence divergence between taxa in the NW Pacific than between taxa in the NE Pacific. This contrast may be due to differences in the climatic histories in two regions. Greater amounts of coastal glaciation in the

NE Pacific likely led to isolations in coastal refugia, but also to extinctions that prevented the appearance of deeply divided lineages.

4.1 Genetic relationships among taxa

The current taxonomy of *Alaria* is largely based on the foundational studies of Widdowson (1971a, b), who used 66 traits to devise a taxonomic key for species' identifications. In this approach to the taxonomy of *Alaria*, a major problem is the large amount of morphological variability produced by environment-genetic interactions. In support of this conclusion are plants assigned to the same species, on the basis of morphology, that appeared in different major lineages. For example, sequences from plants identified as *A. taeniata* appeared in lineages A, B and C. Haplotypes from the geographically widely distributed *A. nana* were distributed among lineages, A, C & D. Plants from a single location at Bamfield, Vancouver Island, BC fell into lineages, A & D, that were separated by 17 mutational steps in the network (Fig. 2), indicating that they are unlikely to belong to the same species. The results of the present study do not support the validity of *A. nana* as a distinct species. It appears to be a convergent ecotype of several species.

Haplotypes of *A. marginata* appeared in only in lineages A and D but were most numerous in lineage D, which in our population study was found at only two sites, Afognak Island (5) and Sitka (14). It was also identified in collections from southeastern Alaska, the south central coast of Alaska and Kachemak Bay (Lane et al., 2007). This species is apparently most common along southern shores of British Columbia, Washington, and Oregon, reaching to southern California (Widdowson, 1971a). Hence, populations in Alaska appear to be at the northern extreme of the geographical range of this species. In a tree analysis of *rbcSP*, *COI*, and *ITS* sequences, Lane et al. (2007) found evidence for northern and southern groups of this species. However the groups were polyphyletic with respect to both *A. taeniata* and *A. tenuifolia*. The relationships in Fig. 2 confirm this relationship.

The *COI*-5P lineages in the present study correspond somewhat to *COI*-5P groups in Lane et al. (2007). Lineage A in the present study appears to correspond to group 'E' in Lane et al., and an un-named, cluster in group 'E' corresponds to lineage C. Lineage B corresponds to group 'D', and lineage D corresponds to group 'F'. Plants in lineage E, collected on the southern coast of the Alaska Peninsula, were apparently not sampled by Lane et al. (2007). The analysis of several species with the chloroplast DNA gene *rbc*Sp, the mitochondrial DNA gene *COI*-5P, and a nuclear gene *ITS* failed to clearly differentiate among the species of *Alaria* (Lane et al., 2007). The inclusion of *COI*-5P sequences from these species with the *COI*-5P database in this study also did not provide clear separation between taxa (Fig. 2). However, conclusions based on *COI*-5P must be tempered with caution because of small sample sizes of identified plants added to the study and because of the use of a single molecular marker.

4.2 Pleistocene ice ages and speciation

The process of speciation in the marine realm is poorly understood, but geographical isolation and adaptive divergence is thought to be fundamental mechanisms (Rundle and Nosil, 2005; Miglietta et al., 2011). The study of divergence between species and con-specific populations in the Northeastern Pacific Ocean are of particular interest because this region experienced extreme climate changes during the Pleistocene Epoch (Kaufman and Manley, 2004). Imprints of these ice-age disturbances are apparent in the limited amount of genetic variability among evolutionary lineages in the NE Pacific, compared to well-defined species in the Northwestern Pacific, where coastal glaciers were a minor feature during ice-age cycles. The climatic history of the NW Pacific differs from the history of the NE Pacific in several ways. Because of the west wind drift over the North Pacific, moisture-laden clouds produced large glaciers across the northern portion of North America. The prevailing weather pattern in the NW Pacific has been influenced by dry air masses that have traversed the Eur-Asian continent. Since these masses of air carried little moisture, there was little buildup of glaciers on land and no incursion of terrestrial glaciers into the shorelines around the NW Pacific.

Many populations are fixed for different lineages that are unlikely to have arisen in situ. The deep separations between the lineages indicate that the isolations predate the last glacial maximum about 20 000 years ago. A molecular-clock calibration of 0.4786% per million years was estimated by Zhang et al. (2015) for *Saccharina japonica*, based on fossil evidence for nodes in a phylogenetic reconstruction of brown algal evolution (Silberfeld et al., 2010). This calibration overestimates times of separation between recently diverged lineages because of the non-linearty of apparent mutation rates (Ho et al., 2005). In any case, temporal estimates of divergence between lineages range from about 10 000 years between A & C to 41 000 years between B & D. Estimates of divergence time between all of the taxa, including the five lineages and five Northwestern Pacific and North Atlantic species for which *COI*-P5 sequence where available ranged from 12 000 years between lineage C and *A. paradisea* to 194,000 years between lineage D and *A. crispa*. These point estimates have huge errors associated with them, but neverthless indicate that present levels of diversity likely arose within the last two Milankovitch climate cycles.

The abupt genetic transitions between populations in the NE Pacific can also be explained as legacies, at least in part, of ice-age isolations and post-glacial colonizations in which established populations retard gene flow through high-density blocking or local adaptatinon discourages the reproductive sucesses of plants originating from populations adapted to other environments (Grant and Bringloe, in preparation). The mosaic genetic pattern that we see today among five major lineages may have been produced by multiple divergences and multiple refugia. Each glacial advance along the shores of the NE Pacific shuffled the lineages geographically since the locations of refugia likely shifted with glacial each advance.

The 100 000-year timeframe for the ages of species in the genus contrasts sharply with the longevity of the *Alaria* lineage with diverge from its sister genus *Undaria* about 16 million years ago (approximate HPD: 22–10 my) (Silberfeld et al., 2010). The lack of deeply separated lineages in a long-lived genus points to a ‘recent’ bottleneck in the number of species and an episode of speciation, or more likely to a history of species turnover prompted by periodic environmental disturbances from glacial expansions into coastal areas. The recent origins of present-day populations in the Northeastern Pacific Ocean is supported by the small values of nucleotide diversity in both the location samples and lineages (Grant and Bringloe, in preparation). Eleven of the 16 populations sampled around the Gulf of Alaska had no, or little, diversity, as they were fixed or nearly fixed for a single *COI*-5P haplotype. The remaining 5 populations had larger diversities, but this was due to the mixing of plants from divergent COI lineages. Theses low levels of diversity are typical of recently established populations that have not existed long enough for mutations to accummulate (Grant and Bowen, 1998).

The low levels of species diversity and divergence between lineages and nominal species in the Northeastern Pacific contrast sharply with the greater diversity of *Alaria* and larger divergences between species in the Northwestern Pacific Ocean (Fig. 1b, and Fig. 3 in Lane et al. 2007). The reason for this contrast is likely to be found in differences in the environmental histories of the two oceanic regions. While Northeastern Pacific shores were covered with glacial ice, the rim of the Northwestern Pacific was less glaciated and may have provided a greater number of ice-age habitats for *Alaria*. In the Okhotsk Sea, for example, the spatial and temporal distributions of ice-rafted debris (IRD) indicated the calving of ice bergs from sites along the western Kamchatka Peninsula into the eastern Kamchatka Sea during stadials but seasonally ice-free shores along the western Okhotsk Sea (Nürnberg et al., 2011).

4.3 Future prospects

The delineation of evolutionary units with molecular markers is the first step in understanding the origins of diversity among taxa of *Alaria* in the N Pacific. The next step is to understand the role that local adaptation plays in diversification. Two approaches are possible. First, an eco-genetic approach involves field and laboratory work with plants from a range of habitats to establish the extent that local adaptation drives, or maintains, the abrupt genetic transitions between populations. Laboratory culture studies might be useful in some cases to estimate the heritabilities of the adaptive phenotypes.

The resolution of the five lineages of *Alaria* in the N Pacific was made with a presumably selectively neutral molecular marker, so that these lineages may not consitute adaptive units.

Hence, the chaotic pattern among populations detected with this marker may be the result of selectively neutral processes that have nothing to do with adaptation. Repeated isolations during each glacial advance into coastal areas followed by disperals during warm periods and isolation again with the next glacial advance. About 80% of the time over the last 100 thousand year glacial cycle sea levels were 50 meters or more lower than present-day sea level, and much as low as 120 meters lower during glacial maxima. Hence, the lineages evolved in environmental conditions quite different from conditions over the past 10 000 years of the Holocene. The key to understanding divergence mechanisms may not lie in studies of populations during the present anomalously warm period with a coastline that differs from it long-term configuration. Research question have to be framed with some thought.

Another approach is to attempt to identify genes or gene expression patterns that are correlated with various environmental variables. Genomic methods can now be used to screen an incredibly large number of genes across an entire genome and to relate differences in gene profiles or gene expression among locations to adaptative responses to environmental variables. The results of genomic studies have shown that the units of adaptation are generally a small set of interacting genes rather than the whole genome (Wolf and Ellegren, 2017). For example, Coyer et al. (2011) found a genomic signature in *Fucus serratus* that was associated with adaptaton along a salinity gradient. In a contemporary view, reproductive isolation is a by product of adaptation to particular environmental variables, so that differentiation across an entire genome is not a central criterion of speciation (Wu 2001). Empirical data tends to indicate that genes, not the genome as a whole, are the units enabling speciation (Wolf and Ellegren, 2017).

Acknowledgements

This study was supported by the North Pacific Research Board Project 1618.

References

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., 2013. Hybridization and speciation. J. Evol. Biol., 26, 229–246.

Adams, K.L. and Wendel, J.F., 2005. Polyploidy and genome evolution in plants. Curr. Opin. Plant Biol., 8, 135–141.

Butlin, R., 1987. Speciation by reinforcement. Trend. Ecol. Evol., 2, 8–13.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1660.

Coyer, J.A., Hoarau, G., Pearson, G., Mota, C., Jüterbock, A., Alpermann, T., John, U., Olsen, J.L., 2011. Genomic scans detect signature of selection among a salinity gradient in populations of the intertidal seaweed *Fucus serratus* on a 12 km scale. Mar. Genom. 4, 41–49.

Coyne, J.A., Orr, H.A., 2004. Speciation Sinauer Associates. Sunderland, MA, pp. 276–281.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.

Grant, W.S., Bowen, B.W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J. Hered. 89, 415–426.

Grant, W.S., Bringloe, T.T. In preparation. Chaotic geographical structure of five cryptic lineages of ribbon kelp *Alaria* in Alaska reflects post-glacial isolation-by-colonization.

Ho, S.Y., Phillips, M.J., Cooper, A., Drummond, A.J., 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Mol. Biol. Evol. 22, 1561–1568.

Hoskin, C.J., Higgie, M., McDonald, K.R., Moritz, C., 2005. Reinforcement drives rapid allopatric speciation. Nature, 437, 1353.

Kaufman, D.S., Manley, W.F., 2004. Pleistocene maximum and Late Wisconsinan glacier extents across Alaska, USA. Develop. Quat. Sci. 2, 9–27.

Klimova, A.V., Klochkova, N.G., Klochkova, T.A., Kim, G.H., 2018. Morphological and molecular identification of *Alaria paradisea* (Phaeophyceae, Laminariales) from the Kurile Islands. Algae 33, 37–48.

Kraan, S., Guiry, M., 2000. Molecular and morphological character inheritance in hybrids of *Alaria esculenta* and *A. praelonga* (Alariaceae, Phaeophyceae). Phycologia 39, 554–559.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Lane, C.E., Mayes, C., Druehl, L.D., Saunders, G.W., 2006. A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. J. Phycol. 42, 493–512.

Lane, C.E., Lindstrom, S.C., Saunders, G.W., 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. Mol. Phyl. Evol. 44, 634–648.

Linder, C.R. and Rieseberg, L.H., 2004. Reconstructing patterns of reticulate evolution in plants. Am. J. Botany, 91, 1700–1708.

Mayr, E., 1942. Systematics and the origin of species. Columbia Univ. Press, New York.

McDevit, D.C., Saunders, G.W., 2009. On the utility of DNA barcoding for species differentiation among brown macroalgae (Phaeophyceae) including a novel extraction protocol. Phycol. Res. 57, 131–141.

Miglietta, M.P., Faucci, A., Santini, F., 2011. Speciation in the sea: overview of the symposium and discussion of future directions. Integ. Compar. Biol. 51, 449–455.

Nosil, P., 2012. Ecological speciation. Oxford Univ. Press.

Nürnberg, D., Dethleff, D., Tiedemann, R., Kaiser, A., Gorbarenko, S.A., 2011. Okhotsk Sea ice coverage and Kamchatka glaciation over the last 350 ka—Evidence from ice-rafted debris and planktonic δ18O. Palaeogeogr. Palaeoclim. Palaeoecol. 310, 191–205.

Orr, M.R., Smith, T.B., 1998. Ecology and speciation. Trend. Ecol. Evol., 13, 502–506.

Rieseberg, L.H., 2001. Chromosomal rearrangements and speciation. Trend. Ecol. Evol., 16, 351–358.

Rundle, H.D., Nosil, P., 2005. Ecological speciation. Ecol. Letts 8, 336–352.

Saunders, G.W., McDevit, D.C., 2014. A DNA barcode survey of Haida Gwaii kelp (Laminariales, Phaeophyceae) reveals novel ecological and distributional observations and *Saccharina druehlii* sp. nov. *Botany* 92, 821–826.

Servedio, M.R., Noor, M.A., 2003. The role of reinforcement in speciation: theory and data. Ann. Rev. Ecol. Evol. Systemat., 34, 339–364.

Silberfeld, T., Leigh. J.W., Verbruggen, H., Cruaud, C., De Reviers, B., Rousseau F., 2010. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the “brown algal crown radiation”. Mol. Phylogen. Evol. 56, 659–674.

Soltis, P.S., Soltis, D.E., 2009. The role of hybridization in plant speciation. Ann. Rev. Plant Biol., 60, 561–588.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Phyl. Evol. 10, 512–526.

Widdowson, T.B., 1971a. A taxonomic revision of the genus *Alaria* Greville. Syesis 4, 11–49.

Widdowson, T.B., 1971b. A statistical analysis of variation in the brown alga *Alaria*. Syesis 4, 125–143.

Wolf, J.B., Ellegren, H. 2017. Making sense of genomic islands of differentiation in light of speciation. *Nat. Revs Genet.*, 18, 87–100.

Wu, C.-I., 2001. The genic view of the process of speciation. J. Evol. Biol. 14, 851–865.

Zhang, J., Yao, J.-T., Sun, Z.-M., Fu, G., Galanin, D.A., Nagasato, C., Motomura, T., Hu, Z.-M., Duan, D.-L. 2015. Phylogeographic data revealed shallow genetic structure in the kelp *Saccharina japonica* (Laminariales, Phaeophyta). BMC Evol. Biol. 15, e237.

TABLE 1.

Genbank *COI*-5P sequences used in this study

Species	Genbank Nos	Location	Reference
A. crispa	EF218901	Bering Sea	Lane et al. 2007
	MH593100	Bering Sea	Bringloe and Saunders 2019
A. crassifolia	AB775220	Japan	Kawai et al. 2013
A. esculenta	EF218856, EF218857, EF218858, EF218859, EF218860, EF218879	N Atlantic	Lane et al. 2007
A. marginata	EF218867, EF218868, EF218869, EF218870, EF218875, EF218880, EF218890, EF218891, EF218892, EF218893, EF218894, EF218895, EF218896, EF218899	NE Pacific	Lane et al. 2007
	FJ409129, FJ409130, FJ409131, FJ409132, FJ409133	NE Pacific	McDevit and Saunders 2009
	KJ960252, KJ960258		Saunders and McDevit 2014
A. nana	EF218871, EF218872, EF218873, EF218888, EF218897, EF218898, EF218900	NE Pacific	Lane et al. 2007
A. paradisea	MG913352, MG913353, MG913354, MG913355,	Russia	Klimova et al. 2018
A. praelonga	EF218902	Japan	Lane et al. 2007
A. taeniata	EF218861, EF218862, EF218863, EF218864, EF218865, EF218866, EF218874, EF218876, EF218877, EF218881, EF218886, EF218889	Alaska	Lane et al. 2007
A. tenuifolia	EF218882, EF218883, EF218884, EF218885, EF218887	Alaska	Lane et al. 2007

TABLE 2

Locations and collection dates of samples from the Gulf of Alaska and Aleutian Islands

Sample	Location	Habitat	N Latitude	W Longitude
1	Morris Cove, Unalaska Island	Protected	53.919	166.438
2	Sand Point, Popf Island, Alaska Peninsula	Protected	55.309	160.513
3	Table Island, Kodiak Island	Exposed	57.188	152.925
4	Near Island, Kodiak Island	Protected	57.788	152.388
5	Black Cape, Afognak Island	?	58.402	152.882
6	Kasitsna Bay, Kachemak Bay	Protected	59.468	151.553
7	Kayak Beach, Kachemak Bay	Semi-exposed	59.497	151.472
8	Homer Spit, Kachemak Bay	Protected	59.604	151.418
9	Lowell Point, Resurrection Bay	Semi-exposed	60.064	149.443
10	Cordova, Prince William Sound	Protected	60.545	145.768
11	Bridge Site, Yakutat	Current swept	60.056	149.443
12	Auke Bay, North of Juneau	Protected	58.374	134.728
13	Magoun Island, W Baranof Island	Protected	57.157	135.567
14	Watson Point, W Baranof Island	Semi-Exposed	57.070	135.368
15	Little Port Walter, SE Baranof Island	Protected	56.384	134.641
16	Tokeen Bay, Prince of Wales Island	?	55.993	133.464

TABLE 3

Average sequence divergence (Tamura and Nei 1993) between populations and species of *Alaria*.

2	0.0223															
3	0.0176	0.0292														
4	0.0174	0.0288	0.0004													
5	0.0322	0.0216	0.0394	0.0390												
6	0.0175	0.0291	0.0005	0.0003	0.0394											
7	0.0102	0.0117	0.0222	0.0219	0.0187	0.0221										
8	0.0171	0.0286	0.0003	0.0001	0.0386	0.0002	0.0217									
9	0.0028	0.0201	0.0140	0.0137	0.0288	0.0139	0.0092	0.0136								
10	0.0044	0.0225	0.0162	0.0159	0.0311	0.0161	0.0113	0.0157	0.0015							
11	0.0028	0.0201	0.0140	0.0137	0.0288	0.0139	0.0092	0.0136	0.0000	0.0015						
12	0.0029	0.0203	0.0141	0.0139	0.0291	0.0141	0.0094	0.0137	0.0001	0.0016	0.0001					
13	0.0079	0.0147	0.0198	0.0195	0.0223	0.0197	0.0032	0.0193	0.0064	0.0083	0.0064	0.0066				
14	0.0277	0.0210	0.0362	0.0359	0.0059	0.0362	0.0170	0.0355	0.0244	0.0266	0.0244	0.0247	0.0197			
15	0.0103	0.0118	0.0223	0.0220	0.0188	0.0222	0.0001	0.0218	0.0093	0.0114	0.0093	0.0094	0.0033	0.0171		
16	0.0043	0.0226	0.0160	0.0157	0.0306	0.0159	0.0109	0.0155	0.0012	0.0027	0.0012	0.0013	0.0079	0.0261	0.0109	
mar	0.0223	0.0218	0.0313	0.0310	0.0141	0.0313	0.0168	0.0307	0.0196	0.0218	0.0196	0.0198	0.0180	0.0144	0.0169	0.0212
nan	0.0150	0.0225	0.0277	0.0273	0.0220	0.0276	0.0133	0.0271	0.0136	0.0155	0.0136	0.0138	0.0136	0.0204	0.0133	0.0152
tae	0.0114	0.0228	0.0101	0.0099	0.0319	0.0101	0.0134	0.0098	0.0085	0.0105	0.0085	0.0087	0.0121	0.0286	0.0135	0.0102
ten	0.0043	0.0184	0.0156	0.0154	0.0268	0.0155	0.0074	0.0152	0.0018	0.0034	0.0018	0.0020	0.0058	0.0229	0.0074	0.0032
pra	0.1269	0.0827	0.0713	0.0724	0.1502	0.0729	0.0949	0.0718	0.1157	0.1201	0.1157	0.1167	0.1021	0.1463	0.0951	0.1120
cra	0.0940	0.0867	0.1011	0.1025	0.0734	0.1033	0.0716	0.1017	0.0965	0.1024	0.0965	0.0973	0.0802	0.0762	0.0717	0.1048
cri	0.1664	0.0966	0.0964	0.0977	0.1853	0.0985	0.1142	0.0970	0.1532	0.1591	0.1532	0.1544	0.1277	0.1818	0.1144	0.1682
par	0.0213	0.0165	0.0276	0.0277	0.0234	0.0279	0.0112	0.0275	0.0195	0.0223	0.0195	0.0197	0.0142	0.0225	0.0113	0.0217
esc	0.0699	0.0573	0.0810	0.0823	0.0794	0.0804	0.0462	0.0816	0.0644	0.0681	0.0644	0.0650	0.0526	0.0766	0.0463	0.0709
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Sample number															

Table 3 continued to the right

nan	0.0203							
tae	0.0247	0.0203						
ten	0.0190	0.0135	0.0095					
pra	0.1373	0.1241	0.0902	0.1115				
cra	0.0849	0.0794	0.0923	0.0916	0.1017			
cri	0.1733	0.1530	0.1182	0.1454	0.0120	0.0962		
pra	0.0235	0.0196	0.0220	0.0178	0.0762	0.0579	0.0901	
esc	0.0750	0.0662	0.0667	0.0608	0.0717	0.0698	0.0860	0.0309
	mar	nan	tae	ten	pra	cra	cri	par
	Species abbreviation							

TABLE 4

Average sequence divergence (Tamura and Nei 1993) between lineages and species of *Alaria* (lower triangle), and average sequence divergence within taxa (diagonal)

A	0.0015									
B	0.0150	0.0003								
C	0.0101	0.0219	0.0010							
D	0.0308	0.0398	0.0195	0.0020						
E	0.0214	0.0289	0.0122	0.0217	0.0004					
pra	0.1183	0.0721	0.0959	0.1510	0.0827	0.0000				
cra	0.0986	0.1021	0.0728	0.0739	0.0867	0.1017	0.0000			
cri	0.1589	0.0973	0.1158	0.1861	0.0966	0.0120	0.0962	0.0000		
par	0.0208	0.0277	0.0117	0.0236	0.0165	0.0762	0.0579	0.0901	0.0000	
esc	0.0672	0.0812	0.0470	0.0793	0.0573	0.0717	0.0698	0.0860	0.0309	0.0002
	A	B	C	D	E	pra	cra	cri	par	esc
	Lineage					Species				

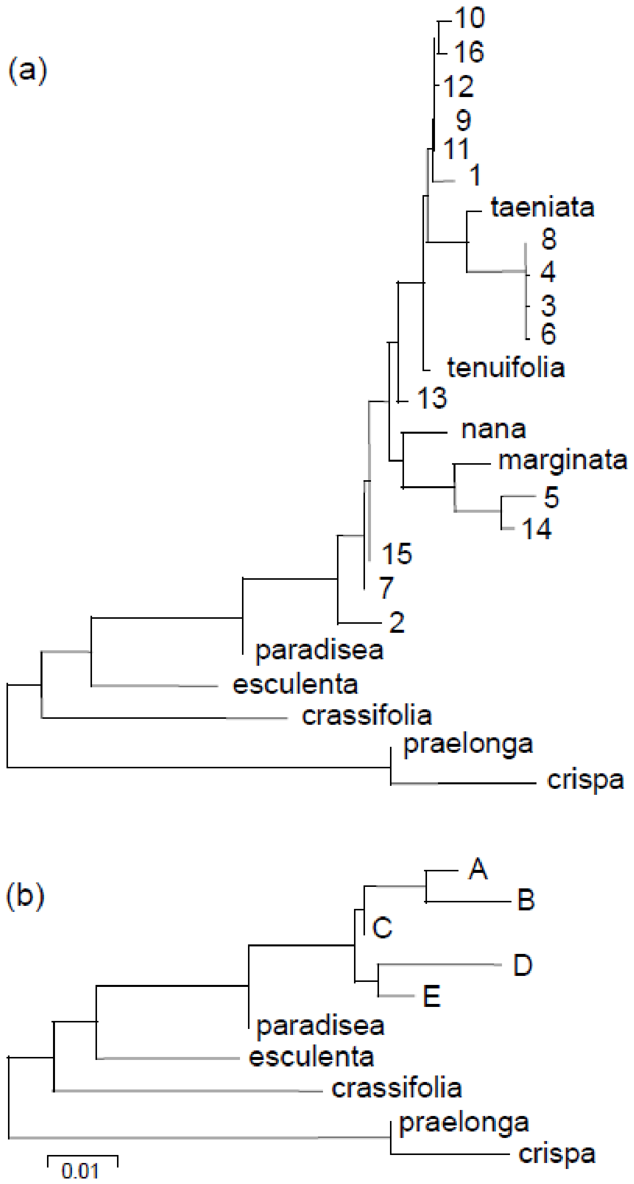


Fig. 1. Neighbor-joining tree of Tamura and Nei (1993) of *COI*-5P sequence divergences Alaskan populations and species of *Alaria*. (a) Sequences grouped by collection location or species. (b) Sequences grouped by haplotype lineage and species. Sample numbers as in Table 2 and lineage designations as in Figure 2c.

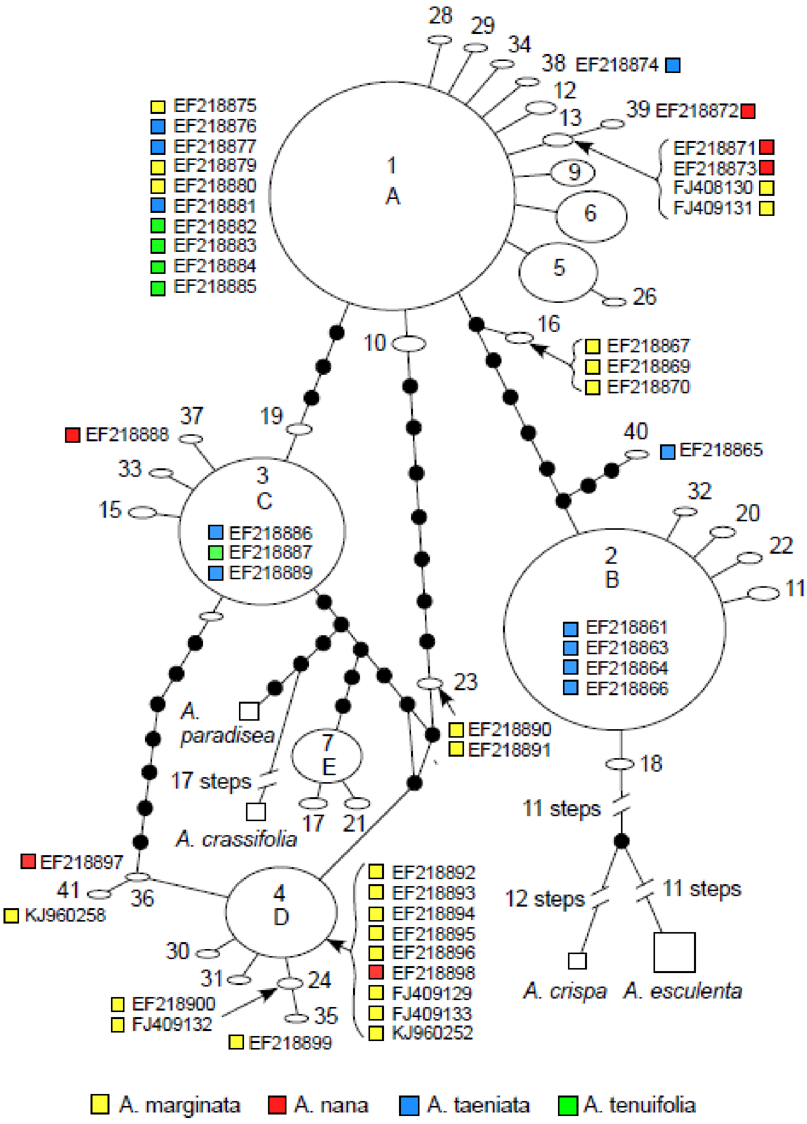
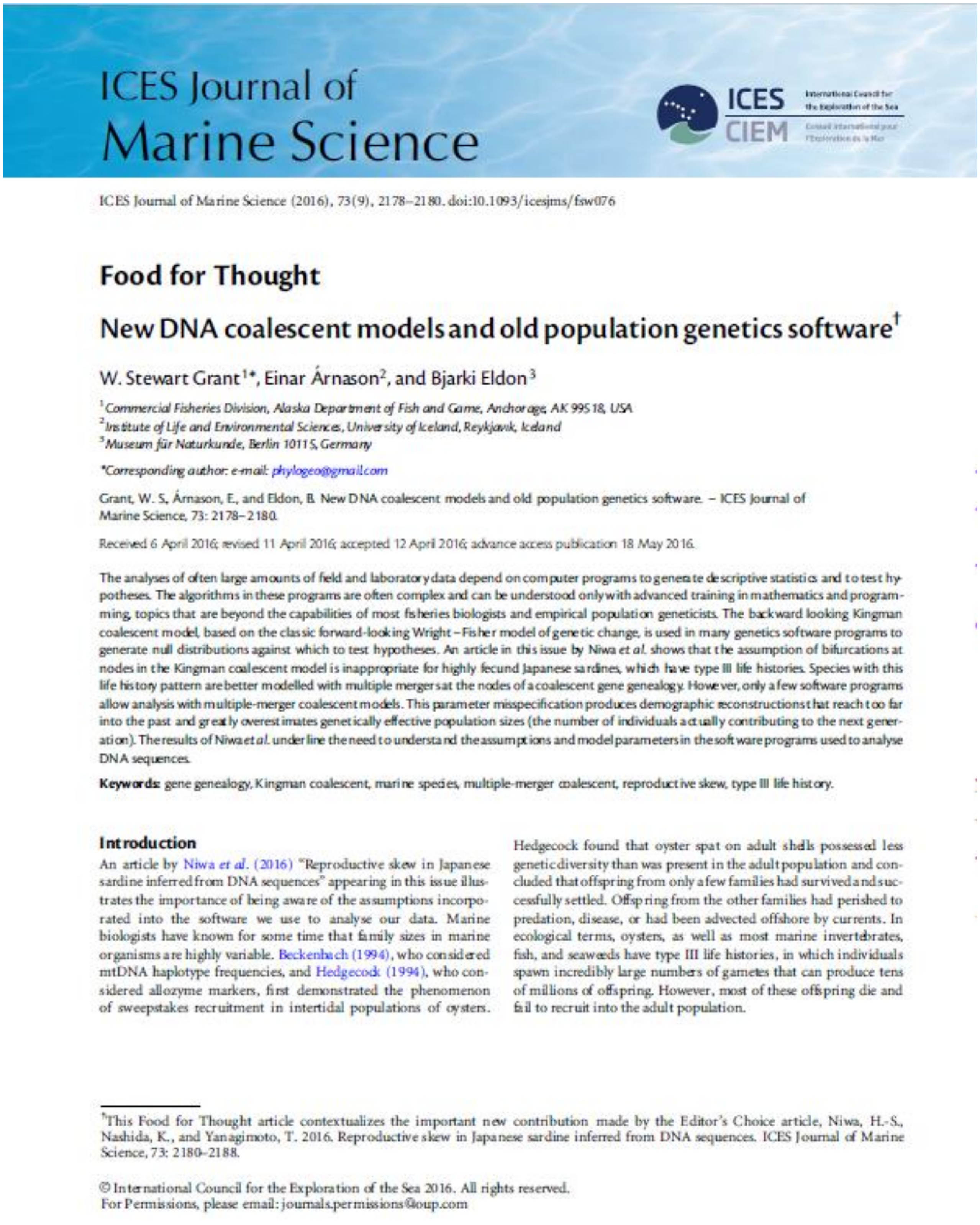


Fig. 2. Unedited parsimony network of cytochrome oxidase I-5P haplotypes (634 bp) showing relationships of published sequences (Lane et al., 2007) with species identifications with haplotype lineages in the present study (total *n* = 658). Published sequences indicated by Genbank Accession Numbers. Numbers indicate haplotypes for this dataset, and closed circles represent hypothetical, or unobserved haplotypes. Adjoining observed or hypothetical haplotypes separated from each other by one nucelotide mutation step. Lineage A corresponds with group E in Lane et al. (2007), lineage B with group D, lineage C with E-southern, and lineage D with group F.

Chapter 8



ICES Journal of Marine Science

ICES CIEM

ICES Journal of Marine Science (2016), 73(9), 2178–2180. doi:10.1093/icesjms/fsw076

Food for Thought

New DNA coalescent models and old population genetics software[†]

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Grant, W. S., Árnason, E., and Eldon, B. New DNA coalescent models and old population genetics software. – ICES Journal of Marine Science, 73: 2178–2180.

Received 6 April 2016; revised 11 April 2016; accepted 12 April 2016; advance access publication 18 May 2016.

The analyses of often large amounts of field and laboratory data depend on computer programs to generate descriptive statistics and to test hypotheses. The algorithms in these programs are often complex and can be understood only with advanced training in mathematics and programming, topics that are beyond the capabilities of most fisheries biologists and empirical population geneticists. The backward looking Kingman coalescent model, based on the classic forward-looking Wright–Fisher model of genetic change, is used in many genetics software programs to generate null distributions against which to test hypotheses. An article in this issue by Niwa *et al.* shows that the assumption of bifurcations at nodes in the Kingman coalescent model is inappropriate for highly fecund Japanese sardines, which have type III life histories. Species with this life history pattern are better modelled with multiple mergers at the nodes of a coalescent gene genealogy. However, only a few software programs allow analysis with multiple-merger coalescent models. This parameter misspecification produces demographic reconstructions that reach too far into the past and greatly overestimates genetically effective population sizes (the number of individuals actually contributing to the next generation). The results of Niwa *et al.* underline the need to understand the assumptions and model parameters in the software programs used to analyse DNA sequences.

Keywords: gene genealogy, Kingman coalescent, marine species, multiple-merger coalescent, reproductive skew, type III life history.

Introduction

An article by Niwa *et al.* (2016) "Reproductive skew in Japanese sardine inferred from DNA sequences" appearing in this issue illustrates the importance of being aware of the assumptions incorporated into the software we use to analyse our data. Marine biologists have known for some time that family sizes in marine organisms are highly variable. Beckenbach (1994), who considered mtDNA haplotype frequencies, and Hedgecock (1994), who considered allozyme markers, first demonstrated the phenomenon of sweepstakes recruitment in intertidal populations of oysters. Hedgecock found that oyster spat on adult shells possessed less genetic diversity than was present in the adult population and concluded that offspring from only a few families had survived and successfully settled. Offspring from the other families had perished to predation, disease, or had been advected offshore by currents. In ecological terms, oysters, as well as most marine invertebrates, fish, and seaweeds have type III life histories, in which individuals spawn incredibly large numbers of gametes that can produce tens of millions of offspring. However, most of these offspring die and fail to recruit into the adult population.

[†] This Food for Thought article contextualizes the important new contribution made by the Editor's Choice article, Niwa, H.-S., Nashida, K., and Yanagimoto, T. 2016. Reproductive skew in Japanese sardine inferred from DNA sequences. ICES Journal of Marine Science, 73: 2180–2188.

Reproductive skew

The effects of highly variable family sizes and sweepstakes recruitment (reproductive skew) on genetic diversity have been documented in the fisheries literature. Most notably, estimates of genetically effective population size, N_e, from surveys of genetic variability, or from the analyses of historical and contemporary DNA samples, show that N_e is as much as five orders of magnitude smaller than estimates of census size, N_c (Hedgecock, 1994). N_e is the size of a hypothetical population, usually with Wright–Fisher sampling, with the same amount of genetic diversity as the real population (Crow and Kimura, 1970). The reason that the concept of N_e is not more firmly rooted in the stock assessment literature is that it can be estimated only indirectly with molecular markers. Further, estimates of N_e have a large variance because they depend on allele-frequency estimates, which themselves have large variances because of finite sampling. However, the prevalence of profoundly skewed N_e/N_c ratios in highly fecund marine species is now universally accepted by marine and fishery biologists.

The prevalence of reproductive skew in marine species has implications for how genetic data are used to infer the dynamics and evolutionary histories of natural populations. Genetic data are now largely analysed with simulations based on the data themselves that produce distributions used to determine the statistical significances of observed patterns. A common approach for producing sample distributions is to use a backward-looking gene coalescence model to produce samples of possible hypothetical gene genealogies. Since the coalescence time of two genes to a common ancestor is random and continuous, an infinite number of gene genealogies are possible. The computational powers of desktop and laptop computers support a growing toolbox of coalescence-based computer programs to analyse genetic data.

Coalescent analysis

The widely used Kingman coalescent model (Kingman, 1982; Hudson, 1990) stems from the Wright–Fisher model of forward-in-time allele-frequency change. The Kingman coalescent assumes a bifurcation at nodes in the gene genealogy, an assumption which may be valid for low fecundity species, but which is clearly not valid for highly fecund marine species with skewed offspring distribution. An article in this issue by Niwa *et al.* explores the effect of this parameter mis-specification on the analysis of mitochondrial DNA variability in Japanese sardines. These authors use the model and resulting Beta-coalescent derived by Schweinsberg (2003) that allows multiple mergers at nodes in the gene genealogy, which more closely mimic the dynamics of type III marine species. This model leads to significantly different conclusions about the genetic structure of sardines from analyses made with the Kingman coalescence model.

An almost universal signature in mitochondrial DNA sequences of marine species is the occurrence of "star-shaped" genealogies, in which an abundant haplotype is connected to low-frequency and singleton haplotypes by only one or two mutations. Several mechanisms can produce these star-shaped genealogies, but the most widely invoked mechanism is that an excess of low-frequency mutations is retained in rapidly growing populations to a greater degree than in stable or declining populations (Rogers and Harpending, 1992; Drummond *et al.*, 2005). Climate warming after the last glacial maximum 18–20k years ago provides a plausible mechanism for the apparently widespread signal of recent population expansion in marine species.

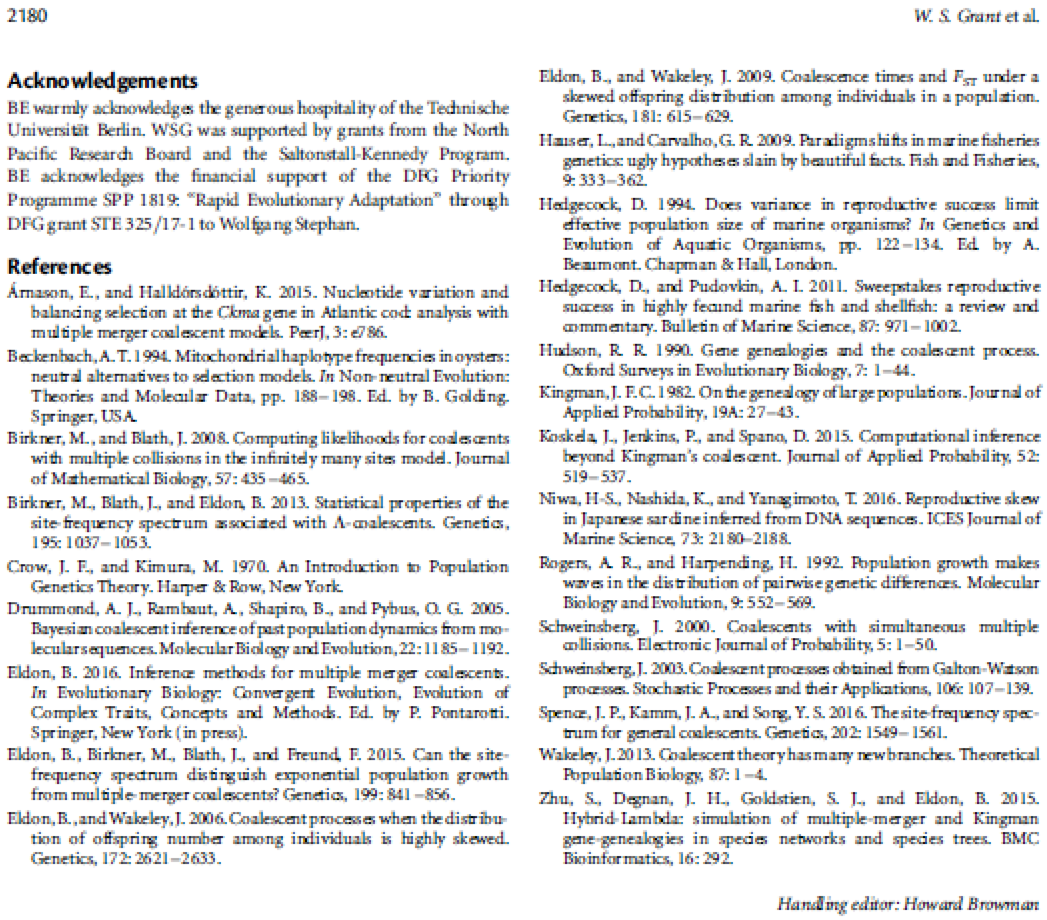
However, other mechanisms can also produce star-shaped genealogies. For example, selective sweeps in un-recombining mtDNA can produce genealogies that are indistinguishable from those created by a population expansion. Selective sweeps conferring greater fitness to some colonizers may be confounded with consequential expansions of populations into newly available areas. An excess of low-frequency mutants can also arise from the accumulation of slightly deleterious alleles that are prevented by selection from reaching high frequencies. Unfortunately, the nature of selection and its intensity are difficult to measure in natural populations. Hence, most researchers revert to interpreting star-shaped genealogies in terms of the neutral demographic processes, genetic drift and population growth.

Recent developments in multiple-merger coalescence theory (reviewed by Wakeley, 2013) have generated two sets of models: one involving a single multiple-merger at any time [Λ coalescents which include the Beta $(2-\alpha,\alpha)$ model used by Niwa *et al.*] and more general models allowing simultaneous multiple mergers at any time (Ξ coalescents) (Schweinsberg, 2000). Both sets of models predict a site-frequency spectrum (SFS) with a higher frequency of singletons than predicted by the Kingman coalescent model (Birkner *et al.*, 2013; Eldon *et al.*, 2015). Statistical inference methods are now available for the new coalescent models (Birkner and Blath, 2008; Koskela *et al.*, 2015; Zhu *et al.*, 2015; Eldon, 2016; Spence *et al.*, 2016), which may be appropriate as neutral null models for high-fecundity organisms with skewed offspring distribution (Árnason and Halldórsdóttir, 2015), many marine organisms, plants, and possibly even micro-organisms, including viruses.

The results of Niwa *et al.* lead to an important alternative conclusion about the demographic history of Japanese sardines, which is thought to have experienced a post-glacial population expansion. The SFS of simulated sequences under a model of a population expansion differed from the SFS under a multiple-merger coalescence model, which produced a greater proportion of low-frequency mutations. This difference was also recently noted in a study of autosomal sequences of Atlantic cod (Árnason and Halldórsdóttir, 2015). In Japanese sardines, the resemblance of the observed mtDNA sequences to sequences simulated under a multiple-merger coalescence model implies the absence of a recent demographic expansion in Japanese sardines.

Conclusion

The prevalence of reproductive skew and low N_e/N_c values in marine species has been confirmed with genetic markers in many marine species (Hauser and Carvalho, 2009; Hedgecock and Pudovkin, 2011). Despite these results, the idea has still not been incorporated into the management of marine species. The effects of reproductive skew on genetic structure have also not been used in the interpretations of DNA sequence patterns, although the effect has been studied theoretically over the last several years (Eldon and Wakeley, 2006, 2009). This is due in part to the inertia created by the pervasive use of software for data analysis that employs the Kingman coalescent to create null distributions. Given the results of Niwa *et al.*, and the theoretical predictions of multiple-merger coalescence, many studies will have to be reinterpreted to account for the effect that reproductive skew has on allele- or nucleotide-site-frequency distributions. These reinterpretations will be especially important for evolutionary biologists wanting to understand the relative roles of population history and adaptation in producing species diversity. Apart from genetic analysis, the prevalence of reproductive skew in marine species influences management strategies of exploited fishery stocks.



Acknowledgements

BE warmly acknowledges the generous hospitality of the Technische Universität Berlin. WSG was supported by grants from the North Pacific Research Board and the Saltonstall-Kennedy Program. BE acknowledges the financial support of the DFG Priority Programme SPP 1819: "Rapid Evolutionary Adaptation" through DFG grant STE 325/17-1 to Wolfgang Stephan.

References

Árnason, E., and Halldórsdóttir, K. 2015. Nucleotide variation and balancing selection at the *Ckma* gene in Atlantic cod: analysis with multiple merger coalescent models. PeerJ, 3: *e*786.

Beckenbach, A. T. 1994. Mitochondrial haplotype frequencies in oysters: neutral alternatives to selection models. *In* Non-neutral Evolution: Theories and Molecular Data, pp. 188–198. Ed. by B. Golding. Springer, USA.

Birkner, M., and Blath, J. 2008. Computing likelihoods for coalescents with multiple collisions in the infinitely many sites model. Journal of Mathematical Biology, 57: 435–465.

Birkner, M., Blath, J., and Eldon, B. 2013. Statistical properties of the site-frequency spectrum associated with Λ-coalescents. Genetics, 195: 1037–1053.

Crow, J. F., and Kimura, M. 1970. An Introduction to Population Genetics Theory. Harper & Row, New York.

Drummond, A. J., Rambaut, A., Shapiro, B., and Pybus, O. G. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution, 22: 1185–1192.

Eldon, B. 2016. Inference methods for multiple merger coalescents. *In* Evolutionary Biology: Convergent Evolution, Evolution of Complex Traits, Concepts and Methods. Ed. by P. Pontarotti. Springer, New York (in press).

Eldon, B., Birkner, M., Blath, J., and Freund, F. 2015. Can the site-frequency spectrum distinguish exponential population growth from multiple-merger coalescents? Genetics, 199: 841–856.

Eldon, B., and Wakeley, J. 2006. Coalescent processes when the distribution of offspring number among individuals is highly skewed. Genetics, 172: 2621–2633.

Eldon, B., and Wakeley, J. 2009. Coalescence times and F_{ST} under a skewed offspring distribution among individuals in a population. Genetics, 181: 615–629.

Hauser, L., and Carvalho, G. R. 2009. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish and Fisheries, 9: 333–362.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population size of marine organisms? *In* Genetics and Evolution of Aquatic Organisms, pp. 122–134. Ed. by A. Beaumont. Chapman & Hall, London.

Hedgecock, D., and Pudovkin, A. I. 2011. Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. Bulletin of Marine Science, 87: 971–1002.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Oxford Surveys in Evolutionary Biology, 7: 1–44.

Kingman, J. F. C. 1982. On the genealogy of large populations. Journal of Applied Probability, 19A: 27–43.

Koskela, J., Jenkins, P., and Spano, D. 2015. Computational inference beyond Kingman's coalescent. Journal of Applied Probability, 52: 519–537.

Niwa, H-S., Nashida, K., and Yanagimoto, T. 2016. Reproductive skew in Japanese sardine inferred from DNA sequences. ICES Journal of Marine Science, 73: 2180–2188.

Rogers, A. R., and Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution, 9: 552–569.

Schweinsberg, J. 2000. Coalescents with simultaneous multiple collisions. Electronic Journal of Probability, 5: 1–50.

Schweinsberg, J. 2003. Coalescent processes obtained from Galton-Watson processes. Stochastic Processes and their Applications, 106: 107–139.

Spence, J. P., Kamm, J. A., and Song, Y. S. 2016. The site-frequency spectrum for general coalescents. Genetics, 202: 1549–1561.

Wakeley, J. 2013. Coalescent theory has many new branches. Theoretical Population Biology, 87: 1–4.

Zhu, S., Degnan, J. H., Goldstien, S. J., and Eldon, B. 2015. Hybrid-Lambda: simulation of multiple-merger and Kingman gene-genealogies in species networks and species trees. BMC Bioinformatics, 16: 292.

Handling editor: Howard Browman

Chapter 9

Comparative phylogeography of the ocean planet

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Edited by John C. Avise, University of California, Irvine, CA, and approved April 19, 2016 (received for review February 11, 2016)

Understanding how geography, oceanography, and climate have ultimately shaped marine biodiversity requires aligning the distributions of genetic diversity across multiple taxa. Here, we examine phylogeographic partitions in the sea against a backdrop of biogeographic provinces defined by taxonomy, endemism, and species composition. The taxonomic identities used to define biogeographic provinces are routinely accompanied by diagnostic genetic differences between sister species, indicating interspecific concordance between biogeography and phylogeography. In cases where individual species are distributed across two or more biogeographic provinces, shifts in genotype frequencies often align with biogeographic boundaries, providing intraspecific concordance between biogeography and phylogeography. Here, we provide examples of comparative phylogeography from (*i*) tropical seas that host the highest marine biodiversity, (*ii*) temperate seas with high productivity but volatile coastlines, (*iii*) migratory marine fauna, and (*iv*) plankton that are the most abundant eukaryotes on earth. Tropical and temperate zones both show impacts of glacial cycles, the former primarily through changing sea levels, and the latter through coastal habitat disruption. The general concordance between biogeography and phylogeography indicates that the population-level genetic divergences observed between provinces are a starting point for macroevolutionary divergences between species. However, isolation between provinces does not account for all marine biodiversity; the remainder arises through alternative pathways, such as ecological speciation and parapatric (semiisolated) divergences within provinces and biodiversity hotspots.

biogeography | coral reefs | evolution | marine biodiversity | speciation

Phylogeography has roots in biogeography, wherein geographic provinces are identified by concordant shifts in species composition. If the partitions defined by taxonomy are regarded as first-order approximations of evolutionary genetic separations, then continuity between biogeography and phylogeography is apparent. Marine biogeography, the study of species' distributions and evolutionary processes in the sea, began in the mid-19th century based on taxonomic distinctions. Dana (1) divided the surface waters of the world into several temperature zones based on the distributions of corals and crustaceans. Woodward (2) identified a series of marine provinces based on the distributions of mollusks. Forbes (3) made three enduring observations: (*i*) each biogeographic province is a center of origin for new species, (*ii*) these new species tend to migrate outward from the center of origin, and (*iii*) provinces, like species, must be traced back to their historical origins to be understood. These three fundamental contributions appeared in the same decade in which Darwin and Wallace (4) and Darwin (5) identified geography and natural selection as agents of evolutionary change.

It is remarkable that five essential publications in the 1850s (1–5) set the stage for 150 y of biogeographic research. Subsequent effort was devoted to species descriptions, geographic ranges, and relationships. Evolutionary hypotheses were formulated by examining the morphology and distribution of organisms. However, not until the advent of molecular technologies in the 1970s did biogeography transition through another fundamental change (6).

A primary theme emerging from marine biogeography is concordant levels of endemism in very diverse taxa. For example, endemism in Hawai'i is 25% for red algae and fishes (7, 8) and 20% for mollusks (9). The Caribbean Province has 33% endemism for fishes (10), 32% for decapod crustaceans (11), and 37% for corals (12). In the Red Sea, endemism is 13% for fishes and polychaetes, 8% for echinoderms, 17% for ascidians, and 5.5% for corals (13). This concordance across diverse taxonomic groups indicates unifying evolutionary processes.

Here, we demonstrate concordance between biogeographic provinces defined by taxonomy and phylogeographic clusters identified with DNA sequences. At the level of interspecific comparisons, this concordance is obvious; genetic partitions between sister species are expected. However, below this level, at the inception of speciation, it is still unclear how genetic partitions within species (defined by allele-frequency shifts and significant *F*-statistics) translate into species-level divergences (reciprocal monophyly and morphological distinction). Concordance between taxonomy-based biogeography and genetic-based phylogeography would indicate a continuum from population isolation to morphological divergence to evolutionary innovation. In this review, we examine comparative phylogeography, first across biogeographic provinces and second across taxonomic groups with widely divergent life histories.

A second goal is to summarize aspects of comparative phylogeography that illuminate the origins of marine biodiversity. As in terrestrial and freshwater systems, phylogeographic comparisons among species often reveal a diversity of outcomes, attributed to the idiosyncrasies of individual taxa (14, 15). However, the comparative approach can reveal insights unavailable from any one example (16), as illustrated by the terrestrial biota of Hawai'i (17). Finally, illuminating the origins of new species at biodiversity hotspots and centers of endemism can illustrate conservation priorities for the ocean, the cradle of life on our beleaguered planet.

Biogeographic Provinces

Tropical Oceans. Tropical oceans are characterized by biodiversity hotspots, including the Caribbean and the Coral Triangle (between the Philippines, Indonesia, and New Guinea) (Fig. 1*A*) and endemism hotspots, such as Hawai'i and the Red Sea on the periphery of the Indo-Pacific. The evolutionary role of biodiversity

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This paper results from the Arthur M. Sackler Colloquium of the National Academy of Sciences, "In the Light of Evolution X: Comparative Phylogeography," held January 8–9, 2016, at the Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering in Irvine, CA. The complete program and video recordings of most presentations are available on the NAS website at www.nasonline.org/ILE_X_Comparative_Phylogeography.

Author contributions: B.W.B., M.R.G., J.D.D., M.I., K.R.A., W.S.G., R.J.T., and J.C.B. designed research, performed research, and wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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www.pnas.org/cgi/doi/10.1073/pnas.1602404113

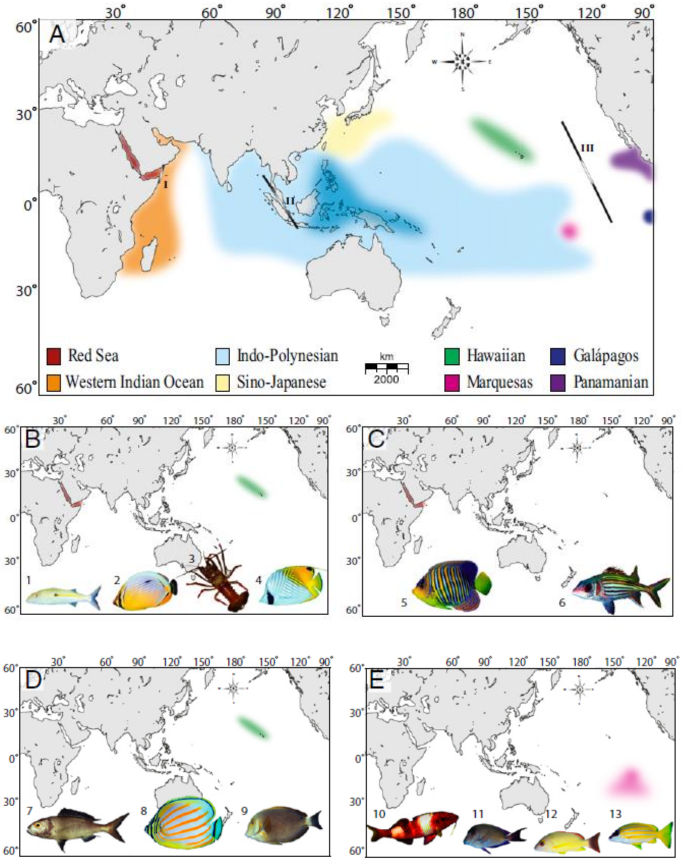

Fig. 1. (*A*) Biogeographic provinces of the tropical Indo-Pacific as defined by >10% endemism (18). Coral triangle is indicated in dark blue. Primary barriers include (site I) Red Sea Barrier, (site II) Indo-Pacific Barrier, and (site III) East Pacific Barrier. (*B–E*) Minimaps illustrating widespread species with phylogeographic separation (strong allele-frequency shifts and significant *F*-statistics) at peripheral provinces. For each panel, the peripheral region(s) of phylogeographic distinction is highlighted in color, and photos are of the species with genetic evidence for that pattern as follows: (*B*) Hawai'i and the Red Sea [1, *Mulloidichthys flavolineatus* (19); 2, *Corallochaetodon* species complex (20); 3, *Panulirus penicillatus* (21); 4, *Chaetodon auriga* (22)]; (*C*) Red Sea only [5, *Pygoplites diacanthus* (23); 6, *Neoniphon sammara* (24)]; (*D*) Hawai'i only [7, *Pristipomoides filamentosus* (25); 8, *Chaetodon ornatissimus* (26); 9, *Acanthurus nigroris* (27)]; (*E*) Marquesas/French Polynesia [10, *Parupeneus multifasciatus* (28); 11, *Acanthurus nigrofuscus* (29); 12, *Lutjanus fulvus* (30); 13, *Lutjanus kasmira* (30)]. Photo credits: J. E. Randall/FishBase (photograph 7); Tane Sinclair-Taylor (all other fish photographs); Matthew Iacchei (photograph of *Panulirus penicillatus*).

hotspots versus endemism hotspots is contentious although biodiversity hotspots are widely recognized as evolutionary incubators producing new species (31, 32).

The Coral Triangle has been a stable reef habitat for tens of millions of years, and this persistence is believed to be key to the production and export of species (33). Pervasive signals of population structure indicate that novel species are arising by parapatric means within the Coral Triangle, wherein partial isolation between subregions reinforces isolation along ecological gradients (34–37). Based on phylogenies of three reef fish families, Cowman and Bellwood (38) estimate that 60% of Indo-Pacific reef fauna have origins in the Coral Triangle. In contrast, peripheral endemism hotspots were previously regarded as evolutionary dead ends (39, 40), in which rare colonization events can produce endemic species, but with no further evolutionary radiations. This assumption has been challenged in recent years because phylogeographic studies show that both Hawaiian and Red Sea provinces can export novel biodiversity (24, 41).

The dominant feature of tropical marine biogeography is the vast Indo-Polynesian Province (IPP), spanning almost half the planet (Fig. 1*A*). Concomitant with this large province are unusually large range sizes, averaging 9 million km^2 for reef fishes, roughly the size of mainland China (42). Genetic surveys of reef organisms are generally consistent with the boundaries of the IPP, showing little genetic structure across broad areas with a few important exceptions (e.g., Indo-Pacific Barrier) (41). Schultz et al. (43) use bathymetry profiles to demonstrate that dispersal across most of this range (Polynesia to Western Australia) requires no deep-water traverse greater than 800 km. Undoubtedly, this continuity of shallow habitat contributes to the cohesiveness of the IPP.

At the center of this vast province is an intermittent barrier around the Indo-Malay Archipelago, known as the Indo-Pacific Barrier (Fig. 1*A*). In the mid-Miocene (16–8 Ma), the Australian and Eurasian plates collided and reduced water flow between the Pacific and Indian Oceans (44). During Pleistocene glacial cycles, sea level dropped as much as 130 m below present levels, further constricting connections between these ocean basins. Evidence for interruptions of gene flow can be found in the distributions of sister species, coupled with phylogeographic partitions (as defined by reciprocal monophyly or $\Phi_{ST} > 0.10$) in green turtles (45), dugongs (46), and ~80% of surveyed reef species (Fig. 2) (30, 52). Given the cyclic nature of this barrier, phylogeographic partitions driven by Pleistocene glacial fluctuations are expected to be concordant in terms of geography, but not necessarily concordant in terms of chronology.

On the eastern periphery of the enormous IPP are three isolated provinces with high endemism in reef fishes: (*i*) the Hawaiian Islands with 25% endemism (8), (*ii*) the Marquesas Islands with 13.7% endemism (53), and (*iii*) Easter Island with 21.7%

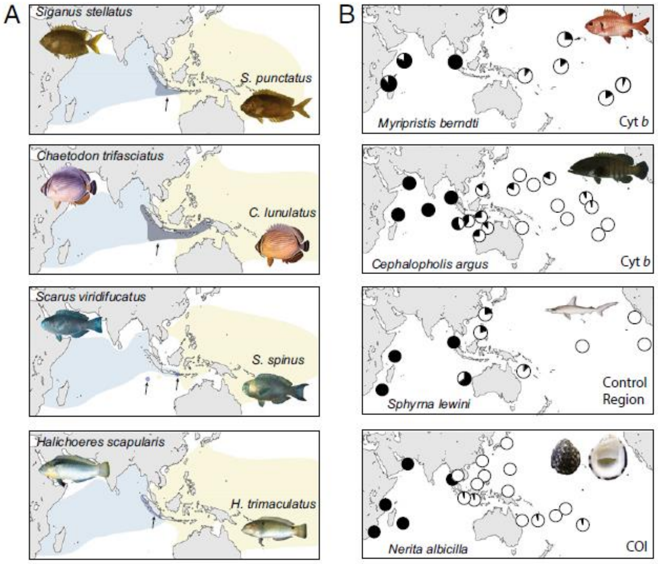

Fig. 2. Evidence of isolation across the Indo-Pacific Barrier. (*A*) Distribution patterns of sister species pairs. Distributions shaded in purple (and indicated by arrows) represent areas of species overlap. (*B*) Phylogeographic studies demonstrating divergent genetic lineages within species. Black and white in pie diagrams indicate distribution of mtDNA phylogroups separated by at least three mutations. In all cases, there is evidence of population expansion with overlap in the Indo Malay-Philippine biodiversity hotspot (Coral Triangle) (47). *Myripristis berndti* data from Craig et al. (48), *Cephalopholis argus* data from Gaither et al. (49), *Sphyrna lewini* data from Duncan et al. (50), and *Nerita albicilla* data from Crandall et al. (51). COI, cytochrome oxidase subunit 1; Cyt *b*, cytochrome *b*. Photo credit: J. E. Randall for fishes, Wikimedia commons/Harry Rose for *Nerita albicilla*.

endemism (54). Phylogeographic studies of the first two provinces show strong concordance with biogeographic partitions (Fig. 1*B*). In Hawai'i, 11 of 16 fishes surveyed are genetically distinct from conspecifics elsewhere in the Pacific (reviewed in ref. 25). At the Marquesas, three of five studies reveal divergences that range from $F_{ST} \geq 0.24$ at allozyme loci to reciprocal monophyly at mtDNA (28, 30), and a RADSeq study reveals strong divergence between a Marquesan surgeonfish and a widespread sister species (55).

On the western side of the IPP lies the Red Sea biogeographic province, an endemism hotspot characterized by a shallow connection to the Indian Ocean and latitudinal gradients in temperature, salinity, and nutrient load (13, 56). Many Red Sea endemics have sister species in the adjacent Western Indian Ocean (56). This interspecific pattern aligns with mtDNA partitions within species ranging from haplotype frequency shifts to reciprocal monophyly in fishes and invertebrates (table 2 in ref. 56). For example, the Indo-Pacific damselfish (*Dascyllus aruanus*; 57) and yellowstripe goatfish (*Mulloidichthys flavolineatus*; 19) both demonstrate similar divisions in mtDNA sequences ($\Phi_{ST} > 0.65$) and microsatellite genotypes ($F_{ST} > 0.03$). In some cases, coalescence analyses reveal that Red Sea lineages are older than those in the Indian Ocean, indicating that the former can export biodiversity to adjacent waters (24).

For widely distributed species, genetic divergences at peripheral locations may be the inception of speciation. The pronghorn spiny lobster, *Panulirus penicillatus*, with a 9-mo pelagic larval duration and a distribution across the entire tropical Indo-Pacific, illustrates genetic diversification at both ends of its range. Iacchei et al. (21) found fixed differences in mtDNA of East Pacific and Red Sea populations ($\Phi_{ST} = 0.74$), corroborated by morphological differentiation in the East Pacific (58). Speciation in peripheral provinces is apparent in *Thalassoma* wrasses (59), *Anampses* wrasses (60), *Acanthurus* surgeonfishes (55), *Mulloidichthys* goatfishes (19), and *Montastraea* corals (61).

The East Pacific Barrier (EPB) limits the distribution of tropical species (62), with few taxa able to maintain population connectivity across the EPB, as evidenced by the lobster *P. penicillatus* (21), and the coral *Porites lobata* (63, 64). However, some fishes (65) and the echinoderm *Echinothrix diadema* (66) have low or insignificant Φ_{ST} values across the EPB.

Atlantic and Indo-Pacific Connections. Two geological events isolated the tropical Atlantic from the Indo-Pacific: (*i*) closure of the Tethys Sea ~13 Ma, brought about by the collision of Africa and Eurasia, and (*ii*) the rise of the Isthmus of Panama ~3.5 Ma that separated the Atlantic from the East Pacific Ocean (67). For the latter, some species diverged well before the final closure although the timing of partitions remain controversial (68) (a fruitful topic for genomic studies). Since the closure of the Tethys Sea, natural dispersal between the Atlantic and Indian Oceans has been limited to the hydrographically complex waters around southern Africa (69). A warm-water corridor here was curtailed ~2.5 Ma by the advent of modern glacial cycles and upwelling in the Benguela Current on the Atlantic side (70). However, the Agulhas Current on the Indian Ocean side occasionally forces warm-water gyres into the Atlantic (71), a potential route of colonization. Phylogeographic studies confirm sporadic dispersal along this route over the last 2.5 My, primarily from the Indian to Atlantic Ocean (72, 73).

Summary. In conclusion: (*i*) Biodiversity hotspots and peripheral centers of endemism both produce and export novel evolutionary lineages. (*ii*) Phylogeographic partitions, as defined by mtDNA monophyly or strong population structure, align well with the biogeographic provinces defined by taxonomy. (*iii*) Sporadic dispersal around southern Africa is the primary avenue of colonization between Indo-Pacific and Atlantic oceans.

Temperate and Polar Seas. Northern seas experienced greater extremes in temperature over the Pleistocene than tropical seas, and northern near-shore ecosystems were periodically eradicated by glaciers encroaching onto continental shelves whereas interglacial warming led to colonizations and population expansions. Although phylogeographic structure generally occurs between biogeographic provinces, sub-Arctic shelf fauna have been repeatedly disrupted by glacial cycles (74). Therefore, present-day physical barriers to gene flow may not exert the same influence on phylogeographic patterns as observed in more stable tropical seas. The most notable barriers separating biogeographic domains are the large expanses of ocean waters across the North Pacific and North Atlantic.

North Pacific. Species in the temperate regions on both sides of the North Pacific show a range of evolutionary divergences that largely depend on dispersal capabilities, temperature tolerances, and climate history. Taxa at higher latitudes tend to have distributions that span the North Pacific (versus taxa at midlatitudes). For example, cold-tolerant cods (*Gadus*), herring (*Clupea*), and king crabs (*Lithodes*, *Paralithodes*) occur in both the Northwest and Northeast Pacific. Most of these trans-Pacific species show phylogeographic breaks, centered on the Aleutian Archipelago or eastern Bering Sea, that represent secondary contact zones after repeated isolations (75–77). In contrast, temperate fishes, invertebrates, and seaweeds at midlatitudes are generally limited to one side of the North Pacific, with closely related species on the other side. A notable exception are disjunct populations of Pacific sardines (*Sardinops*) in the Northwest and Northeast Pacific (78).

North Atlantic. This basin is smaller than the North Pacific and has a U-shaped shoreline with Greenland, Iceland, and Faroe Islands in midocean. Populations of fishes, invertebrates, and seaweeds show a range of genetic divergences across the North Atlantic (79–81). Conspecific populations on either side of the North Atlantic were isolated during glacial episodes, and, in some taxa, the Northwest Atlantic was extirpated and reestablished after the Last Glacial Maximum. Some populations in the Northwest Atlantic show closer genetic affiliations to the North Pacific than to the Northeast Atlantic (seagrass and sea urchins) (82). The Baltic, North Sea, and Mediterranean biogeographic provinces are isolated to some extent from the Atlantic by narrow straits, which often coincide with phylogeographic transitions (83, 84).

Arctic biogeographic province. The far northern ocean has served as a pathway for dispersal between the North Atlantic and North Pacific (85). Phylogeographic and taxonomic studies reveal sister species in the North Atlantic and North Pacific, including several fishes (86), invertebrates (85), and seaweeds (87). During ~20% of the Pleistocene, high sea levels breached the 50-m sill across the Bering Strait (88), allowing interocean dispersal as early as 6.4 Ma and again at 3.5 Ma (89). More recent dispersal events have led to the cooccurrence of conspecific populations in both oceans (90).

Antarctic biogeographic province. The Antarctic is relatively old, ~25 My, compared with about 2.5 My for the Arctic. The result of this ancient formulation is high endemism: 88% in fishes (91) and 42–56% in four invertebrate classes (92). The high homogeneity of taxa across this vast region is facilitated by the Antarctic Circumpolar Current, which circles the entire continent. Phylogeographic studies are consistent with a highly connected Antarctic Province, showing little (or no) population structure for two decapods (93), one nemertean (94), and four ice fishes (95).

Patterns Within Biogeographic Provinces. Within the shallow-water provinces, species often share genetic breaks at specific geological features or geographical regions. Examples range from the classic study by Avise (96) on the Carolina Province (Southeast United States), through more recent surveys of the benthic fauna along the coast of New Zealand (97), the northeastern Pacific (98), the Coral Triangle (36), southern Africa (69), and Hawai'i (14). Endemic species confined to a single province tend to show more population structure than widespread species at the same geographic scale (36, 99, 100). Species that lack pelagic development generally show strong genetic structure whereas species with pelagic development are less predictable (101, 102). Regardless of developmental mode, ecological niche, or evolutionary relationships, species showing geographic structuring often have concordant genetic breaks, indicating that shared history or physical factors drive the observed pattern (96). Examination of 47 reef-associated species across the Hawaiian Archipelago reveals that multispecies trends in genetic diversity are driven by a combination of both the dominant physical, historical and ecological features of the seascape, and ecological–genetic feedback within communities (103).

Species that counter these trends may be particularly informative about the process of evolution. For example, Hawaiian limpets of the genus *Cellana* have diversified within the archipelago along a tidal gradient that indicates ecological speciation (104). Certainly, species sharing population structure at unexpected locations within biogeographic provinces (such as Fiji in the tropical Pacific) (21, 105), or other exceptions to those general trends, will provide evolutionary insights.

Summary. In conclusion: (*i*) Species distributions are fundamentally shaped by physiological tolerances to north–south temperature gradients in the North Pacific and North Atlantic. (*ii*) Glacial cycles impact phylogeography by repeatedly altering species distributions, isolating populations, and creating secondary contact zones. (*iii*) Shifting interactions between ocean-climate, coastal configuration, and bottom topography produce barriers to dispersal between ocean basins. (*iv*) Some biogeographic provinces are genetically homogenous, with little opportunity for allopatric divergences, whereas others host heterogeneous habitats that can promote speciation along ecological boundaries.

Taxon-Specific Patterns

Migratory ability and historical dispersal define taxa along a continuum of evolutionary divergence. Clusters of closely related species, each confined to a single biogeographic province, are at one end of the continuum, and highly migratory megafuana are at the other end. Oceanic migrants provide special challenges to both phylogeographic studies and conservation strategies, because both must be conducted on a scale that transcends biogeographic provinces and political jurisdictions (106). Species in the center of the continuum include temperate taxa inhabiting disjunct regions, such as antitropical taxa, sister species separated by the tropics. Comparative phylogeography of these groups provides insights into the roles of dispersal and isolation in contributing to biodiversity.

Antitropical Taxa. Species with disjunct distributions on both sides of the tropics provide fascinating subjects for phylogeographic study. Equatorial surface waters are lethal to these cold-adapted species, so how do they cross the tropics, and how often can this crossing be accomplished? Sister taxa of fishes on each side of the equator reveal divergences ranging from populations to distinct lineages, but without a clear pattern. For example, a single species of anchovy (*Engraulis*) occurs in the North Atlantic, southern Africa, and Japan, but three additional species have more restricted ranges (107, 108). In contrast, a single species

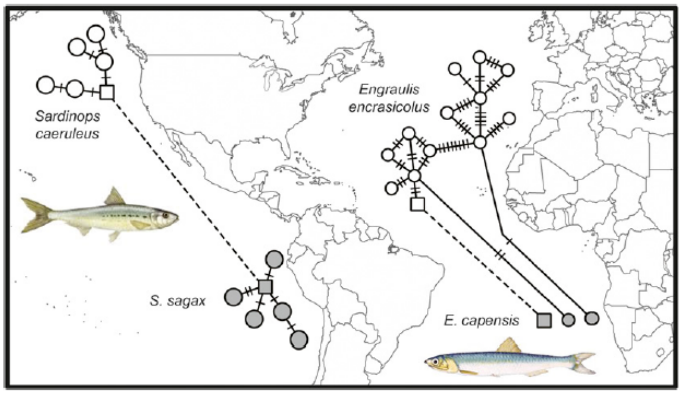

Fig. 3. Sardines (genus *Sardinops*) and Anchovies (genus *Engraulis*) are antitropical species that recently surmounted the warm-water barrier between northern and southern hemispheres, as indicated by mtDNA haplotype networks. For sardines in the East Pacific, transequatorial dispersal is facilitated by a short and steep continental shelf and adjacent deep cold water (78). For anchovies in the East Atlantic, transequatorial dispersal is facilitated by upwelling (cold nutrient-rich water) in low latitudes (107). Light and dark haplotypes indicate northern and southern hemisphere, respectively. Squares connected by a dashed line indicate haplotypes shared between hemispheres. Note that, in the East Pacific sardine, the haplotype shared between northern and southern hemisphere is internal to both networks, indicating an ancient connection. In contrast, the East Atlantic anchovy has connections across the equator that include both interior and peripheral haplotypes in the network.

of sardine (*Sardinops*) extends from southern Africa to Australia to Chile, California, and Japan (78).

Overall results show that the ability to traverse the tropics is species-specific and that these events have not been limited to particular periods of global cooling. However, one possible point of concordance includes the eastern continental margins of the Atlantic (for anchovies) and the Pacific (for sardines). In both cases, colonizations across the equator have been accomplished recently, as indicated by shared mtDNA haplotypes (Fig. 3).

Cetaceans. Patterns of gene flow vary extensively across space and time for cetaceans, driven largely by the wide variety of life history traits (109, 110). Most species exhibit limited gene flow between ocean basins, even in taxa with temperate distributions; but genetic structure within ocean basins varies substantially across species. For Mysticetes (baleen whales), patterns of gene flow are shaped by migratory pathways, with individuals typically exhibiting maternally based site fidelity to tropical breeding and temperate/Arctic feeding areas. This fidelity leads to population genetic separations between ocean basins and among breeding areas, with F_{ST} values of 0.05 to 0.1 for right whales (111), blue whales (112), and humpback whales (113).

In contrast, most Odontocetes (toothed whales) do not undertake large-scale migrations and often exhibit genetic structure over relatively short geographic distances due to site fidelity, resource specialization, and social structure. For example, strong fidelity to narrow ranges can result in genetically divergent populations along continuous coastlines or between adjacent islands, as is the case for spinner dolphins (114), Hector's dolphins (115), and Indo-Pacific humpback dolphins (116). Some Odontocetes have ecologically and behaviorally distinct groups ("ecotypes"), with limited gene flow even in parapatry or sympatry (109). Several dolphin species contain genetically divergent coastal and pelagic ecotypes (117). Killer whales have sympatric ecotypes that differ in prey type, foraging strategy, social structure, and movement (118).

Sea Turtles. The seven species of sea turtles show patterns of population structure within ocean basins defined by natal homing, the habit of females (and sometimes males) to return to the vicinity of their natal beach, after decades of growth in ocean and coastal habitats. This behavior is the basis for defining regional management units (119). On a global scale, occasional wandering provides connections between nesting populations and ocean basins. Cold-tolerant species, such as the leatherback turtle, pass freely between ocean basins (120). Tropical species, such as the green turtle and the hawksbill turtle, make rarer connections between the Atlantic and Indo-Pacific via southern Africa (121, 122). Bowen and Karl (123) note higher genetic divergences between ocean basins in tropical species, providing a signal that allopatric speciation may predominate in this group.

Pelagic Fishes. A primary phylogeographic pattern for these oceanic migrants is low to no genetic structure within ocean basins, and strong genetic structure between the Atlantic and Indo-Pacific. Some pelagic species seem to cross the Benguela Barrier (southern Africa) often enough to preclude the development of evolutionary partitions, including albacore tuna (124, 125), wahoo (126), and the common dolphinfish (127). However, these species are likely exceptions, with many large, vagile species demonstrating structured populations across this barrier, including the scalloped hammerhead shark (50), whale shark (128), and blue marlin (129). For tunas in particular, a recurring pattern is two mtDNA lineages: one confined to the Atlantic and an Indo-Pacific lineage that is also found in the Atlantic (table 6 in ref. 126). This pattern indicates extended periods of isolation, punctuated by dispersal around southern Africa.

Plankton. In the oceanic pelagic zone, where all life stages are planktonic, species' ranges are both extensive and dynamic because adult distributions are not tied to a particular benthic habitat. In turn, biogeographic provinces for the pelagic zone are based on physical and chemical properties (biogeochemical provinces) (130) rather than endemism or species assemblages. Longhurst (131) identified ~55 biogeochemical provinces (BGCPs), nested within four biomes (Polar, Westerly Winds, Trade Winds, Coastal), across four ocean basins (Atlantic, Pacific, Indian, Southern). Like the species they harbor, the boundaries of the BGCPs fluctuate on both seasonal and annual timescales in accordance with changing environmental conditions (132). Our understanding of pelagic community composition is still nascent, but recent studies have shown concordance between BGCPs and community composition in taxa ranging from viruses (133) to phytoplankton (134) to fishes (135).

Cosmopolitan distributions in the pelagic zone initially prompted the conclusion of little to no population structure in the open ocean, a position that has eroded in recent decades (136, 137). Phylogeographic studies reveal that many cosmopolitan taxa are composed of multiple cryptic species (138, 139), including some that are sympatric over part of their ranges (140). Populations of these cosmopolitan species are subdivided in two ways concordant with the BGCP framework: (*i*) by continental land masses separating ocean basins, and (*ii*) by habitat discontinuities in the equatorial region between subtropical gyres in the northern and southern hemispheres (140–142). The few global-scale phylogeographic studies have been restricted to copepods, but evidence from a diversity of other taxa sampled at ocean basin scales indicate that lineages have diverged both in allopatry and sympatry at much smaller geographic distances than anticipated, with examples drawn from chaetognaths (143), euphausiids (144), and mollusks (145).

These combined results indicate that population discontinuities of pelagic species are determined not by the temporal and spatial scales of dispersal, but by habitat characteristics enabling species to maintain viable populations (137, 143). Habitat selection, rather than physical barriers, may be a primary force driving speciation in the pelagic zone (146). Therefore, a biogeographic framework based on water properties is concordant with genetic partitions within species.

Summary. In conclusion: (*i*) Several temperate species show disjunct distributions across the tropics, indicating historical dispersals across warm-water barriers. (*ii*) The deepest phylogeographic separations for oceanic migrants indicate patterns of allopatric isolation between ocean basins, especially for fishes. (*iii*) Migratory sea turtles and cetaceans show population structure based on reproductive site fidelity. (*iv*) An ecological component to speciation is indicated by isolation along behavioral barriers in cetaceans, and by the presence of sympatric sister species in the plankton. (*v*) Planktonic biogeographic provinces are defined by water masses that can change size and position based on oceanographic conditions. (*vi*) Initial plankton studies indicate concordance between biogeochemical provinces and phylogeographic partitions, particularly at the equatorial break between northern and southern subtropics.

Terrestrial vs. Marine Phylogeography

Life began in the oceans, but the field of phylogeography began with continental biota (6, 15), and many of the insights reviewed here have precedents in terrestrial cases. The biogeographic settings have parallels between land and sea, particularly with latitudinal gradients in biodiversity and concordance between biogeographic provinces and phylogeographic partitions (15, 147). Glacial habitat disruptions in northern seas have a strong parallel in continental faunas (148, 149). Biodiversity hotspots in Indo-Pacific reefs, forests of northern Australia, and Neotropical plant communities are all distinguished by periods of stability, habitat heterogeneity, and the ability to export species (33, 150, 151). A primary difference between marine and terrestrial phylogeography is greater dispersal potential and fewer barriers in the oceans. Although a squirrel in Central Park (New York) cannot deposit progeny in Hyde Park (London), a squirrelfish is capable of dispersing on this scale (48). This difference in evolutionary processes is clear in the Hawaiian Archipelago, where rare terrestrial colonists have proliferated into dozens and hundreds of species (17) whereas marine colonists produce one or a few species (104). Therefore, the evolutionary dramas above and below the waterline have the same ingredients (isolation, selection, adaptation, speciation), but markedly different tempos and outcomes (152).

Conclusion

Marine phylogeography encompasses half-billion year separations and the largest habitat on the planet. Given this diversity, generalizations are few, but some are especially robust. First, phylogeography is the new incarnation of spatial biogeography (153). The alignment of population genetic separations and taxonomic distributions reveals that these are part of a continuum. Evolutionary partitions that could previously be described only with taxonomy are now evaluated with the genomic footprints of isolation, selection, and speciation. Second, the model of allopatric speciation that previously dominated evolutionary thought is an incomplete fit to the dispersive aquatic medium. Phylogeography of oceanic migrants indicates a strong role for allopatric speciation whereas heterogeneous coastal habitats provide more opportunity for sympatric/ecological divergences. Phylogeography in high latitudes is defined by shifting habitats in response to glaciation. Finally, both biodiversity hotspots and endemism hotspots are important in producing novel evolutionary lineages and may work in synergy to enhance biodiversity on the ocean planet.

ACKNOWLEDGMENTS. We thank John C. Avise and Francisco J. Ayala, coorganizers of *In the Light of Evolution X: Comparative Phylogeography*, an Arthur M. Sackler Colloquium of the National Academy of Sciences. For stimulating discussions, advice, and editorial prowess, we thank E. A. Hanni, P. Marko, S. A. Karl, J. Eble, L. Rocha, G. Bernardi, M. Berumen, and the B.B./R.J.T. (ToBo) laboratory. We thank T. Sinclair-Taylor, T. Lilley, and A. Cros for assistance with illustrations and editor John Avise and two anonymous reviewers for comments that improved the manuscript. The authors' research reported here was funded by the National Science Foundation, the Seaver Institute, the University of Hawaii Sea Grant Program, NOAA, the North Pacific Research Board, and the Saltonstall–Kennedy Grant Program.

1. Dana JD (1853) On the isothermal oceanic chart: Illustrating the geographical distribution of marine animals. *Am J Sci* 16:314–327.
2. Woodward SP (1851) *A Manual of the Mollusca; or, Rudimentary Treatise of Recent and Fossil Shells* (John Weale, London).
3. Forbes E (1859) *The Natural History of the European Seas* (John Van Voorst, London).
4. Darwin C, Wallace A (1858) On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. *Proc Linn Soc London* 3(9):45–62.
5. Darwin C (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* (John Murray, London).
6. Avise JC, et al. (1987) Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annu Rev Ecol Syst* 18:489–522.
7. Abbott IA (1999) *Marine Red Algae of the Hawaiian Islands* (Bishop Museum, Honolulu).
8. Randall JE (2007) *Reef and Shore Fishes of the Hawaiian Islands* (University of Hawaii Sea Grant College Program, Honolulu).
9. Kay EA (1980) *Little Worlds of the Pacific: An Essay on Pacific Basin Biogeography (Harold L. Lyon Arboretum Lecture)* (Lyon Arboretum, Honolulu), Lecture No. 9.
10. Floeter SR, et al. (2008) Atlantic reef fish biogeography and evolution. *J Biogeogr* 35(1):22–47.
11. Boschi EE (2000) Species of decapod crustaceans and their distribution in the American marine zoogeographic provinces. *Revista de Investigación y Desarrollo Pesquero* 13:1–136.
12. Veron JEN (2000) *Corals of the World* (Australian Institute of Marine Science, Townsville, Australia), Vol 1–3.
13. DiBattista JD, et al. (2016) A review of contemporary patterns of endemism for shallow water reef fauna in the Red Sea. *J Biogeogr* 43(3):423–439.
14. Toonen RJ, et al. (2011) Defining boundaries for ecosystem-based management: A multispecies case study of marine connectivity across the Hawaiian Archipelago. *J Mar Biol* 2011:460173.
15. Riddle BR (2016) Comparative phylogeography clarifies the complexity and problems of continental distribution that drove A. R. Wallace to favor islands. *Proc Natl Acad Sci USA* 113:7970–7977.
16. Bermingham E, Moritz C (1998) Comparative phylogeography: Concepts and applications. *Mol Ecol* 7(4):367–369.
17. Shaw KL, Gillespie RG (2016) Comparative phylogeography of oceanic archipelagos: Hotspots for inferences of evolutionary process. *Proc Natl Acad Sci USA* 113:7986–7993.
18. Briggs JC, Bowen BW (2012) A realignment of marine biogeographic provinces with particular reference to fish distributions. *J Biogeogr* 39(1):12–30.
19. Fernandez-Silva I, et al. (2015) Yellow tails in the Red Sea: Phylogeography of the Indo-Pacific goatfish *Mulloidichthys flavolineatus* reveals isolation in peripheral provinces and cryptic evolutionary lineages. *J Biogeogr* 42(12):2402–2413.
20. Waldrop E, et al. (2016) Phylogeography, population structure and evolution of coral-eating butterflyfishes (Family Chaetodontidae, genus *Chaetodon*, subgenus *Corallochaetodon*). *J Biogeogr* 43(6):1116–1129.

21. Iacchei M, Gaither MR, Bowen BW, Toonen RJ (2016) Testing dispersal limits in the sea: Range-wide phylogeography of the pronghorn spiny lobster *Panulirus penicillatus*. *J Biogeogr* 43(5):1032–1044.
22. DiBattista JD, et al. (2015) Blinded by the bright: A lack of congruence between colour morphs, phylogeography and taxonomy for a cosmopolitan Indo-Pacific butterflyfish, *Chaetodon auriga*. *J Biogeogr* 42(10):1919–1929.
23. Coleman RR, et al. (2016) Regal phylogeography: Range-wide survey of the marine angelfish *Pygoplites diacanthus* reveals evolutionary partitions between the Red Sea, Indian Ocean, and Pacific Ocean. *Mol Phylogenet Evol* 100:243–253.
24. DiBattista JD, et al. (2013) After continents divide: Comparative phylogeography of reef fishes from the Red Sea and Indian Ocean. *J Biogeogr* 40(6):1170–1181.
25. Gaither MR, et al. (2011) High connectivity in the deepwater snapper *Pristipomoides filamentosus* (Lutjanidae) across the Indo-Pacific with isolation of the Hawaiian archipelago. *PLoS One* 6(12):e28913.
26. DiBattista JD, Rocha LA, Craig MT, Feldheim KA, Bowen BW (2012) Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. *J Hered* 103(5):617–629.
27. DiBattista J, Wilcox C, Craig M, Rocha L, Bowen B (2011) Phylogeography of the Pacific blueline surgeonfish, *Acanthurus nigroris*, reveals high genetic connectivity and a cryptic endemic species in the Hawaiian Archipelago. *J Mar Biol* 2011:839134.
28. Szabó Z, Snelgrove B, Craig M, Rocha L, Bowen B (2014) Phylogeography of the Manybar Goatfish, *Parupeneus multifasciatus* reveals moderate structure between the Central and North Pacific and a cryptic endemic species in the Marquesas. *Bull Mar Sci* 90(1):493–512.
29. Eble JA, Rocha LA, Craig MT, Bowen BW (2011) Not all larvae stay close to home: Insights into marine population connectivity with a focus on the brown surgeonfish (*Acanthurus nigrofuscus*). *J Mar Biol* 2011:518516.
30. Gaither MR, Toonen RJ, Robertson DR, Planes S, Bowen BW (2010) Genetic evaluation of marine biogeographic barriers: perspectives from two widespread Indo-Pacific snappers (*Lutjanus* spp.). *J Biogeogr* 37(1):133–147.
31. Briggs JC (2003) Marine centres of origin as evolutionary engines. *J Biogeogr* 30(1):1–18.
32. Bowen BW, Rocha LA, Toonen RJ, Karl SA; ToBo Laboratory (2013) The origins of tropical marine biodiversity. *Trends Ecol Evol* 28(6):359–366.
33. Pellissier L, et al. (2014) Quaternary coral reef refugia preserved fish diversity. *Science* 344(6187):1016–1019.
34. Timm J, Figiel M, Kochzius M (2008) Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. *Mol Phylogenet Evol* 49(1):268–276.
35. Barber PH, Cheng SH, Erdmann MV, Tengardjaja K (2011) Evolution and conservation of marine biodiversity in the Coral Triangle: Insights from stomatopod Crustacea. *Crustac Issues* 19:129–156.
36. Carpenter KE, et al. (2010) Comparative phylogeography of the Coral Triangle and implications for marine management. *J Mar Biol* 2011:396982.
37. Tornabene L, Valdez S, Erdmann M, Pezold F (2015) Support for a 'Center of Origin' in the Coral Triangle: Cryptic diversity, recent speciation, and local endemism in a diverse lineage of reef fishes (Gobiidae: *Eviota*). *Mol Phylogenet Evol* 82(Pt A):200–210.
38. Cowman PF, Bellwood DR (2013) The historical biogeography of coral reef fishes: Global patterns of origination and dispersal. *J Biogeogr* 40(2):209–224.
39. Alison Kay E, Palumbi SR (1987) Endemism and evolution in Hawaiian marine invertebrates. *Trends Ecol Evol* 2(7):183–186.
40. Bellemain E, Ricklefs RE (2008) Are islands the end of the colonization road? *Trends Ecol Evol* 23(8):461–468.
41. Eble JA, Bowen BW, Bernardi G (2015) Phylogeography of coral reef fishes. *Ecology of Fishes on Coral Reefs*, ed Mora C (University of Hawaii Press, Honolulu), pp 64–75.
42. Allen GR (2008) Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conserv: Mar Freshw Ecosyst* 18(5):541–556.
43. Schultz JK, et al. (2008) Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). *Mol Ecol* 17(24):5336–5348.
44. Kennett JP, Keller G, Srinivasan M (1985) Miocene planktonic foraminiferal biogeography and paleoceanographic development of the Indo-Pacific region. *Geol Soc Am* 163:197–236.
45. Dethmers KE, et al. (2006) The genetic structure of Australasian green turtles (*Chelonia mydas*): Exploring the geographical scale of genetic exchange. *Mol Ecol* 15(13):3931–3946.
46. Blair D, et al. (2014) Pleistocene sea level fluctuations and the phylogeography of the dugong in Australian waters. *Mar Mamm Sci* 30(1):104–121.
47. Crandall ED, Sbrocco EJ, Deboer TS, Barber PH, Carpenter KE (2012) Expansion dating: Calibrating molecular clocks in marine species from expansions onto the Sunda Shelf Following the Last Glacial Maximum. *Mol Biol Evol* 29(2):707–719.
48. Craig MT, Eble JA, Bowen BW, Robertson DR (2007) High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). *Mar Ecol Prog Ser* 334(3):245–254.
49. Gaither MR, et al. (2011) Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the Indo-Pacific Barrier with contemporary overlap in The Coral Triangle. *BMC Evol Biol* 11:189.
50. Duncan KM, Martin AP, Bowen BW, De Couet HG (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Mol Ecol* 15(8):2239–2251.
51. Crandall ED, Frey MA, Grosberg RK, Barber PH (2008) Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. *Mol Ecol* 17(2):611–626.
52. Ahti PA, et al. (2016) Phylogeography of Indo-Pacific reef fishes: Sister wrasses *Coris gaimard* and *C. cuvieri* in the Red Sea, Indian Ocean and Pacific Ocean. *J Biogeogr* 43(6):1103–1115.
53. Delrieu-Trottin E, et al. (2015) Shore fishes of the Marquesas Islands: An updated checklist with new records and new percentage of endemic species. *Check List* 11(5):1–13.
54. Randall JE, Cea A (2011) *Shore Fishes of Easter Island* (Univ of Hawaii Press, Honolulu).
55. Gaither MR, et al. (2015) Genomic signatures of geographic isolation and natural selection in coral reef fishes. *Mol Ecol* 24(7):1543–1557.
56. DiBattista JD, et al. (2016) On the origin of endemic species in the Red Sea. *J Biogeogr* 43(1):13–30.
57. Liu SYV, Chang FT, Borsa P, Chen WJ, Dai CF (2014) Phylogeography of the humbug damselfish, *Dascyllus aruanus* (Linnaeus, 1758): Evidence of Indo-Pacific vicariance and genetic differentiation of peripheral populations. *Biol J Linn Soc Lond* 113(4):931–942.
58. George R (2005) Tethys sea fragmentation and speciation of *Panulirus* spiny lobsters. *Crustaceana* 78(11):1281–1309.
59. Bernardi G, Bucciarelli G, Costagliola D, Robertson DR, Heiser J (2004) Evolution of coral reef fish *Thalassoma* spp. (Labridae). 1. Molecular phylogeny and biogeography. *Mar Biol* 144(2):369–375.
60. Hodge JR, Read CI, van Herwerden L, Bellwood DR (2012) The role of peripheral endemism in species diversification: Evidence from the coral reef fish genus *Anampses* (Family: Labridae). *Mol Phylogenet Evol* 62(2):653–663.
61. Budd AF, Pandolfi JM (2010) Evolutionary novelty is concentrated at the edge of coral species distributions. *Science* 328(5985):1558–1561.
62. Gaither MR, Bowen BW, Rocha LA, Briggs JC (2016) Fishes that rule the world: Circumtropical distributions revisited. *Fish Fisheries*, in press.
63. Baums IB, Boulay JN, Polato NR, Hellberg ME (2012) No gene flow across the Eastern Pacific Barrier in the reef-building coral *Porites lobata*. *Mol Ecol* 21(22):5418–5433.
64. Forsman Z, Wellington GM, Fox GE, Toonen RJ (2015) Clues to unraveling the coral species problem: Distinguishing species from geographic variation in *Porites* across the Pacific with molecular markers and microskeletal traits. *PeerJ* 3:e751.
65. Lessios HA, Robertson DR (2006) Crossing the impassable: Genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proc Biol Sci* 273(1598):2201–2208.
66. Lessios HA, Kessing BD, Robertson DR (1998) Massive gene flow across the world's most potent marine biogeographic barrier. *Proc Biol Sci* 265(1396):583–588.
67. Lessios HA (2008) The great American schism: Divergence of marine organims after the rise of the Central American Isthmus. *Annu Rev Ecol Syst* 39:63–91.
68. Marko PB, Eytan RI, Knowlton N (2015) Do large molecular sequence divergences imply an early closure of the Isthmus of Panama? *Proc Natl Acad Sci USA* 112(43):E5766.
69. Teske PR, Von Der Heyden S, McQuaid CD, Barker NP (2011) A review of marine phylogeography in southern Africa. *S Afr J Sci* 107(5-6):43–53.
70. Dwyer GS, et al. (1995) North Atlantic deepwater temperature change during late Pliocene and late Quaternary climatic cycles. *Science* 270(5240):1347–1351.
71. Hutchings L, et al. (2009) The Benguela Current: An ecosystem of four components. *Prog Oceanogr* 83(1):15–32.
72. Reece JS, Bowen BW, Smith DG, Larson A (2010) Molecular phylogenetics of moray eels (Muraenidae) demonstrates multiple origins of a shell-crushing jaw (*Gymnomuraena, Echidna*) and multiple colonizations of the Atlantic Ocean. *Mol Phylogenet Evol* 57(2):829–835.
73. Gaither MR, et al. (2015) Two deep evolutionary lineages in the circumtropical glasseye *Heteropriacanthus cruentatus* (Teleostei, Priacanthidae) with admixture in the south-western Indian Ocean. *J Fish Biol* 87(3):715–727.
74. Marko PB, et al. (2010) The 'Expansion-Contraction' model of Pleistocene biogeography: Rocky shores suffer a sea change? *Mol Ecol* 19(1):146–169.
75. Canino MF, Spies IB, Cunningham KM, Hauser L, Grant WS (2010) Multiple ice-age refugia in Pacific cod, *Gadus macrocephalus*. *Mol Ecol* 19(19):4339–4351.
76. Liu M, et al. (2012) What maintains the central North Pacific genetic discontinuity in Pacific herring? *PLoS One* 7(12):e50340.
77. Grant WS, Zelenina DA, Mugue NS (2014) Phylogeography of red king crab: Implications for management and stock enhancement. *The King Crabs*, ed Stevens B (CRC, Boca Raton, FL), pp 47–72.
78. Bowen B, Grant WS (1997) Phylogeography of the sardines (*Sardinops* spp.): Assessing biogeographic models and population histories in temperate upwelling zones. *Evolution* 51(5):1601–1610.
79. Addison JA, Hart MW (2005) Colonization, dispersal, and hybridization influence phylogeography of North Atlantic sea urchins (*Strongylocentrotus droebachiensis*). *Evolution* 59(3):532–543.
80. Árnason E (2004) Mitochondrial cytochrome *B* DNA variation in the high-fecundity Atlantic cod: Trans-Atlantic clines and shallow gene genealogy. *Genetics* 166(4):1871–1885.
81. McCusker MR, Bentzen P (2010) Historical influences dominate the population genetic structure of a sedentary marine fish, Atlantic wolffish (*Anarhichas lupus*), across the North Atlantic Ocean. *Mol Ecol* 19(19):4228–4241.
82. Olsen JL, et al. (2004) North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Mol Ecol* 13(7):1923–1941.
83. Johannesson K, André C (2006) Life on the margin: Genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Mol Ecol* 15(8):2013–2029.
84. Patarnello T, Volckaert FA, Castilho R (2007) Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographical break? *Mol Ecol* 16(21):4426–4444.
85. Vermeij GJ (1991) Anatomy of an invasion: The trans-Arctic interchange. *Paleobiology* 17(3):281–307.
86. Grant WS (1987) Genetic divergence between congeneric species of Atlantic and Pacific ocean fishes. *Population Genetics and Fishery Management*, eds Ryman N, Utter F (Univ of Washington Press, Seattle), pp 225–246.

87. Lindstrom S (2001) The Bering Strait connection: Dispersal and speciation in boreal macroalgae. *J Biogeogr* 28(2):243–251.
88. Miller KG, et al. (2005) The Phanerozoic record of global sea-level change. *Science* 310(5752):1293–1298.
89. Marincovich L, Gladenkov AY (1999) Evidence for an early opening of the Bering Strait. *Nature* 397(6715):149–151.
90. Carr SM, Kivlichan DS, Pepin P, Crutcher DC (1999) Molecular systematics of gadid fishes: Implications for the biogeographic origins of Pacific species. *Can J Zool* 77(1): 19–26.
91. Eastman JT (2005) The nature of the diversity of Antarctic fishes. *Polar Biol* 28(2): 93–107.
92. Griffiths HJ, Barnes DK, Linse K (2009) Towards a generalized biogeography of the Southern Ocean benthos. *J Biogeogr* 36(1):162–177.
93. Raupach MJ, et al. (2010) Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. *Mar Biol* 157(8):1783–1797.
94. Thornhill DJ, Mahon AR, Norenburg JL, Halanych KM (2008) Open-ocean barriers to dispersal: A test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). *Mol Ecol* 17(23):5104–5117.
95. Janko K, et al. (2007) Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. *BMC Evol Biol* 7(1):220.
96. Avise JC (1996) Toward a regional conservation genetics perspective: Phylogeography of faunas in the southeastern United States. *Conservation Genetics: Case Histories from Nature*, eds Avise JC, Hamrick J (Chapman and Hall, New York), pp 431–470.
97. Ross P, Hogg ID, Pilditch CA, Lundquist C (2009) Phylogeography of New Zealand's coastal benthos. *N Z J Mar Freshw Res* 43(5):1009–1027.
98. Kelly RP, Palumbi SR (2010) Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. *PLoS One* 5(1):e8594.
99. Tenggardjaja KA, Bowen BW, Bernardi G (2014) Vertical and horizontal genetic connectivity in *Chromis verater*, an endemic damselfish found on shallow and mesophotic reefs in the Hawaiian Archipelago and adjacent Johnston Atoll. *PLoS One* 9(12):e115493.
100. Tenggardjaja KA, Bowen BW, Bernardi G (2016) Reef fish dispersal in the Hawaiian Archipelago: Comparative phylogeography of three endemic damselfishes. *J Mar Biol* 2016:3251814.
101. Riginos C, Buckley YM, Blomberg SP, Treml EA (2014) Dispersal capacity predicts both population genetic structure and species richness in reef fishes. *Am Nat* 184(1): 52–64.
102. Liggins L, Treml EA, Possingham HP, Riginos C (2016) Seascape features, rather than dispersal traits, predict spatial genetic patterns in co-distributed reef fishes.
103. Selkoe KA, et al. (2016) Conserving genetic diversity of whole communities: Identifying patterns and landscape drivers. *Proc R Soc B* 283:20160354.
104. Bird CE, Holland BS, Bowen BW, Toonen RJ (2011) Diversification of sympatric broadcast-spawning limpets (*Cellana* spp.) within the Hawaiian archipelago. *Mol Ecol* 20(10):2128–2141.
105. Drew JA, Barber PH (2012) Comparative phylogeography in Fijian coral reef fishes: A multi-taxa approach towards marine reserve design. *PLoS One* 7(10):e47710.
106. Toonen RJ, et al.; Big Ocean Think Tank (2013) One size does not fit all: The emerging frontier in large-scale marine conservation. *Mar Pollut Bull* 77(1-2):7–10.
107. Grant WS, Leslie RW, Bowen BW (2005) Molecular genetic assessment of bipolarity in the anchovy genus *Engraulis*. *J Fish Biol* 67(5):1242–1265.
108. Silva G, Horne JB, Castilho R (2014) Anchovies go north and west without losing diversity: Post-glacial range expansions in a small pelagic fish. *J Biogeogr* 41(6): 1171–1182.
109. Hoelzel A (1998) Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: Implications for conservation policy. *J Hered* 89(5):451–458.
110. Andrews KR (2014) Population genetics in the conservation of cetaceans and primates. *Primates and Cetaceans*, eds Yamagiwa J, Karczmarski L (Springer, New York), pp 289–308.
111. Carroll EL, et al. (2015) Cultural traditions across a migratory network shape the genetic structure of southern right whales around Australia and New Zealand. *Sci Rep* 5:16182.
112. Torres-Florez JP, et al. (2014) Blue whale population structure along the eastern South Pacific Ocean: Evidence of more than one population. *Mol Ecol* 23(24): 5998–6010.
113. Jackson JA, et al. (2014) Global diversity and oceanic divergence of humpback whales (*Megaptera novaeangliae*). *Proc Biol Sci* 281(1786):20133222.
114. Andrews KR, et al. (2010) Rolling stones and stable homes: Social structure, habitat diversity and population genetics of the Hawaiian spinner dolphin (*Stenella longirostris*). *Mol Ecol* 19(4):732–748.
115. Hamner RM, Pichler FB, Heimeier D, Constantine R, Baker CS (2012) Genetic differentiation and limited gene flow among fragmented populations of New Zealand endemic Hector's and Maui's dolphins. *Conserv Genet* 13(4):987–1002.
116. Brown AM, et al. (2014) Population differentiation and hybridisation of Australian snubfin (*Orcaella heinsohni*) and Indo-Pacific humpback (*Sousa chinensis*) dolphins in north-western Australia. *PLoS One* 9(7):e101427.
117. Andrews KR, et al. (2013) The evolving male: Spinner dolphin (*Stenella longirostris*) ecotypes are divergent at Y chromosome but not mtDNA or autosomal markers. *Mol Ecol* 22(9):2408–2423.
118. Hoelzel AR, Dahlheim M, Stern SJ (1998) Low genetic variation among killer whales (*Orcinus orca*) in the eastern north Pacific and genetic differentiation between foraging specialists. *J Hered* 89(2):121–128.
119. Wallace BP, et al. (2010) Regional management units for marine turtles: A novel framework for prioritizing conservation and research across multiple scales. *PLoS One* 5(12):e15465.
120. Dutton PH, Bowen BW, Owens DW, Barragan A, Davis SK (1999) Global phylogeography of the leatherback turtle (*Dermochelys coriacea*). *J Zool (Lond)* 248(3): 397–409.
121. Bourjea J, et al. (2007) Phylogeography of the green turtle, *Chelonia mydas*, in the Southwest Indian Ocean. *Mol Ecol* 16(1):175–186.
122. Vargas SM, et al. (2016) Phylogeography, genetic diversity, and management units of hawksbill turtles in the Indo-Pacific. *J Hered* 107(3):199–213.
123. Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. *Mol Ecol* 16(23):4886–4907.
124. Vinas J, Alvarado Bremer J, Pla C (2004) Inter-oceanic genetic differentiation among Albacore (*Thunnus alalunga*) populations. *Mar Biol* 145(2):225–232.
125. Montes I, et al. (2012) Worldwide genetic structure of albacore *Thunnus alalunga* revealed by microsatellite DNA markers. *Mar Ecol Prog Ser* 471:183–191.
126. Theisen TC, Bowen BW, Lanier W, Baldwin JD (2008) High connectivity on a global scale in the pelagic wahoo, *Acanthocybium solandri* (tuna family Scombridae). *Mol Ecol* 17(19):4233–4247.
127. Díaz-Jaimes P, et al. (2010) Global phylogeography of the dolphinfish (*Coryphaena hippurus*): The influence of large effective population size and recent dispersal on the divergence of a marine pelagic cosmopolitan species. *Mol Phylogenet Evol* 57(3): 1209–1218.
128. Castro ALF, et al. (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Mol Ecol* 16(24):5183–5192.
129. Buonaccorsi VP, McDowell JR, Graves JE (2001) Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). *Mol Ecol* 10(5):1179–1196.
130. Longhurst A (1995) Seasonal cycles of pelagic production and consumption. *Prog Oceanogr* 36(2):77–167.
131. Longhurst A (2007) *Ecological Geography of the Sea* (Academic, San Diego), 2nd Ed.
132. Reygondeau G, et al. (2013) Dynamic biogeochemical provinces in the global ocean. *Global Biogeochem Cycles* 27(4):1046–1058.
133. Brum JR, et al. (2015) Patterns and ecological drivers of ocean viral communities. *Science* 348(6237):1261498.
134. Alvain S, Moulin C, Dandonneau Y, Bréon F-M (2005) Remote sensing of phytoplankton groups in case 1 waters from global SeaWiFS imagery. *Deep-Sea Res* 52(11):1989–2004.
135. Reygondeau G, et al. (2012) Biogeography of tuna and billfish communities. *J Biogeogr* 39(1):114–129.
137. Norris RD (2000) Pelagic species diversity, biogeography, and evolution. *Paleobiology* 26(sp4):236–258.
138. Miyamoto H, Machida RJ, Nishida S (2012) Global phylogeography of the deep-sea pelagic chaetognath *Eukrohnia hamata*. *Prog Oceanogr* 104:99–109.
139. Hirai J, Tsuda A, Goetze E (2015) Extensive genetic diversity and endemism across the global range of the oceanic copepod *Pleuromamma abdominalis*. *Prog Oceanogr* 138:77–90.
140. Andrews KR, Norton EL, Fernandez-Silva I, Portner E, Goetze E (2014) Multilocus evidence for globally distributed cryptic species and distinct populations across ocean gyres in a mesopelagic copepod. *Mol Ecol* 23(22):5462–5479.
141. Goetze E (2011) Population differentiation in the open sea: Insights from the pelagic copepod *Pleuromamma xiphias*. *Integr Comp Biol* 51(4):580–597.
142. Norton EL, Goetze E (2013) Equatorial dispersal barriers and limited population connectivity among oceans in a planktonic copepod. *Limnol Oceanogr* 58(5): 1581–1596.
143. Peijnenburg KT, Fauvelot C, Breeuwer JA, Menken SB (2006) Spatial and temporal genetic structure of the planktonic *Sagitta setosa* (Chaetognatha) in European seas as revealed by mitochondrial and nuclear DNA markers. *Mol Ecol* 15(11):3319–3338.
144. Bucklin A, et al. (2007) DNA barcodes for species identification of euphausiids (Euphausiacea, Crustacea). *J Plankton Res* 29(6):483–493.
145. Burridge AK, Goetze E, Raes N, Huisman J, Peijnenburg KT (2015) Global biogeography and evolution of *Cuvierina* pteropods. *BMC Evol Biol* 15(1):39.
146. Peijnenburg KT, Goetze E (2013) High evolutionary potential of marine zooplankton. *Ecol Evol* 3(8):2765–2781.
147. Schluter D (2016) Speciation, ecological opportunity, and latitude (American Society of Naturalists Address). *Am Nat* 187(1):1–18.
148. Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol J Linn Soc Lond* 58(3):247–276.
149. Bernatchez L, Wilson CC (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Mol Ecol* 7(4):431–452.
150. Moritz C, Ens E-J, Potter S, Catullo R (2013) The Australian monsoonal tropics: An opportunity to protect unique biodiversity and secure benefits for Aboriginal communities. *Pac Conserv Biol* 19(4):343–355.
151. Antonelli A, et al. (2015) An engine for global plant diversity: Highest evolutionary turnover and emigration in the American tropics. *Front Genet* 6:130.
152. Bowen BW (2016) The three domains of conservation genetics: Case histories from Hawaiian waters. J Hered 107(4):309–317.
153. Arbogast BS, Kenagy G (2001) Comparative phylogeography as an integrative approach to historical biogeography. *J Biogeogr* 28(7):819–825.

Chapter 10

Rev Fish Biol Fisheries (2017) 27:615–649
DOI 10.1007/s11160-017-9489-7

REVIEWS

Responsible genetic approach to stock restoration, sea ranching and stock enhancement of marine fishes and invertebrates

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Received: 15 January 2017 / Accepted: 22 June 2017 / Published online: 29 June 2017

Abstract The origins of agriculture date to about 9000 years, but commercial culture and supplementation of marine populations reach back only a few centuries. Hence, wild populations still play a major role in seafood production. Closed culture, stock restorations, sea ranching and stock enhancements of marine fishes and invertebrates have been implemented with various outcomes. A review of the literature indicates that considerable effort has been directed toward culture technologies to maximize production, but scant attention has been given to genetic risks to wild populations. Genetic risks from stock enhancements can be substantial, because of inattention to brood-stock sizes, and because hybridization between hatchery-reared and wild individuals can lower the fitness or lead to the extinction of a natural population. In many cases, small brood-stock sizes have led to the loss of genetic diversity. In some cases, hatchery-reared individuals appear to have replaced, rather than supplemented, wild populations. Here, we outline a responsible approach to managing genetic resources that includes six steps: (1) assess the costs and benefits of a stock restoration or enhancement, (2) set goals and genetic benchmarks, (3) use appropriate brood stock and limit domestication, (4) design release strategies that maximize the effectiveness of supplementation efforts, (5) track individuals after release and (6) minimize genetic impacts on wild populations. Stock supplementation is often viewed as an immediate solution to a stock decline, but should be undertaken as a last resort because of the high cost of implementation and the substantial ecological and genetic risks to wild populations.

Keywords Effective population size · Genetic diversity · Hatchery brood stock · Marine species · Stock supplementation

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Introduction

Efforts to increase production of marine fishes and invertebrates commonly focus on closed culture, sea ranching, stock restorations and stock enhancements.

These activities have increased sharply in the past few decades, largely because overexploited capture fisheries can no longer meet the demand for seafood (Jackson et al. 2001; Garibaldi 2012). While ecological impacts of enhanced production on wild populations have been recognized (Tegner and Butler 1989; Blankenship and Leber 1995; Pauly et al. 2002, 2003; Lorenzen 2008; Zohar et al. 2008; Lorenzen et al. 2010), the genetic effects of supplementation have received much less attention (Taniguchi 2003). Genetic influences on the viabilities of wild populations are more difficult to gauge than ecological influences, because genetic effects may not be as apparent as short-term shifts in abundance or age structure. Genetic principles have to be considered in stock supplementation, because numerous studies show that natural disturbances and human manipulations of populations can lead to microevolutionary changes on short time scales that may be irreversible (Law 2000; Ashley et al. 2003; Hendry et al. 2011). A growing body of research shows that domestication and inadvertent selection in culture changes the genetic profiles of hatchery-reared individuals and that hybridizations between hatchery-reared and wild individuals can adversely affect wild populations (Araki et al. 2007; Hedgecock and Coykendall 2007; spp.) populations (Busack and Currens 1995; Mobrand et al. 2005; Naish et al. 2008), and these guidelines provide a starting point for developing genetic guidelines for marine fishes and invertebrates. The effects of hatchery culture on salmon have been studied in depth, because of their short life span and moderate fecundities (2000–5000 eggs), which facilitate experimental matings to understand the effects of hatchery culture. However, little attention has been directed at the effects of genetic diversity and effect on population vigour of marine species (Hoban et al. 2013; Mimura et al. 2017). Additional guidelines are needed for marine species, because most marine organisms spawn millions or tens of millions of eggs, making populations vulnerable to recruitment sweepstakes (Hedgecock 1994; Sun and Hedgecock 2017). Large variances in family size lead to rapid genetic changes and amplify the effects of supplementations with hatchery-reared individuals. Hence, the goal of this review is to outline the potential genetic effects of cultured individuals on wild populations and to suggest actions to lessen these effects.

Attempts to increase the productions of marine stocks are generally prompted by drops in the abundances of stocks resulting from overharvesting

Young et al. 2008).

The first attempts to enhance marine populations on a large scale took place in the late 1800s for Atlantic cod (*Gadus morhua*) (Shelbourne 1964; Kirk 1987) and for European (*Homarus gammarus*) and American (*Homarus americanus*) clawed lobsters (Addison and Bannister 1994; Nicosia and Lavalli 1999). A comprehensive assessment of stock enhancements for European lobster and Atlantic cod indicated that enhancements were not viable tools for the fishery management of these species (Svåsand et al. 2004). One economic success has been stock enhancements of Japanese Flounder (*Paralichthys olivaceus*), which led to a 253% return on investment (Okouchi et al. 2004). However, environmental and genetic costs were excluded from this measure of success. The variable successes of supplementations have led to a debate on the value of supplementation programs (Secor et al. 2002), as most attempted stock enhancements of marine species have met with limited or no demonstrated success (Masuda and Tsukamoto 1998).

Several policy guidelines have focused on the supplementations of Pacific salmon (*Oncorhynchus* (Munro and Bell 1997; Botsford et al. 1997; Jackson et al. 2001; Christensen et al. 2003), from environmental regime shifts (Anderson and Piatt 1999), or from both (Bechtol and Kruse 2009b). Depleted populations often show heritable changes in demography with shifts to younger ages at maturity and to smaller sizes for a given age (Sharpe and Hendry 2009; Longhurst 2010; Audzijonyte et al. 2013). Overharvested populations can lose genetic diversity (Smith et al. 1991; Hauser et al. 2002; McCusker and Bentzen 2010) and show shifts in genotypic frequencies, as noted by drops in growth rates and early maturity (Olsen et al. 2004; Hutchings 2005; Longhurst 2010). The protection of genetic diversity and adaptive potential in wild populations has to be a primary concern in any supplementation program, because of the potential for harming not only the target species (Conover 1998), but also the community of individuals interacting with the target species (Moya-Laraño 2011; Vellend 2006).

While this is not a systematic review (Haddaway et al. 2015), we have, nevertheless, attempted to

include a broad swath of topics that bear on understanding how stock supplementations in their various forms influence genetic processes in wild populations. We draw largely on primary research and principles in conservation biology, resource management, fisheries science and population genetics to assemble genetic guidelines to promote a responsible genetic approach to sea ranching, stock restorations and stock supplementations:

1. Assess biological and economic cost and benefits of a stock restoration or enhancement.
2. Set goals and genetic benchmarks.
3. Use appropriate brood stock and limit domestication.
4. Design release strategies that maximize the effectiveness of supplementation efforts.
5. Track individuals after release.
6. Minimize genetic impacts on wild populations.

We provide a theoretical and empirical basis for these steps, with the fundamental assumption that the protection of genetic diversity within and among wild populations of an exploited species is the highest priority not only in supplementation programs, but also in overall harvest management.

the wild populations (Waples et al. 2012; Svåsand et al. 2007; Karlsson et al. 2016).

Sea farming, sea ranching

Sea ranching consists of releasing hatchery-reared individuals into the wild, but with the expectation that individuals are harvested before they reproduce or mate with wild individuals. These operations are typically called ‘put-grow-and-take’ fisheries of such species as oysters (*Crassostrea* spp.), abalone (*Haliotis* spp.), or geoduck clams (*Panopea generosa*). Hatchery technologies for rearing individuals are the same as for stock restorations and enhancements, but a fundamental assumption of responsible sea ranching is the reproductive segregation of hatchery-reared individuals from wild individuals.

Stock restoration

Stock restorations are designed to replenish a depressed population to a previous level of abundance when natural population growth has failed to increase numbers (Rose et al. 2001). Individuals from the remnant population are generally used as brood stock, and larvae or juveniles are released into the wild until

Forms of stock supplementations and restorations

Supplementation programs are defined by the amount of time individuals are propagated in captivity and by the objective of a project. Hatchery culture and supplementation can consist of closed culture, sea ranching, stock restoration or stock enhancement. Each kind of supplementation can influence wild populations through ecological or genetic interactions.

Closed culture

In closed culture, progeny of brood stock are grown in a contained system to maturity and used as brood stock for the following generation. This approach is used for many aquaculture systems and for the captive propagation of endangered species or populations (FAO 2014). In captive propagation for conservation, every attempt is made to prevent domestication so individuals remain genetically similar to wild individuals. In commercial culture, however, individuals are often selectively bred for traits that increase hatchery production. An enduring problem is the effect of escaped cultured individuals on the population recovers to a pre-determined level of abundance. Stock restorations might be used to counter an Allee effect in which a threshhold level of abundance is needed for successful reproduction and recruitment (e.g. Stoner and Ray-Culp 2000; Berec et al. 2006; Kuparinen et al. 2014). Restorations are unlikely to be successful if physical variables or predators limit the survival of larvae or juveniles released into the wild, or if harvests continue.

Stock enhancement

Stock enhancements, on the other hand, continue indefinitely to boost the production of a self-sustaining population that has not reached carrying capacity. Stock enhancements require that hatchery-reared individuals survive and reproduce in the wild. Enhancements may be motivated by an attempt to mitigate for habitat deterioration from pollution by coastal industries, or to enhance (augment, replenish, supplement) stocks above present levels of abundance. However, massive programs have not always led to large increases in abundances (Coleman et al. 1998; Svåsand et al.

1989, 2000; Secor et al. 2002; Leber 2004). One reason is that hatcheries often produce individuals that are genetically less fit than wild individuals because they are protected from natural selection in a hatchery setting. Consequently, hatchery-reared individuals often do not survive as well as wild fish after release or are less productive (Atlantic cod, Svåsand et al. 1989; Howell 1994; Japanese flounder, Blaxter 2000; Miyazaki et al. 2000). Additionally, high levels of production may prevent a large hatchery from appropriately conditioning individuals before release, resulting in high levels of predation or inappropriate foraging behaviour (e.g. Purcell and Grover 1990; van der Meeren 2000).

Why protect wild populations?

Domestication of marine species

Unlike terrestrial plants and animals that were initially domesticated for food production about 12,000 year ago (Mannion 1999; Zeder 2006; Vigne 2011), marine species have largely not been domesticated, so that food production largely depends on harvests of wild populations (Teletchea and Fontaine 2014). Although fish were cultured in ancient Egypt about 1500 years ago and in Asia about 1000 years ago (Nash 2011; Liao 2000), commercial-scale aquaculture has only recently expanded to meet global market demands. The domestication of aquatic organisms is in its infancy, so the conservation of genetic resources in wild populations is not only possible, it is essential to ensure sustained harvests and aquaculture potential (Teletchea and Fontaine 2014). With domestication comes the loss of genetic diversity, as illustrated by the world's massive production of monocultures of only a few species of grain (Tilman 1999). The risk of relying on a limited set of genotypes for much of the world's food supply has stimulated recent efforts to find the original land races of plants and animals to recover genetic diversity (Brush 1995; FAO 2013). Hence, pro-active conservation of reaction norms and adaptive potentials of wild marine populations has to be a high priority, especially in the light of climate change.

Eco-evolutionary community dynamics

Fishery managers recognize the need to move beyond single-species management by also considering the effects of exploitation on non-targeted species and on the food-web dynamics in an ecosystem (Jennings et al. 1999; Conover and Munch 2002; Hopper et al. 2005; Link 2002; Pikitch et al. 2004; Gamble and Link 2012). Genetic diversity and adaptation play key roles in community dynamics by supporting ecosystem functions (Hoffman and Merilä 1999; Worm et al. 2006; Hughes et al. 2008; Whitham et al. 2008; Bailey et al. 2009; Matthews et al. 2011; Hoban et al. 2013). For example, genetic diversity can enhance the resilience of a keystone species, and thereby preserve ecosystem structure (Reynolds et al. 2012). Indirect genetic effects within species can also have evolutionary and ecological consequences on managed populations by shifting phenotypes in response to the genotypes of conspecific individuals (Wolf et al. 1998; Wolf 2003; Bailey et al. 2014). The genes influencing the phenotype of an individual may be indirectly influenced by the expression of genes in interacting individuals. Indirect genetic effects can be seen, for example, in the cannibalism of small individuals by fast growing individuals in a hatchery setting, but can also extend to interactions in the wild that influence phenotype. While not always appreciated, the demise of a key species through genetic decline can produce major shifts in a community of interdependent species (Hopper et al. 2005; Kahilainen et al. 2014).

Genetic guidelines must be informed by ecosystem-based approaches to management, because stock restorations and supplementations have become an integral part of fishery management (Francis et al. 2007; Olsen et al. 2008). Supplementations can greatly impact an ecosystem food web, because economically important species are often dominant predators. Predators affect local species diversity and ultimately have top-down influences on nutrient flux and primary productivity (Heithaus et al. 2008; Hughes et al. 2008; Moya-Laraño 2011). These interactions collectively contribute to community stability and resilience to environmental variability and enhance the stabilities of harvested dominant species. When life-history variables in a fishery species are altered by selective harvests, environmental changes, or genetic transformations from supplementation activities, changes ripple through the ecosystem and may diminish the capacity of an ecosystem to support upper trophic-level species. Ecological impacts influence ecosystem health by moderating competition for space and food, by changing patterns of predation or herbivory and, in some cases, by altering the physical structure a foundation species may provide (Hughes and Stachowicz 2004; Whitham et al. 2006; Lankau and Strauss 2007). For these reasons, the protection of genetic resources in wild populations is an overarching goal in planning and implementing stock supplementations.

Why protect genetic diversity?

The information encoded in genes underpins all aspects of a species' life-history cycle, including growth, fecundity, immune responses to pathogens and behaviour. The genetic integrity of a wild population and its adaptive potential can be influenced by activities at several stages in a supplementation project. Hatchery culture practices can influence the genetic makeup of individuals being released, and these individuals can influence wild populations. The genetic conditions of individuals collectively influence the ability of a population to persist, to play a role in the ecological dynamics of a community and to support sustained harvests (Vellend and Geber 2005; Reusch et al. 2005; Agashe 2009). The preservation of genetic diversity among populations is especially important for the long-term persistence of a species (Schindler et al. 2010).

Genetic diversity and adaptive potential

Adaptive potential is the ability of wild populations to change genetically in response to ecological challenges (Conover 1998; Hughes and Stachowicz 2004), but also the ability to respond to artificial selection (Tave 1993). The capacity for adaptive change is influenced by several elements, but most importantly, by the additive genetic variance, which underpins ecologically important quantitative traits (Bürger and Lynch 1995; Hill et al. 2008; Frankham et al. 2014). These variance components are correlated with short-term responses to selection, whereas allelic diversity is associated with long-term adaptation (Caballero and Garcia-Dorado 2013; Pearse 2016). Adaptive potential is influenced by both beneficial and deleterious mutations at individual genes with large effects and by numerous low-frequency alleles (Lynch et al. 1995).

A common proxy for adaptive potential is genetic diversity (*heterozygosity*) as measured by molecular markers (Box 1). However, heterozygosity is insensitive to low frequency alleles that can play important roles in quantitative traits (Falconer and Mackay 1996). The number of alleles standardized by sample size (*allelic richness*) offers an additional, and more sensitive, measure of genetic diversity (Kalinowski 2005: Hoban et al. 2014). Gene diversities are not highly correlated with diversities at quantitative trait loci (Reed and Frankham 2001, 2003; Evans et al. 2004) or with fitness (David 1998; Hansson and Westerberg 2002). Genomic methods are now available to be able to identify specific genes or gene clusters associated with adaptive traits and will become increasingly applied to problems in fishery management (Pearse 2016).

The loss of genetic diversity as measured by neutral molecular markers likely also indicates the loss of genetic variation at quantitative trait loci influencing

Box 1 Measuring genetic diversity within populations

Effective population size (N_e) is the most important variable influencing adaptive potential. N_e is the size of an ideal population that loses genetic diversity at the same rate as a real population (Crow and Kimura 1970). Genetic diversity can be measured by the expected proportion of heterozygous individuals in the population (H) based on the Hardy–Weinberg relation between allelic and genotypic frequencies

$$h = 1 - \sum_{i=1}^{k} p_i^2$$

where p is the frequency of an allele in a sample and k is the number of alleles at a locus. Locus heterozygosities are averaged over several gene markers to yield a general estimate of genetic diversity in a population (H). The loss of genetic diversity from a population in one generation is proportional to the inverse of N_e

$\Delta H = H\left(\frac{1}{2N_e}\right)$, where $2N_e$ is the number of genes for a locus in a population of diploid individuals. Since the loss of genetic diversity is cumulative over generations, the loss over t generations is

At small population sizes, genetic diversity is lost rapidly. For example, when $N_e = 500$, 1% of the diversity is lost over ten generations. However, when $N_e = 50$, 9.4% of the diversity is lost over the same period. Long-term losses of genetic diversity can be substantial adaptive potential, but the absence of a reduction in molecular genetic diversity does not necessarily mean that additive genetic variance is unaffected. Nevertheless, surveys of neutral or nearly neutral molecular markers have detected low levels of genetic diversity in some wild populations, and this has been attributed in large part to reduced population sizes from overharvesting (Hauser et al. 2002; Sekino et al. 2002; Yu and Chu 2006; Porta et al. 2007; Lind et al. 2009; Novel et al. 2013). Natural selection can lead to adaptive differences between populations more rapidly than the rate of divergence from random drift at neutral molecular markers (Conover 1998; Conover et al. 2006; Swain et al. 2004; Barrett and Schluter 2008). For example, common-garden experiments showed that larval growth rates, survival and reaction norms differed among four populations of Atlantic cod that could not be distinguished with selectively neutral microsatellite markers (Hutchings et al. 2007).

Even modest declines in marine population sizes may lead to the loss of low-frequency alleles that may be important for adaptation and resilience to the decadal and multi-decadal environmental changes that characterize marine environments (Ryman et al. 1995; Mantua and Hare 2002; Griffies and Bryan 1997). The loss of genetic diversity in small populations through overharvesting or genetic swamping from hatchery releases can lead to inbreeding (Keller and Waller 2002). Selective pressures from environmental variability can produce short-term plastic changes that shift a phenotype along a reaction norm. Long-term selection over several generations can lead to genetic changes that shift the reaction norm itself (Hutchings et al. 2007; Olsen et al. 2008). The importance of genetic diversity comes from observations and experiments on marine organisms showing that genetic diversity can influence survival at particular life-history stages. For example, higher levels of genetic diversity led to greater successful settlement in an intertidal barnacle (*Balanus improvisus*) (Gamfeldt et al. 2005). Genetic diversity was also important for survival and population stability in manipulated populations of amphipods (*Gammarus* spp., Gamfeldt and Källström 2007). High levels of genetic diversity also provide resistance to bacterial and viral infections in many species (Harvell et al. 1999; Arkush et al. 2002; Suttle 2007).

Genetic effects of supplementation

Supplementations can influence wild populations in several ways. First, the use of a small number of individuals for brood stock inevitably produces a cohort of individuals that differs genetically from the wild population. Hybridizations between hatchery and wild individuals have the potential to lower the fitness of the wild population. The fitness effects of hatchery-wild hybridizations have been studied in salmonids because homing behavior is conducive to experiments that assess traits influencing survival and reproductive success (Kostow 2004; Araki et al. 2007, 2008). These studies show that even a single generation in a hatchery can diminish fitness.

Second, supplementations have the potential to reduce the effective size of a wild population, which can lead to the loss of genetic diversity through increased amounts of random drift. The Ryman–Laikre model (Ryman and Laikre 1991) and its extensions (Waples et al. 2016) predict the total effective size of a wild population after a generation of supplementation (Box 2). This model can also be used to determine appropriate brood-stock sizes needed to avoid a drop in the effective size of a supplemented wild population.

Third, the persistent release of individuals into the wild from a single source can homogenize genetic variability among populations. However, the preservation of among-population diversity is important because diversity enhances the ability of a regional group of populations to track environmental changes. It is not possible to predict which populations harbour the components of genetic diversity that would be important in a changing environment (Hilborn et al. 2003; Schindler et al. 2010).

Responsible genetic approaches

Here, we offer six guidelines to foster a responsible genetic approach to stock supplementations. In addition to factors directly influencing genetic variability, these guidelines also include social, biological and economic considerations in deciding to implement a stock supplementation. Stock supplementations in their various forms always have the potential to

Box 2 The Ryman–Laikre model

This model is derived from the concept of inbreeding effective population size and assumes that hatchery individuals recruit into the adult wild population and augment its spawning biomass. The effective size of a wild population after one generation of supplementation ($N_{e(WH)}$) depends on the effective size of hatchery brood stock ($N_{e(H)}$), the effective size of the wild population before supplementation ($N_{e(W)}$), and the proportion of hatchery-reared individuals successfully reproducing in the wild population [$x = N_H/(N_H + N_W)$], where N_H and N_W are the census numbers of brood stock and individuals in the wild, respectively

$$\frac{1}{N_{e(WH)}} = \frac{x^2}{N_{e(H)}} + \frac{(1-x)^2}{N_{e(W)}}$$

The genetic effective size of a wild population can be estimated before supplementation begins with a single sample of molecular data (Tallmon et al. 2008; Waples and Do 2008) or with generational samples (Wang et al. 2016). The theoretical upper limit of x can be estimated from hatchery production and stock assessments of abundance of a wild stock, but the actual value of x depends on the reproductive success of hatchery individuals in the wild. Important considerations are the spatial extent and genetic structure of a population, which influence estimates of $N_{e(W)}$

By rearrangement the effective population size of the combined wild-hatchery population is

$$N_{e(WH)} = \frac{N_{e(W)}N_{e(H)}}{N_{e(H)}x^2 + N_{e(W)}(1-x)^2}$$

This equation can be used to show the effects of $N_{e(H)}$ and$x$ on the combined wild-hatchery population $N_{e(WH)}$ (Fig. 2). At large values of x and small values of $N_{e(H)}$, hatchery introductions into the wild reduce the effective population size of the combined wild-hatchery population

influence genetic resources in a wild population, and this risk must be weighed against potential benefits.

Assess biological and economic cost and benefits of a stock restoration or enhancement

A fishery stock may be depressed for several reasons, including overfishing and ecosystem changes that impact habitats and thereby affect fecundity, survival or recruitment. A full-scale stock supplementation should be preceded by studies designed to understand the reason for the decline and whether the release of larvae or juveniles would beneficially increase population abundance without adversely influencing wild populations (Wilson et al. 1998; Molony et al. 2004; Waples and Drake 2004; Støttrup and Sparrevohn 2007). A careful evaluation of the biological and economic benefits is needed before a supplementation is initiated, because supplementation programs are often impossible to stop once underway, even if biological or economic benefits are minimal. A risk-benefit analysis should factor in unexpected events that may not be beneficial to wild populations. Tradeoffs have to be considered in a risk analysis, as actions taken to reduce one risk often increase another risk, so that it is impossible to implement a risk-free supplementation program (Waples and Drake 2004). Several criteria should be considered before embarking on a stock supplementation program in its several forms (Table 1) (Støttrup and Sparrevohn 2007).

Table 1 Criteria to consider before embarking on the supplementation of natural populations (after Støttrup and Sparrevohn 2007)

Criterion	Comment
Reduced recruitment not attributable to harvests	When the goal of a supplementation is to restore wild spawning, limits on harvest should be the first step to achieve an increase in biomass. Habitat improvement may also be more effective than supplementation in achieving production goals. The implementation of sea ranching depends on other criteria
Population below habitat carrying capacity	Spawner biomass is largely controlled by food availability. The addition of hatchery-reared fish may exceed the carrying capacity of a habitat, leading not only to poor growth of hatchery individuals, but also of wild individuals
Low predation on life history stage to be released	Early life-history stages generally experience high levels of predation. When a decline in a stock is due to predation supplementation efforts may not be effective
Cost-benefit analysis	The costs of research, hatchery construction and operation, and monitoring may exceed the monetary return on the supplementation effort

Recruitment limitation

Supplementation is predicated on the twin assumptions that inadequate natural recruitment is limiting abundance, and that the release of hatchery-produced individuals can enhance the size of a natural population. Before supplementations are considered, the initial focus of a restocking program has to be on activities that improve recruitment (Doherty 1999; Morgan and Botsford 2001; Botsford et al. 2003; Gascoigne and Lipcius 2004). Recruitment may be restricted by several factors: the failure of individuals to progress through life-history stages (Walters and Juanes 1993; Caley et al. 1996; Chesson 1998) (Table 2); physical and biological constraints that limit spawning, egg and larval development, growth, maturity and reproduction (Hughes 1990; Neill et al. 1994; Hunt and Scheibling 1997; Wahle 2003; Seitz et al. 2008); and environmental factors imposing controls on population abundances (Masuda and Tsukamoto 1998; Kitada et al. 1992).

Populations decline not only because of overexploitation, but also because of environmental and biological changes, so that a depressed stock may not respond to supplementation efforts, even when fishing is reduced or suspended. Environmental changes influencing local population abundances can arise from events on several temporal and spatial scales (Table 2). The causes of environmental changes that interrupt ecosystem services and limit suitable habitats and food availability and quality may be local, regional or global (e.g. Olson and Olson 1989). For example, large-scale climate changes, such as those driven by the Pacific Decadal Oscillation, the North Atlantic Oscillation and the El Niño-Southern Ocean cycles (Mantua and Hare 2002), led to major shifts in species composition and food-web dynamics and even local extinctions (Alheit and Hagen 1997).

The effects of regional changes cascade down to local habitats by changing patterns of energy flow through an ecosystem. After a shift, primary and secondary production may be insufficient to support local populations at pre-shift levels. A supplementation may not be successful, because environmental or ecosystem shifts have altered habitat carrying capacity. For example, food limitation was a key variable that led to the failure of supplementations along the coast of Norway to increase Atlantic cod stock abundances (Smedstad et al. 1994; Svåsand et al. 2000). Even if carrying capacity has not changed, new competitors, predators or pathogens may limit the size of a local population. For example, a climate regime shift occurred in 1977–1978 that lead to a change from a crustacean dominated ecosystem in the Gulf of Alaska, supporting large shrimp and crab fisheries, to a fish-dominated ecosystem (Anderson and Piatt 1999). The closure of a fishery on populations of red king crabs (*Paralithodes camtschaticus*) around Kodiak Island in 1984 (Bechtol and Kruse 2009b) has not led to the recovery of these populations, most likely because of predation on juveniles in shallow-water nurseries (Bechtol and Kruse 2009a).

In some cases, depressed stocks represent geographically marginal populations (Leggett and Frank 1997) or non self-sustaining sink populations (Crowder et al. 2000; Fogarty and Botsford 2007; Chen et al. 2014). Local habitats are often part of larger ecosystems that expand and contract with climate variability, so that declines in local abundance of a species may be due to a larger process (MacCall 1990). During periods of high abundance, geographic range increases and populations may occupy less optimal habitats to escape density-dependent effects on food availability, competition, or habitat space. For example, the geographic range of yellowtail flounder (*Pleuronectes ferruginea*) on the Grand Banks contracted during periods of low abundance and expanded at high abundances (Simpson and Walsh 2004). Declines in local abundance may trigger stock supplementation efforts, but the previous occurrence of a species in a marginal habitat at the edge of its distribution is no guarantee that stock supplementations will be successful. As populations expand from core habitats during large-scale environmental changes, the abundances of local populations may return without intervention and without genetic risk to a possible remnant population.

A fundamental step in stock enhancements is to improve the passage through early life-history stages with protected hatchery culture. For example, likelihoods of restrictions at each life-history stage were identified in American lobsters and used to plan supplementation strategies (Aiken and Waddy 1986). In Dungeness crabs (*Metacarcinus magister*) along the Oregon and California coasts, the size of the mature population was strongly associated with the successful passage of larvae into the juvenile stage (Shanks and Roegner 2007) (Fig. 1). The enhanced passage

Table 2 Studies showing restrictions on various life-history stages that limit recruitment into the adult population

Species	Location	Life-history stage	Limiting factors	References
Spiny lobster *Panulirus argus*	Florida	Early juvenile	Small-scale artificial shelters increased survival of small, sedentary juveniles, but not larger, mobile juveniles	Butler and Herrnkind (1997)
American lobster *Homarus americanus*	New England	Settling larvae	Asymptotic relation between final larval stage and stock size 5–7 years later indicating possible density-dependent control of recruitment	Fogarty and Idoine (1986)
	Maine	Juvenile	Post-larval supply was controlled by wind-driven surface currents. When settlement location became crowded, lobsters moved to low density areas	Wahle and Incze (1997)
Dungeness crabs *Cancer magister*	Oregon-California	Planktonic larval stage	Timing of the spring transition influenced survival of the planktonic megalope larvae stage returning to shore to settle. Higher recruitment occurred with earlier spring transitions	Shanks and Roegner (2007)
	Washington	Juvenile	The strength of recruitment of juveniles on the bottom was correlated with the presence of shell substrates and with reduced predation	Eggleston and Armstrong (1995)
Tropical sea urchin *Diadema antillarum*	Caribbean		Recruitment and mortality were independent of local densities and food did not appear to limiting. Hence, depressed population abundances appear to result in recruitment limitation from the lack of larvae	Karlson and Levitan (1990)
Anchoveta *Engraulis ringens*	Peru	Planktonic larvae	In areas of upwelling with water current turbulence, reduced opportunities for feeding by larvae limit growth and survival, and hence limit recruitment	Cury and Roy (1989)
Pacific Sardine *Sardinops sagax*	North Pacific			
Olympia oyster *Ostrea lurida*	Washington	Post-settlement stage	Post-settlement mortality appears to limit population recovery, rather than the production of larvae. Spat appear to be plentiful	Trimble et al. (2009)
Olympia oyster *Ostrea lurida*	Florida	Settlement stage	Suitable settlement space appears to be limiting population growth	Brumbaugh and Coen (2009)
Mussels *Aulacohya moariana* *Mytilus galloprovincialis* *Perna canaliculus*	New Zealand	Planktonic larvae	Current patterns and food availability appear to explain lower levels of recruitment into outer coastal populations, relative to bay abundant bay populations	Helson and Gardner (2004)

Table 2 continued

Species	Location	Life-history stage	Limiting factors	References
Barnacles (Cirripedia) Mussels (*Mytilus*) Crab (*Cancer* spp.) Crab (*Cancermagister*) Crab (Porcellanidae) Hermit crab (Paguridae) Crab (Grapsidae) Crab (Majidae)	California	Planktonic larvae	Larval dispersal to a suitable settlement site is influenced by the interaction between larval behaviour and ocean current, upwelling relaxation and unknown mechanisms	Morgan et al. (2009)
Alaska plaice *Pleuronectes quadrituberculatus*	Gulf of Alaska, eastern Bering Sea	Juvenile	The size of the adult population was positively related to the size of the shallow-water nursery habitat	Bailey et al. (2003)
Fishes: Benthic (7 species) Proxima-benthic (7 species) Pelagic (8 species)	Global	Juvenile	Population sizes in species with partitioned juvenile and adult habitats tended to be controlled by the size of the juvenile habitat and not the size of the adult habitat	Iles and Beverton (2000)
Plaice *Pleuronectes platessa*	United Kingdom	Planktonic larvae	Temperature variation February–June had strong effect on recruitment, likely because of increase in larval predation	Fox et al. (2000)

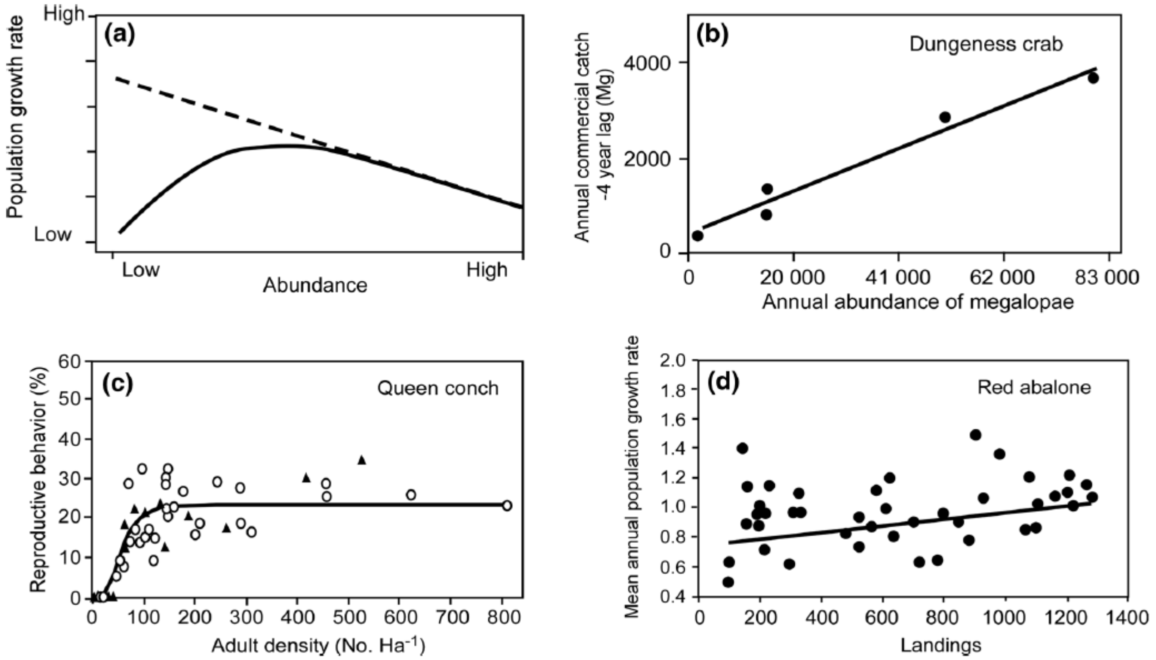


Fig. 1 The Allee effect. **a** *Lower curve* shows the Allee effect when population growth declines at low population abundances (redrawn from Hutchings 2014). **b** Relationship between larval (megalopae) abundance (proxy for reproductive output) and commercial catch with a four-year time lag to account for individual growth (redrawn from Shanks and Roegner 2007). **c** Allee effect in the marine queen conch (redrawn from Stoner and Ray-Culp 2000). **d** Allee effect in red abalone (redrawn from Gascoigne and Lipcius 2004)

through early juvenile stages, however, may not improve recruitment into the adult population because of restrictions on subsequent life-history stages. In populations of spiny lobsters (*Panulirus argus*) in Florida, artificial shelters increased the numbers of non-mobile juveniles by protecting them from predation, but did not increase the abundances of more mobile, larger lobsters (Butler and Herrnkind 1997).

Even when recruitment into a population is limited, ecological and genetic risks to a depressed population may outweigh potential benefits from supplementation. Alternatives should be considered prior to commencing supplementation. Changes in fishery management, the establishment of marine reserves (Roberts et al. 2005) or habitat enhancements may be more effective in restoring a stock to previous levels than the release of hatchery-reared individuals. If reductions in exploitation do not lead to an increase in the abundance of a stock, supplementations are likely to be ineffective. Only some depressed stocks may be helped by supplementation, in particular, those in which limited spawning success does not produce enough larvae, or those in which passage of individuals through a life-history stage is hampered by food limitation, inhospitable temperatures, predation or disease. Some species with late maturation may have such low survival to first reproduction that the release of hatchery-reared juveniles does little to increase adult population size (Heppell and Crowder 1998).

The special case of depensation

Recruitment limitation may lead to depensation in which a depleted population does not expand into usable habitat space (Liermann and Hilborn 1997). Depensation is the circumstance in which per-capita success, either survival or reproduction, decreases when the population is at low abundance. It is a rare circumstance in which supplementation may be both effective and less risky than in other cases. Social animals, broadcast spawners, or organisms that modify their environment are likely to suffer such effects (Navarrete et al. 2005). Such populations, once driven to low abundance, may be trapped there by low survival or recruitment. Supplementation can be less risky in such cases because it can be stopped when survival increases or reproductive success recovers. In many cases, however, supplementations continue so that genetic risks accumulate and further jeopardize the population.

Economic costs and benefits

A fundamental consideration is whether a supplementation program would yield a positive economic outcome. Stock enhancements and restorations are expected to produce economic benefits to fishermen, fish processors, wholesalers, retailers and coastal communities. Before embarking on a program, project planners must consider whether economic returns exceed the costs of hatchery infrastructure, of developing larval culture methods, of finding a suitable release site and of monitoring activities after release (Sproul and Tominaga 1992; Moksness et al. 1998; Wilson et al. 1998; Mustafa et al. 2003; Ye et al. 2005). The cost of hatchery infrastructure required to culture early life-history states in a genetically friendly fashion can be considerable. A positive balance sheet for marine stock supplementations is seldom achieved (Bannister and Addison 1998; Hilborn 1998; Secor erable cost has not always led to increases in the abundances of Atlantic cod and European lobster (Coleman et al. 1998; Svåsand et al. 1989, 2000). For abalone supplementation in Japan, the income:cost ratio per individual was often less than 1.0, ranging from 0.4 to 6.2 at various localities (Hamasaki and Kitada 2008b).

Setting goals and genetic benchmarks

Goals and benchmarks measuring success differ among the various kinds of supplementations. In stock restorations, hatchery individuals are released to replenish wild populations that are at low levels of abundance, or have been extirpated. In both cases, the biological and genetic characteristics of hatchery releases will largely define the biological characteristics of the restored population after releases become established. Hence, the quality of hatchery releases and the adaptive fit to the habitat are of crucial importance. The preservation of adaptive potential in hatchery-reared individuals depends importantly on (1) large brood-stock sizes to maintain genetic diversity, (2) the origins of the brood stock relative to the target population and (3) intentional or un-anticipated phenotypic and genotypic changes during culture.

In sea farming, hatchery-origin individuals are harvested before maturity and interactions with wild populations are generally considered to be of little concern. The ability to selectively harvest hatchery-origin individuals is crucial. However, with long-term sea ranching, at least some individuals escape harvest, mature and join the wild spawning biomass. Again, the quality of brood stock producing individuals for grow-out is a major concern, not only for maintaining high levels of commercial production, but also for the eventuality of hybridizations between hatchery-reared and wild individuals. The use of large brood-stock sizes to lessen the loss of genetic diversity and of local brood stocks to lessen genetic contrasts with wild populations is important in brood-stock management. Responsible sea farming requires enforcing scientifically based limits on the contribution of hatchery-origin individuals to the wild breeding population.

The success of stock enhancements cannot be measured by increases in stock size alone, but must also be evaluated by the effects of enhancement on genetic diversity within and between wild populations requires that (1) a large proportion of the released individuals survive and grow to harvestable sizes, (2) hatchery releases effectively supplement the abundance of next generation of spawners above pre-supplementation levels, (3) hatchery-cultured individuals supplement the wild population, rather than replace it and (4) the release of hatchery-reared individuals does not lead to detrimental ecological and genetic impacts on wild populations (Secor et al. 2002).

The effectiveness of a stock enhancement depends not only on the performance of hatchery-reared individuals after release and but also on the harvest management of the enhanced population. A critical benchmark is to ensure that the effective size of the combined wild-hatchery population does not fall below the original size of the wild population, which in most cases is difficult to achieve (see step 6). A supplementation program may be successful in the short-term by increasing harvest opportunities, but detrimental in the long-term by changing the genetic characteristics of the stock, or altering ecosystem structure. Other benchmarks of successful population recoveries have been defined by various management agencies (Hutchings et al. 2012; Kahilainen et al. 2014). However, these cost-benefit analyses generally focus on economic returns and often do not consider environmental and genetic costs (e.g. Taylor et al. 2005).

Brood-stock management

Hatchery practices are central to successful stock restorations and supplementations. Most efforts in the early stages of a project are generally focused on the development of culture conditions that promote growth and survival of larvae and juveniles. However, the histories of countless hatchery projects show that little attention is generally given to genetic concerns. These concerns fall into two areas: the selection of brood-stock individuals and the size of a brood stock.

Origin of brood stock

The choice of brood-stock individuals relative to the genetic profiles of a wild individuals influences genetic risk. Hence, population genetic and life history data are essential to guide the choice of hatchery brood stock. Surveys to assess genetic variability within and among wild populations are essential before embarking on culture experiments (e.g. Uthicke and Purcell 2004), because populations may differ genetically from one another and the use of genetically different individuals at a site may disrupt adaptation in local populations (Sanford and Kelly 2011). Hence, brood stock must be drawn from populations with individuals that are genetically compatible with individuals at the release site. Hybrids between exogenous hatchery individuals released into the wild and individuals showing adaptations to local environmental conditions may show reduced levels of fitness. For example, hybrids between anadromous pink salmon populations separated by 1000 km showed a reduction in survival to maturity relative to local fish (Gilk et al. 2004). In another study of pink salmon, fry from early-spawning fish showed lower freshwater survival because of harsh physical conditions and disturbance from late spawners, but late-emerging fry showed lower marine survival (Gharrett et al. 2013).

In the marine realm, examples of local adaptation in marine species are generally based on correlations between fitness traits and environmental variables (Chaoui et al. 2012; Jensen et al. 2016; Le Moan et al. 2016), on the identification of 'outlier' genes in population surveys (Nielsen et al. 2009: Wang et al. 2013; Bekkevold et al. 2016) and on comparisons between quantitative genetic traits among populations with selectively neutral genetic markers (Gagnaire and Gaggiotti 2016). For example, a linear regression model indicated that temperature differences, rather than geographical distance or larval drift patterns, best explained patterns of genetic differentiation among populations of northern shrimp, *Panadalus borealis*, in the North Atlantic (Jorde et al. 2015). In a few studies, adaptive variability among populations has been detected in common garden experiments (Sussarellu et al. 2015). Genetically heritable shell shape in the broadcast spawning clam, *macoma baltica*, differed between populations separated by only tens of kilometers in the North Sea, even though allozyme and mitochondrial DNA frequencies were not significantly different among populations (Luttikhuizen et al. 2003).

The development of molecular markers that can probe deeply into the genomes of marine species are showing that local adaptation is more common than expected from population surveys using selectively neutral molecular markers (Gagnaire et al. 2015). A surprising number of marine species with large potentials for dispersal through pelagic larvae or adult movement are showing evidence of local adaptation (Sanford and Kelly 2011). These studies together demonstrate that the choice of brood-stock individuals and release sites have to be important considerations to protect the adaptive potentials of wild populations.

In stock restorations, it may be best to use individuals from the depressed population itself or from a genetically similar population, so that mixing between hatchery and wild individuals does not introduce maladaptive genes (Tringali et al. 2008). Even so, cultured individuals often diverge from wild populations by shifts in genetic traits that enhance survival and growth in culture (domestication). For example, hatchery-reared individuals can develop behavioral deficits, such as in predator avoidance or appropriate foraging behaviours, that lead to poor survival in the wild (Brown and Day 2002). Domestication becomes more prevalent when brood-stock individuals come from previous generations of the hatchery population.

When wild individuals are used for brood stock each generation, the introduction of new genetic material reduces the loss of genetic diversity. Periodically replenishing brood stock with wild individuals minimizes genetic changes resulting from hatchery domestication (Mobrand et al. 2005; Ford et al. 2006). One challenge is to select individuals in the wild that do not have hatchery ancestry, as hatchery-origin individuals used for brood stock may hasten the replacement of a wild population with hatchery-reared individuals (Ryman and Laikre 1991).

Brood-stock size

Brood-stock size determines the amount of genetic diversity retained in a hatchery cohort. Most marine fishes and invertebrates spawn enormous numbers of offspring (Oosthuizen and Daan 1974; Suquet et al. 2016) so that the use of only a few brood stock individuals may be economically efficient to reduce hatchery costs and may satisfy the production goals of a project. However, small brood stock sizes can quickly alter the genetic make-up of a hatchery population. The loss of genetic diversity through random genetic drift can be substantial, and this loss is irreversible. The loss of genetic diversity, and additive genetic variance, can impact traits such as growth, tissue quality, and survival, and can reduce the potential for artificial selection to improve production traits. Numerous studies show that the loss of genetic diversity through small brood-stock size can adversely affect hatchery production (Taniguchi et al. 1983; Macaranas and Fujio 1990).

The loss of genetic diversity can also influence the outcome of a stock restoration when a remnant population is small or extirpated. Founder effects from a small number of colonizers have been well documented in the medical records of human populations. These records show the predicted increase in the frequencies of deleterious recessive genes through rapid population growth and random drift and the corresponding increase in inherited diseases and reduced life expectancy (e.g. Ramachandran et al. 2005; Geldenhuys et al. 2014). Although similar documentation is generally unavailable for marine species, the same genetic processes are expected to operate in stock restorations (Anderson and Hedgecock 2010).

Conservation biology and population genetics theory are starting points for understanding the relationship between brood-stock size and genetic diversity (Crow and Kimura 1970; Allendorf et al. 2013). Theory indicates that small population sizes can erode a substantial amount of genetic diversity in a single generation. In many conservation efforts to save endangered populations with captive propagation, the loss of 2% of genetic diversity ($n = 50$) is acceptable in the short term, but less than 0.2% ($n = 500$) in the long term (Frankham 1995, 2005). However, the use of these parameters can still lead to a substantial loss of adaptive potential because of the loss of genetic diversity (Frankham et al. 2014) and to the accumulation of deleterious mutations (Charlesworth et al. 1993; Lynch et al. 1995). Several instances of the loss of genetic diversity have been documented in hatchery populations used for enhancements of marine populations (Table 3).

The additive variance underpins variability at quantitative trait loci and is influenced by small effects of numerous loci with low-frequency alleles (Lande 1981; Falconer and Mackay 1996). The loss of low-frequency alleles can be severe in small brood stocks. For example, the use of only four individuals leads to a 66% chance of losing an allele with a frequency of 0.05, but with the use of 100 individuals this chance drops to 0.004% (Tave 1993). The loss of lower-frequency alleles is even greater at these brood-stock sizes; the expected chance of losing an allele with a frequency of 0.01 is 92% with a brood stock size of four individuals.

The Ryman–Laikre model can be used to estimate the effect of stock supplementations on a wild population by predicting the effective size of the combined hatchery-wild population (Box 3). The model is based on three variables: the effective size of the brood stock, $N_{e(H)}$, the effective size of the wild population before supplementation, $N_{e(W)}$, and the proportion of hatchery-reared individuals that successfully spawn in the wild, x. Effective population sizes (N_e) of marine species are typically 3–6 orders of magnitude smaller than census size because of reproductive sweepstakes (Hedgecock 1994; Turner et al. 2002; Nakajima et al. 2014; Montes et al. 2016; Rugger et al. 2016). This range of effective wild population sizes encompasses most of the effective population sizes estimated for marine species (Turner et al. 1999, 2002; Hauser et al. 2002; Ovenden et al.

Table 3 Comparisons of genotypic diversity in hatchery and relative to wild populations

Species	Locality	Comment	Reference
Marine fishes			
Dover sole *Solea solea*	Europe	Loss of genetic diversity in hatchery-reared larvae and juveniles	Exadactylos et al. (1999)
European sea bass *Dicentrarchus labrax*	Spain	No loss of genetic diversity, but microsatellite markers indicated that family sizes were uneven in brood stock selected for larger growth rates	Novel et al. (2013)
Haddock *Melanogrammus aeglefinus*	Eastern Canada	Microsatellite markers indicated that brood stock at two hatcheries were highly inbred, with 99.5 and 88.7% of the individuals related as sibs or half-sibs	Martin-Robichaud et al. (2007)
Pacific herring *Clupea pallasii*	Japan	No reduction in diversities of low-frequency alleles between hatchery and wild populations. Wild individuals used as hatchery brood stock each year	Kitada et al. (2009)
Red drum *Sciaonops ocellatus*	Gulf of Mexico	Estimates of effective hatchery brood stock size ranged from 5 to 50 fish	McEachron et al. (1998), Tringali and Bert (1998), Turner et al. (1999)
Red sea bream *Pagrus major*	Japan	Loss of low-frequency microsatellite DNA alleles in hatchery populations, but heterozygosity remained unchanged. Hatchery-reared individuals used as brood stock for several generations	Kitada et al. (2009)
Red sea bream *Pagrus major*	Japan	Large releases of hatchery offspring, about 50% of total population, were spawned by 63.7 fish on average	Perez-Enriquez et al. (1999)
Senegalese sole *Solea senegalensis*	Spain, Portugal	Substantial loss of microsatellite allele diversity in one generation in brood stock at four hatcheries founded by related individuals	Porta et al. (2007)
Spanish mackerel *Scomberomorus niphonius*	Japan	Significantly lower levels of genetic diversity in hatchery-reared fish, because of small brood stock sizes (~10 fish)	Nakajima et al. (2014)
White sea bass *Atractoscion nobilis*	California	The effective number of breeders producing offspring for release averaged 2.95–20 fish	Hedgecock (2004), Hedgecock and Coykendall (2007)
Marine invertebrates			
Pearl oyster *Pinctada fucata*	Southern China	Loss of AFLP polymorphisms in hatchery individuals, substantial genetic divergence between hatchery and wild populations	Yu and Chu (2006)
Silver-lipped pearl oyster *Pinctada maxima*	Indonesia, West Paupa	Reduced number of microsatellite alleles in hatchery population relative to wild populations. Pedigree analysis indicated that 40% of brood stock individuals came from a single family	Lind et al. (2009)
Asian suminoe oyster *Crassostrea ariakensis*	Asia, USA	A significant drop in genetic diversity in hatchery populations in the USA compared to source populations in Asia. Hatchery stocks also had diverged genetically to a large extent	Xiao et al. (2011)
South African abalone *Haliotis midae*	Southern Africa	Loss of microsatellite low-frequency alleles compared to wild populations (35–62%). Estimated $N_e = 75$. Significant genetic divergence between hatchery and wild population	Evans et al. (2004)
South African abalone *Haliotis midae*	Southern Africa	A survey of microsatellite loci did not detect a drop in genetic diversity in hatchery brood stock, but the hatchery population had diverged significantly from wild populations	Rhode et al. (2012)

Table 3 continued

Species	Locality	Comment	Reference
Blacklip abalone *Haliotis rubra*	Tasmania, Australia	Loss of microsatellite low-frequency alleles compared to wild populations (37.5–40%) after single hatchery spawning of wild brood stock. Estimate $N_e = 22–27$. Significant genetic divergence from wild populations.	Evans et al. (2004)
Blue abalone *Haliotis fulgens*	Baja California, Mexico	Microsatellite analysis showed that released offspring had genetic diversities that were similar to hatchery brood stock. About 80% of the brood stock contributed to offspring that were released	Gutierrez-Gonzalez and Perez-Enriquez (2005)
Bay scallop *Argopecten irradians*	Western North Atlantic, China	Loss of mtDNA genetic diversity in cultured populations in Virginia ($h = 0.0$) and China ($h = 0.55$) relative to wild populations in the western North Atlantic ($h = 0.69–0.91$)	Blake et al. (1997)
Japanese scallop *Patinopecten yessoensis*	Japan	Drop in allozyme diversity in hatchery-reared scallops	Fujio and von Brand (1991)
King scallop *Pecten maximus*	Irish Sea	Survey of 15 microsatellite loci in wild populations and hatchery brood stock showed similar levels of heterozygosity, but hatchery stock had lost low-frequency alleles	Hold et al. (2013)
Purple sea urchin *Paracentrotus lividus*	Ireland, Spain	Survey of 9 microsatellite loci in wild and hatchery populations showed significant drop in allelic richness	Segovia-Viadero et al. (2016)
Abalone *Haliotis tuberculata*	Ireland	Survey of variation at three allozyme loci indicated that heterozygosities were similar in two hatchery stocks and the originating wild population, but a drop in rare alleles was detected in a hatchery stock in culture for 3 generations	Mgaya et al. (1995)
Geoduck clams *Panopea generosa*	Puget Sound	Five microsatellite loci showed reduced genetic diversity in hatchery broodstock relative to wild populations and several full-sib groups, indicating a large variance in family size	Straus et al. (2015)

Allelic richness, not heterozygosity, provides the best indicator of the loss of genetic diversity (Allendorf 1986; Hoban et al. 2014)

2007). A reduction of four orders of magnitude was used to illustrate possible outcomes of gene flow from hatchery populations.

An overall goal in a responsible approach is to avoid depressing the effective size of a wild population below its original size. Table 4 shows minimal brood-stock sizes needed to avoid a drop in $N_{e(WH)}$ for wild-population effective sizes ranging from 400 to 12,000 and for proportional contributions of hatchery-reared individuals of 0.01–0.3, which includes most if not all examples of hatchery production.

Unfortunately, most hatchery supplementations lead to a decline in the combined wild-hatchery population. Figure 2 shows values of $N_{e(WH)}$ for various brood-stock sizes $N_{e(H)}$ and proportions of the wild-hatchery populations produced by hatchery-reared individuals, x, for effective wild-population sizes ranging from 100 to 12,800 individuals. These results show that brood-stock effective sizes should be at least 100 individuals to avoid reducing the combined wild-hatchery effective population size. When thousands of hatchery-reared individuals are introduced into a wild population and when they constitute 10–20% to the spawning population, effective brood-stock sizes should be on the order of 200–1000 individuals. When hatchery effective population sizes are less than about 100–200 individuals, supplementation always reduces the effective size of the wild-hatchery population, and the effect is greater for large supplementations (x) for a given brood-stock size. Positive increases in the wild-hatchery effective size are achieved in only a small window of large brood-stock sizes and with small to moderate wild effective population sizes. High levels of production magnify

Box 3 Genetically effective size of hatchery brood stock

The Ryman–Laikre equation can be solved for the necessary hatchery brood-stock effective size $N_{e(H)}$, given a target value of the effective wild-hatchery size after supplementation $N_{e(WH)}$

An important genetic benchmark is to keep the ratio $N_{e(WH)}/N_{e(W)}$ at values greater than 1.0. Table 1 shows a hypothetical case to estimate the effective brood-stock size of geoduck clams needed to maintain the wild-hatchery effect size at or above the pre-supplementation size of the wild population. Several combinations of total census size ($N_{e(W)}$) (400–12,000) and the proportion of hatchery-origin individuals successfully mating with wild individuals ($x = 0.01$ to 0.30). The value of x increases as the size of a local wild population decreases. In this example, an effective population size four orders of magnitude smaller than census size was assumed for these calculations

The effective size of a brood stock is influenced by events in a hatchery. Most hatcheries use communal spawning to produce larvae because of the added costs of spawning individual pairs and maintaining individual families. Communal spawning can first lead to a departure of a 1:1 sex ratio among breeders. In this case, the effective size of a population can be estimated by

$$N_e = \frac{4N_f N_m}{N_f + N_m}$$

For example, when one male is used to fertilize the eggs of two females, a 2:1 sex ratio, the effective population size drops 11%. When a 10:1 sex ratio is used, the effective population size drops by 73%

Another factor influencing the effective size of a brood stock is a disparity in the relative contributions to offspring among breeders. Most invertebrates are capable of producing large numbers of gametes and larvae, which can lead to large differences the number of offspring among families. When the variance of the number of successful offspring per individual, k, averages two [$\mathrm{var}(k) = 2.0$] the effective size of the broodstock is calculated as above. However, when the variance in family size is larger, $\mathrm{var}(k) > 2.0$, effective broodstock size (N_e) is

$$N_e = \frac{4N - 2}{2 + \mathrm{var}(k)}$$

where N is the number of offspring, and $\mathrm{var}(k)$ is the variance of offspring number among individuals; for example, if communally spawned individuals produce an unequal number of gametes or offspring survival differs among families so that $\mathrm{var}(k) > 2.0$. When the variance among families is $\mathrm{var}(k) = 4$, the effective size of the brood stock drops by 33%. When the variance among families is $\mathrm{var}(k) = 8$, which may be a conservative estimate of for highly fecund species (Hedgecock 1994), the effective brood-stock size drops by 60%

the genetic profiles of a limited number of brood stock, and offspring have the potential of diluting genetic diversity in the hatchery-wild population. Large, persistent supplementations have the potential to genetically swamp or displace a natural population. For example, persistent introductions of juvenile pearl oysters from genetically divergent populations led to a reduction of among-population diversity in French Polynesia (Arnaud-Haond et al. 2004).

Sex ratio

The ratio of males and females also influences the effective size of the brood stock. For example, the use of a single male to fertilize the eggs of ten highly fecund females may produce hundreds of thousands of larvae but the effective size is only 3.6 individuals, even though 11 breeders were used. When the sex ratio is highly skewed, the inbreeding coefficient can be large. In the example, a substantial inbreeding coefficient $F = 0.138$ results in a single generation. Effective brood-stock size is maximized by using an equal number of males and females.

Equal family sizes

Genetic considerations continue beyond mating design. Even with a 1:1 sex ratio, other variables influence the numbers of offspring among families, including timing of gamete maturity, quality of gametes among parents and variation in fecundity because of parental size or genetic legacy (Keller and Reeve 1994; Hedgecock and Pudovkin 2011). Unequal contributions among parents in a hatchery setting can also arise because some genotypes lead to faster growth or better survival. For example, hatchery brood stock of geoduck clams was found to be composed of only full-sib groups, indicating a large variance in reproductive success among spawners (Straus et al. 2015). In another example, pedigree tracing among the offspring of cultured Japanese flounder showed that in a mass spawning most of the offspring came from a single male, and half of the

Table 4 Minimal broodstock effective sizes needed to avoid a drop in the effective size of the wild-hatchery population $N_{e(WH)}$

$N_{e(W)}$	$N_{e(WH)}$	x				
		0.01	0.05	0.1	0.2	0.3
400	400	2	10	21	44	71
1000	1000	5	26	53	111	176
2000	2000	10	51	105	222	353
3000	3000	15	77	158	333	529
4000	4000	20	103	211	444	706
5000	5000	25	128	263	556	882
6000	6000	30	154	316	667	1059
7000	7000	35	179	368	778	1235
8000	8000	40	205	421	889	1412
9000	9000	45	231	474	1000	1588
10,000	10,000	50	256	526	1111	1765
12,000	12,000	60	308	632	1333	2118

The range of effective sizes of wild populations is typical for marine species. An effective to census wild population size ratio was assumed to be 10^{-4}. Minimal brood stock sizes were estimated with the Ryman–Laikre model (Ryman and Laikre 1991). $N_{e(W)}$ is the effective size of a wild population. $N_{e(WH)}$ is the effective size of the combined wild-hatchery population. x is the proportion of hatchery-reared individuals successfully reproducing in the wild

females failed to produce any offspring (Sekino et al. 2003). Reproductive skew greatly influences effective population size and the retention of adaptive potential.

Domestication

Domestication occurs when intentional, or inadvertent, selection changes genotypic frequencies in cultured cohorts. Un-intentional genetic shifts may not be entirely avoidable, because the rationale for culturing early life-history stages is to provide a protected environment to enhance the survivals of individuals that would be eliminated in a wild setting. In closed culture, selective breeding can improve the financial foundation of an operation by enhancing food efficiency, accelerating growth, or improving the quantity and quality of the end-product. Even without artificial selection, enhanced survival in a hatchery often produces shifts in the frequencies of some genotypes and leads to phenotypic changes that enhance the survivals of some genotypes over others. These shifts can lead to changes in growth, morphology and behavior that are not adaptive in the wild. For example, hatchery-reared blue crabs (*Callinectes sapidus*) in Chesapeake Bay were similar to wild blue crabs in several variables, in some behaviours and growth, but differed in having shorter spines and in lacking predator response behaviours that influenced survival in the wild (Davis et al. 2004). As another example, juveniles of Europeans lobsters cultured in a hatchery had two scissor claws instead of having one scissor claw and one crusher claw (Agnalt et al. 2004).

Many marine organisms show phenotypic plasticity in response to environmental variability. Behaviour and morphology, for example, can change in response to food availability, temperature, salinity or predation, which are variables that can be controlled during hatchery culture and which inevitably differ from natural environments. The conventional wisdom is that shifts in gene frequencies precede phenotypic changes (Schlichting and Pigliucci 1998), but in some cases, phenotypes shifts along a reactive norm toward an optimum that enhances fitness and that consequently facilitates genotypic shifts in a new environment (Ghalambor et al. 2007). Even when hatchery rearing does not lead to a genetic shift, indirect genetic effects can occur, because the survival or dominance of some individuals over others may result in the propagation of only a portion of the genetic variability.

Hatchery culture often changes morphologies and behaviours relative to wild counterparts, but without necessarily changing genotypic frequencies (Olla et al. 1998). For example, hatchery-reared juveniles of queen conchs in the Bahamas had thinner shells, shorter spines and lower burial frequencies than wild queen conchs, and hence were more susceptible to predation (Stoner and Davis 1994). However, as the conchs grew they began to resemble wild individuals in these traits. In a mesocosm study of red drum, hatchery-reared juveniles chose less structured habitats than did wild juveniles, even in the presence of a predator (Stunz et al. 2001). This behaviour was most likely the result of conditioning to hatchery conditions and did not reflect a shift in genotype. These examples represent variability along a reaction norm, but not a shift in the reaction norm itself.

Release design

Following the culture of early life-history stages in a protective hatchery environment, young individuals are released into marine waters to supplement the

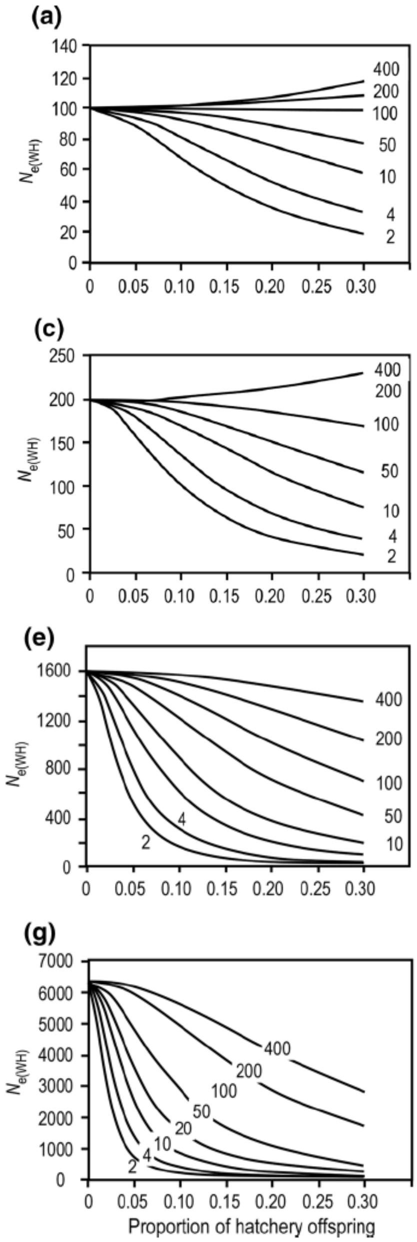


Fig. 2 *Curves* represent the combined wild-hatchery effective population sizes $N_{e(WH)}$ in relation to brood stock effective size (*number in curve*) and to the proportion of hatchery-reared individuals successfully reproducing in the wild (x). Sets of curves were produced for wild populations with effective sizes N_W, typical for local populations of marine species assuming a census: effective population size ration of 10^{-4}. **a** $N_{e(W)} = 100$; **b** $N_{e(W)} = 400$; **c** $N_{e(W)} = 200$; **d** $N_{e(W)} = 800$; **e** $N_{e(W)} = 1600$; **f** $N_{e(W)} = 3200$; **g** $N_{e(W)} = 6400$; **h** $N_{e(W)} = 12{,}800$

abundance of a stock. Successful strategies depend on two considerations. First, a match between genotype and local environment is essential because reaction norms differ not only among populations because of genetic differences in fitness traits among local habitats (Hutchings et al. 2007; Le Vay et al. 2007), but can also be altered by hatchery culture. Hence, the preservation of genetic variability during hatchery culture is essential to preserve the ability to respond to short-term environmental changes, especially for stock restorations with the goal of establishing a viable population.

Second, a release strategy has to consider numerous physical and biological variables, including carrying capacity, seasonal cycles of temperature and salinity, and habitat and food availability, as well as predation intensity. A particular life-history stage has to be released at a location that meshes with corresponding ontogenetic migrations in wild populations, as juvenile nursery areas are often separated from adult foraging areas. Both the timing and location of release are important to the success of enhancement.

Size at release

The size of hatchery-reared individuals at release is the single most important variable influencing the survival in the wild. Numerous studies showed an inverse allometric relationship between mortality and size (Lorenzen 2000; Beyer et al. 1999) so that larger individuals foraged more efficiently and better defended themselves against predators (Cowx 1994; Lorenzen 2000). However, additional hatchery costs may preclude raising individuals to larger sizes before release. For example, stock enhancements of striped bass in Chesapeake Bay were more cost-effective by releasing larvae experiencing high mortality than by releasing larger juveniles with lower mortality in years when the survival of wild larvae was high (Secor and Houde 1998; Johnson et al. 2008). In another example, a field study of blue crab stock enhancement showed that out-planting of early life-history stages was unsuccessful and that success required the use of older crabs (Eggleston et al. 2008). However, increasing the time in captivity to increase release size also increases hatchery costs and the potential for artificial selection or the expression of phenotypic traits unsuited for the natural environment.

Match between life-history stage and ecology

The timing of a release in the life cycle can influence the success of a project. The release of hatchery-cultured planktonic larvae has met with success in stock enhancements of striped bass, abalone and bay scallops, but not with Atlantic cod and hard clams (Arnold 2006). Hatchery-reared Pacific threadfin (*Polydactylus sexfilis*) were released experimentally at different sizes, at different sites and times in the years (Leber et al. 1998). Large fish survived better when released in winter, but small fish did better when released in summer and fall. In an enhancement of blue crab populations, the challenge was to balance poor survival of older juveniles in the hatchery because of cannibalism with the release of older juveniles that grew rapidly and matured in as little as two months (Davis et al. 2005). Crabs held longer in the hatchery before release recruited into the spawning population a year earlier than crabs released at an earlier age.

Life-history stages are often geographically segregated, and a release strategy must accommodate ontogenetic migrations. Seasonal migration patterns and dispersal ability of a species will influence the effectiveness of targeting a particular area. For example, gravid blue crab females in Chesapeake Bay migrate to high-salinity areas in the lower part of the bay where they spawn in early spring (Hines et al. 2008). Larvae settle into benthic habitats and metamorphose, and after feeding on spring and summer plankton blooms, juveniles migrate to the upper bay to overwinter. Juveniles mature in their second year and return to the lower part of the bay to mate and spawn. Higher stocking levels may be needed when the life-history stage being released is dispersive (Polacheck 1990). Experimental releases of blue crab larvae at the megalopae stage were not effective because the small larvae dispersed rapidly from the release site (Eggleston et al. 2008). For many marine species, spawning, nursery and adult foraging habitats are separated often by considerable distances. Atlantic cod move onshore during spring and summer and offshore in fall to aggregate along the continental shelf in winter.

Location

Release location is also of importance. If the goal of a project is to boost the abundance of a wild population, hatchery-reared individuals must be released into habitats that enhance survival and growth. Hence, the need to distinguish between self-sustaining populations in source habitats and sink populations that rely on recruits from source populations. For stock restorations, the release into ‘sink’ habitats will be successful only if the objective is to grow out juveniles for harvest and if the habitat provides shelter and food (Lipicius et al. 2008). When the objective of a project is to supplement or establish a self-recruiting population, the identification of an optimal habitat is critical for success. For example, populations of whelks (*Trochus niloticus*) in the Pacific (Heslinga et al. 1984) and hard-shell clam (*Mercenaria mercenaria*) in the Atlantic (McCay 1988) were depleted by overexploitation and were restocked along with habitat improvements. However, not all populations recovered because some of the restocking had occurred in marginal habitats.

For many marine species, recruitment may be limited by small nursery habitats (Beverton 1995; Iles and Beverton 2000; Halpern et al. 2005). A comparison of populations of the flatfish, Alaska plaice, in the Gulf of Alaska and Bering Sea showed that the size of the adult population was related to the size of the juvenile nursery habitat (Bailey et al. 2003). Even when an area is biologically and physically suitable for stocking, small-scale habitats and current patterns must be considered, because larvae or juveniles can be advected out of suitable habitats (Lipicius et al. 2008).

The availability of small-scale habitat heterogeneity may also contribute to the success of a population supplementation (Herrnkind and Cobb 2007; Ochwada-Doyle et al. 2012b). Survival of small spiny lobsters in Florida State was increased by providing artificial shelters for non-mobile juveniles, but shelter did not improve the survival of more mobile, larger juveniles (Butler and Herrnkind 1997). In Washington State, recruitment success of Dungeness crabs was associated with shelled substrates and low levels of predation (Eggleston and Armstrong 1995). Unless suitable habitats are available for the life-history stage being released, a supplementation may not succeed even though adult habitats are unsaturated.

Track individuals after release

Tracking the fates of hatchery-reared individuals in the wild is essential to provide feedback on the effectiveness of various hatchery practices and release strategies (Lorenzen 2006). Many supplementations focus on mass production with little attention to the effectiveness of supplementation (Solemdal et al. 1984; Secor and Houde 1998; Secor et al. 2002). Monitoring success is often difficult when climate variability counters or augments supplementation efforts. Several approaches have been used to measure the results of a stock enhancement.

Demographic analysis

For stock restorations, the abundance of spawning biomass is key to the success of a project. The assumption that the abundance of a stock should increase in proportion to the size of the supplementation can be subverted by the confounding effects of environmental variability and harvest intensity (e.g. Serafy et al. 1999). Additionally, density-dependent controls of abundance may come into play when a habitat is close to carrying capacity (Lorenzen 2005). For example, stock enhancements of bay scallops (*Argopecten irradians*) were tracked with larval recruitment collectors and juvenile quadrate surveys, but with little evidence of recruitment success (Leverone et al. 2010). It was uncertain whether an increase in abundance was due to stock-enhancement efforts or to changing environmental conditions. Meta-analyses often show that substantial supplementation effort does not always lead to larger stock abundance (Masuda and Tsukamoto 1998). Marine populations tend to vary naturally because small environmental changes can precipitate a cascade of trophic events that influence survival, fecundity and recruitment into the adult population.

Genetic population structure

Genetic population surveys have been used to assess the impact of hatchery releases by searching for shifts in allele frequencies. Several seeding attempts of the red abalone (*Haliotis rufenscens*) in California were made in the 1970s with little success, because of predation and emigration (Gaffney et al. 1996). However, one large-scale out-planting appeared to have been successful, because samples from the area of supplementation showed allele-frequency differences from wild populations, departures from Hardy–Weinberg proportions and significant disequilibria between loci. In another example, the analysis of microsatellite loci in red sea bream (*Pagrus major*) indicated that stocked populations showed larger values of differentiation and significant departures from Hardy–Weinberg proportions that were interpreted to reflect supplementation (Perez-Enriquez et al. 2001). Surveys of microsatellite variability among populations of black (*Acanthopagrus schlegelii*) and red (*P. major*) sea bream around Japan showed that hatchery releases produced patches of genetic differentiation in an otherwise panmictic species (Blanco Gonzalez and Umino 2009; Blanco Gonzalez et al. 2015). However, no genetic population changes were observed in Pacific herring (*Clupea pallasii*) (Sugaya et al. 2008) or in Ezo abalone (*Haliotis discus hannai*) (Sekino et al. 2005) after large supplementations. The analysis of genetic population structure provides only a small amount of power to detect the effects of supplementation because the effects of environmental change, random drift and migration can override small genetic signals of supplementation.

Phenotypic markers

Hatchery culture often leaves phenotypic marks that distinguish hatchery-reared from wild individuals. For example, marks on fish scales and otoliths have been used to distinguish hatchery-reared fish from wild fish (Butcher et al. 2003). Starvation and an abrupt shift to lower salinity created checks in the scales of hatchery-cultured mulloway (*Argyrosomus japonicus*) in Australia that easily identified cultured fish after release (Taylor and Piola 2008). In Japan, hatchery-origin red sea bream could be identified in commercial catches by a hatchery-induced deformity of the inter-nostril epidermis, and hatchery flounder could be identified by induced pigmentation on the bottom side of a fish (Kitada and Kishino 2006). Hatchery-reared individuals of the queen conch are distinguishable after release by their thin shells (Stoner and Davis 1994). For most species, however, phenotypic markers cannot be reliably used to identify hatchery-reared individuals after release so that physical or genetic tags must be used.

Physical tags

A variety of physical tags has been used to mark hatchery-reared individuals. Cultured prawns in Japanese waters were marked with coded wire tags, along with clipped uropods to identify individuals with tags (Hamasaki and Kitada 2008a). North Sea European lobsters were implanted with fluorescent tags, which indicated that after about 4 years 3–8% of captured lobsters were of hatchery origin (Schmalenbach et al. 2011). Internal tags and external marks have also been used to identify hatchery-reared lobsters (Linnane and Mercer 1998; van der Meeren 2000). Chemical markers have been useful for marking hatchery individuals of some species. The survivals of hatchery juveniles of sea cucumbers marked with fluorochromes varied greatly among locations (Purcell and Simutoga 2008). While physical tags can potentially track hatchery-reared individuals over a lifetime, they cannot measure reproductive successes of the hatchery-reared individuals.

Genetic markers

Molecular genetic markers have been used to monitor several aspects of supplementation, including levels of genetic diversity, effective number of hatchery parents, survival rates, contribution to harvests and importantly the extent of hybridization between hatchery and wild individuals. In sea ranching projects, in which individuals are released into the wild but are expected to be harvested before maturity, genetic markers can identify first generation hatchery-reared individuals or hybridizations between hatchery-reared and wild individuals. However, this is possible only when hatchery cohorts have genetically diverged from wild populations, or when brood stock have been genotyped so that parentage-based tagging can be used to identify individuals of hatchery origins (Anderson and Garza 2006; Tringali 2006). Mimura et al. (2017) and Bruford et al. (2017) provide reviews of methods to monitor intraspecific genetic diversity with markers at neutral and functional genes.

Genetic markers lend themselves to identifying hatchery-reared individuals. For example, individual assignment tests, based on 8 microsatellites and mtDNA, indicated that about 10% of 1579 eastern oysters (*Crassostrea virginica*) were first generation hybrids between a disease tolerant hatchery stock and wild individuals (Hare et al. 2006). In a supplementation of European lobsters in Norway, family genotypic marking with microsatellites indicated that only 60% of cultured larvae survived relative to wild larvae and that survival of offspring varied greatly among families (Jøstad et al. 2005). In another project, a survey of mtDNA SNPs in a sample of 3545 oyster spat after an out-planting into Chesapeake Bay of over four million Gulf Coast oysters from Louisiana indicated that only 0.08% of the 3545 spat had the Gulf Coast haplotype (Milbury et al. 2004).

When the goal of a supplementation is to enhance the spawning biomass of a wild population, genetic markers can additionally be used to estimate effective population size and to track introgressive hybridizations in subsequent generations (Pritchard et al. 2000; Nielsen et al. 2006; Karlsson et al. 2008; Saillant et al. 2009). For example, genetic markers indicated that the effective size of hatchery-supplemented populations of red drum had increased over 20 years (Carson et al. 2009). However, a survey of microsatellite and mtDNA variability in Japanese populations of Spanish mackerel (*Scomberomorus niphonius*) indicated that the effective size of a wild population after supplementation was only 430–970 fish (Nakajima et al. 2014). A study of chum salmon in Alaska showed that hatchery strays had hybridized with wild salmon at several locations (Jasper et al. 2013).

Minimize genetic impacts on wild populations

The goals of stock supplementation are two-fold: first, to increase the abundance of the target stock and second to avoid disrupting the genetic integrity of local stocks. Even though individuals of many marine species potentially disperse long distances, several recent studies show local adaptations on much smaller spatial scales than expected (Lamichhaney et al. 2012) that can be disrupted by introgressive hybridization. The release of large numbers of individuals can influence the genetic structure of a target species by leading to drops in genetic diversity and by disrupting genetic population integrity (Arnaud-Haond et al. 2006; Bekkevold et al. 2006).

Ecological effects of supplementation

The displacement of wild individuals can occur with persistent releases of hatchery-reared offspring when release numbers are large and habitats are near carrying capacity (Tringali and Bert 1998). It was not clear whether the long-term enhancements of American and European lobster populations augmented, or displaced, wild populations (Addison and Bannister 1994; Hilborn 1998). Even when wild populations are not displaced by hatchery supplementation, the persistent release of hatchery-reared individuals can still influence wild populations when the carrying capacity is exceeded. Density-dependent factors can intensify competition for food or space and can alter growth patterns and reproductive output, which in turn can reduce the effective population sizes and potentially lead to the loss of adaptive potential (Ochwada-Doyle et al. 2012a). Fishery harvests can intensify replacement of wild-origin individuals, as successful supplementation programs produce many more offspring per individual than occurs in the wild; consequently, any harvest rate removes a larger fraction of the wild than the hatchery surplus offspring.

Hatchery-wild hybridization

A variety of genetic influences can ensue when hatchery individuals interbreed with wild individuals. First-generation hybrids may not be as fit as wild individuals in a natural setting and may not contribute to the next generation because of reduced survival. In this case, reproductive potential is lost in the wild population. When hybrids survive and successfully reproduce, maladaptive genes may be introduced into the wild population. Theoretical results show that introgressive hybridizations can lead to drops in fitness in only a few generations and the magnitudes of these drops depend, in part, on the number of generations a brood stock has been cultivated in a hatchery under relaxed or positive selection for maladaptive or domestication genes (Lynch and O'Hely 2001).

Highly fecund species

Apart from reductions in fitness from hatchery-wild hybridizations, supplementations have the potential to reduce the effective population size of wild populations, because most invertebrates produce enormous numbers of offspring. Limited hatchery capacity for maintaining large numbers of brood stock also encourages the used of only a few individuals for brood stock. For example, brood stock of large supplementations of Spanish mackerel in Japan consisted of only about 10 fish, because of the short timeframe of maturity and the logistics of artificially mating fish at sea (Nakajima et al. 2014). The large number of offspring from these few fish was able to satisfy the production goals of the hatchery, but represented only a fraction of the genetic diversity in the population.

The loss of genetic diversity from small brood-stock sizes can lead to inbreeding depression (Charlesworth and Charlesworth 1987; Crnokrak and Roff 1999). Experiments with purple sea urchins (*Strongylocentrotus purpuratus*) showed that inbred larvae were smaller and showed greater variation in size than did outcrossed individuals (Anderson and Hedgecock 2010). Segovia-Viadero et al. (2016) found that hatchery-reared populations of the European purple sea urchin (*Paracentrotus lividus*) were inbred and had genetically diverged from wild populations.

Effective size of wild population after supplementation

An important goal is to ensure that the effective population size of a wild population does not drop below its original size (Box 4). Successful supplementations that boost the effective size of a wild population depend on several factors. One is the effective size of the hatchery brood stock relative to the effective size of the wild population (Waples et al. 2016). The ratio of the effective to census size of hatchery brood stock is commonly less than 1.0 because of hatchery culture practices. Supplementation boosts the effective size of a wild population only when the hatchery effective size is large relative to the number of offspring and the effective size of the wild population is small relative to the number of individuals. Supplementations with individuals from a hatchery population with a small effective size cannot increase the effective size of the wild population when the effective size of the wild population is large.

A second factor is the proportion of hatchery individuals incorporated into a wild population: the greater the supplementation effort, the greater supplementation will influence a wild population. A small effective brood-stock size will dilute genetic diversity in wild populations with most effective brood-stock sizes and the dilution effect increases with an increase in the magnitude of the supplementation. Generally, when hatchery-origin individuals make up more than 10% of the wild population, its total effective population size will drop precipitously unless the ratio of the effective size to the census size of the wild population is small.

Discussion

Many marine fish and invertebrate stocks around the globe are threatened by over harvesting and habitat degradation, hence the growing interest in stock enhancements with hatchery-reared individuals. Declining harvests of wild populations have stimulated the culture and release of billions of fish for over 300 species annually (Welcomme and Bartley 1998). While most enhancements have been of freshwater species, a growing number of marine species are now under culture. Marine stock enhancements are relatively recent compared to the birth of agriculture 9000 years ago. The use of hatchery-reared invertebrates and fishes for sea ranching, stock restoration

Box 4 Genetic dilution effect of hatchery supplementation

Waples et al. (2016) provide refinements of the Ryman–Laikre model by resolving the variables into sub-components. First, the effective size of a wild population can drop when a substantial number of individuals are removed for brood stock. This may be especially important for population restorations, attempting to restore a depleted population to its previous size. A large brood stock size, relative to wild population size, reduces the effective size of the wild population ($N_{e(W)}$). In this case, the total effective size of the wild population after supplementation ($N_{e(HW)}$) would otherwise be overestimated

A further refinement shows that the combined wild-hatchery effective size ($N_{e(HW)}$) can be influenced by the ratios of the relative effective sizes of hatchery $\alpha_H = N_{e(H)}/N_{c(H)}$ and wild $\alpha_W = N_{e(W)}/N_{c(W)}$ populations, where N_c is census size. Only when the ratio of these values, $\beta = \alpha_H/\alpha_W$, is larger than 1.0 can supplementation increase the effective size of the total population. The ratio β can be thought of as a dilution factor: only the addition of a more concentrated solution of a substance can increase the concentration of the substance in another solution

As the magnitude of a supplementation (x) increases, the value of β becomes more important in determining the effective size of the supplemented wild population. Large supplementations with small values of β can lead to large drops in the effective size of the wild population, because of the dilution effect. However, when α_W is small, as appears to be the case for many marine species, β is larger than 1.0 for a given value of α_H, and supplementation can lead to an increase in the effective size of the wild-hatchery population. Alternatively, for a given value of α_W, an increase in the value of α_H can lead to a positive supplementation. Hence, the need to provide sufficiently large hatchery facilities to be able to implement culture practices that maximize $\alpha_H = N_{e(H)}/N_H$

and stock supplementation will become more common as the abundances of wild populations decline from exploitation and climate change and as markets for seafoods continue to grow.

Unlike agricultural production on land, the production of seafood depends on wild populations as primary sources of exploitable biomass and as sources for the development of semi-domesticated and fully domesticated strains for aquaculture and sea ranching. The developments of hatchery technology and procedures are at early stages and, while these developments parallel the inception of land agriculture nine thousand years ago, they are progressing much more rapidly at a scale that has the potential to harm wild populations. The conservation of not only the abundances of wild populations, but also the genetic foundations for population sustainability and adaptive potential, is important for prudent development of seafood husbandry.

Despite supplementation efforts for several species, post-release evidence does not show unequivocal success in enhancing wild populations of marine species. Enhancement efforts in marine waters began in the late 1800s with the release of Atlantic cod (*G. morhua*) larvae. Restocking with larvae has been met with mixed success. Many enhancement attempts with Atlantic cod and hard clams, *Mercenaria* spp. were unsuccessful, but some successes were achieved with enhancements of abalone (*Haliotis* spp.) and bay scallops (*A. irradians*) (Arnold 2006). In some cases, stock enhancements may have led to the replacement rather than supplementation of a stock (Leber et al. 1995).

Mariculture in its various forms has the potential to influence wild populations in several ways. Large-scale operations can adversely influence the local ecosystems by displacements and extinctions of local species, by resource depletion and competition, by pollution that shifts nutrient and oxygen availability and by genetic risks that influence the fitnesses of local populations and their adaptive potentials. The development of these guidelines focuses on the dynamics of genetic risks, which are not as easily tracked as environmentally induced shifts in abundance and in market quality of seafood products. Nevertheless, genetic influences on sustainable exploitation are just as important.

The release of hatchery-reared individuals to rebuild a depleted stock should be used as a last resort after other measures have failed, after it is certain that the stock is recruitment limited and when the ecosystem is not at carrying capacity. It is depressingly common that the benefits of a hatchery program are assumed, and that success is measured by the number of hatchery-produced individuals released. Evaluation of the program's effectiveness in increasing population abundance and any negative effects on the wild population are episodic, often undertaken by independent groups. Continual monitoring and regular review of hatchery programs are necessary to ensure that objectives are being met and that ecological and genetic impacts on the wild population are within acceptable limits. This monitoring and review should be part of the design and the budget of any hatchery program.

Three strategies can be used to rebuild a depleted stock (Molony et al. 2004). First, the most ecologically friendly is to limit harvests of the stock by restricting gear, reducing fishery openings, lowering quotas, or restricting entry into the fishery. This approach is the least genetically intrusive. Fishing reductions, however, are often hard to implement because of the push-back from fishing and fish-processing communities. Recovery of a stock after fishery restrictions may take several generations and may be unacceptably slow from a fishing community's perspective.

Second, a restriction on a fishery may not be enough when a decline has resulted from habitat deterioration or loss. Hence, habitat restoration or modification (e.g. with artificial reefs, Buckley 1989) may be a way of increasing abundance by increasing carrying capacity. While habitat modifications on small scales have been effective in increasing local diversity, habitat restorations on large scales in the marine environment are difficult to achieve (Bohnsack et al. 1991). Regional habitat shifts and concurrent drops in fishery populations may be due to large-scale, uncontrollable climate changes.

Third, stock enhancements can potentially increase the abundance of a stock, but only when the release of hatchery-reared individuals circumvents a choke point in the flow of individuals through various life-history stages. Stock supplementations are often viewed as an immediate solution to a stock decline, but should be undertaken only as a last resort because of the generally high cost of implementation and the substantial ecological and genetic risks to wild populations (Heppell and Crowder 1998). Several facets of supplementation must be evaluated before a program begins, including the availability of hatchery technology, survival of hatchery-reared individuals once they are released, economic benefits and market trends. The objective of a supplementation is best defined before the start of an operation, and importantly planners must consider the risks from supplementation not only to the target species, but also to the community and ecosystem.

The best genetic practice is to culture individuals for the fewest number of generations as possible to minimize genetic changes and to use large brood stock sizes to reduce the loss of genetic diversity. Some culture practices, such as the use of small brood-stock sizes, unequal sex ratios, or unequal family sizes, tend to reduce the effective population size of the cultured population and can lead to rapid genetic shifts from random drift and to the loss of genetic diversity. Even though large numbers of offspring can be produced from only a few parents, the important variable is brood stock size. Large enhancements with small brood-stock sizes can greatly reduce the effective population size of a wild-hatchery population.

Genetic principles play important roles, directly and indirectly, in determining not only the economic success of a supplementation project, but also in maintaining the ecological vigor of wild populations. The costs of infrastructure and culture may be larger than economic returns on investments and the long-term ecological and genetic costs of supplementations may preclude embarking on a stock restoration or enhancement. Adaptive potential can be influenced by brood-stock choice, hatchery practices, domestication, timing of release, release location and magnitude of the supplementation. While the genetic effects of supplementations are difficult to measure and are not always immediately apparent, they have the potential for long-term impacts on wild genetic resources.

Acknowledgements C. Habicht, H. Liller, W. Templin and R.S. Waples provided comments on various drafts of the manuscript. The writing of this review was supported by Saltonstall-Kennedy Grant 15AKR009 and by North Pacific Research Board Projects 1526 and 1618.

References

Addison JT, Bannister RCA (1994) Re-stocking and enhancement of clawed lobster stocks: a review. Crustaceana 67:131–155

Agashe D (2009) The stabilizing effect of intraspecific genetic variation on population dynamics and in novel and ancestral habitats. Am Nat 174:255–267

Agnalt A-L, Jøstad KE, Kristiansen T, Nøstvold E, Farestveit E, Næss H, Paulsen OI, Svåsand T (2004) Enhancing the European lobster (*Homarus gammarus*) stock at Kvitsøy Islands: perspectives of rebuilding Norwegian stocks. Ch 30. In: Leber KM, Kitada S, Svåsand T, Blankenship HL (eds) Stock enhancement and sea ranching, 2nd edn. Blackwell, Oxford, pp 415–426

Aiken DE, Waddy SL (1986) Environmental influence on recruitment of American lobster, *Homarus americanus*: a perspective. Can J Fish Aquat Sci 43:2258–2270

Alheit J, Hagen E (1997) Long-term climate forcing of European herring and sardine populations. Fish Oceanogr 6:130–139

Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. Zoo Biol 5:181–190

Allendorf FW, Luikart G, Aitken SN (2013) Conservation and the genetics of populations, 2nd edn. Wiley, Oxford

Anderson EC, Garza JC (2006) The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics 172:2567–2582

Anderson D, Hedgecock D (2010) Inbreeding depression and growth heterosis in larvae of the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson). J Exp Mar Biol Ecol 384:68–75

Anderson PJ, Piatt JF (1999) Community reorganization in the Gulf of Alaska following ocean climate regime shift. Mar Ecol Prog Ser 189:117–123

Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318:100–103

Araki H, Berejikian BA, Ford MJ, Blouin MS (2008) Fitness of hatchery-reared salmonids in the wild. Evol Appl 1:342–355

Arkush KD, Giese AR, Mendonca HL, McBride AM, Marty GD, Hedrick PW (2002) Resistance to three pathogens in the endangered winter-run Chinook salmon (*Oncorhynchus tshawytscha*): effects of inbreeding and major histocompatibility complex genotypes. Can J Fish Aquat Sci 59:966–975

Arnaud-Haond S, Vonau V, Bonhomme F, Boudry P, Blanc F, Prou J, Seaman T, Goyard E (2004) Spatio-temporal variation in the genetic composition of wild populations of pearl oyster (*Pinctada margaritifera comingii*) in French Polynesia following 10 years of juvenile translocation. Mol Ecol 13:2001–2007

Arnaud-Haond S, Teixeira S, Massa SI, Billot C, Saenger P, Coupland G, Duarte CM, Serrao EA (2006) Genetic structure at range edge: low diversity and high inbreeding in Southeast Asian mangrove (Avicennia marina) populations. Mol Ecol 15(12):3515–3525

Arnold WS (2006) Application of larval release for restocking and stock enhancement of coastal marine bivalve populations. Rev Fish Sci 16:65–71

Ashley MV, Willson MF, Pergams ORW et al (2003) Evolutionarily enlightened management. Biol Conserv 111:115–123

Audzijonyte A, Kuparinen A, Gorton R, Fulton EA (2013) Ecological consequences of body size decline in harvested fish species: positive feedback loops in trophic interactions amplify human impact. Biol Lett 9:20121103

Bailey KM, Brown ES, Duffy-Anderson JT (2003) Aspects of distribution, transport and recruitment of Alaska plaice (*Pleuronectes quadrituberculatus*) in the Gulf of Alaska and eastern Bering Sea: comparison of marginal and central populations. J Sea Res 50:87–95

Bailey JK, Schweitzer JA, Úbeda F et al (2009) From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. Philos Trans R Soc B 364:1607–1616

Bailey JK, Genung MA, Ware I, Gorman C, van Nuland ME et al (2014) Indirect genetic effects: an evolutionary mechanism linking feedbacks, genotypic diversity and coadaptation in a climate change context. Funct Ecol 28:87–95

Bannister RCA, Addison JT (1998) Enhancing lobster stocks: a review of recent European methods, results and future prospects. Bull Mar Sci 62:369–387

Barrett RD, Schluter D (2008) Adaptation from standing genetic variation. Trends Ecol Evol 23:38–44

Bechtol WR, Kruse GH (2009a) Analysis of a stock-recruit relationship for red king crab off Kodiak Island, Alaska. Mar Coast Fish 1:29–44

Bechtol WR, Kruse GH (2009b) Reconstruction of historical abundance and recuitment of red king crab during 1960–2004 around Kodiak, Alaska. Fish Res 100:86–98

Bekkevold D, Hansen MM, Nielsen EE (2006) Genetic impact of gadoid culture on wild fish populations: predictions, lessons from salmonids, and possibilities for minimizing adverse effects. ICES J Mar Sci 63:198–208

Bekkevold D, Gross R, Arula T, Helyar SJ, Ojaveer H (2016) Outlier loci detect intraspecific biodiversity amongst Spring and Autumn spawning herring across local scales. concentration hypothesis. Neth J Sea Res 34:1–6

Beyer JE, Kirchner CH, Holtzhausen JA (1999) A method to determine size-specific natural mortality applied to west coast steenbras (*Lithognathus aureti*) in Namibia. Fish Res 41:133–153

Blake SG, Black NJ, Oesterling MJ, Graves JE (1997) Genetic divergence and loss of diversity in two cultured populations of the bay scallop, *Argopecten irradians* (Lamarck, 1819). J Shellfish Res 16:55–58

Blanco Gonzalez E, Umino T (2009) Fine-scale genetic structure derived from stocking black sea bream *Acanthopagrus schlegelii* (Bleeker, 1854), in Heroshima Bay, Japan. Jpn J Appl Ichthyol 25:407–410

Blanco Gonzalez E, Aritaki M, Knutsen H, Taniguchi N (2015) Effects of large-scale releases on the genetic structure of red sea bream (*Pagrus major*, Temminck et Schlegel) populations in Japan. PLoS ONE 10:e0125743

Blankenship HL, Leber KM (1995) A responsible approach to marine stock enhancement. Am Fish Soc Symp 15:167–175

Blaxter JHS (2000) The enhancement of marine fish stocks. Adv Mar Biol 38:2–54

Bohnsack JA, Johnson DL, Ambrose RF (1991) Ecology of artificial reef habitats and fishes. In: Seaman WR Jr, Sprague LM (eds) Artificial habitats for marine and freshwater fisheries. Academic Press, London, pp 61–107

Botsford LW, Castilla JC, Peterson CH (1997) The management of fisheries and marine ecosystems. Science 277:509–515

Botsford LW, Nicheli F, Hastings A (2003) Principles for the design of marine reserves. Ecol Appl 13:25–31

Brown C, Day RL (2002) The future of stock enhancements: lessons for hatchery practice from conservation biology. Fish Fish 3:79–94

Bruford MW, Davies N, Dulloo ME, Faith DP, Walters M (2017) Monitoring changes in genetic diversity. In: Walters M, Scholes RJ (eds) The GEO handbook on biodiversity observation networks. Springer International Publishing, pp 107–128

Brumbaugh RD, Coen LD (2009) Contemporary approaches for small-scale oyster reef restoration to address substrate *versus* recruitment limitation: a review and comments relevant for the Olympia oyster, *Ostrea lurida* Carpenter 1864. J Shellfish Res 28:47–161

Brush SB (1995) In situ conservation of landraces in centers of crop diversity. Crop Sci 35:346–354

Buckley RM (1989) Habitat alterations as a basis for enhancing marine fisheries. CalCOFI Rep 30:40–45

Bürger R, Lynch M (1995) Evolution and extinction in a changing environment: a quantitative genetic analysis. Evolution 49:151–163

Busack CA, Currens KP (1995) Genetic risks and hazards in hatchery operations: fundamental concepts and issues. Am Fish Soc Symp 15:71–80

Butcher A, Mayer D, Willet D, Johnston M, Smallwood D (2003) Scale pattern analysis is preferable to OTC marking of otoliths for differentiating between stocked and wild dusky flathead, *Platycephalus fuscus*, and sand whiting, *Sillago ciliata*. Fish Manag Ecol 10:163–172

Caballero A, Garcia-Dorado A (2013) Allelic diversity and its implications for the rate of adaptation. Genetics 195:1373–1384

Caley MJ, Carr MH, Hixon MA, Hughes TP, Jones GP, Menge BA (1996) Recruitment and the local dynamics of open marine populations. Ann Rev Ecol Syst 27:477–500

Carson EW, Karlsson S, Saillant E, Gold JR (2009) Genetic studies of hatchery-supplemented populations of red drum in four Texas bays. N Am J Fish Manag 29:1502–1510

Chaoui L, Gagnaire PA, Guinand B, Quignard JP, Tsigenopoulos C, Kara MH, Bonhomme F (2012) Microsatellite length variation in candidate genes correlates with habitat in the gilthead sea bream Sparus aurata. Mol Ecol 21:5497–5511

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Ann Rev Ecol Syst 18:237–268

Charlesworth D, Morgan MT, Charlesworth B (1993) Mutation accumulation in finite outbreeding and inbreeding populations. Genet Res 55:39–56

Chen K, Ciannelli L, Decker MB et al (2014) Reconstructing sour-sink dynamics in a population with a pelagic dispersal phase. PLoS ONE 9:e95316

Chesson P (1998) Recruitment limitation: a theoretical perspective. Austral J Ecol 23:234–240

Christensen V, Guenette S, Heymans JJ et al (2003) Hundred-year decline of North Atlantic predatory fishes. Fish Fish 4:1–24

Coleman F, Travis J, Thistle AB (1998) Marine stock enhancement: a new perspective. Bull Mar Sci 62:303

Conover DO (1998) Local adaptation in marine fishes: evidence and implications for stock enhancement. Bull Mar Sci 62:477–493

Conover DO, Munch SB (2002) Sustaining fisheries yields over evolutionary time scales. Science 297:94–96

Conover DO, Clarke LM, Munch SB, Wagner GN (2006) Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. J Fish Biol 69:21–47

Cowx IG (1994) Stocking strategies. Fish Manag Ecol 1:15–30

Crnokrak P, Roff DA (1999) Inbreeding depression in the wild. Heredity 83:260–270

Crow JF, Kimura M (1970) An introduction to population genetics theory. Harper and Row, New York

Crowder LB, Lyman SJ, Figueira WF, Priddy J (2000) Source-sink population dynamics and the problem of siting marine reserves. Bull Mar Sci 66:799–820

Cury P, Roy C (1989) Optimal environmental window and pelagic fish recruitment success in upwelling areas. Can J Fish Aquat Sci 46:670–680

David P (1998) Heterozygosity-fitness correlations: new perspectives on old problems. Heredity 80:531–537

Davis JLD, Young AC, Aguilar R (2004) Differences between hatchery-raised and wild blue crabs (*Callinectes sapidus*): implications for stock enhancement potential. Trans Am Fish Soc 133:1–14

Davis JLD, Young-Williams AC, Hines AH, Zohar Y (2005) Assessing the potential for stock enhancement in the case of the Chesapeake Bay blue crab (*Callinectes sapidus*). Can J Fish Aquat Sci 62:109–122

Doherty PJ (1999) Recruitment limitation is the theoretical basis for stock enhancement in marine populations. In: Howell BR, Moksness E, Svåsand T (eds) Stock enhancement and sea ranching. Fishing News Books, Oxford, pp 9–21

Eggleston D, Armstrong DA (1995) Pre- and post-settlement determinants of estuarine Dungeness crab recruitment. Ecol Monogr 65:193–216

Eggleston DB, Johnson EG, Kellison GT, Plaia GR, Huggett CL (2008) Pilot evaluation of early juvenile blue crab stock enhancement using a replicated BACI design. Rev Fish Sci 16:91–100

Evans D, Bartlett J, Sweijd N, Cook P, Elliott NG (2004) Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*). Aquaculture 233:109–127

Exadactylos A, Geffen AJ, Thorpe JP (1999) Growth and genetic variation in hatchery-reared larval and juvenile Dover sole, *Solea solea* (L.). Aquaculture 176:209–226

Falconer DF, Mackay TFC (1996) Introduction to quantitative genetics. Prentice Hall, New York

FAO (UN Food and Agriculture Organization) (2013) In vivo conservation of animal genetic resources. FAO Animal Production and Health Guidelines. No. 14, Rome

FAO (UN Food and Agriculture Organization) (2014) The state of world fisheries and aquaculture. No. 3, Rome

Fogarty MJ, Botsford LW (2007) Population connectivity and spatial management of marine fisheries. Oceanography 20:112–123

Fogarty MJ, Idoine JS (1986) Recruitment dynamics in an American lobster (*Homarus americanus*) population. Can J Fish Aquat Sci 43:2368–2376

Ford MJ, Fuss H, Boelts B, LaHood E, Hard J, Miller J (2006) Changes in run timing and natural smolt production in a naturally spawning coho salmon (*Oncorhynchus kisutch*) population after 60 years of intensive hatchery supplementation. Can J Fish Aquat Sci 63:2343–2355

Fox CJ, Planque BP, Darby CD (2000) Synchrony in the recruitment time-series of plaice (*Pleuronectes platessa* L.) around the United Kingdom and the influence of sea temperature. J Sea Res 44:159–168

Francis RC, Hixon MA, Clarke ME, Murawski SA, Ralston S (2007) Ten commandments for ecosystem-based fisheries scientists. Fisheries 32:217–233

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. Genet Res 66:95–107

Frankham R (2005) Genetics and extinction. Biol Conserv 126:131–140

Frankham R, Bradshaw C, Brook RW (2014) Genetics in conservation management: revised recommendations for the 50/500 rule, Red List criteria and population viability analyses. Biol Conserv 170:56–63

Fujio Y, von Brand E (1991) Differences in degree of homozygosity between seed and sown populations of the Japan Scallop *Patinopecten yessoensis*. Nipp Suis Gakk 57:45–50

Gaffney PM, Rubin VP, Hedgecock D, Powers DA, Morris G, Hereford L (1996) Genetic effects of artificial propagation: signals from wild and hatchery populations of red abalone in California. Aquaculture 143:257–266

Gagnaire P-A, Broquet T, Aurelle D, Viard F, Souissi A, Bonhomme F, Arnaud-Haond S et al (2015) Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. Evol Appl 8:769–786

Gagnaire P-A, Gaggiotti OE (2016) Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. Curr Zool 62(6):603–616

Gamble RJ, Link JS (2012) Using an aggregate production simulation model with ecological interactions to explore effects of fishing and climate on a fish community. Mar Ecol Prog Ser 459:259–274

Gamfeldt L, Källström B (2007) Increasing intraspecific diversity increased predictability in population survival in the face of perturbations. Oikos 116:700–705

Gamfeldt L, Wallén J, Jonsson PR, Bentsson KM, Havenhand JN (2005) Increasing intraspecific diversity enhances settling success in a marine invertebrate. Ecology 86:3219–3224

Garibaldi L (2012) The FAO global capture production database: a six-decade effort to catch the trend. Mar Policy 36:760–768

Gascoigne J, Lipcius RH (2004) Allee effects in marine systems. Mar Ecol Prog Ser 269:49–59

Geldenhuys G, Glanzmann B, Lombard D, Moolay S, Carr J, Bardien S (2014) Identification of a common found couple for 40 South African Afrikaner families with Parkinson's disease. S Afr Med J 104:413–419

Ghalambor CJ, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol 21:394–407

Gharrett AJ, Joyce J, Smoker WW (2013) Fine-scale temporal adaptation within a salmonid population: mechanism and consequences. Mol Ecol 22:4457–4469

Gilk SE, Wang IA, Hoover CL, Smoker WW, Taylor SG, Gray AK, Gharrett AJ (2004) Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. Environ Biol Fish 69:287–297

Griffies SM, Bryan K (1997) Predictability of North Atlantic multidecadal climate variability. Science 275:181–184

Gutierrez-Gonzalez JL, Perez-Enriquez R (2005) A genetic evaluation of stock enhancement of blue abalone *Haliotis fulgens* in Baja California, Mexico. Aquaculture 247:233–242

Haddaway NR, Woodcock P, Macura B, Collins A (2015) Making literature review more reliable through application of lessons from systematic reviews. Conserv Biol 29:1596–1605

Halpern BS, Gaines SD, Warner RR (2005) Habitat size recruitment, and longevity as factors limiting population size in stage-structured species. Am Nat 165:82–94

Hamasaki K, Kitada S (2008a) Potential of stock enhancement for decapod crustaceans. Rev Fish Sci 16:164–174

Hamasaki K, Kitada S (2008b) The enhancement of abalone stocks: lessons from Japanese case studies. Fish Fish 9:243–260

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. Mol Ecol 11:2467–2474

Hare MP, Allen SK Jr, Bloomer P, Camara MD, Carnegie RB, Murfree J, Luckenbach M et al (2006) A genetic test for recruitment enhancement in Chesapeake Bay oysters, *Cassostrea virginica*, after population supplementation with a disease tolerant strain. Conserv Genet 7:717–734

Harvell CD, Kim K, Burkholder JM et al (1999) Emerging marine diseases: climate Links and anthropogenic factors. Science 285:1505–1510

Hauser L, Adcock GH, Smith PJ, Bernal Ramirez JH, Carvalho GR (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proc Natl Acad Sci USA 99:1742–11747

Hedgecock D (1994) Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont AR (ed) Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp 122–134

Hedgecock D (2004) Quantifying and minimizing risk that hatchery-enhancement will reduce genetic diversity of white seabass. Final report, California Sea Grant College Program, University of California, San Diego. http://repositories.cdlib.org/csgc/rcr/Fisheries04_03

Hedgecock D, Coykendall K (2007) Genetic risks of marine hatchery enhancement: the good, the bad, and the unknown. In: Bert TM (ed) Ecological and genetic implications of aquaculture activities. Springer, Dordrecht, pp 85–101

Hedgecock D, Pudovkin AI (2011) Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. Bull Mar Sci 87:971–1002

Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines. Trends Ecol Evol 23:202–210

Helson JG, Gardner JPA (2004) Contrasting patterns of mussel abundance at neighbouring sites: does recruitment limitation explain the absence of mussels on Cook Strait (New Zealand) shores? J Exp Mar Biol Ecol 312:285–298

Hendry AP, Kinnison MT, Heino M et al (2011) Evolutionary principles and their practical application. Evol Appl 4:159–183

Heppell SS, Crowder LB (1998) Prognostic evaluation of enhancement programs using population models and life history analysis. Bull Mar Sci 62:495–507

Herrnkind WF, Cobb JS (2007) Artificial shelters for clawed and spiny lobsters: a critical review of enhancement efforts. Am Fish Soc Symp 49:587–594

Heslinga GA, Orak O, Ngiramengior M (1984) Coral reef sanctuaries for Trochus shells. Mar Fish Rev 46:73–80

Hilborn R (1998) The economic performance of marine stock enhancement projects. Bull Mar Sci 62:661–674

Hilborn R, Quinn T, Schindler D, Rogers D (2003) Biocomplexity and fisheries sustainability. Proc Natl Acad Sci USA 100:6564–6568

Hill WG, Goddard ME, Visscher PM (2008) Data and theory point to mainly additive genetic variance for complex traits. PLoS Genet 4:e1000008

Hines AH, Johnson EG, Young AC, Aguilar R, Kramer MA, Goodison M, Zmora O, Zohar Y (2008) Release strategies for estuarine species with complex migratory life cycles: stock enhancement of Chesapeake blue crabs (*Callinectes sapidus*). Rev Fish Sci 16:175–185

Hoban S, Hauffe HC, Pérez-Espona S, Arntzen JW, Bertorelle JW, Bryja J, Frith K et al (2013) Bringing genetic diversity to the forefront of conservation policy and management. Conserv Genet Resour 5:593–598

Hoban S, Arntzen JA, Bruford MW et al (2014) Comparative evaluation of potential indicators and temporal sampling protocols for monitoring genetic erosion. Evol Appl 7:984–998

Hoffman AA, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol Evol 14:96–101

Hold N, Murray LG, Kaiser MJ, Hinz H, Beaumont AR, Taylor MI (2013) Potential effects of stock enhancement with hatchery-reared seed on genetic diversity and effective population size. Can J Fish Aquat Sci 70:330–338

Hopper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH et al (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol Monogr 75:3–35

Howell BR (1994) Fitness of hatchery-reared fish for survival in the sea. Aquac Fish Manag 25(supplement):3–17

Hughes TP (1990) Recruitment limitation, mortality, and population regulation in open systems: a case study. Ecology 71:12–20

Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a sea grass ecosystem to disturbance. Proc Natl Acad Sci USA 101:8998–9002

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. Ecol Lett 11:609–623

Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruiting of benthic marine invertebrates. Mar Ecol Prog Ser 155:269–301

Hutchings JA (2005) Life history consequences of overexploitation to population recovery in Northwest Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 62:824–832

Hutchings JA (2014) Renaissance of a caveat: Allee effects in marine fish. ICES J Mar Sci 71:2152–2157

Hutchings JA, Swain DP, Rowe S, Eddington JD, Puvanendran V, Brown JA (2007) Genetic variation in life-history reaction norms in marine fish. Proc R Soc B 274:1693–1699

Hutchings JA, Butchart SHM, Collen B, Schwartz MK, Waples RS (2012) Red flags: correlates of impaired species recovery. Trends Ecol Evol 27:542–546

Iles TC, Beverton RJH (2000) The concentration hypothesis: the statistical evidence. ICES J Mar Sci 57:216–227

Jackson JBC, Kirby MX, Berger WH et al (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629–638

Jasper JR, Habicht C, Moffitt S, Brenner R, Marsh J, Lewis B, Creelman Fox E et al (2013) Source-sink estimates of genetic introgression show influence of hatchery strays on wild chum salmon populations in Prince William Sound, Alaska. PLoS ONE 8(12):e81916

Jennings S, Greenstreet SPR, Reynolds JD (1999) Structural change in an exploited fish community: a consequence of differential fishing effects on species with contrasting life histories. J Anim Ecol 68:617–627

Jensen JD, Foll M, Bernatchez L (2016) The past, present and future of genomic scans for selection. Mol Ecol 25:1–4

Johnson EG, Hines AH, Kramer MA, Young AC (2008) Importance of season and size of release to stocking success for the blue crab in Chesapeake Bay. Rev Fish Sci 16:243–253

Jorde PE, Søvik G, Westgaard J-I, Albretsen J, André C, Hvingel C, Johansen T et al (2015) Genetically distinct populations of northern shrimp *Pandalis borealis*, in the North Atlantic: adaptation to different temperatures as an isolation factor. Mol Ecol 24:1742–1757

Jøstad KE, Prodöhl PA, Kristiansen TS, Hughes M, Farestveit E, Taggart JB, Agnalt A-L et al (2005) Communal larval rearing of European lobster (*Homarus gammarus*): family identification by microsatellite DNA profiling and offspring fitness comparisons. Aquaculture 247:275–285

Kahilainen A, Puurtinen M, Kotiaho JS (2014) Conservation implications of species-genetic diversity correlations. Glob Ecol Conserv 2:315–323

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189

Karlson RH, Levitan DR (1990) Recruitment-limitation in open populations of *Diadema antillarum*: an evaluation. Oecologia 82:40–44

Karlsson S, Saillant E, Bumguardner BW, Vega RR, Gold JR (2008) Genetic identification of hatchery-released red drum in Texas bays and estuaries. N Am J Fish Manag 28:1294–1304

Karlsson S, Diserud OH, Fiske P, Hindar K (2016) Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES J Mar Sci 73:2488–2498

Keller L, Reeve HK (1994) Partitioning of reproduction in animal societies. Trends Ecol Evol 9:98–103

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241

Kirk RA (1987) A history of marine fish culture in Europe and North America. Fishing News Books, Oxford

Kitada S, Kishino H (2006) Lessons learned from Japanese marine finfish stock enhancement programmes. Fish Res 80:101–112

Kitada S, Taga Y, Kishino H (1992) Effectiveness of stock enhancement program evaluated by a two-stage sampling survey of commercial landings. Can J Fish Aquat Sci 49:1573–1582

Kitada S, Shishidou H, Sugaya T, Kitakado T, Hamasaki K, Kishino H (2009) Genetic effects of long-term stock enhancement programs. Aquaculture 290:69–79

Kostow KE (2004) Differences in juvenile phenotypes and survival between hatchery stocks and a natural population provide evidence for modified selection due to captive breeding. Can J Fish Aquat Sci 61:577–589

Kuparinen A, Kieth DM, Hutchings JA (2014) Allee effect and the uncertainty of population recovery. Conserv Biol 28:790–798

Lamichhaney S, Martinez Barrio A, Rafati N et al (2012) Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. Proc Natl Acad Sci USA 109:19345–19350

Lande R (1981) The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99:541–553

Lankau RA, Strauss SY (2007) Mutual feedbacks maintain both genetic and species diversity in a plant community. Science 317:1561–1563

Law R (2000) Fishing, selection, and phenotypic evolution. ICES J Mar Sci 57:659–668

Le Moan A, Gagnaire PA, Bonhomme F (2016) Parallel genetic divergence among coastal–marine ecotype pairs of European anchovy explained by differential introgression after secondary contact. Mol Ecol 25:3187–3202

Le Vay L, Carvalho GR, Quinitio ET, Lebata JH, Ut VN, Fushimi H (2007) Quality of hatchery-reared juveniles for marine fisheries enhancement. Aquaculture 268:168–180

Leber KM (2004) Summary of case studies of the effectiveness of stocking aquacultured fishes and invertebrates to replenish and enhance coastal fisheries. In: Bartley DM, Leber KM (eds) Marine Ranching, FAO Fish Technical Papers 429, pp 203–213

Leber KM, Brennan NP, Arce SM (1995) Marine enhancement with striped mullet: are hatchery releases replenishing or displacing wild stocks? Am Fish Soc Symp 15:376–387

Leber KM, Brennan NP, Arc SM (1998) Recruitment patterns of cultured juvenile Pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. Bull Mar Sci 62:389–408

Leggett WC, Frank KT (1997) A comparative analysis of recruitment variability in North Atlantic flatfishes—testing the species range hypothesis. J Sea Res 37:281–299

Leverone JR, Geiger SP, Stephenson SP, Arnold WS (2010) Increase in bay scallop (*Argopecten irradians*) populations following releases of competent larvae in two west Florida estuaries. J Shellfish Res 29:395–406

Liao IC (2000) The state of finfish diversification in Asian aquaculture. Cah Opt Méd 47:109–125

Liermann M, Hilborn R (1997) Depensation in fish stocks: a hierarchic Bayesian meta-analysis. Can J Fish Aquat Sci 54:1976–1984

Lind CE, Evans BS, Knauer J, Taylor JJU, Jerry DR (2009) Decreased genetic diversity and a reduced effective population size in cultured silver-lipped pearl oysters (*Pinctada maxima*). Aquaculture 286:12–19

Link JS (2002) What does ecosystem-based fisheries management mean? Fisheries 27:18–21

Linnane A, Mercer JP (1998) A comparison of methods for tagging juvenile lobsters (*Homarus gammarus* L.) reared for stock enhancement. Aquaculture 163:195–202

Lipicius RN, Schreiber S, Wang H, Shen J, Sisson M (2008) Metapopulation source-sink dynamics and stock enhancement of marine species. Rev Fish Sci 16:101–110

Longhurst A (2010) Mismanagement of marine fisheries. Cambridge University Press, Cambridge

Lorenzen K (2000) Allometry of natural mortality as a basis for assessing optimal release size in fish-stocking programmes. Can J Fish Aquat Sci 57:2374–2381

Lorenzen K (2005) Population dynamics and potential of fisheries stock enhancement: practical theory for assessment and policy analysis. Philos Trans R Soc B 360:171–189

Lorenzen K (2006) Population management in fisheries enhancement: gaining key information from release experiments through use of a size-dependent mortality model. Fish Res 80:19–27

Lorenzen K (2008) Understanding and managing enhancement fisheries systems. Rev Fish Sci 16:10–23

Lorenzen K, Leber KM, Blankenship HL (2010) Responsible approach to marine stock enhancement: an update. Rev Fish Sci 18:189–210

Luttikhuizen PC, Drent J, van Delden W, Piersma T (2003) Spatially structure genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. J Evol Biol 16:260–272

Lynch M, O'Hely M (2001) Captive breeding and the genetic fitness of natural populations. Conserv Genet 2:363–378

Lynch M, Conery J, Bürger R (1995) Mutation accumulation and the extinction of small populations. Am Nat 146:489–518

Macaranas J, Fujio Y (1990) Strain differences in cultured fish–isozymes and performance traits as indicators. Aquaculture 85:69–82

MacCall AD (1990) Dynamic geography of marine fish populations. University of Washington Press, Seattle

Mannion AM (1999) Domestication and the origins of agriculture: an appraisal. Prog Phys Geogr 23:37–56

Mantua NJ, Hare SR (2002) The Pacific decadal oscillation. J Oceanogr 58:35–44

Martin-Robichaud DJ, Haché R, Pernet F, Ritchie R (2007) Genomic evaluation of haddock (*Melanogrammus aeglefinus* L.) broodstock. Can Tech Rep Fisher Aquat Sci 2704:iv+10

Masuda R, Tsukamoto K (1998) Stock enhancement in Japan: review and perspective. Bull Mar Sci 62:337–358

Matthews B, Narwani A, Hausch S et al (2011) Toward an integration of evolutionary biology and ecosystem science. Ecol Lett 14:690–701

McCay BJ (1988) Muddling through the clam beds: cooperative management of New Jersey's hard clam spawner sanctuaries. J Shellfish Res 7:327–340

McCusker MR, Bentzen P (2010) Positive relationships between genetic diversity and abundance in fishes. Mol Ecol 19:4852–4862

McEachron LW, Colura RL, Bumguardner BW, Ward R (1998) Survival of stocked red drum in Texas. Bull Mar Sci 62:359–368

Mgaya YD, Gosling EM, Mercer JP, Donlon J (1995) Genetic variation at three polymorphic loci in wild and hatchery stocks of the abalone, *Haliotis tuberculata* Linnaeus. Aquaculture 136:71–80

Milbury CA, Meritt DW, Newell RIE, Gaffney PM (2004) Mitochondrial DNA markers allow monitoring of oyster stock enhancement in the Chesapeake Bay. Mar Biol 145:351–359

Mimura M, Yahara T, Faith DP, Vázquez-Dominguez E, Colautti RI et al (2017) Understanding and monitoring the consequences of human impacts of intraspecific variation. Evol Appl 10:121–139

Miyazaki T, Masuda R, Furutra S, Tsukamoto K (2000) Feeding behaviour of hatchery-eared juveniles of the Japanese flounder following a period of starvation. Aquaculture 190:129–138

Mobrand LE, Barr J, Blankenship L, Campton DE, Evelyn TT, Flagg TA, Mahnken CV, Seeb LW, Seidel PR, Smoker WW (2005) Hatchery reform in Washington State: principles and emerging issues. Fisheries 30:11–23

Moksness E, Stole R, van der Meeren G (1998) Profitability analysis of sea ranching with Atlantic salmon (*Salmo salar*), Arctic charr (*Salvelinus alpinus*) and European lobster (*Homarus gammarus*) in Norway. Bull Mar Sci 62:689–699

Molony BW, Lenanton R, Jackson G, Norriss J (2004) Stock enhancement as a fisheries management tool. Rev Fish Biol Fish 13:409–432

Montes I, Iriondo M, Manzano C, Santis M, Conklin D, Carvalho G, Irigoien X et al (2016) No loss of genetic diversity in the exploited and recently collapsed population of Bay of Biscay anchovy (*Engaulis encrasicholus*, L.). Mar Biol 163:1–10

Morgan LE, Botsford LW (2001) Managing with reserves: modeling uncertainty in larval dispersal for a sea urchin fishery. In: Spatial processes and management of marine populations. Alaska Sea Grant College Program, Fairbanks, Alaska

Morgan SG, Fisher JL, Mace AJ (2009) Larval recruitment in a region of strong, persistent upwelling and recruitment limitation. Mar Ecol Prog Ser 394:79–99

Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure. Philos Trans R Soc B 366:1425–1437

Munro JL, Bell JD (1997) Enhancement of marine fisheries resources. Rev Fish Sci 5:185–222

Mustafa S, Saad S, Rahman RA (2003) Species studies in sea ranching: an overview and economic perspectives. Rev Fish Biol Fish 13:165–175

Naish KA, Taylor JE, Levin PS, Quinn TP, Winton JP, Huppert D, Hilborn R (2008) An evaluation of the effects of conservation and fishery enhancement hatcheries on wild populations of salmon. Adv Mar Biol 53:61–194

Nakajima K, Kitada S, Habara Y, Sano S, Yokoyama E, Sugaya T, Iwamoto A et al (2014) Genetic effects of marine stock enhancement: a case study based on the highly piscivorous Japanese Spanish mackerel. Can J Fish Aquat Sci 71:301–314

Nash CE (2011) The history of aquaculture. Wiley, Ames

Navarrete SA, Weiters EA, Broitman BR, Castilla JC (2005) Scales of benthic-pelagic coupling and the intensity of species interactions: from recruitment limitation to top-down control. Proc Natl Acad Sci USA 102:18046–18051

Neill WH, Miller JM, van der Veer HW, Weinmiller KO (1994) Ecophysiology of marine fish recruitment: a conceptual framework for understanding interannual variability. Neth J Sea Res 32:135–152

Nicosia F, Lavalli K (1999) Homarid lobster hatcheries: their history and role in research, management, and aquaculture. Mar Fish Rev 61:1–57

Nielsen EE, Bach LA, Kotlicki P (2006) Hybidlab (version 1.0): a program for generating simulated hybrids from population samples. Mol Ecol Notes 6:971–973

Nielsen EE, Hemmer-Hansen J, Poulsen NA, Loeschcke V, Moen T, Johansen T, Mittelholzer C et al (2009) Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). BMC Evol Biol 9:276

Novel P, Porta J, Fernández J, Méndez T, Gallardo-Gálvez JB (2013) Critical points for the maintenance of genetic variability over a production cycle in the European sea bass, *Dicentrarchus labrax*. Aquaculture 416–417:8–14

Ochwada-Doyle F, Loneragan NR, Gray CA, Southers IM, Taylor MD (2012a) Competition between wild and captive-bred *Penaeus plebejus* and implications for stock enhancement. Mar Ecol Prog Ser 450:115–129

Ochwada-Doyle F, Loneragan N, Gray C, Southers I, Taylor M (2012b) Complexity affects habitat preference and predation mortality in postlarval *Penaeus plebejus*: implications for stock enhancement. Mar Ecol Prog Ser 380:161–171

Okouchi H, Kitada S, Iwamoto A, Fukunaga T (2004) Flounder stock enhancement in Miyako Bay, Japan. FAO Fisheries Technical Paper 429, p 179

Olla BL, Davis MW, Ryer CH (1998) Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. Bull Mar Sci 62:531–550

Olsen EM, Heino M, Lilly GR, Morgan MJ, Brattey J, Ernande B, Dieckman U (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature 428:932–935

Olsen EM, Knutsen H, Gjøsæter J, Jorde PE, Knutsen JA, Stenseth NC (2008) Small-scale biocomplexity in coastal Atlantic cod supporting a Darwinian perspective on fisheries management. Evol Appl 1:524–533

Olson RR, Olson MH (1989) Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? Ann Rev Ecol Syst 20:225–247

Oosthuizen E, Daan N (1974) Egg fecundity and maturity of North Sea cod, *Gadus morhua*. Neth J Sea Res 8:378–397

Ovenden JR, Peel D, Street R et al (2007) The genetic effective and adult census size of an Australian population of tiger prawns (*Penaeus esculentus*). Mol Ecol 16:127–138

Pauly D, Christensen V, Guénette S et al (2002) Towards sustainability in world fisheries. Nature 418:689–695

Pauly D, Alder J, Bennett E, Christensen V, Tyedmers P, Watson R (2003) The future for fisheries. Science 302:1359–1361

Pearse DE (2016) Saving the spandrels? Adaptive genomic variation in conservation and fisheries management. J Fish Biol 89:2697–2716

Perez-Enriquez R, Takagi M, Taniguchi N (1999) Genetic variability and pedigree tracing of a hatchery-reared stock of red sea bream (*Pagrus major*) used for stock enhancement, based on microsatellite DNA markers. Aquaculture 173:413–423

Perez-Enriquez R, Takaemura M, Tabata K, Taniguchi N (2001) Genetic diversity of red sea bream *Pagrus major* in western Japan in relation to stock enhancement. Fish Sci 67:71–78

Pikitch EK, Santora C, Babcock EA et al (2004) Ecosystem-based fishery management. Science 305:346–347

Polacheck T (1990) Year around closed areas as a management tool. Nat Res Modell 4:327–354

Porta J, Porta JM, Cañavate P, Martínez-Rodríguez G, Alvarez MC (2007) Substantial loss of genetic variation in a single generation of Senegalese sole (*Solea senegalensis*) culture: implications in the domestication process. J Fish Biol 71(Supplement B):223–234

Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Purcell JE, Grover JJ (1990) Predation and food limitation as causes of mortality in larval herring at a spawning ground in British Columbia. Mar Ecol Prog Ser 59:55–61

Purcell SW, Simutoga M (2008) Spatio-temporal and size-dependent variation in the success of releasing cultured sea cucumbers in the wild. Rev Fish Sci 16:204–214

Ramachandran S, Deshpande O, Roseman CC, Rosenberg NA, Feldman MW, Cavalli-Sforza LL (2005) Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. Proc Nat Acad Sci USA 102:15942–15947

Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17:230–237

Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc Natl Acad Sci USA 102:2826–2831

Reynolds LA, McGlathery KJ, Waycott M (2012) Genetic diversity enhances restoration success by augmenting ecosystem services. PLoS ONE 7:e38397

Rhode C, Hepple J-A, Jansen S, Davis T, Vervalle J, Bester-van der Merwe AE, Roodt-Wildig R (2012) A population genetic analysis of abalone domestication events in South Africa: implications for the management of abalone resource. Aquaculture 356–357:235–242

Roberts CM, Hawkins JP, Gell FR (2005) The role of marine reserves in achieving sustainable fisheries. Philos Trans R Soc B 360:123–132

Rose KA, Cowan JH, Winemiller KO, Myers RA, Hilborn R (2001) Compensatory density dependence in fish populations: importance, controversy, understanding and prognosis. Fish Fish 2:293–327

Rugger P, Spendani A, Occhipinti G, Fioravanti T, Santojanni A, Leonori I, DeFelice A, Arneri E, Procaccini G, Catanese G, Tičina V, Bonanno A, Cerioni PN, Giovannotti M, Grant WS, Barucchin VC (2016) Biocomplexity in populations of European anchovy in the Adriatic Sea. PLoS ONE 11(4):e0153061

Ryman N, Laikre L (1991) Effects of supportive breeding on the genetically effective population size. Conserv Biol 5:325–329

Ryman N, Utter F, Lairkre L (1995) Protection of intraspecific biodiversity of exploited fishes. Rev Fish Biol Fish 5:417–446

Saillant E, Renshaw MA, Gatlin Lii DM, Neill WH, Vega RR, Gold JR (2009) An experimental assessment of genetic tagging and founder representation in hatchery-reared red drum (*Sciaenops ocellatus*) used in stock enhancement. J Appl Ichthyol 25:108–113

Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. Annu Rev Mar Sci 3:509–535

Schindler D, Hilborn R, Chasco B, Boatright C, Quinn T, Rogers L, Webster M (2010) Population diversity and the portfolio effect in an exploited species. Nature 465:609–613

Schlichting CD, Pigliucci M (1998) Phenotypic evolutions: a reaction norm perspective. Sinauer Associates, Sunderland

effect in an exploited species. Nature 465:609–613

Schlichting CD, Pigliucci M (1998) Phenotypic evolutions: a reaction norm perspective. Sinauer Associates, Sunderland

Schmalenbach I, Mehrtens F, Janke M, Buchholz F (2011) A mark-recapture study of hatchery-reared juvenile European lobsters, *Homarus gammarus*, released at the rocky island of Hegoland (German Bight, North Sea) from 2000 to 2009. Fish Res 108:22–30

Secor DH, Houde ED (1998) Use of larval stocking in restoration of Chesapeake Bay striped bass. ICES J Mar Sci 55:228–239

Secor DH, Hines AH, Place AR (2002) Japanese hatchery-based stock enhancement: lessons for the Chesapeake Bay blue crab. Publication Number UM-SG-TS-2002-02, Maryland Sea Grant Program, College Park, MD, p 46

Segovia-Viadero M, Serrão EA, Canteras-Jordana JC, Gonzalez-Wangüemert M (2016) Do hatchery-reared sea urchins pose a threat to genetic diversity in wild populations? Heredity 116:378–383

Seitz RD, Lipcius RN, Knick K, Seebo M, Long WC (2008) Stock enhancement and carrying capacity of blue crab nursery habitats in Chesapeake Bay. Rev Fish Sci 16:329–337

Sekino M, Hara M, Taniguchi N (2002) Loss of microsatellite and mitochondrial DNA variation in hatchery strains of Japanese flounder *Paralichthys olivaceus*. Aquaculture 213:101–122

Sekino M, Saitoh K, Yamada T, Kumagai A, Hara M, Yamashita Y (2003) Microsatellite-based pedigree tracing in a Japanese flounder *Paralichthys olivaceus* hatchery strain: implications for hatchery management related to stock enhancement program. Aquaculture 221:255–263

Sekino M, Saido T, Fujita T, Kobayashi T, Takami H (2005) Microsatellite DNA markers of ezo abalone (*Haliotis discus hannai*): a preliminary assessment of natural populations samples from heavily stocked areas. Aquaculture 243:33–47

Serafy JE, Ault JS, Capo TR, Schultz DR (1999) Red drum, *Sciaenops ocellatus* L., stock enhancement in Biscayne Bay, FL, USA: assessment of releasing unmarked early juveniles. Aquac Res 30:737–750

Shanks AL, Roegner GC (2007) Recruitment limitation in Dungeness crab populations is driven by variation in atmospheric forcing. Ecology 88:1726–1737

Sharpe DMT, Hendry AP (2009) Life history change in commercially exploited fish stocks: an analysis of trends across studies. Evol Appl 2:260–275

Shelbourne JE (1964) The artificial propagation of marine fish. Adv Mar Biol 2:1–83

Simpson MR, Walsh SJ (2004) Changes in the spatial structure of Grand Bank yellowtail flounder: testing MacCall's basin hypothesis. J Sea Res 51:199–210

Smedstad OM, Salvanes AGV, Fosså JH, Nordeide JT (1994) Enhancement of cod, *Gadus morhua* L. in Masfjorden: an overview. Aquac Fish Manag 25:117–128

Smith PJ, Francis R, McVeagh M (1991) Loss of genetic diversity due to fishing pressure. Fish Res 10:309–316

Solemdal P, Dahl E, Danielssen DS, Moksness E (1984) The cod hatchery in Flødevigen – background and realities. In: Dahl E, Danielssen DS, Moksness E, Solemdal P (eds) The propagation of cod, Flødevigen rapportser, Arendal, Norway, pp 17–45

Sproul JT, Tominaga O (1992) An economic review of the Japanese flounder stock enhancement project in Ishikari Bay, Hokkaido. Bull Mar Sci 5:75–88

Sproul JT, Tominaga O (1992) An economic review of the Japanese flounder stock enhancement project in Ishikari Bay, Hokkaido. Bull Mar Sci 5:75–88

Stoner AW, Davis M (1994) Experimental outplanting of juvenile queen conch, *Strombus gigas*: comparison of wild and hatchery reared stocks. Fish Bull US 92:390–411

Stoner AW, Ray-Culp M (2000) Evidence for Allee effects in an over-harvested marine gastropod: density-dependent mating and egg production. Mar Ecol Prog Ser 202:297–302

Støttrup JG, Sparrevohn CR (2007) Can stock enhancement enhance stocks? J Sea Res 57:104–113

Straus KM, Vadopalas B, Davis JP, Friedman CS (2015) Reduced genetic variation and decreased effective number of breeders in five year-classes of cultured geoducks (*Panopea generosa*). J Shellfish Res 34:163–169

Stunz GW, Levin PS, Minello TJ (2001) Selection of estuarine nursery habitats by wild-caught and hatchery-reared juvenile red drum in laboratory mesocosms. Environ Biol Fish 61:305–313

Sugaya T, Sato M, Yokoyama E, Nemoto Y, Fujita T, Okouchi H, Hamasaki K et al (2008) Population genetic structure and variability of Pacific herring *Clupea pallasii* in the stocking area along the Pacific coast of northern Japan. Fish Sci 74:579–588

Sun X, Hedgecock D (2017) Temporal genetic change in North American Pacific oyster populations suggests caution in seascape genetics analyses of high gene-flow species. Mar Ecol Prog Ser 565:79–93

Suquet A, Malo F, Quere C, Ledu C, LeGrand J et al (2016) Gamete quality in triploid Pacific oyster (*Crasostrea gigas*). Aquaculture 451:11–15

Sussarellu R, Huvet A, Lepègue S, Quillen V, Lelong C, Cornette F, Jensen LF et al (2015) Additive transcriptomic variation associated with reproductive traits suggest local adaptation in a recently settled population of Pacific oyster, *Crassostrea gigas*. BMC Genom 16:808

Suttle CA (2007) Marine viruses—major players in the global ecosystem. Nat Rev Microbiol 5:801–812

Svåsand T, Skilbrei OT, van der Meeren GI, Holm M (1989) Review of morphological and behavioural differences between reared and wild individuals: implications for searanching of Atlantic salmon, *Salmo salar* L., Atlantic cod, *Gadus morhua* L. and European lobster, *Homarus gammarus* L. Fish Manag Ecol 5:1–18

Svåsand T, Kristiansen TS, Pedersen T, Salvanes AGV, Engelsen R, Nævdal G, Nødtvedt M (2000) The enhancement of cod stocks. Fish Fish 1:173–205

Svåsand T, Agnalt A-L, Skilbrei OT, Bothen J, Heggberget T (2004) An integrated development programme for marine stocking: The Norwegian example. In: Bartley DM, Leber KM (eds) Marine Ranching, FAO Fisheries Technical Paper, No. 429, FAO, Rome, pp 19–72

Svåsand T, Crosetti D, García-Vázquez E, Verspoor E (eds) (2007) Genetic impact of aquaculture activities on native populations. Genimpact final scientific report. EU contract RICA-CT-2005-022802. http://www.imr.no/genimpact/filarkiv/2007/07/genetic_impact_of_aquaculture.pdf/en. Accessed 30 Sept 2014

Swain DP, Hutchings JA, Foote CJ (2004) Environmental and genetic influences on stock identification characters. In: Cadrin SX, Friedland KD, Waldman J (eds) Stock identification methods. Academic Press, New York, pp 43–83

Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008) ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. Mol Ecol Res 8:299–301

Taniguchi N (2003) Genetic factors in broodstock management for seed production. Rev Fish Biol Fish 13:177–185

Taniguchi N, Sumantadinata K, Iyama S (1983) Genetic change in the first and second generations of hatchery stock of black seabream. Aquaculture 35:309–320

Tave D (1993) Genetics for fish hatchery managers. Van Nostrand Reinhold, New York

Taylor MD, Piola RF (2008) Scale stocking checks to differentiate between wild and hatchery-reared mulloway (*Argyosomus japonicus*). Fish Manag Ecol 15:211–216

Taylor MD, Palmer PJ, Fielder DS, Southers IM (2005) Responsible estuarine finfish stock enhancement: an Australian perspective. J Fish Biol 67:329–331

Tegner MJ, Butler RA (1989) Abalone seeding. In: Hahn K (ed) Handbook of culture of abalone and other marine gastropods. CRC Press, Boca Raton, pp 157–182

Teletchea F, Fontaine P (2014) Levels of domestication in fish: implications for the sustainable future of aquaculture. Fish Fish 15:181–195

Tilman D (1999) Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proc Natl Acad Sci USA 96:5995–6000

Trimble AC, Ruesink JL, Dumbauld BR (2009) Factors preventing the recovery of historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864. J Shellfish Res 28:97–106

Tringali MD (2006) A Bayesian approach for the genetic tracking of cultured and released individuals. Fish Res 77:159–172

Tringali MD, Bert TM (1998) Risk to genetic effective population size should be an important consideration in fish stock-enhancement programs. Bull Mar Sci 62:641–659

Tringali MD, Seyoum S, Wallace EM et al (2008) Limits to the use of contemporary genetic analyses in delineating biological populations for restocking and stock enhancement. Rev Fish Sci 16:111–116

Turner TF, Richardson LR, Gold JR (1999) Temporal genetic variation of mitochondrial DNA and the female effective population size of red drum (*Sciaenops ocellatus*) in northern Gulf of Mexico. Mol Ecol 8:1223–1229

Turner TF, Wares JP, Gold JR (2002) Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162:1329–1339

Uthicke S, Purcell S (2004) Preservation of genetic diversity in restocking of the sea cucumber *Holothuria scabra* investigated by allozyme electrophoresis. Can J Fish Aquat Sci 61:519–528

van der Meeren G (2000) Predation on hatchery-reared lobsters released into the wild. Can J Fish Aquat Sci 57:1794–1803

Vellend M (2006) The consequences of genetic diversity in competitive communities. Ecology 87:304–311

Vellend M, Geber MA (2005) Connections between species diversity and genetic diversity. Ecol Lett 8:767–781

Vigne J-D (2011) The origins of animal domestication and husbandry: a major change in the history of humanity and the biosphere. Compt Rend Biol 334:171–181

Wahle RA (2003) Revealing stock–recruitment relationships in lobsters and crabs: is experimental ecology the key? Fish Res 65:3–32

Wahle RA, Incze LS (1997) Pre- and post-settlement processes in recruitment of the American lobster. J Exp Mar Biol Ecol 217:179–207

Walters CJ, Juanes F (1993) Recruitment limitation as a consequence of natural selection for use of restricted feeding habitats and predation risk taking by juvenile fishes. Can J Fish Aquat Sci 50:2058–2070

Wang L, Liu S, Zhuang Z, Guo L, Meng Z, Lin H (2013) Population genetic studies revealed local adaptation in a high gene-flow marine fish, the small yellow croaker (*Larimichthys polyactis*). PLoS ONE 8:e83493

Wang J, Santiago E, Caballero A (2016) Prediction and estimation of effective population size. Heredity 117:193–206

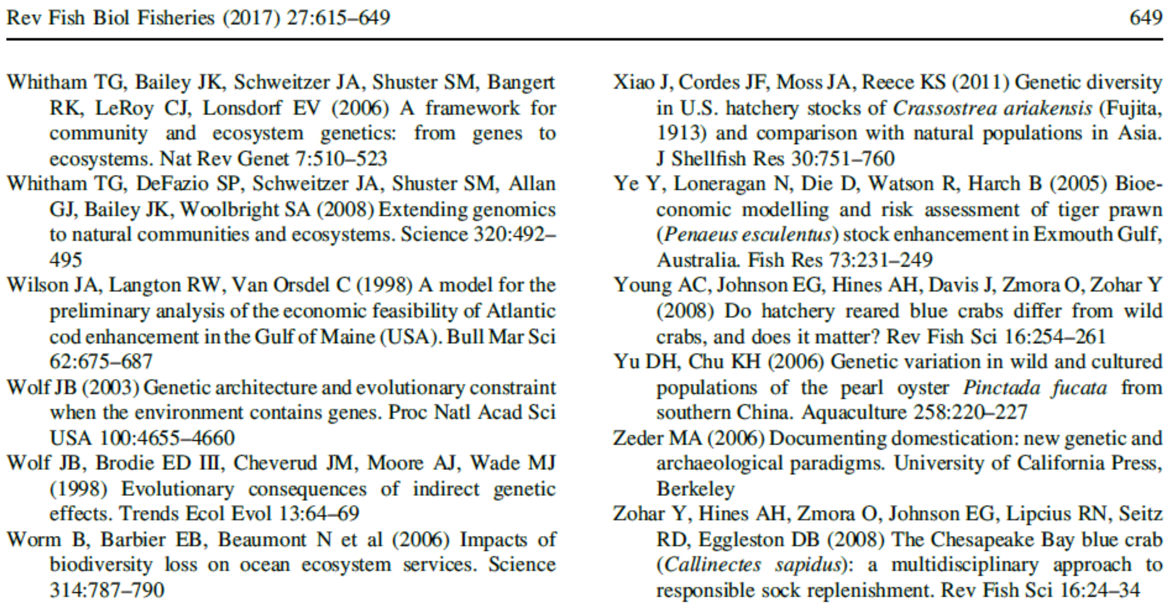
Waples RS, Do C (2008) ldne: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8:753–756

Waples RS, Drake J (2004) Risk-benefit considerationfor marine stock enhancement: a Pacific salmon perspective. In: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds) Stock enhancement and sea ranching, 2nd edn. Blackwell, Oxford, pp 160–306

Waples RS, Hindar K, Hard JJ (2012) Genetic risks associated with marine aquaculture. NOAA Technical Memorandum NMFS-NWFSC-119, Seattle, WA

Waples RS, Hindar K, Karlsson S, Hard JJ (2016) Evaluating the Ryman–Laikre effect for marine stock enhancement and aquaculture. Curr Zool 62:617–627

Welcomme RL, Bartley DM (1998) An evaluation of present techniques for the enhancement of fisheries. FAO Fisheries Technical Paper 374, Rome



Rev Fish Biol Fisheries (2017) 27:615–649 649

Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf EV (2006) A framework for community and ecosystem genetics: from genes to ecosystems. Nat Rev Genet 7:510–523

Whitham TG, DeFazio SP, Schweitzer JA, Shuster SM, Allan GJ, Bailey JK, Woolbright SA (2008) Extending genomics to natural communities and ecosystems. Science 320:492–495

Wilson JA, Langton RW, Van Orsdel C (1998) A model for the preliminary analysis of the economic feasibility of Atlantic cod enhancement in the Gulf of Maine (USA). Bull Mar Sci 62:675–687

Wolf JB (2003) Genetic architecture and evolutionary constraint when the environment contains genes. Proc Natl Acad Sci USA 100:4655–4660

Wolf JB, Brodie ED III, Cheverud JM, Moore AJ, Wade MJ (1998) Evolutionary consequences of indirect genetic effects. Trends Ecol Evol 13:64–69

Worm B, Barbier EB, Beaumont N et al (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314:787–790

Xiao J, Cordes JF, Moss JA, Reece KS (2011) Genetic diversity in U.S. hatchery stocks of *Crassostrea ariakensis* (Fujita, 1913) and comparison with natural populations in Asia. J Shellfish Res 30:751–760

Ye Y, Loneragan N, Die D, Watson R, Harch B (2005) Bioeconomic modelling and risk assessment of tiger prawn (*Penaeus esculentus*) stock enhancement in Exmouth Gulf, Australia. Fish Res 73:231–249

Young AC, Johnson EG, Hines AH, Davis J, Zmora O, Zohar Y (2008) Do hatchery reared blue crabs differ from wild crabs, and does it matter? Rev Fish Sci 16:254–261

Yu DH, Chu KH (2006) Genetic variation in wild and cultured populations of the pearl oyster *Pinctada fucata* from southern China. Aquaculture 258:220–227

Zeder MA (2006) Documenting domestication: new genetic and archaeological paradigms. University of California Press, Berkeley

Zohar Y, Hines AH, Zmora O, Johnson EG, Lipcius RN, Seitz RD, Eggleston DB (2008) The Chesapeake Bay blue crab (*Callinectes sapidus*): a multidisciplinary approach to responsible sock replenishment. Rev Fish Sci 16:24–34

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Chapter 11: **Management Implications**

Abstract: Alaska’s long, ‘pristine’ coastline provides an opportunity to develop a commerical seaweed industry and to add to the State’s economic base. This project on the genetics of three species of kelp represents further progress in developing a seaweed industry in Alaska. The genetic population structures of these kelps are highly subdivided in the Gulf of Alaska and are unlike the structures of other marine organisms in the region that have been studied. Marine species tend to have substantial dispersal capabilities, either as adults or planktonic larvae, that tend to produce genetically homogeneous populations with divergences generally less than $F_{ST} = 0.05$. In contrast, divergences between populations of kelps were generally larger than $F_{ST} = 0.40$. Even though kelp populations show strong differences from one another, defining production units is complicated by the mosaic nature of the population structure. A much greater sampling effort is needed to be able to resolve the genetic population structures of these kelps on small geographical scales. Further study of incipient species in of winged kelp, *Alaria*, are needed to unravel the taxonomies of this species complext. The results of this study further show that Alaska’s kelp populations have low levels of genetic diversity that reflect the evolutionary legacies of population turnover during a long history of Pleistocene ice ages. The development of a viable seaweed industry in Alaska requires a research program focused on understanding the physiological and ecological responses to environmental variability. Environmental variables, including temperature, salinity and nutrient availablity, control growth rates and the chemical

compositions of the kelps. The development of aquaculture strains through traditional agricultural methods of selective breeding or other genetic tools is needed to improve product quality and maximize growth. These genetic improvements are essential for Alaska to be able to participate in global markets. The initiative to develop a seaweed industry in Alaska comes at a time of profound climate change that is leading to warmer seawater temperatures and to an increase in ocean acidification. Both warming and acidification will affect the distributions and local abundances of kelps.

11.1 Introduction

The term ‘seaweed’ describes marine macroalgae that grow in shallow subtidal areas within the photic zone and intertidal zones along a coast. Seaweeds include kelps (phaeophyceae), red algae (rhodophyceae) and green algae (chlorophyceae). A wide range of seaweeds have been used as food since prehistorical times (Mouritsen *et al*. 2013). Presently, seaweed harvests represent about 25% of global aquacuture production with a value of about six billion USD (FAO 2018a). About 85% of this production is consumed by humans directly as fresh or dried products or is processed as food additives in the form of alginates, agar, and carrageenan (FAO 2018a). The rest is used in fertilizers, livestock feed, pet foods, cosmetics, pharmaceuticals and numerous other products (Holdt & Kraan 2011; Ugarte & Sharp 2012; Rebours *et al*. 2014; Wu *et al*. 2016, among numerous industrial studies). Global seaweed production comprises *Eucheuma* (35%, tropical red alga), *Saccharina japonica* (27%m temperature brown alga), *Gracilaria* (14%, warm temperate red alga), and *Undaria* (7%, warm temperate brown alga) (FAO 2018b). Smaller amounts of the red algae *Palmaria*, *Porphyra* and *Chondrus*, and the green alga, *Ulva* are also harvested from the wild or cultivated for commercial use.

The harvest and culture of seaweeds represent a substantial global industry that has grown over the past few decades from a few thousands of tons in 1950 to over 30 million tons wet weight in 2017 (FAO http://www.fao.org/fishery/statistics/en) (Figure 1a). Most of this production takes place in Asia with China as a leading producer (Figure 1b). Nevertheless, production still represents a considerable source of economic revenue in other Asian countries (Figure 2) and to a lesser extent in Europe. Eight Asian countries produce 99% of seaweed harvests. Most seaweeds are cultured for commercial use, but a small amount is harvested from the wild (Figure 3). The present production of seaweeds in Alaska is minuscule by comparison to global production, consisting of only a few tons predominantly of sugar kelp (*Saccharina latissima*). Nevertheless, permitted operations have grown from one farm in 1992 to 13 in 2018 (Figure 4) and are likely to grow in number over the coming decades.

The startup and operation of seaweed farming in Alaska is viewed by industry as cumbersome because of layers of regulation from several sources that entail 1) leasing of aquaculture sites, 2) regulatory policies affecting operations, and 3) other policies, including taxes, subsidies, support of research and marketing, infrastructure, among many others (Knapp & Rubino 2016). While the principle of private property across landscapes is widely accepted, marine waters are generally perceived as being a public resource owned in common. Governmental mechanisms for the leasing of public properties for logging, mining, oil extraction and grazing are well established, but harvests from shorelines adjoining private property and the use of public waterways for aquaculture often meets resistance (Feeney 2001). Waterfront transformations by aquaculture are generally perceived to be negative because of possible economic competition with the fishing industry, loss of access to water, changes in natural views, loss of value of waterfront property, or ecosystem harm from organic pollution (Knapp & Rubino 2016).

In addition to their commercial value, seaweeds are also of considerable ecological importance. Kelp canopies in shallow subtidal and intertidal areas provide ecosystem services for numerous invertebrates and maturing fishes (Dean *et al*. 2000; Daly & Konar 2008; Christie *et al*. 2009). The detrital decomposition of seaweeds injects considerable secondary organic productivity into nearshore ecosystems (Duggins *et al*. 1989). Without these services nearshore ecosystems would be much less productive and diverse. Seaweed culture and harvest activities can have both beneficial and negative consequences for these near-shore ecosystems that merit consideration in the development of a seaweed industry (Hasselström *et al*. 2018). The culture of seaweeds in large kelp farms has been suggested as mitigation for the world's output of the greenhouse carbon gases, because of large seaweed growth rates and biomasses that extract carbon dioxide from sea water for photosynthesis (World Bank Group 2016; Sondak *et al*. 2017; Chung *et al*. 2013).

The goal of this review is to provide a conceptual foundation for devising a management strategy for the development of seaweed farming in Alaska. This foundation has to be built on evolutionary principles that can be used to maximize production over the long term without impacting natural populations. The relevant foundational concepts fall into four areas: 1) genetic diversity within and among populations, 2) natural selection and 'local adaptation', 3) connectivity among populations, and 4) eco-evolutionary dynamics (Hendry *et al*. 2011). Central to a management plan are the results of theoretical and genetic studies of seaweed populations in Alaska waters and elsewhere that address questions in these four areas. What features of genetic population structure are relevant to resource management? Is there evidence for local adaptation that requires special attention? How important is the protection of wild populations to the long-term viability of a seaweed industry? How imporant is dispersal in bringing about gene flow and enhancing colonizations of suitable, but empty habitats patches? How should reproductive biologies, often peculiar to plants, be incorporated into management strategies? In the case of Alaskan kelps, how to account for the unusual mosaic genetic structure in management?

Among these important topics is the need to understand possible effects of domestication and selective breeding of farmed seaweeds on wild populations. Studies of kelps in the Northwestern Pacific and in North American and European waters provide opportunities to explore the effects of seaweed culture on wild populations, as countries in these areas have had a long history of seaweed harvesting and farming. The application of evolutionary principles to seaweed resource managment requires several assumptions from a multitude of disciplines, including population genetics, biogeography, evolutionary biology, ecology, and conservation biology. Finally, the high latitude waters of Alaska are changing rapidly from the rising temperatures and ocean acidification driven by the warming effects of greenhouse gases. Climate warming will lead to poleward shifts in species' distributions and to ecosystem reorganizations along Alaska's coasts.

Hence, evolutionary thinking is important to the conservation and management of natural resources. Naïve or inappropriate management can lead to unexpected outcomes and to the degradation of a natural resource (Vitousek *et al*. 1997).

11.2 Genetic diversity

Genetic diversity can be estimated with molecular markers in several ways. One measure is the proportion of polymorphic loci in a set of genes sampled from a population. A related measure is the average number of alleles among loci in a sample of individuals. One of the most common measures is average heterozygosity among a group of individuals for a set of gene loci. This measure can be estimated as the observed proportion of heterozygous individuals in a population sample, or as the number of heterozygous loci in an average individual. Additionally, expected heterozygosity can be estimated from allele frequencies by assuming Hardy-Weinberg genotypic proportions. Differences between observed and expected heterozygosity can reveal some aspects of population structure. When observed heterozygosity is significantly less than expected heterozygosity, inbreeding may be at play, non-random mating, or population mixing (Wahlund's effect). Yet other departures from Hardy-Weinberg proportions may be due to the nature of the molecular marker, for example null alleles, and may not directly reflect population processes.

Genetic diversity in Northeastern Pacific kelps examined in this study show some unusual features compared with other kelps. Organellar DNA haplotype diversities (*h)* are generally large in kelps overall, ranging from 0.770 to 0.900 in the seven species of kelps listed in Table 7.1. Nucleotide diversities (θ_π), which incorporates sequence divergences between haplotypes in addition to differences in haplotype frequencies, are also generally large among kelps, ranging from 0.00091 to 0.0152. Gene diversities in *Saccharina latissima* tended to be smaller than diversities in ecologically similar kelps (Table 1). The large contrast between overall diversity (h = 0.781; θ_π% = 0.088) and mean diversity among populations (h = 0.188; θ_π% = 0.0.016) is due to strong haplotype frequency differences between populations. Seven of the 14 populations examined had haplotype diversities ranging from 0.0 to 0.08. Many populations, in fact, are fixed, or nearly fixed, for a single DNA haplotype, so that estimates of diversity averaged over populations are considerably smaller than species-wide diversity. Large genetic diversities in some populations are due to the mixing of distinct haplotype lineages and not to the accumulation of mutations *in situ* in a single DNA lineage.

Microsatellite diversity follows a similar pattern. Northeastern Pacific populations of *Saccharina latissima* tend to have lower levels of average microsatellite diversity among populations (0.350–0.360) than *Saccharina latissima* in the North Atlantic (0.309–0.0690) (Table 2). One caveat in making these comparisons is that the estimates of diversity were based on different sets of microsatellite loci, except for one comparison between North Pacific (this study) and North Atlantic populations (Neiva *et al*. 2018), which included the same loci.

Microsatellite loci for these studies were generally selected for their ability to discriminate among populations and were not randomly selection to estimate levels of genetic diversity. Even so, Northeastern Pacific populations of *Saccharina latissima* appear to harbor less genetic variability than other populations of kelps.

The preliminary results for split kelp, *Hedophyllum nigripes*, populations in the NE Pacific show levels of genetic diversity similar to those in sugar kelp, *Saccharina latissima* (Table 1). Overall values of diversity (*COI*-*rbc*L: h = 0.770, θ_{π}% = 0.091) are larger than mean diversities within populations (h = 0.00.291, θ_{π}% = 0.025), indicating a considerable amount of haplotype-frequency divergence among populations. As for sugar kelp, most of the genetic diversity in this species is due to genetic heterogeneity between populations rather than to differences between plants within populations.

The results for *Alaria* must be considered separately because the samples collected for this study included several ill-defined taxa, which our results resolved by *COI* haplotypes into five deep lineages (Section 6). Genetic diversities within these *COI* lineages (θ_{π}% = 0.03–0.20) are much smaller than overall diversities than diversities pooled over lineages (θ_{π}% = 1.024). These lineages individually harbor much less genetic diversity than other kelps in the North Pacific and North Atlantic oceans (Table 1).

A pervasive theme in the management and conservation of natural populations is the preservation of genetic diversity within and among populations (Ryman 1991; Futuyma 1995; Ryman *et al*. 1995), and the protection of genetic diversity has become central to resource managment (Kenchington *et al*. 2003; Valero *et al*. 2011; Hoban *et al*. 2013), particularly to the management of salmon populations in Alaska (Davis *et al*. 1985). The protection of genetic diversity is deemed important because most aspects of a species' ecology and responses to environmental variability are underpinned by inherited genetic variation. Genetic diversity is also thought to be important because it is the foundation for 'evolutionary potential', which is the ability of a species, or population, to track environmental changes through shifts in genetic attributes. A fundamental assumption in the application of neutral population markers to resource management is that molecular markers can be used to measure genetic diversity as a proxy for mean fitness of a population.

Levels of standing genetic diversity are influenced by a population's demographic history, mutation, and natural selection. Natural selection on organellar DNA and nuclear microsatellites is assumed to be an inconsequential force acting directly on these genetic markers to shape population structure. This assumption is generally invoked in fisheries stock-structure studies, except when evidence for outlier loci is detected that might point to the effects of divergent selection among populations. While mutations in DNA sequences are the ultimate sources of genetic variability, they occur at rates that are much slower than demographic events driven by seasonal, decadal, centennial and millennial climatic instability (Figure 8).

In an evolutionary context, the success of a population or species is measured by ‘fitness’, which is the ability of an individual to successfully reproduce and contribute offspring to the next generation that themselves successfully reproduce (Benton & Grant 2000). Reproductive strategies vary among marine organisms. Some species invest considerable energy into ensuring the survival of a few offspring, for example, by producing large calorically rich eggs or by defending egg nests. Most marine species, however, broadcast large numbers of calorically inexpensive eggs into the water and provide little or no parental care, as do kelps. In both cases, the amount of genetic diversity is thought to increase fitness by enhancing the survival and reproductive output of offspring.

Since the inception of allozyme methods in the 1960s (Hubby & Lewontin 1966; Lewontin & Hubby 1966), biochemical and molecular markers have been used to estimate gene diversities in natural populations. The link between estimates of heterozygosity (and inbreeding) with various molecular methods and fitness can arise in several ways (Hansson & Westerberg 2002). First, variability in the molecular markers themselves may be linked to fitness, especially for markers, such as allozymes, that have metabolic functions that shape an individual’s physiology and phenotype. Heterozygotes at an allozyme locus may have different catalytic properties that provide greater efficiencies than homozygotes (functional overdominance or heterosis). Numerous studies using allozyme markers have invoked overdominance to explain associated between heterozygosity and some aspect of fitness (Mitton & Grant 1984; Britten 1996).

Direct overdominance, however, is unlikely to explain correlations between microsatellite DNA and fitness, because theses markers do not encode functional products and are unlikely to be affected by natural selection (Coltman & Slate 2003). Even so, associations between microsatellite diversity and fitness traits have appeared in several species (Bierne *et al*. 1998) A second explanation for heterozygosity-fitness association in these cases may be that the markers are linked to genes conferring greater fitness (local area hypothesis: Hansson & Westerberg 2002). The markers may not necessarily be physically linked to the fitness genes, but can be associated with fitness traits because of random drift in a small population (associative overdominance: Ohta & Kimura 1970). Associative overdominance is expected to decline with time as populations reach linkage equilibrium and is unlikely to occur in large, panmictic populations.

The correlation between heterozygosity and fitness in natural populations is a pervasive assumption in conservation biology and resource management for animals (Allendorf & Luikart 2007; Bert *et al*. 2007; DeWoody *et al*. 2010) and seaweeds (Valero *et al*. 2011). The concern centers on the loss of fitness that is thought to result from a reduction in genetic diversity or from an increase in homozygosity due to mating between relatives (inbreeding). The loss of heterozygous loci across a genome may lead to the loss of overdominance and heterozygote advantage. The presence of more than one functional allele at a locus provides alternative metabolic pathways to cope with such environmental variables as temperature, salinity, and pH.

An alternative mechanism affecting fitness may be inbreeding and elevated levels of homozygosity of deleterious alleles that reduce fitness (Keller & Waller 2002; Charlesworth 2003).

The amount of genetic diversity in a population is thought to be a critical component of adaptive potential (Lande & Schemske 1985; Kawecki & Ebert 2004) and the protection of this diversity from harvest and aquaculture activities has been a mantra among conservation biologist and fishery managers for the past few decades (Lande & Schemske 1985). Measures of genetic diversity in a sample of genes are often used as a proxy to predict adaptive potential, which is generally affected by numerous quantitative loci throughout the genome. However, theoretical (Ohta & Kimura 1970; Chakraborty 1981) and simulated (Balloux *et al*. 2004) results, together with empirical results (Slate *et al*. 2004), show that locus heterozygosities across the genome are uncorrelated. Surveys of indicator loci indicate nothing more than levels of diversity of the loci that have been surveyed. In any case, estimates of genetic diversity are based on a small fraction of the genome that may act independently of other parts of the genome (Galtier *et al*. 2009). Furthermore, fitness is uncoupled from genetic diversity is uncoupled from fitness in many ecologically successful plants reproduce asexually and have no within-population genetic diversity (*e.g*. eelgrass, Reusch *et al*. 1999). Even though estimates of diversity may not be associated with fitness or evolutionary potential, they have successfully been used to infer demographic events, such as founder effects and bottlenecks in population size (*e.g*. Hauser *et al*. 2002). Assessments of adaptive potential based on the level of genetic diversity at marker loci may not be important when environmental shifts require changes that exceed the reaction norms of genotypically influenced phenotypes (Schlichting & Pigliucci 1995).

The chief limiting factor of genotypic-based responses to environmental change is the slow accumulation of beneficial mutations relative to the pace of climate change. New mutations can be lost by random drift or by counter selection during a developmental or an alternate life-history phase. Genetic diversity in plants is also correlated with mating system so the use of molecular markers as proxies for genome-wide diversity to assess evolutionary potential can be misleading. For example, selfing, which is comon in plants, is associated with a decrease in additive genetic variance in models (Charlesworth & Charlesworth 1995) and in natural populations (Bartkowska & Johnston 2009). A literature survey of plant adpatation showed that both the frequencies of selfing and outcrossing were not correlated with local adaptation (Hereford 2010). In another survey, no difference in local adaptation was detected between self-compatible, which are expected to have low levels of genetic diversity, and self-incompatible species with higher levels of diversity (Leimu & Fischer 2008). Hence, the reduced genetic variability in selfing species and the greater potential for gene flow in outcrossing plants may have little influence on the ability of plant populations to adapt to local conditions.

Most research on selfing has been conducted on terrestrial plants. However, the ecological effects of selfing also appear in kelps. In the giant kelp, *Macrocystis pyrifera*, a selfing rate was estimated to be 30–40% of fertilizations, and this led to a substantial increase in mortality over outcrossed plants (Raimondi *et al*. 2004; Johansson *et al*. 2013). Selfing was more common at low densities where the opportunities for outcrossing were limited (Johansson *et al*. 2013). Reductions in the average fitness of populations with large proportions of selfed plants may be responsible for strong oscillations in abundance that occurs in many kelp beds (Raimondi *et al*. 2004). In the present study, duplicate microsatellite genotypes appeared in some Alaskan populations of *Alaria* and may have been due to selfing. The extent and effects of selfing in Alaskan kelp beds and the effects of selfing on population abundances are important areas of future research.

The patterns of genetic diversity among the kelps in the Northeastern Pacific reflect the effects of population displacements, bottlenecks in population size, extirpations, and colonizations over the last few Milankovitch glacial cycles. During glacial maxima and stadials, the shores of the Aleutian Islands, South Central and Southeastern Alaska were covered by margins of terrestrial glaciers. The mosaic genetic population structure along NE Pacific shores is clear evidence of isolation and divergence in numerous refugia between coastal lobes of the massive Cordilleran ice sheet. Hence, the genomic structures of present-day populations of kelps in the Gulf of Alaska are legacies of natural selection during past cycles of climate change (de Lafontaine *et al*. 2018). In addition to demographic history, the reproductive and recruitment biology's of kelps also contribute to low levels of genetic diversity within populations. Reproductive skew (Eldon & Wakeley 2006), sweepstakes recruitment (Hedgecock & Pudovkin 2011), and incumbency (Hendry 2004; Marshall *et al*. 2010; Waters *et al*. 2012) conspire to produce low levels of genetic diversity within populations.

11.3 Adaptive responses to environmental variability

The rapid pace of climate change in Alaska, past and present, requires rapid phenotypic transformations, if not in gross morphology, in physiology, to meet the challenges of environmental shifts. A discussion of adaptive potential of species in the high-latitude waters of Alaska is of particular important, because the region is experiencing, and will continue to experience, unprecedented increases in sea temperature and with them, ocean acidification (IPCC 2007). The term 'adaptive potential' appears frequently in the literature on natural resource management and biological conservation and has traditionally denoted the genetic variance needed to respond to selection, in much the same way that additive variance of quantitative traits is required to improve plant and animal production (Falconer 1960). Natural selection favors individuals with particular phenotypes over others, so that some individuals are more 'fit' in a particular environmental setting than others. Fitness is ideally measured not just by the proportion of offspring contibuted to the next generation by an individual, but by offspring that reproduce and successfully pass on genes. Individuals with greater fitness are expected to pass on more offspring than less fit individuals. Lifetime reproductive success is a widely used surrogate of fitness (Clutton-Brock 1999; Benton & Grant 2000), but since it is difficult to quantify in the wild, various components of reproductive success, such as sexual dominance, survival or fecundity, are used to measure fitness (Kawecki & Ebert 2004).

Responses to evironmental change depend on phenotypic variability, which can arise from several sources. One source of variability are plastic responses to environmental challenges that occur over the lifetime of an individual (Figure 7.6). These responses represent reactions norms of genotypes in a population, but do no represent changes in the the underlying DNA (Hendry *et al*. 2011). Phenotypic variability without genetic change has been documented in several algae (Shibneva *et al*. 2013; Hu & Lopez-Bautista 2014; Calegario *et al*. 2019). Plastic changes from the extremes of reactions norms may underlie short-term acclimation to altered environmental conditions, but are limited in the ability to produce continuing adaptive responses because they are tied to the underlying DNA. Early work in conservation attempted to measure adaptive potential with molecular markers of genetic diversity, and explained adaptive changes to climate warming by invoking shifts in the frequencies of existing genetic variants. However, genetic change stemming from allele-frequency shifts in standing genetic diversity is limited by the amount of genetic diversity in a population. Many ecologically successful species, including invasives and clonal species, have little genetic diversity, as do the three species of kelps examined in this study. Standing genetic diversity in these species cannot be a continuing source of adaptive change, and the accumulation of beneficial mutations is too slow to keep pace with environmental changes. Hence, non-genetic mechanisms come into play.

11.3.1 Non-genetic variation

For the past few decades, the central dogma of genetics has been the one-way transmission among generations of heritable information encoded in genes. Even though a particular genotype, or combination of genotypes, can respond to environmental variability by producing an array of phenotypes, the underlying genetic information does not change. Contrary to this central assumption, it is now clear that environmental forces can influence heritable variation that can be transmitted across generations (Schmid *et al*. 2018). Transmission of traits can consist of epigenetic changes to DNA and cytoplasmic and somatic alterations. In a broader perspective, nongenetic inheritance bypasses the limitations of genetic inheritance in promoting adaptive fitness by decoupling phenotypic change from the slow process of Mendelian-based genotypic change.

Non-genetic modifications of DNA are implemented through the addition of a methyl group to cytosine or less commonly to adenosine (Figure 11.7). Epigenetic modifications of DNA often involve interference RNA (RNAi), which are RNAs, 20-30 nucleotides in length, involved in the epigenetic modification of DNA (Cerutti *et al*. 2011). This class of RNA likely arose as defences against viruses by degrading viral RNA and against transposable elements by preventing these elements from commandeering DNA encoded genes. Non-genetic variation can reflect epigenetic modifications of gene express, paternal effects in which the phenotype of a parent influences phenotypes of offspring (Bonduriansky & Day 2009). Other forms of non-genetic inheritance include the transmission of a plant's microbiome or transposable DNA elements. Phenotypic variability from these sources interacts with environmental forces to influence the components of adaptation. The boundary between genotype and phenotype is blurred when environmental drivers alter the inheritance of phenotypes through epigenetic mechanisms.

Adaptive responses to evironmental variability through epigenetic modifications of DNA are characterized by five features that have been demonstrated in terrestrial and marine angiosperms (Richards *et al*. 2010, 2017; Schmid *et al*. 2018). The first feature is an uncoupling of epigenetic variation from DNA sequence variation. For example, epialleles in a small, well-studied herbaceous plant, *Arabidopsis* influence morphology that was uncoupled from DNA sequence polymorphisms (Kooke *et al*. 2015). Second, epigentic variation must influence some aspect of a plant's phenotype, and third, environmental selection must influence the epigenetic phenotype. These conditions have been demonstrated in angiosperms. Genomic profiles of methylation showed an association between patterns of methylation and contrasting environment in *Arabidopsis* (Bossdorf *et al*. 2010; Dubin *et al*. 2015) and salt marsh plants (Foust *et al*. 2016). These patterns were interpreted to result from environmental influences on the epigenetic variation. Fourth, the rate of mutation exceeds the mutation rate of the underlying DNA. The rate of mutation of epigenetic variants (epialleles) in plants has been estimated to be as much as five orders of magnitude larger than the mutaton rate in coding DNA (van der Graaf *et al*. 2015).

Fifth and importantly, epialleles are inherited by offspring and continue to shape phenotypes in subsequent generations. Experimental studies of *Arabidopsis* have shown associations between epigenetic changes and flowering time that lasted several generations in a neutral environment without the influence of selection (Schmid *et al*. 2018). Nongenetic inheritance has also been documented in numerous other plants for a variety of phenotype traits (Johannes *et al.* 2008; Holeski *et al.* 2012). Cubas *et al*. (1999) found that a variant producing radial symmetry of a flower, described in the 1700s by Linneaus, was due to the silencing of a gene by epigenetic methylation and has been stable for at least 250 years.

The speed of adaptation to a particular environment is influenced by several factors, including the extent that selection favours a particular genetically influenced phenotype, by the introductions of genes from populations not adapted to local conditions, and by the additive variance in a population (Hereford 2010). Microevolutionary changes can occur over a few decades on time scales corresponding to human environmental disturbance (Dobzhansky 1955; Hendry & Kinnison 1999; Kinnison & Hendry 2001). These changes have been observed in pristine and naturally disturbed habitats, under captive propagation, with accidental or intential introductions of species into non-resident areas (Pergams & Ashley 2001; Boag & Grant 1981; among many others) and in environments modified by human activity (Kettlewell 1973; among numerous other examples).

Numerous studies show that nongenetic inheritance of acquired phenotypes occurs nearly all species. Epigenetic inheritance is more common in plants than in animals, because plant germline arises from somatic cells that have been influenced by the environment. Some plants reproduce asexually without the development gametes. Theoretical models indicate that nongenetic inheritance increases both the rate of phenotypic *and* genetic change and can tweak the direction and speed of evolutionary change (Bonduriansky *et al*. 2012). Attempts to incorporate these broader and important sources of evolutionary change into a new ‘extended synthesis’ of evolutionary biology has been proposed to unite seemingly contradictory results emanating from molecular biology and eco-genetics (Pigliucci 2009; Pigliucci & Müller 2010). The conceptual framework for managing natural resources has to follow suite by recognizing the importance of mechanisms that go beyond Mendelian genetic inheritance.

11.3.2 Adaptive potential in natural populations

Kelps can respond to climatic warming in three ways: by poleward shifts in geographic distribution, by adapting to new environmental conditions, or by becoming extinct locally or regionally. Detecting permanent adaptive shifts due to climate warming is difficult because of the inability to distinguish between genetic, plastic and non-genetic responses to environmental change without breeding experiments. Even when phenotypic changes are documented, it is not always clear whether the changes are adaptive, or whether they arise from other causes, such as a shift in ecosystem structure or predation (Merilä & Hendry 2013). Responses to environmental change through plastic and epigenetic-based phenotypic changes are likely important in kelps in the Northeastern Pacific because of the low levels of genetic diversity and the rapidity of environmental change (Liu 2013; Dodd & Douhovnikoff 2016). Populations of algae in variable environments tend to show more plastic responses than species in stable environments (Schaum & Collins 2014). Fluctuating environments appear to select for plasticity, and this may be the case for kelps in the Northeastern Pacific, given a long history of large environmental changes on seasonal, decadal and millennial time scales (Figure 11.5).

Numerous reciprocal transplant studies have shown that angiosperm plants are often adapted to a particular environment (Turesson 1922; Clausen *et al*. 1940; and numerous subsequent studies). However, other studies show that local populations are not always at an adaptive optimum (Hansen *et al*. 2006; Hereford & Winn 2008). A survey of the literature on local adaptation in natural populations (*n* = 74), as measured by viability and fecundity in reciprocal transplants, showed that genetic differences among populations were positively associated with local adaptation, but phenotypic divergence was not (Hereford 2009). Adaptation of populations plants and animals to local environments produced individuals that were less fit in other environments, so that gene flow and genetic drift limited the effectiveness of natural selection in a particular environment.

Local adaptation has been documented in several kelps. In the Northeastern Atlantic, for example, populations of *S. latissima* in Denmark occur along a salinity gradient that influences the biochemical composition of the kelp (Nielsen *et al*. 2016). Kelp populations in high-salinity habitats had the largest total production potentials, largest protein content, and the greatest capacity for bioremediation of nitrogen. However, plants growing in lower salinities had the largest concentrataions of fermentable sugars and higher concentrations of pigments, qualities that are desirable in both fuel production schemes and for human consumption. Hence, areas producing the largest biomasses of the kelp did not produce plants with the most desirable commerical qualities.

11.4 Connectivity among populations

A fundamental question facing the management of seaweeds is the degree to which populations are connected to one another by gene flow through the dispersals of spores, gametes, or detached sporophytes. A common rationale for identifying stock boundaries is to be able to protect weak populations that may be at risk from human development or that may be decling from the effects of natural environmental changes. In plants, population structure reflects the isolating effects of environmental gradients or of barriers to dispersal. Even in the absence of barriers to dispersal, a pattern of isolation by distance may ensue because of limited dispersal ability. The three species of kelp examined in this project did not appear to track environmental gradients on a large geographical scale, nor did they show isolation by distance (Chapters 3, 5 & 6). Estimates of divergence between populations were large, on average, because of often fixed haplotype- or allele-frequency differences between populations (Figure 11.5).

In Alaskan populations of *Saccharina latissima*, the overall amount of divergence among populations was large for both organellar ($\Phi_{ST} = 0.646$) and microsatellite ($F_{ST} = 0.366$) DNA (Table 11.3; Chapter 3). This amount of divergence was larger than among populations of *Saccharina latissima* in the North Atlantic (organellar DNA $\Phi_{ST} = 0.318$; microsatellite DNA $F_{ST} = 0.015$–0.259), and larger than divergence among populations of *Saccharina japonica* in the Northwestern Pacific (organellar DNA $\Phi_{ST} = 0.284$; microsatellite DNA $F_{ST} = 0.342$). While the amount of sequence divergence between haplotypes was small—not more than 3 mutations between central haplotypes along 1359 base pairs—many populations were fixed or nearly fixed for different haplotypes. These strong differences indicate little genetic exchange between regional groups of populations.

On smaller geographical scales, some populations has similar haplotpic frequencies. In Kachemak Bay similar frequencies between populations may be due to gene flow, but the similarity between samples on larger spatial scales is more likely due to historial post-glacial dispersals from refugia (Chapter 3). For example, contemporary connectivity through gene flow is unlikely to account for the disjunct distribution of C lineage haplotypes, which appear at various frequencies across the Gulf of Alaska from the western Aleutian Islands to Prince of

Wales Island in southeastern Alaska. Similarly, D-lineage haplotypes appear in populations separated by about 1000 km with other lineages appearing in intervening locations.

The superimposition of organellar DNA haplotypes on a principal components analysis of 12 microsatellite loci showed that that the microsatellite alleles clustered more closely with the organellar DNA lineages than they did with the geography of the samples (Figure 3 in Chapter 3). This pattern indicates that the geographical distributions of DNA lineages and microsatellite alleles are not yet in migration-drift equilibrium. Historical events still define the genetic structure of populations of *Saccharina latissima* in the Gulf of Alaska.

A preliminary survey of organellar DNA sequence variability in split kelp, *Hedophyllum nigripes*, showed a simlar mosaic, genetic population structure. Divergences among populations for combined *COI* and *rbc*L sequences ranged from $\Phi_{ST} = 0.151$ to 1.0 and averaged 0.632 (Table 11.3, Chapter 5), reflecting strong haplotype frequency differences between populations. These differences between populations on geographical scales of 100–200 km or less indicate strong restrictions on gene flow. Comparisions with *COI* haplotypes in British Columbia to the south and with haplotypes in the Arctic and Northwestern Atlantic show that populations in the Gulf of Alaska harbor the largest amount of genetic diversity. This center of diversity argues for glacial refugia in the Gulf of Alaska and relative recent dispersal across the Arctic and to southern Northeastern Pacific shores.

The results for the winged kelp, *Alaria*, showed much deeper divergences between the combined *COI*-*rbc*L haplotypes. Overall sequence divergence among populations was large ($\Phi_{ST} = 0.811$) (Table 11.3, Chapter 6) and was due to the inclusion of different taxa, some of which have been described on the basis of morphology. Some of these DNA lineages represent cryptic groups that have not been formally described as species (Widdowson 1971a; Lane *et al*. 2007). As in the case of *Saccharina latissima*, the strong haplotype differences signal little genetic exchange between most populations, which in this case are reproductively isolated taxa in the early stages of speciation.

The geographical patterns of populations within lineages are difficult to explain in terms of gene flow. Haplotypes in lineage A are found from the western Aleutians, across the Gulf of Alaska to the southern part of southeastern Alaska and are mixed with haplotypes from other lineages at some localities. While haplotype-frequency similarities between nearby populations can be accounted for by gene flow (e.g. Kodiak Island and Kachemak Bay), frequency similarities beween widely separated populations are more likely due to post-glacial dispersals from ice-age refugia and ancient colonizations. Some of the differences between populations on small geographical scales are due to ecotypic differences between populations (or between species). For example, a population in Kachemak Bay situated on a wave exposed headland is strongly divergent from populations only tens of kilometers away in both directions at wave-protecrted beaches in Kasistna Bay and at Homer Spit. The superimposition of organellar DNA lineages on a principal components ordination of microsatellite variability showed that plants bearing different lineages tended to hybridize when they occur at the same locality (Figure 3 in Chapter 6). These hybridizations indicate that reproductive isolations between the various lineages are incomplete.

11.5 Eco-evolutionary dynamics of kelps in the Northeastern Pacific

Present-day genetic population structures of kelps in the Northeastern Pacific are legacies of historical population events. Shorelines around the Northeastern Pacific have had a particular turbulent climatic history because of large temperature and sea-level swings (Figure 10.5) and because of episodic incursions of the margins of large terrestial glaciers into coastal areas. The past 10,000 years in the Holocene have had relatively stable temperatures, but climate projections predict that climate warming from greenhouse gases is in store in the coming decades.

11.5.1 Historical biogeography: ice-age upheavals

Climates over the the Pleistocene Epoch beginning 2.6 million years ago have varied considerably, but have been particular unstable in the last 1.26 million years (Morley & Dworetzky 1991; Lisiecki & Raymo 2005). During cold periods, massive sheets of ice accumulated on North American and Euasia and lobes of the glaciers extended onto coastal areas. At the peaks of these glaciations the shores of the Northeastern Pacific and the Aleutian Islands were largely, but not completely, covered with ice that destroyed near-shore habitats and led to the extirpation of kelp populations. The distributions of the kelps may have been displaced to southern unglaciated shores, but the higher levels of genetic diversity in Gulf of Alaska's kelp populations (Sections 4 and 6) than in southern populations, together with the mosaic populations structure, indicates that many populations survived in northern refugia. Refugia may have been located between the major lobes of the terrestrial glaciers as they reached the shore. The mosaic population structures of the three kelps in this study can be explained by dispersals from these refugia and colonizations along post-glacial shorelines.

Under this scenario, refugial populations around the Gulf of Alaska were able to adapt to large temperature shifts. For example, temperatures rose by several degrees centigrade over only a few hundreds of years after the last glacial maximum about 20,000 years ago, and this scenario was repeated over the Pleistocene during stadial-interstadial cycle on hundreds and thousands of years and during major Milankovitch climate cycles every 100,000 years. Marine biota in the Northeastern Pacific have adapted to large temperature swings throughout the Pleistocene, and hence adaptive mechanisms may be in place to cope to some extent with temperature swings predicted in the coming decades. Future global temperatures, however, are predicted to rise above levels not experienced since the last interglacial period about 130,000 years ago.

11.5.2 Climate warming

The evidence for human induced global warming is substantial (Keller 2009; Gleckler *et al*. 2012; IPCC 2015). About 80% of the heat generated by greenhouse gases is absorbed by world's oceans (Domingues *et al*. 2008). This ocean warming has already influenced the abundances and distributions of marine organisms through shifts of ocean isotherms and ocean acidification (Perry *et al*. 2010; Evans *et al*. 2015; Duarte *et al*. 2018; Wahl *et al*. 2015). Seaweeds can respond to global warming in three ways: 1) local populations adapt to new environmental conditions, 2) species' ranges shift into environmentally suitable areas, or 3) regional populations disappear. Phenotypic plasticity in kelps may help to cope with climate warming in the short term before genetic adaptation is possible. Environmental changes may proceed faster than populations can respond with genetically based evolutionary changes. Range shifts and local extirpations are the most likely responses of seaweeds to environmental change in Alaska.

Projections of future climates over the next several decades have been made with simulations of population dynamics under various environmental scarios. The most important variable is the expected concentration of greenhouse gases, which include not only carbon dioxide, but also methane and other gases. Water vapor is also a potent greenhouse gas with concentrations largely determined by evaporation and global temperatures. Other factors also contribute to climate warming, including developments in technology, land use, economic conditions and human population growth (Wayne 2013). To assist in making projections, a standard set of scenarios have been devised to ensure that a standard set of conditions are used in climate projections made in various disciplines.

The latest set of conditions was published by the Intergovernmental Panel on Climate Change (IPCC) in its Fifth Assessment Report (IPCC 2015) and are called *Representative Concentration Pathways* (RCP) with numerical indices indicating particular characteristics of radiation forcings and emissions. Four RCPs, RCP8.5, RCP6, RCP4.5, and RCP2.6, together with additional information for a particular region, are commonly used used to make climate projections. The temporal range of these RCPs extends to the year 2100. RCP8.5 is the most severe environmental scenario and RCP2.6 is most optimistic, apart from a complete cessation of greenhouse gass emission. These scenarios are the lastest in attempts to standardize climate simulations beginning in 1992 with IS92, then SRES in 2000, TAR in 2001, AR4 in 2007, and the latest AR5 in 2015. Modllers in various disciplines use the data in these scenarios as starting points for simulations that are variously designed to address a particular problem or predict change in a particular region. *Extended Concentration Pathways* (ECP) are also available for climate projections to the year 2300. The goal of using RCPs and ECPs for simulations is not to predict future climate conditions with any precision, but to map the range of possible future conditions and to identify uncertainties (IPCC 2015).

In the past 80 years, the Northern Hemisphere has experienced unprecedent increases in temperature (Figure 11.9). Over this time, temperatures in Arctic and subarctic Alaska have increased twice as rapidly as temperatures at temperate latitudes. A consequences of this warming as been a decline in the extent of winter sea ice in both the Alaskan Arctic Ocean and Bering Sea (Figure 11.10; Lader *et al*. 2017). The Bering Sea was nearly ice-free in the 2018-2019 winter and sea surface temperatures are currently 2–3° C warmer than the long-term average (Figure 11.11) (https://uaf-iarc.org/2019/04/11/bering-strait-sea-ice-conditions-winter-2019/ accessed July 2019). A nearly ice-free Arctic ocean is absorbing atmospheric carbon dioxide, and this has led to the acidification of large areas in the western Arctic Ocean north of Alaska (https://www.climate.gov/news-features/features/research-shows-ocean-acidification-spreading-rapidly-arctic accessed July 2019). Ocean warming and acidificaton have also impacted the Gulf of Alaska, where temperatures have risen between 2–4° C in the past few decades (Royer & Grosch 2006; Evans *et al*. 2015; Yang *et al*. 2019). Numerous climate projections indicate that climates will continue to warm in the coming decades, even if present-day levels emissions of green-house is abated (Figures 11.12, 11.13).

The loss of local populations can result from these environmental shifts (Nicastro *et al*. 2013). Many kelps are particular sensitive to high temperatures so that the extents of kelp beds and biogeographical boundaries are regulated by high summer temperatures (Davison 1991; Yesson *et al*. 2015b). The loss of seaweed biomass leads to reductions in primary productivity that ripple through an ecosystem. Macroalgal communities are highly productive primary producers that fuel secondary production in nearshore ecosystems (Mann 1973; Krumhansl & Scheibling 2012). The loss of macroalgae leads not only to the loss of primary productivity and altered foodwebs, but also structural canopies in intertidal and shallow subtidal areas that promote the reproduction, growth, recruitment and survivals of understory species not only of other algae, but also of invertebrates and juvenile fishes (Rangeley & Kramer 1995; Bartsch *et al*. 2008; Christie *et al*. 2019).

Shifts in the abundances of several kelps have been attributed to ocean warming. Gao *et al*. (2015) monitored seasonal changes in biomass, growth and maturation of plants in natural populations of *Saccharina japonica* from 2005 to 2007. In 2006, populations gave several signals of decline: less standing biomass, later appearance of sporophytes, delayed growth and a greater proportion of sporophyte fecundity relative to 2005. In 2007, the population disappeared completely (Kawai *et al*. 2015). The authors concluded that the increase in temperatures at this site over three years exceeded the tolerances of the seaweed. Even in northern areas, populations of *Saccharina japonica* are not immune to the effects of high temperatures. Before 1980, *Saccharina japonica* was the dominant species in the Primorye region of Russia covering the bottom from the low-tide level to about 20 m depth. After 1980, this species declined apparently in response to the warming of the northward flowing Primorye Current that produced marginal conditions for kelp growth, reproduction and recuitment.

In multi-factorial experiments of the microbiome on the kelp *Macrocystis pyrifera*, Minich *et al*. (2018) found that warmer temperatures and elevated CO_2 shifted the bacterial biome associated with kelp mucous production. Warmer temperatures led to reduced growth and to a shift in the microbiome that degraded alginates and sulfated polysaccharides. However, warmer temperatures in combination with increased CO_2 concentrations produced higher growth rates that were also associated with a shift in the kelp's microbiome.

Short term physiological regulation allows plants living in the intertidal zone to cope with large shifts in temperature during a tidal cycle. Phenotypic plasticity allows plants to survive temperature shifts over days and weeks, but genetic adaptation may take hundreds or thousands of years and is largely limited by the appearance of new genetic material through mutation or introductions by gene flow from other populations. Hence, the speed of presesnt-day climate warming does not allow for genetic adaptation. Extinctions at the southern margins of a species' distribution have been observed in the North Atlantic for *Fucus vesiculosus* (Jueterbock *et al*. 2013; Nicastro *et al*. 2103), *Bifurcaria bifurcata*, and *Cystoseira tamariscifolia* (Mieszkowska *et al*. 2006), *Laminaria hyperborea* (Müller *et al*. 2009). Poleward movements of the southern margins of other seaweeds have been observed along the coast of Spain (Fernández 2011, 2016; Diez *et al*. 2012), Portugal (Tuya *et al*. 2012), the United Kingdom (Yesson *et al*. 2015a), German (Pehlke & Bartsch 2008), Norway (Moy & Christie 2012), Australia (Wernberg *et al*. 2011), and Japan (Tanaka *et al.* 2012). Comparable studies searching for range shifts have not been made in the Northeastern Pacific.

Saccharina latissima appears to be more sensitive to high temperatures than other kelps, growing well at 5–17° C (Druehl 1967; Machalek *et al*. 1996). It shows metabolic plasticity to maintain carbon and nitrogen uptake and use at temperatures as high as 20° C (Davison 1987). Even so, the rate of photosynthesis drops and tissues deteriorate at high temperatures (Davison 1991). Short-term acclimation to elevated temperatures involves the upregulation of proteins to protect metabolic processes (Heinrich *et al*. 2012, 2015, 2016). Persistent temperature stress may have led to adaptive ecotypes in some localities (Davison 1987; Gerard *et al*. 1987; Gerard & Dubois 1988), but temperature adaptation at the microscopic gametophytic stage has not been confirmed (Bolton & Lüning 1982). Bolton & Lüning (1982) found no differences in the tolerances of plants from contrasting latitudes to high temperatures and concluded that the successes of populations in different temperatures regimen resulted from metabolic plasticity rather than from the selection of temperature-tolerant ecotypes.

Several studies of seaweeds in the North Atlantic with climate-projection models indicate the inevitability of seaweed community reorganizations, local extinctions at a species' southern boundary, and poleward colonizations. Khan *et al*. (2018) used present day thermal limits and the RCP 4.5 and RCP 8.5 datasets together with atmosphere-ocean general circulation models to predict range shifts for several species of macroalgae in the Northwestern Atlantic. All of the seaweeds examined, except rockweed (*Fucus vesiculosus*), were projected to disappear from the Gulf of St. Lawrence and the Atlantic coast of Nova Scotia. Using a similar approach, but with RCP2.6 and RCP8.5, Neiva *et al*. (2015) found that southern populations of a canopy-forming seaweed in the Northeastern Atlantic were at risk of extinction. The loss of these trailing-edge populations was critical because they harbored unique levels of genetic diversity. Assis *et al*. (2014) also predicted a northern shift in the range of this rockweed with extinctions of populations in the southern North Atlantic range with small extension of the range into the subarctic and arctic waters by the year 2100. Populations of the annual kelp *Saccorhiza polyschides* in the Northeastern Atlantic are predicted to be limited to coastal areas of upwelling by 2100 (Assis *et al*. 2017a). The geographical ranges of an additional eight North Atlantic kelps are expected to shift poleward with large numbers of extinctions among southern populations (Assis *et al*. 2017b). These northward range dispersals will greatly affect commercial harvests, not only of seaweeds, but of crustaceans and fishes that depend on the food webs and physical structures provided by seaweeds. Projections for the distributions of future populations in the Northeastern Pacific have not yet been made, but increases in sea surface temperatures will undoubtedly produce similar shifts in the geographical ranges of seaweeds. Krumhansl *et al*. 2016).

Alaska is unlikely to see southern-edge extinctions as the general distributions of kelps move poleward with warmer waters. The temperature shores of the Northeastern Pacific reach from the western Aleutian Islands to California with the geatest seaweed species diversity in southern British Columbia and northern Washington. The number of species begins to dwindle in northern British Columbia and Alaska, indicating that northern boundaries of some species lie in these northern waters, but few species' southern boundaries. The northern boundaries will likely move poleward and introduce new species to the western shores of the Gulf and into the Bering Sea. Nereocystis, for example appears to be expanding westward along the Aleutians and Macrocystis has appeared on Kodiak Island.

Nevertheless, local bays and headlands and even regions can still lose species of kelps, as local habitats change. For example, about 80% of the biomass of sugar kelp, *Saccharina latissima*, along the southern coast of Norway and about 40% of the biomass along Norway's west coast has been lost (Bekkby & Moy 2011; Moy & Christie 2012). In Japan, a natural population of *Saccharina japonica* disappeared over the course of three years, as local temperatures increased incrementally (Gao *et al*. 2015) Similar declines have occurred in rockweed species in the genus *Fucus* (Coyer *et al*. 2006; Nieva *et al*. 2016). In these cases, the dominant kelps were replaced by small turf algae. In the Gulf of Alaska, the predicted temperatures for the year 2100 will most likely lead to similar local or regional losses or declines in abundance in some kelp species.

11.5.3 Ocean acidification

Since the industrial revolution in the 1800s, the use of fossil fuels and land use practices have led to a large increase in atmospheric CO_2 that has risen from less than 300 parts per million (ppm) over the past 800,000 years to over 409 ppm in 2018 (Gao *et al*. 2019). About 50% of atmosphereic CO_2 has been tied up in terrestrial ecosystems (20%) or absorbed by the world's oceans (30%) (Feely *et al*. 2004; Sabine *et al.* 2004). The oceans are absorbing over 1 million tons of CO_2 each hour (Gao *et al*. 2019). The uptake of CO_2 in the ocean is facilitated by the dissolution of calcium carbonate ($CaCO_3$) into bicarbonate and calcium ions in the reversible reaction

$$CO_2 + CaCO_3 + H_2O \leftrightarrow 2HCO_3^- + Ca^{2+}.$$

The bicarbonate further dissolves into $H_2CO_3 \leftrightarrow HCO_3^- + H^+$, and the excess hydrogen-ion (H^+) concentation leads to lower oceanic pH. Increases in atmospheric CO_2 and absorption by the ocean push both reactions to the right. Sea surface temperatures and CO_2 concentrations are expected to continue to rise in the next several decades (Calderia & Wickett 2005). The greatest effect of ocean acidification on marine organisms is reduced bio-calcification and altered metabolism. However, non-calcifying species, such as kelps, still show a range of effects from ocean acidification on development, growth, survival, and reproduction (Kroeker *et al*. 2010). The loss of soluble a forms of calcium and other minerals, such a magnesium, leads to developmental abnormalities in marine organisms with calcarious structures (Iglesias-Rodriguez 2008; Kurihara 2008). Micro-organisms, such as pteropods at the base of marine food webs, and shellfish are especially affected by ocean acidification (Fabry *et al*. 2008, 2009; Barton *et al*. 2012). Ocean acidificaton is exacerbated at high latitudes of Alaska as the warming of marine waters increases eutrophication and reduces dissolved O_2 in seawater (Gao *et al*. 2019).

Carbon metabolism is key to understanding the effects of ocean acidification on macroalgae (Cornwall *et al*. 2012, 2017). Shifts in ocean CO_2 alter, not only pH, but also the availability of carbon for photosynthesis. The greater availability of CO_2, relavitve to HCO_3^-, at low pH may benefit algae to some extent because of the lower energetic cost of absorbing CO_2 (Carvalho *et al*. 2010). A critical step in kelp metabolism is the uptake of carbon and nutrients across the frond surface. The pH in a diffusion layer on the frond surface is metabolically modified so that the uptake of nutrients, such a nitrogen, are not affected by environmental drops in sea-water pH (Fernández *et al*. 2016). To understand these dynamics, small discs of apical frond near the fast growing meristem from the kelp *Macrocystis pyrifera* were cultured to understand the dynamics of carbon uptake under present (pH 8.00) and future (pH 7.59) acidic conditions (Fernández *et al*. 2015). The results showed an adaptive response by using greater amounts of bicarbonate (HCO_3^-) than CO_2 as a carbon source so that neither photosynthetic nor growth rates were negatively influenced by a reduction in pH. Olischläger *et al*. (2012) examined the effects of external carbonic anhydrase on life-history stages of the kelp *Laminaria hyperborea* in the North Sea. The production of eggs in female gametophytes and the growth of macroscopic sporophytes were enhanced at higher levels of dissolved CO_2.

One of the best studied of the kelps are populations of the giant kelp *Macrocystis pyrifera*, off California and in the southern Hemisphere off Chile and New Zealand. Laboratory experiments with culture conditions typical of present-day environments and with a reduced level of pH expected in the future showed that meiospore mortality increased and gametophyte germination was inhibited at lower pH levels (Gaitán-Espitia *et al*. 2014). In a 15 week culture study of the gametophytes and early sporophytes, Shukla & Edwards (2017) found that gametophyte germling growth declined fourfold at 15° C rather than at 12° C and fivefold under combined elevated temperature and CO_2. Higher temperatures also reduced gametophyte survival. Sporophyte germination and growth were stimulated by higher levels of CO_2. Thus, higher temperatures rather than higher concentrations of seawater CO_2 appeared to be more detrimental to the success of this kelp.

The direct effects of ocean acidifiation are not uniform across species of kelps. CO_2 enrichment may can lead to the reversals of dominance in a kelp community. For example, the replacement of kelps by turf algae is likely due to the positive effect that ocean acidification has on the growth of turf algae (Connell *et al*. 2018). They may explain the replacement of kelps in southern Norway by a diversity of small algae (Bekkby & Moy 2011). In an experiment using a non-calcified brown alga *Cytsoseira compressa* and a calcified brown alga *Padina pavonica* that were cultured under nutrient enriched and non-enriched conditions, Celis-Plá *et al*. (2015) found that both algae benefitted from elevated CO_2 levels. However, the strength of the response depended on light intensity and nutrient availability.

Shifts in temperature and pH and the interaction between them affect various life-history stages of algae. Culture experiments of meiospores of the kelps *Macrocystis pyrifera* and *Undaris pinnatifida* showed that germination was insensitive to sea water warming and reduced pH, but that gametophyte growth increased at higher temperatures (Leal *et al*. 2017a,b). Most studies attempting to understand the effects of elevated ocean temperatures and CO_2 concentrations have used have focused on single species and one or two stressors, ususally temperature and pH. However, natural populations as subject to a variety of stressors, including UV exposure, deoxygentation, nutrient limitation, competition, and herbivory (Boyd *et al*. 2018). Field experiments in a temperate kelp bed showed that increasing sea surface temperatures and higher levels of CO_2 acted synergistically to alter the balance between kelp canopies and understory algal turfs (Connell & Russell 2010). Under historical environmental conditions kelps inhibit the growth of small understory algae, but higher temperatures favor the growth of small turf species, which hinder the recruitment of kelps.

Most insights into the effects of ocean acidification have come from short-term experiments, generally much less than a year, but little is know about how coastal ecosystems are affected because long-term experiments are difficult to persue. However, studies of natural CO_2 vents provide natural experiments to understand the long-term effects of elevated levels of CO_2. Porzio *et al*. (2011) studied marine macroalgal communities near a volcanic CO_2 vent that produced of natural pH gradient ranging 6.07 to 8.20. They found that lower pH led to a decrease in the abundances of some species, especially calcified species, so that a few species dominated a simplier algal assemblage. Another study of seaweeds near CO_2 vents showed that, although calcarious algae recruited into areas of low pH, non-calcarious algae overgrew them and dominated the ecosystem (Kroeker *et al*. 2013a). Succession experiments in the same CO_2 rich environment showed that acidification reduced functional diversity to produce a simpler community dominated by a few algal species (Kroeker *et al*. 2013b).

Responses to ocean acidification and sea water warming can vary among regions, because CO_2 is less soluble in warmer seas than in colder water at high latitudes. Numerous studies show heightened sensitivity to acidification at warmer temperatures (Kroeker *et al*. 2013c). In a study of high-latitude populations of the kelps *Saccharina latissima* and *Laminaria solidungula*, Iñiguez *et al*. (2016) found that both species were unaffected by in an increase in seawater CO_2 concentrations, but that *S. latissima* grew more rapidly at 9° C than at 4° C, indicating that Arctic populations of *S. latissima* may benefit from ocean warming.

Some kelps may be pre-adapted to elevated concentrations of sea-water CO_2. Kelp forests experience daily shifts in pH becauses of photosynthesis, which removes CO_2 from the water, and respiration, which adds CO_2. Laboratory experiments on the juveniles of the kelp *Ecklonia radiata* showed that photosynthetic rates and growth were greater when plants were cultured under normal shifts in pH (8.4 by day and 7.8 at night), but showed no effect on growth and a negative effect on photosynthesis when pH was reduced by only 0.3 units (Britton *et al*. 2016). Seaweeds that tolerate strong diurnal shifts in pH may be better adapted to cope with ocean acidification (Cornwall *et al*. 2012, 2017).

This short review of the effects of ocean acidification on kelps shows that species vary in how they respond to warmer sea water temperature and elevated levels of sea water CO_2. Generally, the increased availability of soluble carbon for photosynthesis and warmer temperatures stimulate growth of macroscopic sporophytes. However, these beneficial effects may be moderated by other interacting environmental variables including nutrient availability and light intensity. Even when warming and increase CO_2 availability have positive effects on kelps, competitive interactions with other algae and herbivore grazing can alter the dynamics of a nearshore community. Ocean acidification can have a diversity of consequences that influence species’ ranges, patterns of dispersal between populations, food-web dynamics, and species’ diversity (Gaylord *et al*. 2015). Little, if any, research has been conducted on the effects of climate change on coastal seaweeds in Alaskan waters.

11.6 Seaweed aquaculture

Macroalgae cultivation and harvest produce a large number of products and provide an economic base for many coastal communities (Wei *et al*. 2013). About 80–90% of these harvests are used as food, or food additives, for human consumption. The high sugar (polysaccharides) content in kelps may make them suitable for the production of bio-fuels through fermentation (Adams *et al.* 2009). Seaweed culture may also play roles in the mitigation of the greenhouse gas CO_2 (Duarte *et al*. 2018) and the bioremediation of organic wastes from coastal fish farms (Broch *et al*. 2013). Up-scaling from experimental culture to large-scale commercial production requires a discussion of risks and benefits to ensure environmental and economic sustainability. Issues include 1) selective breeding to improve production traits 2) genetic interactions between cultivated and wild populations, 3) influence of seaweed cultivation on nearby communities, 4) density dependent epiphytes and diseases, 5) integration of area use, and 6) threats from global climate warming (Stévant *et al*. 2017).

The growth and harvest quality of kelps are influenced by numerous physical and chemical variables, including temperature, salinity, water motion, nutrient availablity, CO_2 and pH, light intensity and quality, and ultra-violet radiation (Kerrison *et al*. 2015). Growth, carbon and nitrogen content also various with age and season (Gevaert *et al*. 2001). Additonally, kelps are influenced by grazers (Dayton 1985) and microbiomes in sea water and on the surface of the fronds (Singh & Reddy 2016; Minich *et al*. 2018) in a network of interactions. Attempts to maximize return on investment in the cultivation of kelps for commerical harvest have to account for these variables. Salinity, for example, affects the biochemical composition of some kelps and hence their commercial value (Nielsen *et al*. 2016), and seaweed microbiomes influence development and physiology (Ramanan *et al.* 2016).

The culture of *Saccharina japonica* in China provides examples of steps taken to improve production. This kelp was introduced to northern China from Hokkaido, Japan in 1927, but has spread to other location naturally or by the transport of cultivars (Shan *et al*. 2017*)*. China produces 1.4 million tons of dry weight of *Saccharina japonica* in a farming area of 130,564 ha, much of which are at the edge of suitable temperatures for culturing this species. Extended grow-out periods are needed to maximize production and require summer sporeling production when temperatures are high. Large-scale cultures must be cooled to 5–10° C to allow gametophytic grow and the production of small sporophytes. Sporophytes can then grow in waters at warm as 20° C at temperatures that would otherwise inhibit the growth of gametophytes. In addition to temperature, the development of improved strains has to include a consideration of other variables, including nutrient availability and light affect the growth of this kelp (Gao *et al*. 2017).

The production of *Saccharina japonica* has been enhanced by the same kind of artificial selection and hybridization used in agriculture (Zhang *et al*. 2007; Zhao *et al*. 2016; Zhang *et al.* 2016). One successful widely used strain, 90-1, was created by hybridizing *Saccharina japonica* and *Saccharina longissima*, maintained by selfing, and artificially selected over generations for desirable traits (Zhang *et al*. 2007). The yield of this strain increased about 50% above other strains used for aquaculture. However, seedlings for production each year were derived from mature sporophytes grown in open waters, a process that led to inbreeding and further hybridization. To avoid these complications another strain, Dongfang no. 7, was created by hybridizing two morpho-types of *Saccharina japonica* that produced desirable wide, smooth dark brown fronds (Li *et al*. 2016). Brood stock for producing young sporophytic plants for grow-out were maintained as gametophytes which were cultured without the problem of inbreeding or genetic contamination from seaweed in the wild. This strain increased dry yield by 43.2% over two widely farmed strains as controls.

Genetic manipulation of plant and animals through artificial selection has been practiced since the beginning of agriculture and has led to improved varieties that increase growth, biomass, nutrition and many other traits of interest. Artificial selection and hybridization have been used to improve commercial strains of seaweeds to increase growth rates and to delay fertility (Charrier *et al*. 2015). The anatomies and developmental pathways of kelps and other farmed seaweeds are much less complex than those of angiosperms and hence can be more easily manipulated. Several mechanisms can lead to morphological and physiological transformations in seaweeds. In culture, dedifferentiation of somatic tissues with nutrient and chemical treatments often induces morphological variants that can be of use in commercial culture (Larkin & Scowcroft 1981). For example, the culture of sporophytic tissues in the kelp *Laminaria* can give rise to gametophyte-like filaments that give rise directly to sporophytes without cycling through meiospores and gametophytes (Gall *et al*. 1996). Such a technique might be useful in maintaining the genetic integrity of a desirable strain.

Other techniques involve selective breeding, hybridization, mutagenesis, and genetic engineering. A US Department of Energy funded project is using selective breeding of *Saccharina latissima* in New England with the goal of developing strains with a 20–30% increase in yield (Lindell *et al*. 2018). Hybridization between strains to induce heterosis has been used extensively for agriculture crops and for seaweeds (Zhong *et al*. 2007). Crossing of parthenogenetic cloned gametophytes in the kelp *Undaria pinnatifida* also led to strains with characteristics that improved the value of the kelp (Shan *et al*. 2013). Mutagenesis in cultured plants with chemicals and UV light has led to improvements in the quality of agar produced (Patwary & van der Meer 1983), increase lipid production (Liu *et al*. 2017) and to understand the molecular basis of development (Coelho *et al*. 2011). Numerous other techniques can be used to manipulation algal genomes (Qin *et al*. 2012) and among them are genetic engineering with zinc fingers and CRISPR technologies (Lin & Qin 2014; Mikami 2014, 2018). Any attempt to produce kelps for biofuels will require rapidly growing strains developed by selective breeding, mutagenesis, or genetic engineering that are adapted to local environments (Kim *et al*. 2017). The development of strains of seaweed adapted to local conditions in Alaska is essential to improve crop yields and quality, and to ensure the viability of seaweed farming.

The development of commercial seaweed culture in Norway parallels the challenges faced by Alaskan kelp farmers. For the past 50 years, production rested on harvests from natural stands of the kelp *Laminaria hyperborea* and the fucoid *Ascophyllum nodosum*. Commercial cultivation in Norway began only in 2014. Harvests of natural populations were often controversial among coastal fisheries, coastal-use planning agencies, and marine conservation groups. Applications to start a kelp-farming operation are vetted by several agencies, including the Directorate of

Fisheries, Norwegian Coastal Administration, Norwegian Food Safety Authority, County Governor's Environmental Department, Norwegian Water Resources and Energy Directorate, and municipal authorities to evaluate cultivation in view of coastal zone planning (Stévant *et al*. 2017). As of 2016, only 277 ha along the entire Norwegian coast had been permitted for seaweed cultivation. The focus of large-scale production from cultivation has been on *Saccharina latissima* because of its rapid growth and saccharide and nutritional content. The cultivation of this seaweed represents 96% of the total cultivated kelp production. Winged kelp, *Alaria esculenta*, makes up the remaining 4% of the production. *Alaria* is generally sold fresh or dried as a high-value food item, whereas *Saccharina latissima* is used for a broader range of products. The production of young sporophytes can be extended throughout the year by controlling the maturity of older sporophytes with light regimen (Forbord *et al.* 2012).

Several layers of regulation by federal, state, and municipalities must be surmounted in Alaska to start a kelp farming operation. Federal entities with regulatory authority of marine waters in Alaska include, the Army Corps of Engineers, the Environmental Protection Agency, the National Oceanic and Atmospheric Administration, the Fish and Wildlife Service, the United States Department of Agriculture, and the Food and Drug Administration. In addition to the Executive and Judicial branches of Alaska's state government, regulatory bodies include the departments of Commerce, Natural Resources, Environmental Conservation, and Fish and Game, each with layers of regulations for the startup and operation of aquaculture farms in Alaska. Compliance issues include several factors (Engle & Stone 2013), but in Alaska's regulations are focused on environmental protection, protection of wild populations, food safety, and legalities of leasing aquaculture sites. The cost in labor and funds to address compliance issues can threaten the survival of marginal operations and discourage investments in new technologies by industry.

Several national and regulatory efforts have been made to produce seaweed products that can enter the organic food market. For example, seaweed farming in China had been poorly regulated until recently when a national effort was made to improve environmental quality by slowing aquaculture production and by addressing pollution and food safety issues. In addition to a push for 'green growth', the aquaculture industry in general is attempting to produce higher quality products that can carry the 'organic' label (Xie *et al*. 2013; Zou & Huang 2015). In the US, the USDA has published a set of criteria to classify food products a 'organic'. These criteria were designed for terrestrial crops, but have been re-interpreted for seaweeds by the Maine Organic Farmers and Gardeners Association (MOFGA) (Table 4). A major criterion is that seaweeds must be grown in clean waters not contaminated by radioactivity from a nuclear reactor, by pollution from waste water treatment plants or industry, or by heavy metals from a commercial boat-building facility. Under the guidelines, nutrients can be used to stimulate the growth of gametophytes and young sporophytes in indoor culture, but cannot be used to enhance growth of plants in open waters. Biofouling and disease must also be controlled without resorting to chemicals or antibiotics. A strong emphasis is also placed on maintaining the ecological integrity of the farmed areas. Similar criteria for organic certification have been established in Europe. For example, the Soil Association of the United Kingdom has published a similar set of criteria for organic labeling of seaweed products, and like those in the state of Maine, emphasize protection of natural ecosystems (Soil Association Standards Seaweeds 2019). Interestingly, the criteria from the UK prohibit the use of GMO seaweed strains for organic certification, but this criterion is missing from the MOFGA organic guidelines. The development of certified organic seaweed products in Alaska would add considerable value to Alaska's seaweed industry.

11.7 Prospects

The global economic value of seaweeds is worth several billion USD annually, and there is considerable interest in Alaska in participating in this market. Alaska has a long coastline without major industrial developments and with pristine water could support the production of premium seaweed products for human consumption and numerous other products, inclulding the production of biofuels.

The development of kelp and seaweed farming in Alaska requires first an understanding of the distributions of managment units and this project was a step in that direction. The results for the three kelps examined in this study show chaotic genetic population structures without regional groups and without isolation by distance. The geographical scale of management small in most cases and will require a much greater sample effort to resolve. Only 5–16 samples per species were collected over a distance of about 2500 kilometers, and this level of resolution is insuffcient to formulate management some aspects of management.

A second step requires an understanding of the adaptive seascape of Alaska's kelps. Temperature, salinity and nutrient availability greatly influence growth rates and biochemical compositions of kelps so that the choice of farming sites will have a large influence on the nature and quality of a product. This study used genetic markers that have traditionally been assumed to be neutral to natural selection and therefore the results of this study do not resolve physiological or adaptive variability among populations of these kelps. This is an active area of research in other regions of the globe and is a necessary second step toward building a viable seaweed industry in Alaska.

A third step is the development of selected strains to enhance seed production, accerlate sporophyte growth, and improve production traits. Chromosome manipulation, as used in the management of some fishes in Alaska, classical agricultural methods of artificial selection, microbiome treatments, and other forms of phenotypic and genetic manipulation can be used to development varieties of kelps with desirable production traits. The development of fast growing strains with desirable charactersitcs is vital if Alaska is to participate in global markets. Organic certification of Alaska's seaweed products consumed by humans or livestock would also add considerable value to Alaska's economy.

The development of a fledging kelp industry in Alaska is taking place at a time of great environmental change that is being driven by global warming from a rise in the concentrations of greenhouse gases. Atmospheric warming leads to the warming of the ocean and the decline in the extents of seasonal sea ice in the Bering Sea and Arctic Ocean. Ocean warming is predicted to lead to poleward shifts in the geographic distributions of kelps and to local population extinctions when local sea temperatures excede the physiological limits of one of the life-history stages of a kelp species. The warming of high latitude seas also triggers a cascade of abiotic and biotic events that lead to ocean acidification that further puts Alaska's aquaculture at risk of failure.

This study focused on three kelps that are of interest to the seaweed industry. However, several other species of algae in Alaska's waters can also be developed for commercial exploitation. Among these are the kelps, *Macrocystis pyrifera*, *Nereocystis luetkeana*, *Eualaria fistulosa*, *Costeria costat*a and *Cymathere triplicata*. The first two of these species exhibit tremendous seasonal growth rates, reaching 20–40 meters in length in only a few months. Numerous small species of red and gree algae can also be expoited. These include the red algae *Pyropia* spp, *Palmaria* spp, *Mastocarpus* spp. and the green alga *Ulva lactuca*, all of which are used in high-value food products in global markets. Future research on the population genetics, adaptive physiology and metabolism of these species are needed to support the commerial development of these species.

11.8 References

Adams JM, Gallagher JA & Donnison IS. 2009. Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. *Journal of Applied Phycology* 21: 569–574.

Allendorf FW & Luikart G. 2007. Conservation and the genetics of populations. Blackwell, Maldon, MA.

Ashley MV, Willson MF, PergO'Dowd DJ, Gende SM & Brown JS. 2003. Evolutionarily enlightened managment. *Biological Conservation* 111: 115–123.

Assis J, Araújo MB & Serrão EA. 2017b. Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. *Global Change Biology* 24: e55–e66.

Assis J, Coelho NC, Lamy T, Valero M, Alberto F & Serrão EA. 2016. Deep reefs are climatic refugia for genetic diversity of marine forests. *Journal of Biogeography* 43:833–844.

Assis J, Lucas AV, Bárbara I & Serrão EA. 2016. Future climate change is predicted to shift long-term persistence zones in the cold-temperate kelp *Laminaria hyperborea*. *Marine Environmental Research* 113: 174-182.

Assis J, Berecibar E, Claro B, Alberto F, Reed D, Raimondi P & Serrão EA. 2017a. Major shifts at the range edge of marine forests: the combined effects of climate changes and limited dispersal. *Scientific Reports* 7: e44348.

Assis J, Serrão EA, Claro B, Perrin C & Pearson GA. 2014. Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. *Molecular Ecology* 23: 2797–2810.

Augyte S, Yarish C, Redmond S & Kim JK. 2017. Cultivation of a morphologically distinct strain of the sugar kelp, *Saccharina latissima* forma *angustissima*, from coastal Maine, USA, with implications for ecosystem services. *Journal of Applied Phycology* 29: 1967–1976.

Balloux F, Amos W & Coulson T. 2004. Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology* 13: 3021–3031.

Barradas A, Alberto F, Engelen AH & Serrào EA. 2011. Fast sporophyte replacement after removal suggests banks of latent microscopic stages of *Laminaria ochroleuca* (Phaeophyceae) in tide pools in northern Portugal. *CBM-Cahiers De Biologie Marine* 52: 435–439.

Bartkowska MP & Johnston MO. 2009. Quantitative genetic variation in populations of *Amsinckia spectabilis* that differ in rate of self-fertilization. *Evolution* 63: 1103–1117.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F & Wiese J. 2008. The genus *Laminaria sensu lato*: recent insights and developments. *European Journal of Phycology* 43: 1–86.

Barton A, Hales B, Waldbusser GG, Langdon C & Feely RA. 2012. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels:

Implications for near-term ocean acidification effects. *Limnology and oceanography* 57: 698–710.

Bekkby T & Moy FE. 2011. Developing spatial models of sugar kelp (*Saccharina latissima*) potential distribution under natural conditions and areas of its disappearance in Skagerrak. *Estuarine, Coastal and Shelf Science* 95: 477–483.

Benton TG & Grant A. 2000. Evolutionary fitness in ecology: comparing measures of fitness in stochastic, density-dependent environments. *Evolutionary Ecology Research* 2: 769–789.

Bert TM, Crawford CR, Tringali MD, Seyoum S, Galvin JL, Higham M & Lund C. 2007. Genetic management of hatchery-based stock enhancement Chapter 8, *In Ecological and Genetic Implications of Aquaculture Activities*, TM Bert (ed.), Springer. pp123–174.

Bierne NS, Launey Y, Naciri-Graven & Bonhomme F. 1998. Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. *Genetics* 148: 1893–1906.

Billot C, Engel CR, Rousvoal S, Kloareg B & Valero M. 2003. Current patterns, habitat discontinuities and population genetic structure: the case of the kelp *Laminaria digitata* in the English Channel. *Marine Ecology Progress Series* 253: 111–121.

Bintanja R, van de Wal RS & Oerlemans J. 2005. Modelled atmospheric temperatures and global sea levels over the past million years. *Nature* 437: 125–128.

Boag PT & Grant PR. 1981. Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galapagos. *Science* 214: 82–85.

Bolton JJ & Lüning K. 1982. Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. *Marine Biology* 66: 89–94.

Bond G, Heinrich H, Broecker W, Labeyrie L, McManus J, Andrews J, Huon S, Jantschik R, Clasen S, Simet C, Tedesco K. Evidence for massive discharges of icebergs into the North Atlantic Ocean during the last glacial period. Nature. 1992 Nov;360(6401):245.

Bonduriansky R & Day T. 2009. Nongenetic inheritance and its evolutionary implications. *Annual Review of Ecology, Evolution, and Systematics* 40: 103–125.

Bonduriansky R, Crean AJ & Day T. 2012. The implications of nongenetic inheritance for evolution in changing environments. *Evolutionary Applications* 5: 192–201.

Borum J, Pedersen MF, Krause-Jensen D, Christensen PB & Nielsen K. 2002. Biomass, photosynthesis and growth of *Laminaria saccharina* in a high-arctic fjord, NE Greenland. *Marine Biology* 141: 11–19. [*Laminaria saccharina* is now *Saccharina latissima*]

Bossdorf O, Arcuri D, Richards CL & Pigliucci M. 2010. Experimental alteration of DNA methylation affects the phenotypic plasticity of ecologically relevant traits in *Arabidopsis thaliana*. *Evolutionary Ecology* 24: 541–553.

Boyd PW, Collins S, Dupont S, Fabricius K, Gattuso JP, Havenhand J, Hutchins DA, Riebesell U, Rintoul MS, Vichi M & Biswas H. 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—a review. *Global Change Biology* 24: 2239–2261.

Breton TS, Nettleton JC, O'Connell B & Bertocci M. 2018. Fine-scale population genetic structure of sugar kelp, *Saccharina latissima* (Laminariales, Phaeophyceae), in eastern Maine, USA. *Phycologia* 57: 32–40.

Britten HB. 1996. Meta-analyses of the association between multilocus heterozygosity and fitness. *Evolution* 50: 2158–2164.

Britton D, Cornwall CE, Revill AT, Hurd CL & Johnson CR. 2016. Ocean acidification reverses the positive effects of seawater pH fluctuations on growth and photosynthesis of the habitat-forming kelp, *Ecklonia radiata*. *Scientific Reports* 6: e26036.

Broch OJ & Slagstad D. 2012. Modelling seasonal growth and composition of the kelp Saccharina latissima. *Journal of Applied Phycology* 24: 759–776.

Broch OJ, Ellingsen IH, Forbord S, Wang X-X, Volent Z, Alver MO, Handå A, Andresen K, Slagstad D, Reitan KI, Olsen Y & Skjermo J. 2013. Modelling the cultivation and bioremediation potential of the kelp *Saccharina latissima* in close proximity to an exposed salmon farm in Norway. *Aquaculture Environment Interactions* 4: 187–206.

Brodie J, Williamson CJ, Smale DA, Kamenos NA, Mieszkowska N, Santos R, Cunliffe M, Steinke M, Yesson C, Anderson KM & Asnaghi V. 2014. The future of the northeast Atlantic benthic flora in a high CO_2 world. *Ecology and Evolution* 4: 2787–2798.

Bruhn A, Janicek T, Manns D, Nielsen MM, Balsby TJS, Meyer AS, Rasmussen MB, Hou X, Saake B, Göke C & Bjerre B. 2017. Crude fucoidan content in two North Atlantic kelp species, *Saccharina latissima* and *Laminaria digitata*—seasonal variation and impact of environmental factors. *Journal of Applied Phycology* 29: 3121–3137.

Caldeira K & Wickett ME. 2005. Oceanography: anthropogenic carbon and ocean pH. *Nature* 425: 365.

Calegario G, Freitas L, Santos E, Silva B, Oliveira L, Garcia G, Omachi C, Pereira R, Thompson C & Thompson F. 2019. Environmental modulation of the proteomic profiles from closely phylogenetically related populations of the red seaweed *Plocamium brasiliense*. *PeerJ* 7: e6469.

Calvin NI & Ellis RJ. 1978. Quantitative and qualitative observations on *Laminaria dentigera* and other subtidal kelps of southern Kodiak Island, Alaska. *Marine Biology* 47: 331–336.

Calvin NA & Ellis RJ. 1981. Growth of subtidal *Laminaria groenlandica* in southeastern Alaska related to season and depth. *Botanica Marina* 24: 107–114. [Note: the nomenclature of *Laminaria groenlandica* is now Hedophyllum *nigripes*, Starko *et al.* 2019]

Campbell I, Macleod A, Sahlmann C, Neves L, Funderud J, Øverland M, Hughes AD & Stanley M. 2019. The environmental risks associated with the development of seaweed farming in Europe–prioritizing key knowledge gaps. *Frontiers in Marine Science* 6: e107.

Carvalho M, Hayashizaki KI & Ogawa H. 2010. Effect of pH on the carbon stable isotope fractionation in photosynthesis by the kelp *Undaria pinnatifida*. *Coastal Marine Science* 34: 135–139.

Celis–Plá PSM, Hall-Spencer JM, Horta PA, Milazzo M, Korbee N, Cornwall CE & Figueroa FL. 2015. Macroalgal responses to ocean acidification depend on nutrient and light levels. *Frontiers of Marine Science* 2: e26.

Cerutti H, Ma X, Msanne J & Repas T. 2011. RNA-mediated silencing in algae: biological roles and tools for analysis of gene function. *Eukaryotic Cell* 10: 1164–1172.

Chakraborty R. 1981. The distribvution of the number of heterozygous loci in an individual in natural populations. *Genetics* 98: 461–466.

Charlesworth D. 2003. Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 358: 1051–1070.

Charlesworth D & Charlesworth B. 1995. Quantitative genetics in plants: the effect of the breeding system on genetic variability. *Evolution* 49: 911–920.

Charrier B, Rolland E, Gupta V & Reddy CRK. 2015. Production of genetically and developmentally modified seaweeds: exploiting the potential of artificial selection techniques. *Frontiers in Plant Science* 6: e127.

Christie H, Norderhaug KM, Fredriksen S. 2009. Macrophytes as habitat for fauna. *Marine Ecology Progress Series* 396: 221–233.

Christie H, Andersen GS, Bekkby T, Fagerli CW, Gitmark JK, Gunderson H & Rinde E. 2019. Shifts between sugar kelp and turf algae in Norway: regime shifts or fluctuations between different opportunistic seaweed species? *Fontiers in Marine Science* 6: e72.

Chung IK, Oak JH, Lee JA, Shin JA, Kim JG & Park KS. 2013. Installing kelp forests/seaweed beds for mitigation and adaptation against global warming: Korean Project Overview. *ICES Journal of Marine Science* 70: 1038–1044.

Clarke L, Edmonds J, Krey V, Richels R, Rose S &, Tavoni M. 2010. International climate policy architectures: overview of the EMF 22 international scenarios. *Energy Economics* 31(suppl 2): S64–S81.

Clausen J, Keck DD & Hiesey HM. 1940. *Experimental studies of the nature of species. I. effect of varied environments on western North American plants*. Carnegie Insitute of Washington, Washington, DC.

Clutton-Brock J. 1999. *A natural history of domesticated mammals*. Cambridge University Press.

Coelho SM, Godfroy O, Arun A, Le Corguillé G, Peters AF & Cock JM. 2011. Genetic regulation of life cycle transitions in the brown alga *Ectocarpus*. *Plant Signaling & Behavior* 6:1858–1860.

Coltman DW & Slate J. 2003. Microsatellite measures of inbreeding: a meta-analysis. *Evolution* 57: 971–983.

Connell SD & Russell BD. 2010. The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proceedings of the Royal Society B: Biological Sciences* 277: 1409–1415.

Connell SD, Doubleday ZA, Foster NR, Hamlyn SB, Harley CD, Helmuth B, Kelaher BP, Nagelkerken I, Rodgers KL, Sarà G & Russell BD. 2018. The duality of ocean acidification as a resource and a stressor. *Ecology* 99: 1005–1010.

Conover DO, Clarke LM, Munch SB & Wagner GN. 2006. Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of Fish Biology* 69: 21–47.

Cornwall CE, Revill AT & Hurd CL. 2015. High prevalence of diffusive uptake of CO 2 by macroalgae in a temperate subtidal ecosystem. *Photosynthesis Research* 124: 181–190.

Cornwall CE, Hepburn CD, Pritchard D, Currie KI, McGraw CM, Hunter KA & Hurd CL. 2012. Carbon-use strategies in macroalgae: differential responses to lowered pH and implications for ocean acidification. *Journal of Phycology* 48: 137–144.

Cornwall CE, Revill AT, Hall-Spencer JM, Milazzo M, Raven JA & Hurd CL. 2017. Inorganic carbon physiology underpins macroalgal responses to elevated CO 2. *Scientific Reports* 7: e46297.

Côté IM & Reynolds JD. 2012. Meta-analysis at the intersection of evolutionary ecology and conservation. *Evolutionary ecology* 26: 1237–1252.

Coyer JA, Hoarau G, Sjøtun K & Olsen JL. 2008. Being abundant is not enough: a decrease in effective population size over eight generations in a Norwegian population of the seaweed, *Fucus serratus*. *Biology Letters* 4: 755–757.

Coyer JA, Hoarau G, Oudot-Le Secq M-P, Stam WT & Olsen JL. 2006. A mtDNA-based phylogeny of the brown algal genus *Fucus* (Heterokontophyta; Phaeophyta). *Molecular Phylogenetics and Evolution* 39: 209–222.

Cubas P, Vincent C & Coen E. 1999. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401: 157–161.

Daly B & Konar B. 2008. Effects of macroalgal structural complexity on nearshore larval and post-larval crab composition. *Marine Biology* 153: 1055–1064.

Damasceno R, Strangas ML, Carnaval AC, Rodrigues MT & Moritz C. 2014. Revisiting the vanishing refuge model of diversification. *Frontiers in Genetics* 5: e353.

Davis G, Allee B, Amend D, Bachen B, Davidson B, Gharrett T, Marshall S & Wertheimer A. 1985. *Alaska Department of Fish & Game Genetics Policy*. Document Alaska Department of Fish & Game, Junealy, AK, 13 p.

Davison IR. 1987. Adaptation of photosynthesis in Laminaria saccharina (Phaeophyta) to changes in growth temperature. *Journal of Phycology* 23: 273–283.

Davison IR. 1991. Environmental effects on algal photosynthesis: temperature. *Journal of Phycology* 27: 2–8.

Davison IR, Greene RM & Podolak EJ. 1991. Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Marine Biology* 110: 449–454.

Dawson MN, Hays CG, Grosberg RK & Raimondi PT. 2014. Dispersal potential and population genetic structure in the marine intertidal of the eastern North Pacific. *Ecological Monographs* 84: 435–456.

Dayton PK. 1985. Ecology of kelp communities. *Annual Review of Ecology and Systematics* 16: 215–245.

Dean TA, Haldorson L, Laur DR, Jewett SC & Blanchard A. 2000. The distribution of nearshore fishes in kelp and eelgrass communities in Prince William Sound, Alaska: associations with vegetation and physical habitat characteristics. *Environmental Biology of Fishes* 57: 271–287.

de Lafontaine G, Napier JD, Petit RJ & Hu FS. 2018. Invoking adaptation to decipher the genetic legacy of past climate change. *Ecology* 99: 1530–1546.

Deshpande O, Batzoglou S, Feldman MW & Cavalli-Sforza LL. 2009. A serial founder effect model of human settlement out of Africa. *Proceedings of the Royal Society* B 276: 291–300.

DeWoody J, Bickham JW, Michler CH, Nichols KM, Rhodes OE Jr & Woeste KE (eds.). 2010. *Molecular Approaches in Natural Resource Conservation and Management*. Cambridge University Press.

Díez I, Muguerza N, Santolaria A, Ganzedo U & Gorostiaga JM. 2012. Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. *Estuarine Coastal and Shelf Science* 99: 108–120.

Dlugosch KM & Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the of multiple introductions. *Molecular Ecology* 17: 431–449.

Dobzhansky, T., 1951. *Genetics and the origins of species*. Columbia Univ. Press, New York'

Dodd RS & Douhovnikoff V. 2016. Adjusting to global change through clonal growth and epigenetic variation. *Frontiers in Ecology and Evolution* 4: e86.

Domingues CM, Church JA, White NJ, Gleckler PJ, Wijffels SE, Barker PM & Dunn JR. 2008. Improved estimates of upper-ocean warming and multi-decadal sea-level rise. *Nature* 453: 1090–1093.

Drinan DP, Gruenthal KM, Canino MF, Lowry D, Fisher MC & Hauser L. 2018. Population assignment and local adaptation along an isolation-by-distance gradient in Pacific cod (*Gadus macrocephalus*). *Evolutionary applications* 11: 1448–1464.

Druehl LD. 1967. Distribution of two species of *Laminaria* as related to some environmental factors. *Journal of Phycology* 3: 103–108.

Duarte B, Martins I, Rosa R, Matos AR, Roleda M, Reusch TB, Engelen AH, Serrão EA. Pearson GA, Marques JC & Caçador I. 2018. Climate change impacts on seagrass meadows and macroalgal forests: an integrative perspective on acclimation and adaptation potential. *Frontiers in Marine Science* 5: e190.

Dubin MJ, Zhang P, Meng D, Remigereau MS, Osborne EJ, Casale FP, Drewe P, Kahles A, Jean G, Vilhjalmsson B & Jagoda J. 2015. DNA methylation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation. *elife* 4: e5255.

Duggins DO, Simenstad CA & Estes JA. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. *Science* 245: 170–173.

Eggert A. 2012. Seaweed responses to temperature. In *Seaweed biology*. C Wiencke & K Bischof (eds), Springer, Berlin, Heidelberg. Pp 47–66.

Ehlers A, Worm B & Reusch TB. 2008. Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. *Marine Ecology Progress Series* 355: 1–7.

Eldon B & Wakeley J. 2006. Coalescent processes when the distribution of offspring number among individuals is highly skewed. *Genetics* 172: 2621–2633.

Engle CR & Stone NM. 2013. Competitiveness of US aquaculture within the current US regulatory framework. *Aquaculture Economics & Management* 17: 251–280.

Evankow AM. 2015. *Genetics of Norwegian kelp forests: Microsatellites reveal the genetic diversity, differentiation, and structure of two foundation kelp species in Norway* (Master's thesis).

Evankow A, Christie H, Hancke K, Brysting AK, Junge C, Fredriksen S & Thaulo J. 2019. Genetic heterogeneity of two bioeconomically important kelp species along the Norwegian coast. *Conservation Genetics* 20: 615–628

Evans W, Mathis JT, Ramsay J & Hetrick J. 2015. On the frontline: tracking ocean acidification in an Alaskan shellfish hatchery. *PLoS One* 10: e130384.

Excoffier L & Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.

Fabry VJ, Seibel BA, Feely RA & Orr JC. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science* 65: 414–432.

Fabry VJ, McClintock JB, Mathis JT & Grebmeier JM. 2009. Ocean acidification at high latitudes: the bellwether. *Oceanography* 22: 160–171.

Falconer DS. 1960. *Introduction to quantitative genetics.* Oliver & Boyd, Edinburgh/London. [followed by numerous revised editions]

FAO. 2018a. *The Global Status of Seaweed Production, Trade and Utililzation*. Globefish Research Programme of the United Nations, volume 124, Rome, 120 p.

FAO. 2018b. *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals*. Food and Agriculture Organization of the United, Rome, 227 p.

Faurby S & Barber PH. 2012. Theoretical limits to the correlation between pelagic larval duration and population genetic structure. *Molecular Ecology* 21: 3419–3432.

Feely R A, Sabine C L, Lee K, Berelson W, Kleypas J, Fabry V J, Millero FJ. 2004. Impact of Anthropogenic CO_2 on the $CaCO_3$ System in the Oceans. Science 305: 362–366.

Feeney MW. 2001. Regulating seaweed harvesting in Maine: the public and private interests in an emerging marine resource industry. *Ocean & Coastal Law Journal* 7: 329–352.

Fernández C. 2011. The retreat of large brown seaweeds on the north coast of Spain: the case of Saccorhiza polyschides. *European Journal of Phycology* 46: 352–360.

Fernández C. 2016. Current status and multidecadal biogeographical changes in rocky intertidal algal assemblages: the northern Spanish coast. *Estuarine, Coastal and Shelf Science* 171: 35–40.

Fernández PA, Roleda MY & Hurd CL. 2015. Effects of ocean acidification and the photosynthetic performance, carbonic anhydrase activity and growth of the giant kelp *Macrocystis pyrifera*. *Photosynthesis Research* 124: 293–304.

Fernández PA, Roleda MY, Leal PP & Hurd CL. 2017. Seawater pH, and not inorganic nitrogen source, affects pH at the blade surface of *Macrocystis pyrifera*: implications for responses of the giant kelp to future oceanic conditions. *Physiologia Plantarum* 159: 107–119.

Forbord S, Skjermo J, Arff J, Handå A, Reitan KI, Bjerregaard R & Lüning K. 2012. Development of Saccharina latissima (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. *Journal of Applied Phycology* 24: 393–399.

Foust CM, Preite V, Schrey AW, Alvarez M, Robertson MH, Verhoeven KJF & Richards CL. 2016. Genetic and epigenetic differences associated with environmental gradients in replicate populations of two salt marsh perennials. *Molecular Ecology* 25: 1639–1652.

Futuyma DJ. 1995. The uses of evolutionary biology. *Science* 267: 41–42.

Gaitán-Espitia, JD, Hancock JR, Padilla-Gamiño JL, Rivest EB, Blanchette CA, Reed DC & Hofmann GE. 2014. Interactive effects of elevated temperature and pCO2 on early-life-history stages of the giant kelp *Macrocystis pyrifera*. *Journal of Experimental Marine Biology and Ecology* 457: 51–58.

Gall EA, Asensi A, Marie D & Kloareg B. 1996. Parthenogenesis and apospory in the Laminariales: a flow cytometry analysis. *European Journal of Phycology* 31: 369–380.

Galtier N, Nabholz B, Glémin S & Hurst GDD. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* 18: 4541–4550.

Gao X, Endo H & Agatsuma Y. 2015. Effect of increased seawater temperature on biomass, growth, and maturation of *Saccharina japonica* near its southern limit in northern Japan. *Journal of Applied Phycology* 27: 1263–1270.

Gao X, Endo H, Nagaki M & Agatsuma Y. 2017. Interactive effects of nutrient availability and temperature on growth and survival of different size classes of *Saccharina japonica* (Laminariales, Phaeophyceae). *Phycologia* 56: 253–260.

Gao K, Beardall J, Häder DP, Hall-Spencer JM, Gao G & Hutchins DA. 2019. Effects of ocean acidification on marine photosynthetic organisms under the concurrent influences of warming, UV radiation and deoxygenation. *Frontiers in Marine Science* 6: e322.

Gaylord B, Reed DC, Raimondi PT, Washburn L & McLean SR. 2002. A physically based model of macroalgal spore dispersal in the wave and current-dominated nearshore. *Ecology* 83: 1239–1251.

Gaylord B, Reed DC, Washburn L & Raimondi PT. 2004. Physical-biological coupling in spore dispersal of kelp forest macroalgae. *Journal of Marine Systems* 49: 19–39.

Gaylord B, Reed DC, Raimondi PT & Washburn L. 2006. Macroalgal spore dispersal in coastal environments: mechanistic insights revealed by theory and experiment. *Ecological Monographs* 76: 481–502.

Gaylord B, Kroeker KJ, Sunday JM, Anderson KM, Barry JP, Brown NE, Connell SD, Dupont S, Fabricius KE, Hall-Spencer JM & Klinger T. 2015. Ocean acidification through the lens of ecological theory. *Ecology* 96: 3–15.

Gedalof Z & Smith DJ. 2001. Interdecadal climate variability and regime-scale shifts in Pacific North America. *Geophysical Research Letters* 28: 1515–1518.

Gerard VA. 1987. Hydrodynamic streamlining of Laminaria saccharina Lamour. in response to mechanical stress. *Journal of Experimental Marine Biology and Ecology* 107: 237–244.

Gerard VA & Du Bois KR. 1988. Temperature ecotypes near the southern boundary of the kelp Laminaria saccharina. *Marine Biology* 97: 575–80.

Gevaert F, Davoult D, Creach A, Kling R, Janquin MA, Seuront L & Lemoine Y. 2001. Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel: biometrics and seasonal variations. *Journal of the Marine Biological Association of the United Kingdom* 81: 727–734.

Gilpin M. 1991. The genetic effective size of a metapopulation. *Biological Journal of the Linnean Society* 42: 165–175.

Gleckler PJ, Santer BD, Domingues CM, Pierce DW, Barnett TP, Church JA, Taylor KE, AchutaRao KM, Boyer TP, Ishii M & Caldwell PM. 2012. Human-induced global ocean warming on multidecadal timescales. *Nature Climate Change* 2: 524–529.

Grant WS, Grauvogel Z, Chenoweth E & Cheng W. in preparation. Isolations in northern ice-age refugia shaped phylogeography of Sugar kelp *Saccharina latissima* in the Gulf of Alaska. To be submitted to *Molecular Ecology*.

Guzinski J, Mauger S, Cock JM & Valero M. 2016. Characterization of newly developed expressed sequence tag-derived microsatellite markers revealed low genetic diversity within and low connectivity between European *Saccharina latissima* populations. *Journal of Applied Phycology* 28: 3057–3070.

Hansson B & Westerberg L. 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology* 11: 2467–2474.

Hansen TF, Carter AJR & Pelabon C. 2006. On adaptive accuracy and precision in natural populations. *American Naturalist* 168; 168–181.

Harrison S. 1991. Local extinction in a metapopulation context: an empirical evaluation. *Biological journal of the Linnean Society* 42: 73–88.

Hasselström L, Visch W, Gröndahl F, Nylund GM & Pavia H. 2018. The impact of seaweed cultivation on ecosystem services-a case study from the west coast of Sweden. *Marine Pollution Bulletin* 133: 53–64.

Hauser L, Adcock GJ, Smith PJ, Ramírez JHB & Carvalho GR. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences* 99: 11742–11747.

Hedgecock D & Pudovkin AI. 2011. Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. *Bulletin of Marine Science* 87: 971–1002.

Heinrich S, Valentin K, Frickenhaus S & Wiencke C. 2015. Temperature and light interactively modulate gene expression in *Saccharina latissima* (Phaeophyceae). *Journal of Phycology* 51: 93–108.

Heinrich S, Valentin K, Frickenhaus S & Wiencke C. 2016. Origin matters—Comparative transcriptomics in Saccharina latissima (Phaeophyceae). *Journal of Experimental Marine Biology and Ecology* 476: 22–30.

Heinrich S, Valentin K, Frickenhaus S, John U & Wiencke C. 2012. Transcriptomic analysis of acclimation to temperature and light stress in *Saccharina latissima* (Phaeophyceae). *PLoS One* 7: e44342.

Helyar SJ, Hemmer-Hansen J, Bekkevold D, Taylor MI, Ogden R, Limborg MT, Cariani A, Maes GE, Diopere E, Carvalho GR & Nielsen EE. 2011. Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology Resources* 11: 123–136.

Hendry AP. 2004. Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evolutionary Ecology Research* 6: 1219–1236.

Hendry AP & Kinnison MT. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution* 53: 1637–1653.

Hendry AP, Kinnison MT, Heino M, Day T, Smith TB, Fitt Gary, Bergstrom CT, Oakeshott J, Jørgensen S, Zalucki MP, Gilchrist G, Southerton S, Sih A, Strauss S, Denison RF & Carroll SP. 2011. Evolutionary principles and their practical application. *Evolutionary Applications* 4: 159–183.

Hereford J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist* 173: 579–588.

Hereford J. 2010. Does selfing or outcorssing promote local adaptation? *American Journal of Botany* 97: 298–302.

Hereford J & Winn AA. 2008. Limits to local adaptation in six populations of the annual plant *Diodia teres*. *New Phytologist* 178: 888–896.Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological journal of the Linnean Society* 58: 247–276.

Hoban SM, Hauffe HC, Pérez-Espona S, Arntzen JW, Bertorelle G, Bryja J, Frith K, Gaggiotti OE, Galbusera P, Godoy JA & Hoelzel AR. 2013. Bringing genetic diversity to the forefront of conservation policy and management. *Conservation Genetics Resources* 5: 593–598.

Holdt SL & Kraan S. 2011. Bioactive compounds in seaweed: functional food applications and legislation. *Journal of applied phycology* 23: 543–597.

Holeski LM, Jander G & Agrawal AA. 2012. Transgenerational defense induction and epigenetic inheritance in plants. *Trends in Ecology & Evolution* 27: 618–626.

Hörandl E & Paun O. 2007. Patterns and sources of genetic diversity in apomictic plants: implications for evolutionary potentials. *Apomixis: Evolution, mechanisms and perspectives. ARG Gantner Verlag KG, Lichtenstein*, pp.169–194.

Hu, Z.M. and Lopez-Bautista J. 2014. Adaptation mechanisms and ecological consequences of seaweed invasions: a review case of agarophyte *Gracilaria vermiculophylla*. *Biological invasions* 16: 967–976.

Hubby JL & Lewontin RC. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. *Genetics* 54: 577–594.

Hughes AR & Stachowicz JJ. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences* 101: 8998–9002.

Iglesias-Rodriguez MD, Halloran PR, Rickaby RE, Hall IR, Colmenero-Hidalgo E, Gittins JR, Green DR, Tyrrell T, Gibbs SJ, von Dassow P & Rehm E. 2008. Phytoplankton calcification in a high-CO2 world. *Science* 320: 336–340.

Iñiguez C, Carmona R, Lorenzo MR, Niell FX, Wiencke C & Gordillo FJ. 2016. Increased temperature, rather than elevated CO 2, modulates the carbon assimilation of the Arctic kelps *Saccharina latissima* and *Laminaria solidungula*. *Marine Biology* 163: e248.

IPCC. 2007. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge UK.

IPCC. 2015. *Climate Change 2014: Synthesis Report*. *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Johannes F, Colot V & Jansen RC. 2008. Epigenome dynamics: a quantitative genetics perspective. *Nature Reviews Genetics* 9: 883–890.

Johansson ML, Raimondi PT, Reed DC, Coelho NC, Serrão EA & Alberto FA. 2013. Looking into the black box: simulating the role of self-fertilization and mortality in the genetic structure of *Macrocystis pyrifera*. *Molecular Ecology* 22: 4842–4854.

Johansson ML, Alberto F, Reed DC, Raimondi PT, Coelho NC, Young MA, Drake PT, Edwards CA, Cavanaugh K, Assis J & Ladah LB. 2015. Seascape drivers of *Macrocystis pyrifera* population genetic structure in the northeast Pacific. *Molecular Ecology* 24: 4866–4885.

Jouzel J, Masson-Delmotte V, Cattani O, Dreyfus G, Falourd S, Hoffman G, Minster B, Nouet J, Barnola JM, Chapellaz J, Fischer H, Gallet JC, Johnsen S, Leuenberger M, Loulergue L, Luethi D, Oerter H, Parrenin F, Raisbeck G, Raynaud D, Schilt A, Schwander J, Selmo E, Souchez R, Spanhi R, Stauffer B, Steffensen JP, Stenni B, Stocker TF, Tison JL, Werner M & Wolff EW. 2007. Orbital and millennial Antarctic climate variability over the past 800,000 years. *Science* 317: 793–796.

Jueterbock A, Tyberghein L, Verbruggen H, Coyer JA, Olsen JL & Hoarau G. 2013. Climate change impact on seaweed meadow distribution in the North Atlantic rocky intertidal. *Ecology and evolution* 3: 1356–1373.

Khan AH, Levac E, van Guelphen L, Pohle G & Chmura GL. 2018. The effect of global climate change on the future distribution of economically important macroalgae (seaweeds) in the northwest Atlantic. *Facets* 3: 275–286.

Karl SA, Toonen RJ, Grant WS & Bowen BW. 2012. Common misconceptions in molecular ecology: echoes of the modern synthesis. *Molecular Ecology* 21: 4171–4189.

Kawai T, Galanin D, Krupnova T & Yotsukura N. 2015. Harvest and cultivation of *Saccharina japonica* in Northern Hokkaido, Japan, and southern Sakhalin and Primorye, Russia: a review. *Algal Resources* 8: 155–163.

Kawecki TJ & Ebert D. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7: 1225–1241.

Keller CF. 2009. Global warming: a review of this mostly settled issue. *Stochastic Environmental Research and Risk Assessment* 23: 643–676.

Keller LF & Waller DM. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution* 17: 230–241.

Keller TE, Lasky JR & Yi SV. 2016. The multivariate association between genomewide DNA methylation and climate across the range of *Arabidopsis thaliana*. *Molecular Ecology* 25: 1823–1837.

Kelly MW & Hofmann GE. 2013. Adaptation and the physiology of ocean acidification. *Functional Ecology* 27: 980–990.

Kelly DW, Macisaac HJ, Heath DD & Crandall K. 2009. Vicariance and dispersal effects on phylogeographic structue and speciation in a widespread estuarine invertebrate. *Evolution* 60: 257–267.

Kenchington E, Heino M & Nielsen EE. 2003. Managing marine genetic diversity: time for action? *ICES Journal of Marine Science* 60: 1172–1176.

Kerrison PD, Stanley MS, Edwards MD, Black KD & Hughes AD. 2015. The cultivation of European kelp for bioenergy: site and species selection. *Biomass and Bioenergy* 80: 229–242.

Kettlewell B. 1973. *The evolution of melanism: the study of a recurring necessity*. Oxford: Clarendon Press.

Khan AH, Levac E, Van Guelphen L, Pohle G & Chmura GL. 2018. The effect of global climate change on the future distribution of economically important macroalgae (seaweeds) in the northwest Atlantic. *Facets* 3: 275–286.

Kim JK, Yarish C, Hwang EK, Park M & Kim Y. 2017. Seaweed aquaculture: cultivation technologies, challenges and its ecosystem services. *Algae* 32: 1–13.

Kinnison MT & Hendry AP. 2001. The pace of modern life II: from rates of contemporary microevolution to pattern and process. In *Microevolution rate, pattern, process* (pp. 145–164). Springer, Dordrecht.

Knapp G & Rubino MC. 2016. The political economics of marine aquaculture in the United States. *Reviews in Fisheries Science & Aquaculture* 24: 213–229.

Kooke R, Johannes F, Wardenaar R, Becker F, Etcheverry M, Colot V, Vreugdenhil D & Keurentjes JJ. 2015. Epigenetic basis of morphological variation and phenotypic plasticity in *Arabidopsis thaliana*. *The Plant Cell* 27: 337–348.

Kroeker KJ, Kordas RL, Crim RN & Singh GG. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters* 13: 1419–1434.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM & Gattuso JP. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* 19: 1884–1896.

Kroeker KJ, Micheli F & Gambi MC. 2013a. Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nature Climate Change* 3: e156.

Kroeker KJ, Gambi MC & Micheli F. 2013b. Community dynamics and ecosystem simplification in a high-CO2 ocean. *Proceedings of the National Academy of Sciences* 110: 12721–12726.

Krueger-Hadfield SA, Roze D, Mauger S & Valero M. 2013. Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed *Chondrus crispus*. *Molecular Ecology* 22: 3242–3260.

Krumhansl KA & Scheibling RE. 2012. Production and fate of kelp detritus. *Marine Ecology Progress Series* 467: 281–302.

Krumhansl KA, Okamoto DK, Rassweiler A, Novak M, Bolton JJ, Cavanaugh KC, Connell SD, Johnson CR, Konar B, Ling SD & Micheli F. 2016. Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences* USA 113: 13785–13790.

Kurihara H. 2008. Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series* 373: 275–284.

Kusumo HT & Druehl LD. 2000. Variability over space and time in the genetic structure of the winged kelp *Alaria marginata*. *Marine Biology* 136: 397–409.

Kusumo HT, Pfister CA, Wootton JT. 2006. Small-scale genetic structure in the sea palm *Postelsia palmaeformis* Ruprecht (Phaeophyceae). *Marine Biology* 149: 731–742.

Lader R, Walsh JE, Bhatt US & Bieniek PA. 2017. Projections of twenty-first-century climate extremes for Alaska via dynamical downscaling and quantile mapping. *Journal of Applied Meteorology and Climatology* 56: 2393–2409.

Lande R & Schemske DW. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39: 24–40.

Lane CE, Lindstrom SC & Saunders GW. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Molecular Phylogenetics and Evolution* 44: 634–648.

Larkin PJ & Scowcroft WR. 1981. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics* 60: 197–214.

Leal PP, Hurd CL, Fernández PA & Roleda MY. 2017a. Meiospore development of the kelps *Macrocystis pyrifera* and *Undaria pinnatifida* under ocean acidification and ocean warming: independent effects are more important than their interaction. *Marine Biology* 164: e7.

Leal PP, Hurd CL, Fernández PA & Roleda MY. 2017b. Ocean acidification and kelp development: reduced pH has no negative effects on meiospore germination and gametophyte development of *Macrocystis pyrifera* and *Undaria pinnatifida*. *Journal of phycology* 53: 557–566.

Lee KM, Yang EC, Coyer JA, Zuccarello GC, Wang WL, Choi CG & Boo SM. 2012. Phylogeography of the seaweed *Ishige okamurae* (Phaeophyceae): evidence for glacial refugia in the northwest Pacific region. *Marine Biology* 159: 1021–1028.

Leimu R & Fischer M. 2008. A meta-analysis of local adaptation in plants. *PloS One* 3: e4010.

Levins, R. (1969) Some demographic and genetic consequences of environmental heterogeneity for biological control *Bulletin of the of the Entomology Society of America* 15: 237–240.

Lewontin RC & Hubby JL. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. *Genetics* 54: 595–609.

Li X, Zhang Z, Qu S, Liang G, Sun J, Zhao N, Cui C, Cao Z, Li Y, Pan J & Yu S. 2016. Improving seedless kelp (*Saccharina japonica*) during its domestication by hybridizing gametophytes and seedling-raising from sporophytes. *Scientific Reports* 6: e21255.

Li JJ, Hu ZM, Gao X, Sun ZM, Choi HG, Duan DL & Endo H. 2017. Oceanic currents drove population genetic connectivity of the brown alga *Sargassum thunbergii* in the north-west Pacific. *Journal of Biogeography* 44: 230–242.

Lin H & Qin S. 2014. Tipping points in seaweed genetic engineering: scaling up opportunities in the next decade. *Marine Drugs* 12: 3025–3045.

Lima FP, Ribeiro PA, Queiroz N, Hawkins SJ & Santos AM. 2007. Do distributional shifts of northern and southern species of algae match the warming pattern? *Global Change Biology* 13: 2592–2604.

Limborg MT, Helyar SJ, De Bruyn M, Taylor MI, Nielsen EE, Ogden ROB, Carvalho GR, FPT Consortium & Bekkevold D. 2012. Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). *Molecular Ecology* 21: 3686–3703.

Lindeberg MR & Lindstrom SC. 2010. *Field Guide to Seaweeds of Alaska*. Sea Grant College Program, University of Alaska Fairbanks.

Lindell S, Yarish C & Augyte S. 2018. Assessing the risk of experimental kelp breeding and farming in New England. DOE project DE-AR0000915, Selective breeding technologies for scalable offshore seaweed farming. 8 pp.

Lindstrom SC. 1977. An annotated bibliography of the marine algae of Alaska. Alaska Department of Fish and Game. Technical Data Report No. 31, 172 pp.

Lisiecki LE & Raymo ME. 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic δ18O records. *Paleoceanography* 20: PA001071.

Liu QA. 2013. The impact of climate change on plant epigenomes. *Trends in Genetics* 29: 503–505.

Liu F, Yao J, Wang X, Repnikova A, Galanin DA & Duan D. 2012. Genetic diversity and structure within and between wild and cultivated *Saccharina japonica* (Laminariales, Phaeophyta) revealed by SSR markers. *Aquaculture* 358: 139-145.

Liu Y, Wang Z, Yan K, Wang Z, Torres OL, Guo R & Chen J. 2017. A new disposal method for systematically processing of ceftazidime: the intimate coupling UV/algae-algae treatment. *Chemical Engineering Journal* 314: 152–159.

Liu S, Zhao Y, Liu L, Ao X, Ma L, Wu M & Ma F. 2015. Improving cell growth and lipid accumulation in green microalgae *Chlorella* sp. via UV irradiation. *Applied Biochemistry and Biotechnology* 175: 3507–3518.

Luttikhuizen PC, van den Heuvel FHM, Rebours C, Witte HJ, van Bleijswijk JDL, Timmermans K. 2018. Strong population structure but no equilibrium yet: Genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). *Ecology and Evolution* 8: 4265–4277.

Mackas DL, Batten S & Trudel M. 2007. Effects on zooplankton of a warmer ocean: recent evidence from the Northeast Pacific. *Progress in Oceanography* 75: 223–252.

Machalek KM, Davison IR, Falkowski PG. 1996. Thermal acclimation and photoacclimation of photosynthesis in the brown alga Laminaria saccharina. *Plant, Cell & Environment* 19: 1005–1016.

Mann KH. 1973. Seaweeds: their productivity and strategy for growth. *Science* 182: 975–981.

Mantua NJ & Hare SR. 2002. The Pacific decadal oscillation. *Journal of Oceanography*. 58: 35–44.

Marshall DJ, Monro K, Bode M, Keough MJ & Swearer S. 2010. Phenotype–environment mismatches reduce connectivity in the sea. *Ecology Letters* 13: 128–140.

Matala AP, Gray AK, Heifetz J & Gharrett AJ. 2004. Population structure of Alaskan shortraker rockfish, *Sebastes borealis*, inferred from microsatellite variation. *Environmental Biology of Fishes* 69: 201–210.

Merilä J & Hendry AP. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications* 7: 1–14.

Mieszkowska N, Kendall MA, Hawkins SJ, Leaper R, Williamson P, Hardman-Mountford NJ & Southward AJ. 2006. Changes in the range of some common rocky shore species in Britain—a response to climate change? In *Marine Biodiversity*, Pp. 241–251). Springer, Dordrecht.

Mikami K. 2014. A technical breakthrough close at hand: feasible approaches toward establishing a gene-targeting genetic transformation system in seaweeds. *Frontiers in Plant Science* 5: e498.

Mikami K. 2018. Recent Developments in Nuclear Reverse-Genetic Manipulation that Advance Seaweed Biology in the Genomic Era. *Journal of Aquatic Research and Marine Sciences* 1: 39–42.

Mineur F, Arenas F, Assis J, Davies AJ, Engelen AH, Fernandes F, Malta EJ, Thibaut T, Van Nguyen T, Vaz-Pinto F & Vranken S. 2015. European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of sea research* 98: 91–108.

Minich JJ, Morris MM, Brown M, Doane M, Edwards MS, Michael TP & Dinsdale EA. 2018. Elevated temperature drives kelp microbiome dysbiosis, while elevated carbon dioxide induces water microbiome disruption. *PloS One* 13: e192772.

Mitton JB & Grant MC. 1984. Associations among protein heterozygosity, gowth rae, and developmental homeostasis. *Annual Reviews in Ecology and Systematics* 15: 479–499.

Monagail, MM, Cornish L, Morrison L, Araújo R & Critchley AT. 2017. Sustainable harvesting of wild seaweed resources. *European Journal of Phycology* 52: 371–390.

Mooney KM, Beatty GE, Elsäßer B, Follis ES, Kregting L, O'Connor NE, Riddell GE & Provan, J. 2018. Hierarchical structuring of genetic variation at differing geographic scales in the cultivated sugar kelp *Saccharina latissima*. *Marine Environmental Research* 142: 108–115.

Moore TC Jr, Burckle LH, Geitzenauer K, Luz B, Molina-Cruz A, Roberston JH, Sachs H, Sancetta C, Thiede J, Thompson P & Wenkam C. 1980. The reconstruction of sea surface temperatures in the Pacific Ocean of 18,000 B.P. *Marine Micropaleontology* 5: 215–247.

Morley JJ & Dworetzky BA. 1991. Evolving Pliocene-Pleistocene climate: A North Pacific perspective. *Quaternary Science Reviews* 10: 225–237.

Morris AB & Shaw J. 2018. Markers in time and space: a review of the last decade of plant phylogeographic approaches. *Molecular Ecology* 27: 2317–2333.

Mortensen LM. 2017. Diurnal carbon dioxide exchange rates of *Saccharina latissima* and *Laminaria digitata* as affected by salinity levels in Norwegian fjords. *Journal of Applied Phycology* 29: 3067–3075.

Mouritsen OG, Dawczynski C, Duelund I, Jhreis G, Wettre W & Schröder M. 2013. On the human consumption of the red seaweed dulse (*Palmaria palmata* (L.) Weber & Mohr). *Journal of Applied Phycology* 25: 1777–1791.

Moy FE & Christie H. 2012. Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. *Marine Biology Research* 8: 357–369.

Müller R, Laepple T, Bartsch I & Wiencke C. 2009. Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Botanica Marina*, *52*(6), pp.617-638.

Müller J, Werner K, Stein R, Fahl K, Moros M & Jansen E. 2012. Holocene cooling culminates in sea ice oscillations in Fram Strait. *Quaternary Science Reviews* 47: 1–14.

Nicastro KR, Zardi GI, Teixeira S, Neiva J, Serrão EA & Pearson GA. 2013. Shift happens: trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga Fucus vesiculosus. *BMC Biology* 11: e6.

Neiva J, Assis J, Coelho NC, Fernandes F, Pearson GA & Serrão EA. 2015. Genes left behind: climate change threatens cryptic genetic diversity in the canopy-forming seaweed *Bifurcaria bifurcata*. *PLoS One* 10: e0131530.

Neiva J, Serrão EA, Assis J, Pearson GA, Coyer JA, Olsen JL, Hoarau G & Valero, M. 2016. Climate oscillations, range shifts and phylogeographic patterns of North Atlantic Fucaceae. In *Seaweed Phylogeography*, pp. 279–308. Springer, Dordrecht.

Neiva J, Paulino C, Nielsen MM, Krause-Jensen D, Saunders GW, Assis J, Bárbara I, Tamigneaux É, Gouveia L, Aires T & Marbà N. 2018. Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. *Scientific Reports* 8: e1112.

Ng PK, Chiou YS, Liu LC, Sun Z, Shimabukuro H & Lin SM. 2019. Phylogeography and genetic connectivity of the marine macro-alga *Sargassum ilicifolium* (Phaeophyceae, Ochrophyta) in the northwestern Pacific. *Journal of Phycology* 55: 7–24.

Nielsen MM, Mans D, D'Este M, Krause-Jensen D, Rasmussen MB, Larsen MM, Alvarado-Morales M, Angelidaki I & Bruhn A. 2016. Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters. *Algal Research* 13: 235–245.

Niwa K & Sakamoto T. 2010. Allopolyploidy in natural and cultivated populations of *Porphyra* (Bangiales, Rhodophyta) 1. *Journal of phycology* 46: 1097–1105.

Niwa HS, Nashida K & Yanagimoto T. 2016. Reproductive skew in Japanese sardine inferred from DNA sequences. *ICES Journal of Marine Science* 73: 2181–2189.

Nunes J, McCoy SJ, Findlay HS, Hopkins FE, Kitidis V, Queirós AM, Rayner L & Widdicombe S. 2015. Two intertidal, non-calcifying macroalgae (*Palmaria palmata* and *Saccharina latissima*) show complex and variable responses to short-term CO2 acidification. *ICES Journal of Marine Science* 73: 887–896.

Olischläger M, Bartsch I, Gutow L & Wiencke C. 2012. Effects of ocean acidification on different life-cycle stages of the kelp *Laminaria hyperborea* (Phaeophyceae). *Botanica Marina* 55: 511–525.

Ohta T & Kimura M. 1970. Development of associative overdominance through linkage disequilibrium in finite populations. *Genetics Research* 16: 165–177.

Park J, Kim JK, Kong J-A, Depuydt S, Brown MT & Han T. 2017. Implications of rising temperatures for gametophyte performance of two kelp species from Arctic waters. *Botanica Marina* 60: 39–48.

Patwary MU & van der Meer JP. 1983. Genetics of *Gracilaria tikvahiae* (Rhodophyceae). IX: some properties of agars extracted from morphological mutants. *Botanica Marina* 26: 295–300.

Pehlke C & Bartsch I. 2008. Changes in depth distribution and biomass of sublittoral seaweeds at Helgoland (North Sea) between 1970 and 2005. Climate research. 37: 135–47.

Pereira TR, Engelen AH, Pearson GA, Valero M & Serrão EA. 2015. Response of kelps from different latitudes to consecutive heat shock. *Journal of Experimental Marine Biology and Ecology* 463: 57–62.

Pergams OR & Ashley MV. 2001. Microevolution in island rodents. *Genetica* 112: 245–256.

Perry RI, Cury P, Brander K, Jennings S, Möllmann C & Planque B. 2010. Sensitivity of marine systems to climate and fishing: concepts, issues and management responses. *Journal of Marine Systems* 73: 427–435.

Pigliucci M. 2009. An extended synthesis for evolutionary biology. *Annals of the New York Academy of Sciences* 1168: 218–228.

Pigliucci M & Müller GB. 2010. Elements of an extended evolutionary synthesis. In: *Evolution: The extended synthesis*, (M Pigliucci & GB Müller, eds). MIT Press, Cambridge, MA. Pp. 3–17.

Piñeiro-Corbeira C, Barreiro R, Cremades J & Arenas F. 2018. Seaweed assemblages under a climate change scenario: Functional responses to temperature of eight intertidal seaweeds match recent abundance shifts. *Scientific Reports* 8: e12978.

Porzio L, Buia MC & Hall-Spencer JM. 2011. Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology* 400: 278–287.

Qin S, Lin H & Jiang P. 2012. Advances in genetic engineering of marine algae. *Biotechnology Advances* 30: 1602–1613.

Raimondi PT, Reed DC, Gaylord B & Washburn L. 2004. Effects of self-fertilization in the giant kelp, *Macrocystis pyrifera*. *Ecology* 85: 3267–3276.

Ramanan R, Kim BH, Cho DH, Oh HM & Kim HS. 2016. Algae–bacteria interactions: evolution, ecology and emerging applications. *Biotechnology Advances* 34: 14–29.

Rangeley RW & Kramer DL. 1995. Use of rocky intertidal habitats by juvenile pollock *Pollachius virens*. *Marine Ecology Progress Series*. 126: 9–17.

Raven JA, Johnston AM, Kubler JE, Korb R, Mcinroy SG, Handley LL, Scrimgeour CM, Walker DI, Beardall J & Clayton MN. 2002. Seaweeds in cold seas: evolution and carbon acquisition. *Annals of Botany* 90: 525–536.

Rebours C, Marinho-Soriano E, Zertuche-González JA, Hayashi L, Vásquez JA, Kradolfer P, Soriano G, Ugarte R, Abreu MH, Bay-Larsen I & Hovelsrud G. 2014. Seaweeds: an opportunity for wealth and sustainable livelihood for coastal communities. *Journal of Applied Phycology* 26: 1939–1951.

Redmond S, Green L, Yarish C, Kim J & Neefus C. 2014. *New England Seaweed Culture Handbook-Nursery Systems*. Connecticut Sea Grant CTSG-14-01. 92 pp. http://seagrant.uconn.edu/publications/aquaculture/handbook.pdf. 92 pp.

Reusch TB, Boström C, Stam WT & Olsen JL. 1999. An ancient eelgrass clone in the Baltic. *Marine Ecology Progress Series* 183: 301–304.

Reusch TB, Ehlers A, Hämmerli A & Worm B. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences* 102: 2826–2831.

Richards CL, Bossdorf O & Pigliucci M. 2010. What role does heritable epigenetic variation play in phenotypic evolution? *BioScience* 60: 232–237.

Richards CL, Alonso C, Becker C, Bossdorf O, Bucher E, Colomé-Tatché M, Durka W, Engelhardt J, Gaspar B, Gogol-Döring A & Grosse I. 2017. Ecological plant epigenetics: Evidence from model and non-model species, and the way forward. *Ecology Letters* 20: 1576–1590.

Ridgwell A & Valdes PJ. 2009. Climate and climate change. *Current Biology* 19: R563-R566.

Riginos C & Cunningham CW. 2007. Hybridization in postglacial marine habitats. *Molecular Ecology* 16: 3971–3972.

Robuchon M, Le Gall L, Mauger S & Valero M. 2014. Contrasting genetic diversity patterns in two sister kelp species co-distributed along the coast of Brittany, France. *Molecular Ecology* 23: 2669–2685.

Royer TC & Grosch CE. 2006. Ocean warming and freshening in the northern Gulf of Alaska. *Geophysical Research Letters* 33: e6.

Ryman N. 1991. Conservation genetics considerations in fishery management. *Journal of Fish Biology* 39: 211–224.

Ryman N, Utter F & Laikre L. 1995. Protection of intraspecific biodiversity of exploited fishes. *Reviews in Fish Biology and Fisheries* 5: 417–446.

Sabin AL & Pisias NG. 1996. Sea surface temperature changes in the Northeastern Pacific Ocean during the past 20,000 years and their relationship to climate change in Northwestern North America. *Quaternary Research* 46: 48–61.

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CSL, Wallace DW, Tilbrook B & Millero FJ. 2004. The oceanic sink for anthropogenic CO2. *Science* 305: 367–371.

Santelices B. 2014. Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanography and Marine Biology* 28: 177–276.

Schaum CE & Collins S. 2014. Plasticity predicts evolution in a marine alga. *Proceedings of the Royal Society B: Biological Sciences* 281: e20141486.

Schlichting CD & Pigliucci M. 1995. Gene regulation, quantitative genetics and the evolution of reaction norms. *Evolutionary Ecology* 9: 154–168.

Schiel DR & Foster MS. 2006. The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. *Annual Reviews in Ecology Evolution and Systematics* 37: 343–72.

Schiener P, Black KD, Stanley MS & Green DH. 2014. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. *Journal of Applied Phycology* 27: 363–373.

Schmid MW, Heichinger C, Schmid DC, Guthörl D, Gagliardini V, Bruggmann R, Aluri S, Aquino C, Schmid B, Turnbull LA & Grossniklaus U. 2018. Contribution of epigenetic variation to adaptation in *Arabidopsis*. *Nature Communications* 9: e4446.

Shan TF, Pang SJ & Gao SQ. 2013. Novel means for variety breeding and sporeling production in the brown seaweed *Undaria pinnatifida* (P haeophyceae): Crossing female gametophytes from parthenosporophytes with male gametophyte clones. *Phycological Research* 61: 154–161.

Shan T, Pang S, Wang X-M, Li J & Su L. 2018. Assessment of the genetic connectivity between farmed and wild populations of *Undaria pinnatifida* (Phaeophyceae) in a representative traditional farming region of China by using newly developed microsatellite markers. *Journal of Applied Phycology* 30: 2707–2714.

Shan TF, Pang SJ, Zhang YR, Yakovleva IM & Skriptsova AV. 2011. An AFLP-based survey of genetic diversity and relationships of major farmed cultivars and geographically isolated wild populations of *Saccharina japonica* (Phaeophyta) along the northwest coasts of the Pacific. *Journal of Applied Phycology* 23: 35–45.

Shan T, Yotsukura N & Pang S. 2017. Novel implications on the genetic structure of representative populations of *Saccharina japonica* (Phaeophyceae) in the Northwest Pacific as revealed by highly polymorphic microsatellite markers. *Journal of Applied Phycology* 29: 631–638.

Shibneva SY, Skriptsova AV, Shan TF & Pang SJ. 2013. The different morphs of *Undaria pinnatifida* (Phaeophyceae, Laminariales) in Peter the Great Bay (Sea of Japan) are phenotypic variants: direct evidence. *Journal of Applied Phycology* 25: 1909–1916.

Shukla, P. & Edwards, M.S., 2017. Elevated p CO2 is less detrimental than increased temperature to early development of the giant kelp, *Macrocystis pyrifera* (Phaeophyceae, Laminariales). *Phycologia* 56: 638–648.

Singh RP, Reddy CR. 2016. Unraveling the functions of the macroalgal microbiome. *Frontiers in Microbiology*. 6: e1488.

Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE & McEwan JC. 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* 93: 255–265.

Sondak SFA, Ang PO Jr, Beardall J, Bellgrove A, Boo SM, Gerung GS, Hepburn CD, Hong DD, Hu Z, Kawai H, Largo D, Lee JA, Lim P-E, Mayakun J, Nelson WA, Oak JH, Phang S-M, Sahoo D, Peerapornpis Y, Yang Y & Chung IK. 2017. Carbon dioxide mitigation potential of seaweed aquaculture beds (SABs). *Journal of Applied Phycology* 29: 2363–2373.

Spurkland T & Iken K 2011 Kelp bed dynamics in estuarine environments in subarctic Alaska. *Journal of Coastal Research* 27: 133–143.

Stein F, Sjøtun K, Lein TE & Rueness J. 1995. Spore dispersal in *Laminaria hyperborea* (Laminariales, Phaeophyceae). *Sarsia* 80: 47–53.

Stekoll MS. 2019. The seaweed resources of Alaska. *Botanica Marina* 62: 227–235.

Stévant P, Rebours C, Chapman A. 2017. Seaweed aquaculture in Norway: recent industrial developments and future perspectives. *Aquaculture International* 25: 1373–1390.

Su L, Pang SJ, Shan TF & Li X. 2017. Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in northern China. *Journal of Applied Phycology* 29: 3003–3013.

Szulkin M, Bierne N & David P. 2010. Heterozygosity-fitness correlations: a time for reappraisal. *Evolution* 64: 1202–1217.

Tanaka K, Taino S, Haraguchi H, Prendergast G & Hiraoka M. 2012. Warming off southwestern Japan linked to distributional shifts of subtidal canopy-forming seaweeds. *Ecology and Evolution* 2: 2854–2865.

Turesson G. 1922. The genotypical response of the plant species to habitat. *Hereditas* 3: 211–350.

Tuya F, Cacabelos E, Duarte P, Jacinto D, Castro JJ, Silva T, Bertocci I, Franco JN, Arenas F, Coca J & Wernberg T. 2012. Patterns of landscape and assemblage structure along a latitudinal gradient in ocean climate. *Marine Ecology Progress Series* 466: 9–19.

Ugarte, R. & Sharp, G. 2012. Management and production of the brown algae Ascophyllum nodosum in the Canadian maritimes. *Journal of Applied Phycology* 24: 409–416.

Unckless RL & Orr HA. 2009. Dobzhansky–Muller incompatibilities and adaptation to a shared environment. *Heredity* 102: 214.

Uwai S, Kogame K, Yoshida G, Kawai H & Ajisaka T. 2009. Geographical genetic structure and phylogeography of the *Sargassum horneri*/*filicinum* complex in Japan, based on the mitochondrial cox3 haplotype. *Marine Biology* 156: 901–911.

Valero M, Destombe C, Mauger S, Ribout C, Engel CR, Daguin-Thiebaut C & F Tellier. 2011. Using genetic tools for sustainable management of kelps: a literature review and the example of *Laminaria digitata*. *Cahiers de Biologie Marine* 52: 467–483.

van Alstyne KL, McCarthy JJ III, Hustead CL & Duggins DO. 1999. Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds. *Marine Biology* 133: 371–379.

Van Der Graaf A, Wardenaar R, Neumann DA, Taudt A, Shaw RG, Jansen RC, Schmitz RJ, Colomé-Tatché M & Johannes F. 2015. Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *Proceedings of the National Academy of Sciences* 112: 6676–6681.

van Vuuren DP, Edmonds J, Mikiko Kainuma M, Riahi K, Thomson A, Hibbard K, Hurtt GC, Kram T, Krey V, Lamarque J-F, Masui T, Meinshausen M, Nakicenovic N, Smith SJ & Rose SK. 2011. The representative concentration pathways: an overview. *Climatic change* 109: 5–31.

Vitousek PM, Mooney HA, Lubchenco J & Melillo JM. 1997. Human domination of Earth's ecosystems. Science. 277: 494-499.

Wahl M, Molis M, Hobday AJ, Dudgeon S, Neumann R, Steinberg P, Campbell AH, Marzinelli E & Connell S. 2015. The responses of brown macroalgae to environmental change from local to global scales: direct versus ecologically mediated effects. *Perspectives in Phycology* 2: 11–29.

Waters JM, Fraser CI & Hewitt GM. 2012. Founder takes all: density-dependent processes structure biodiversity. *Trends in Ecology & Evolution* 28: 78–85.

Wayne GP. 2013. The beginner’s guide to representative concentration pathways. Skeptical Science (http://gpwayne.wordpress.com)

Wei N, Quarterman J, Jin YS. 2013. Marine macroalgae: an untapped resource for producing fuels and chemicals. *Trends in Biotechnology* 31: 70–77.

Wernberg T, Russell BD, Thomsen MS, Gurgel CF, Bradshaw CJ, Poloczanska ES & Connell SD. 2011. Seaweed communities in retreat from ocean warming. *Current Biology* 21: 1828–1832.

Widdowson TB. 1971a. A taxonomic revision of the genus *Alaria* Greville. *Syesis* 4: 11–49.

Widdowson TB. 1971b. A statistical analysis of variation in the brown alga *Alaria*. *Syesis* 4: 125–143.

Wing BL. 2006. Unusual invertebrates and fish observed in the Gulf of Alaska, 2004–2005. *Pices Press* 14: 26–28.

World Bank Group. 2016. *Seaweed Aquaculture for food security, income generation and environmental health in tropical developing countries*. World Bank, 16 p.

Wu S-Y, Yan M-D, Wu ATH, Yuan KS-P & Liu SH. 2016. Brown seaweed fucoidan inhibits cancer progression by dual regulation of mir-29c/ADAM12 and miR-17-5p/PTEN axes in human breast cancer cells. *Journal of Cancer* 7: 2408–2419.

Xie B, Qin J, Yang H, Wang X, Wang YH & Li TY. 2013. Organic aquaculture in China: a review from a global perspective. *Aquaculture* 414: 243–253.

Yang Q, Cokelet ED, Stabeno PJ, Li L, Hollowed AB, Palsson WA, Bond NA & Barbeaux SJ. 2019. How "The Blob" affected groundfish distributions in the Gulf of Alaska. *Fisheries Oceanography* 28: 434–453.

Yesson C, Bush LE, Davies AJ, Maggs CA & Brodie J. 2015a. Large brown seaweeds of the British Isles: evidence of changes in abundance over four decades. *Estuarine, Coastal and Shelf Science* 155: 167–175.

Yesson C, Bush LE, Davies AJ, Maggs CA & Brodie J. 2015b. The distribution and environmental requirements of large brown seaweeds in the British Isles. *Journal of the Marine Biological Association of the United Kingdom* 95: 669–680.

Yotsukura N, Shimizu T, Katayama T & Druehl LD. 2010. Mitochondrial DNA sequence variation of four *Saccharina* species (Laminariales, Phaeophyceae) growing in Japan. *Journal of Applied Phycology* 22: 243–251.

Yotsukura N, Maeda T, Abe T, Nakaoka M & Kawai T. 2016. Genetic differences among varieties of *Saccharina japonica* in northern Japan as determined by AFLP and SSR analyses. *Journal of Applied Phycology* 28: 3043–3055.

Zhang J, Liu T, Bian D-P, Zhang L, Li X-B, Liu D-O, Liu C, Jingjin Cui J-G & Xiao L-A. 2016. Breeding and genetic stability evaluation of the new *Saccharina* variety “Ailunwan” with high yield. *Journal of Applied Phycology* 28: 3413–3421.

Zhang J, Yao J-T, Sun Z-M, Fu G, Galanin DA, Nagasato C, Motomura T, Min Hu Z-M & De-Lin Duan D-L. 2015. Phylogeographic data revealed shallow genetic structure in the kelp *Saccharina japonica* (Laminariales, Phaeophyta). *BMC Evolutionary Biology* 15:237.

Zhang QS, Tang XX, Cong YZ, Qu SC, Luo SJ & Yang GP. 2007. Breeding of an elite *Laminaria* variety 90-1 through inter-specific gametophyte crossing. *Journal of Applied Phycology* 19: 303–311.

Zhang X & Thomsen M. 2019. Biomolecular composition and revenue explained by interactions between extrinsic factors and endogenous rhythms of *Saccharina latissima*. *Marine Drugs* 17: 107.

Zhao XB, Pang SJ, Liu F, Shan TF, Li J, Gao SQ & Kim HG. 2016. Intraspecific crossing of *Saccharina japonica* using distantly related unialgal gametophytes benefits kelp farming by improving blade quality and productivity at Sanggou Bay, China. *Journal of Applied Phycology* 28: 449–455.

Zimmerman RC & Kremer JN. 1986. In situ growth and chemical composition of the giant kelp, Macrocystis pyrifera: response to temporal changes in ambient nutrient availability. *Marine Ecology Progress Series* 27: 277–285.

Zou L & Huang S. 2015. Chinese aquaculture in light of green growth. *Aquaculture Reports* 2: 46–49.

Table 11.1 Summary diversity statistics for organellar genes in *Alaria* spp, *Saccharina latissima*, and *Hedophyllum nigripes* in the Northeastern Pacific Ocean and in other kelps.

				h			θ_π%					
Species	Gene	N	N_h	overall	mean	Range	overall	mean	Range	D_T	P	*Reference*
Alaria spp	*COI-rbc*L	543	33	0.903	0.290	0.0–0.636	0.547	0.061	0.0–0.264	0.776	0.832	This report
Saccharina latissima	*COI-rbc*L	446	16	0.781	0.188	0.0–0.574	0.088	0.016	0.0–0.047	-0.868	0.208	This report
S. japonica Russia	*COI-trn*W-L	267	22	0.584	0.256	0.0–0.600	0.038	0.019	0.0–0.063	-2.358[2]	<0.01	Zhang *et al* 2015
S. japonica Japan	*COI-trn*W-L	231	30	0.723	0.514	0.177–0.762	0.079	0.051	0.010–0.162	–	–	Zhang *et al* 2015
S. japonica Korea	*COI-trn*W-L	27	4	0.033	0.033	–	0.027	0.027	–	–	–	Zhang *et al* 2015
S. japonica China	*COI-trn*W-L	87	5	0.553	0.222	0.0–0.421	0.051	0.017	0.0–0.028	–	–	Zhang *et al* 2015
Hedophyllum nigripes	*COI-rbc*L	52	12	0.770	0.291	0.0–0.833	0.091	0.025	0.0–0.072	-1.210	0.114	This report
Sargassum thunbergii	*CO3*	810	35	–	0.241	0.0–0.733	–	0.055	0.0–0.224	-0.328[1]	5/35[1]	Li *et al*. 2017
Sargassum ilicifolium	*COI*	268	16	0.821	0.366	0.0–0.732	0.339	0.159	0.0–0.599	-1.23	>0.05	Ng *et al.* 2019
Sargassum horneri	*CO3*	305	33	0.864	–	–	1.520	–	–	–	–	Uwai *et al*. 2009
Ishige okamurae	*CO3*	221	17	0.781	0.338	0.0–0.652	0.961	0.232	0.0–1.385	-0.623	NS	Lee *et al*. 2012

[1] Mean D_T; 5of 35 populations $P < 0.05$

[2] Pooled over four samples

Table 11.2 Summary of genetic diversity estimated with microsatellite markers.

Species	Region	N	No. of samples	No. of loci	H_E overall	H_E mean	H_E range	Reference
Alaria	NE Pacific	448	16	8	0.882	0.563	0.357–0.767	This study
Saccharina latissima	NE Pacific	420	13	12	0.558	0.360	0.132–0.467	This study
Saccharina latissima	NE Pacific	15	2	12	–	0.350	0.324–0.375	Neiva et al. 2018
Saccharina latissima	NW Atlantic	188	5	10	–	0.309	0.273–0.340	Breton et al. 2017
Saccharina latissima	NW Atlantic	234	15	12	–	0.413	0.140–0.563	Neiva et al. 2018
Saccharina latissima	NE Atlantic	171	7	12	–	0.531	0.412–0.632	Neiva et al. 2018
Saccharina latissima	NE Atlantic	225	8	12	–	0.515	0.358–0.609	Nielsen et al. 2016
Saccharina japonica-cultivars	NW Pacific	192	8	13	–	0.374	0.342–0.407	Liu et al. 2012
Saccharina japonica-wild	NW Pacific	32	4	13	–	0.457	0.461–0.490	Liu et al. 2012
Laminaria digitata	NE Atlantic	764	21	11	–	0.488	0.349–0.624	Robuchon et al. 2014
Laminaria hyperborea	NE Atlantic	1031	21	9	–	0.570	0.429–0.613	Robuchon et al. 2014
Macrocystis pyrifera	NE Pacific	2631	62	7	–	0.690	0.125–0.817	Johansson et al. 2015

Table 11.3 Comparison of genetic divergence between populations of kelps

				Organellar DNA			Microsatellite DNA			
Species	Region	Range (km)	No. samples	Gene	Φ_{ST}	Range	No. loci	Mean F_{ST}	Range	Reference
Sacchrina latissima	NE Pacific	2480	14	*COI*-*rbc*L	0.646	0.0–1.0	12	0.366	0.041–0.759	This report
S. latissima	NE Atlantic	2580	8	*COI*	0.318	–	10	0.201	0.071–0.564	Luttikhuizen et al. 2018
S. latissima	NE Atlantic	4000	6	–	–	–	25	0.259	0.077–0.562	Guzinski et al. 2016
S. latissima	NW Atlantic	225	5	–	–	–	10	0.015	0.0–0.032	Breton et al. 2017
S. japonica	NW Pacific	3090	14	–	–	–	11	0.342	0.0–0.762	Shan et al. 2017
S. japonica	NW Pacific	3000	26	*COI*-*tm*W–*tm*L	0.284[1]	0.0–1.0	–	–	–	Zhang et al. 2015
H. nigripes	NE Pacific	930	4	*COI*-*rbc*L	0.842	0.342–1.0	–	–	–	This report
Sargassum thunbergii	NW Pacific	3100	35	*CO3*	0.039	0.0–0.94	–	–	–	Li et al. 2017
Sargassum ilicifolium	NW Pacific	3000	23	*COI*	0.115	–	–	–	–	Ng et al. 2019
Sargassum horneri	NW Pacific	1900	37	*CO3*	0.883	–	–	–	–	Uwai et al. 2009
Ishige okamurae	NW Pacific	3000	14	*CO3*	0.561	0.0–1.0	–	–	–	Lee et al. 2012
Laminaria digitata	NE Atlantic	700	18	–	–	–	7	0.068	–	Billot et al. 2003
Laminaria digitata	NE Atlantic	440	21	–	–	–	11	0.085	0.0–0.094	Robuchon et al. 2014
Laminaria hyperborea	NE Atlantic	440	21	–	–	–	9	0.094	0.0–0.235	Robuchon et al. 2014
Alaria	NE Pacific	2650	16	*COI*-*rbcL*	0.811	0.0–1.0	8	0.264	0.086–0.505	This report

[1] Mean over samples

Table 11.4 Interpretation of USDA National Organic Program (NOP) criteria for organic classification of seaweed provided by the Maine Organic Farmers and Gardeners Association (MOFGA). https://mofgacertification.org/certifying-sea-vegetables/

NOP Stipulation for terrestrial crops	MOFGA interpretation for seaweeds
205.202 Land requirements	Organic sea vegetables must be cultivaed, or harvested from the wild, in defined areas with high ecological quality. Growing areas must be located away from sources of radioactivity, chemical and bacteriological contamnation.
205.203 Soil fertility and crop nutrient management practic standard	Fertility and crop nutrients may be used only during the incubation of young sporophytes in indoor facilities, but may not be used in outdoor growing areas.
205.204 Seed and planting stock standard	Certified organic propagules must be used, unless they are not available in the form, quality, or quantity needed. Male and female gametophytes must be cultured in pure seawater or approve growth medium before fertilization and production of young sporophytes.
205.206 Crop pest, weed and disease management standard	The managment of invasive algae, epiphytes, epizooites and diseases should be by cultural, mechanical, or physical controls. If these controls fail, substances listed under NOP 205.601 or NOP 205.605 may be used.
205.207 Wild-crop harvesting practice standard	Harvesting must be from designated areas free of substances listed in NOP 205.105 for one growing cycle preceding harvest. Culture and harvests should be in an ecologically sustainable manner that is not destructive to the environment and that allows subsequent growth of the seaweed.
205.272 Commingling and contact with prohibited substances practice standard	Prevent the commingling of organic with non-orgainic products, or with prohibited substances, during fertilization, culturing of sporophytic propagules, grow-out, harvest, tranport, processing and storage.
205.103 Record keeping by certified operations	Seaweed farmers must maintain records demonstrating compliance with organic production standards at all stages of the operation.

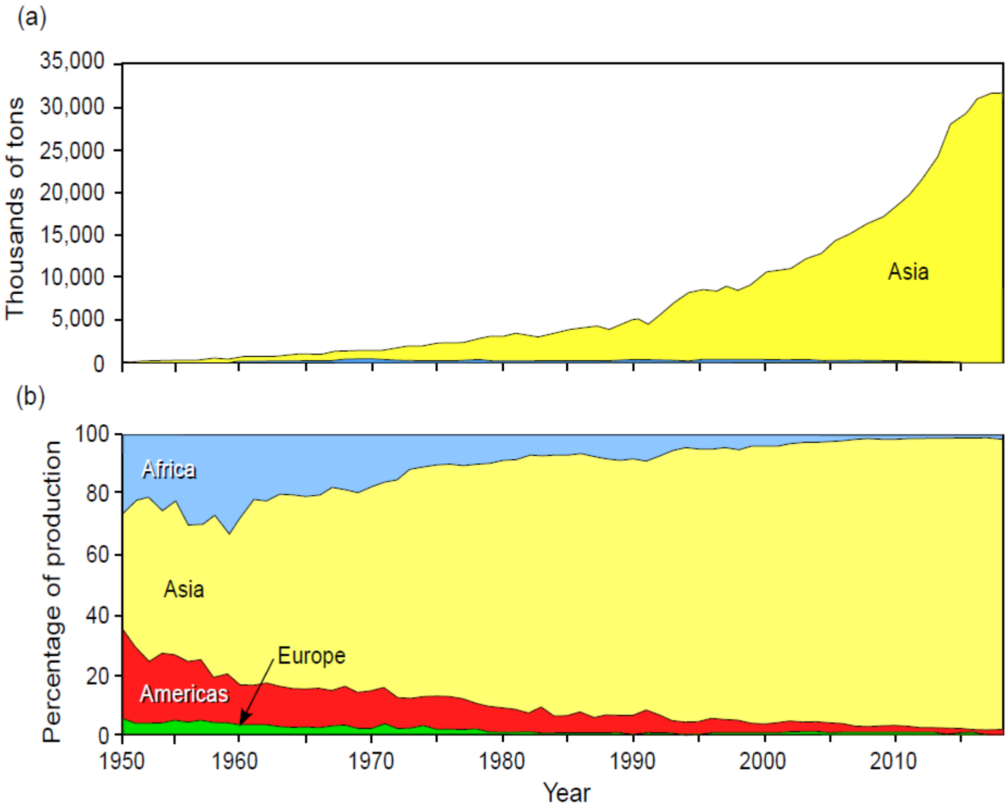

Figure 11.1 Production of seaweeds by wet tonnage globally (a) and by regions (b) from 1950 to 2017. Data from FAO (http://www.fao.org/fishery/statistics/en) accessed July 2019

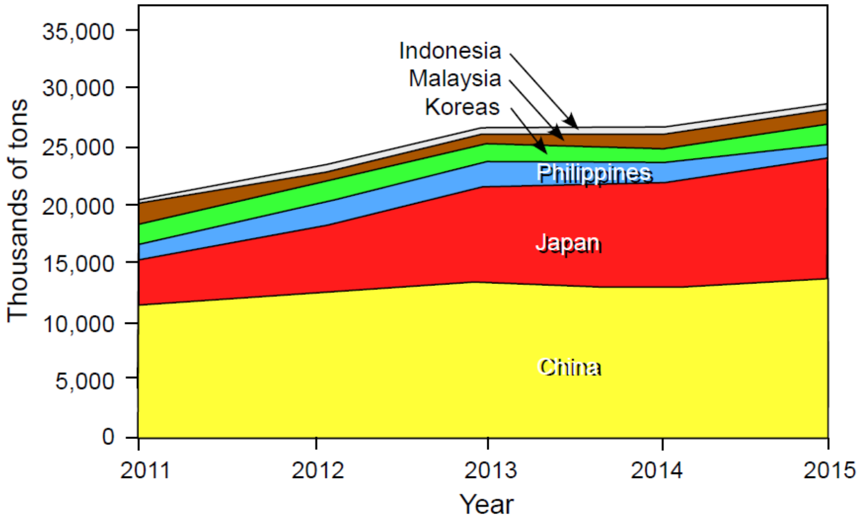

Figure 11.2 Production of seaweeds by Asian countries (wet tonnage). From FAO 2018

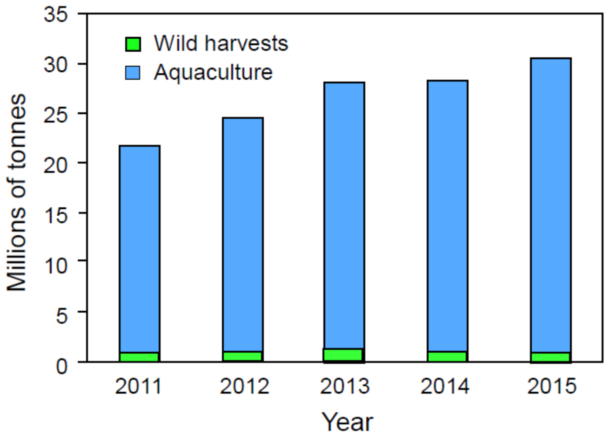

Figure 11.3 Global production of seaweeds from wild harvests and ultivation. From FAO (2018)

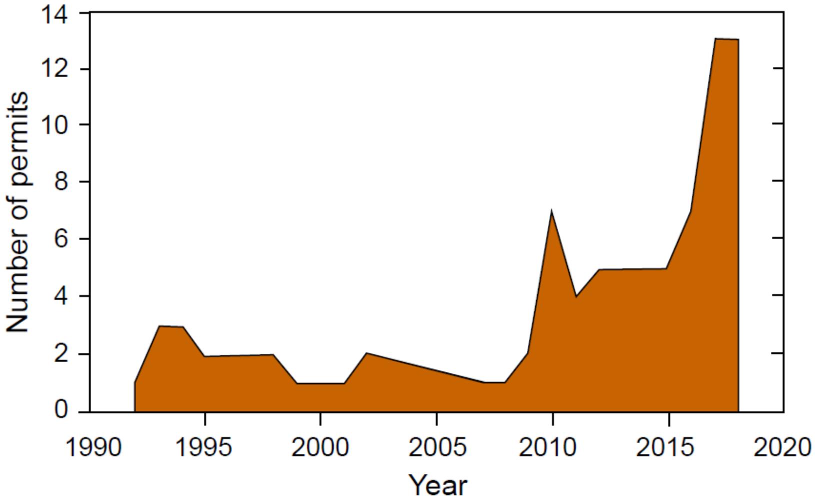

Figure 11.4 Permitted seaweed farm operations in Alaska from 1992 to 2018 (Source: C. Pring-Ham, Alaska Department of Fish & Game)

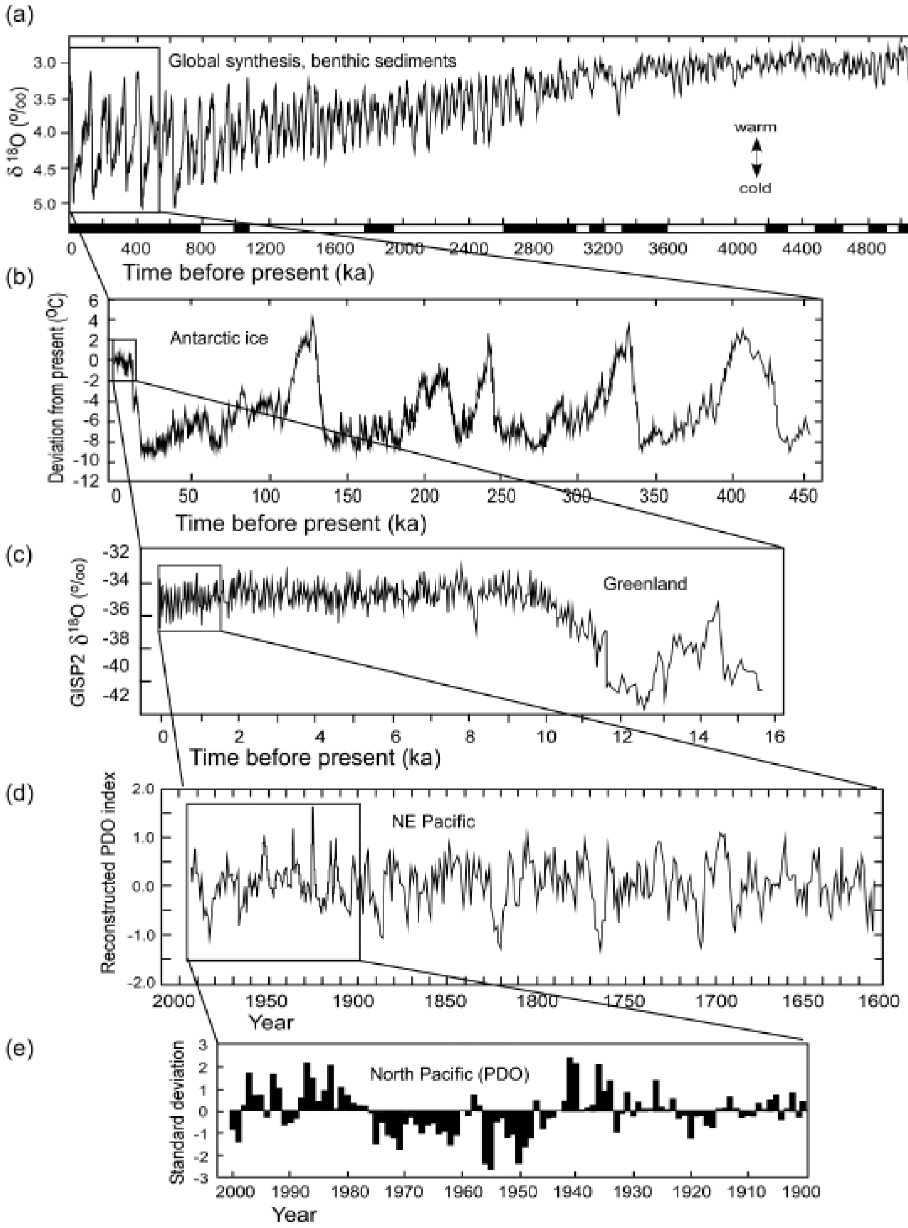

Figure 11.5 Scales of climate variability over the Quaternary 2.6 Ma to present. (a) Temperature proxy δ18O records in 57 benthic sediment cores distributed globally (redrawn from Lisiecki and Raymo 2005). (b) Temperature reconstructions from proxy deuterium isotope profiles in Antarctic ice cores (redrawn from Jouzel et al. 2007). (c) Temperature proxy δ18O records in Greenland ice cores from GISP2 (redrawn from Bond et al. 1997). (d) Extended reconstruction of the Pacific Decadal Oscillation from tree-ring time series along the western coast of North America (redrawn from Gedalof and Smith 2001). (e) Pacific decadal index (redrawn from Mantua and Hare 2002)

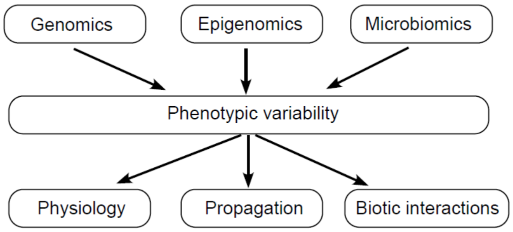

Figure 11.6 Diagram showing relationships between sources of phenotypic variability and its influence on processes that influence adaptation in seaweeds. Modified from Duarte *et al*. (2018)

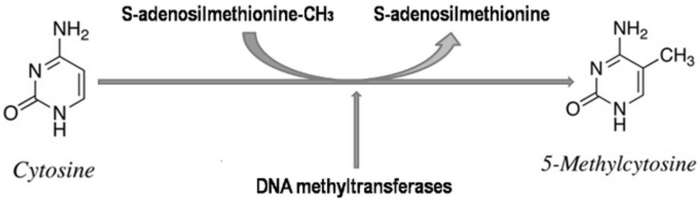

Figure 11.7 Diagram of catalytic reaction fixing a methyl group (CH_3) to the nucleotide cytosine. From: https://www.labome.com/method/DNA-Methylation.html

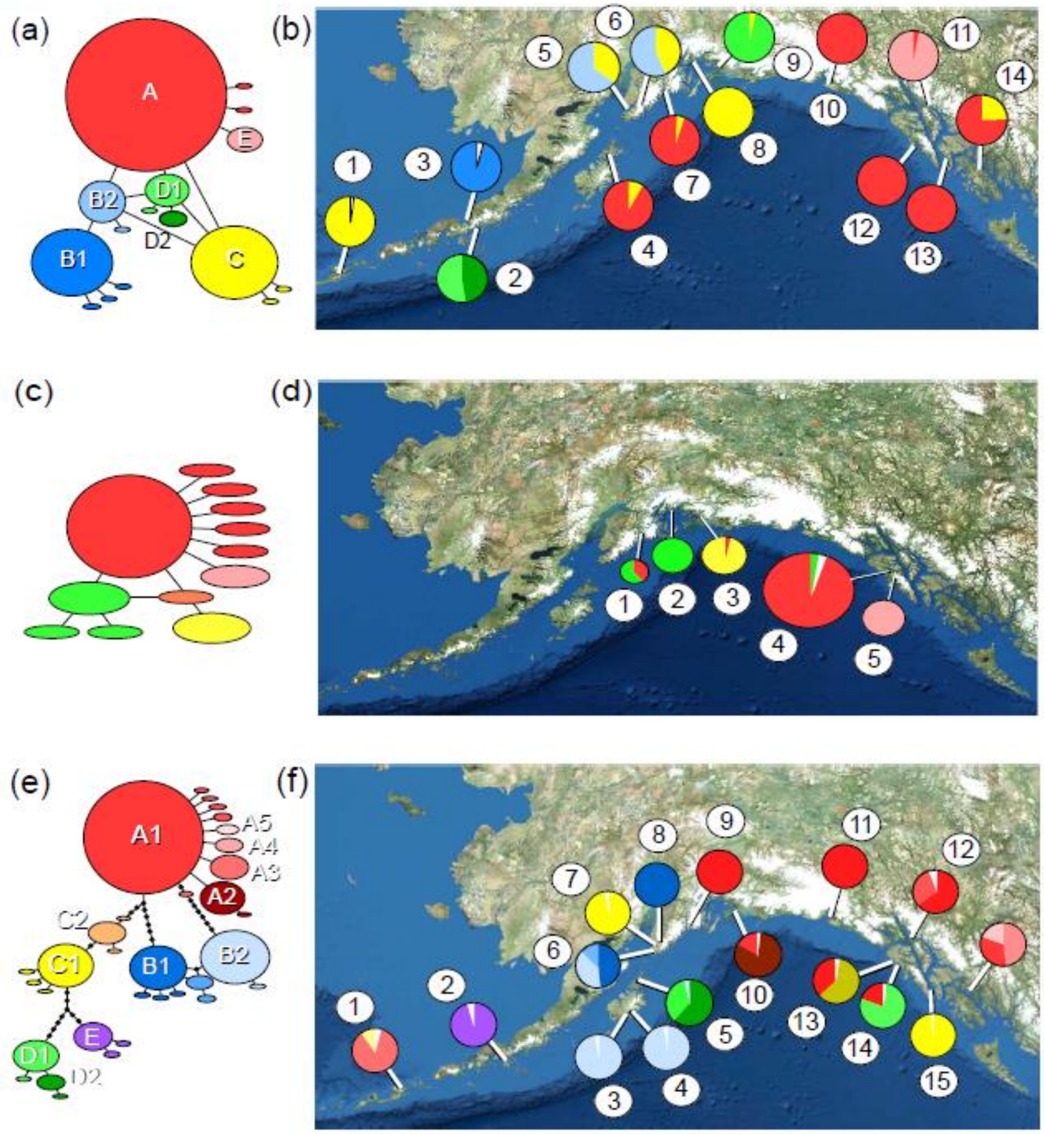

Figure 11.8 Genetic structure (concatenated sequences of *COI* and *rbc*L genes) of three kelps in the Gulf of Alaska. (a, b) haplotype network and distribution of haplotypes of sugar kelp, *Saccharina latissima*. (c, d) haplotype network and distribution of haplotypes of split kelp, *Hedophyllum nigripes*. (e, f) haplotype network and distribution of haplotypes of ribbon or winged kelp, *Alaria* spp. White wedges represent private mutations one step from the most abundant haplotype.

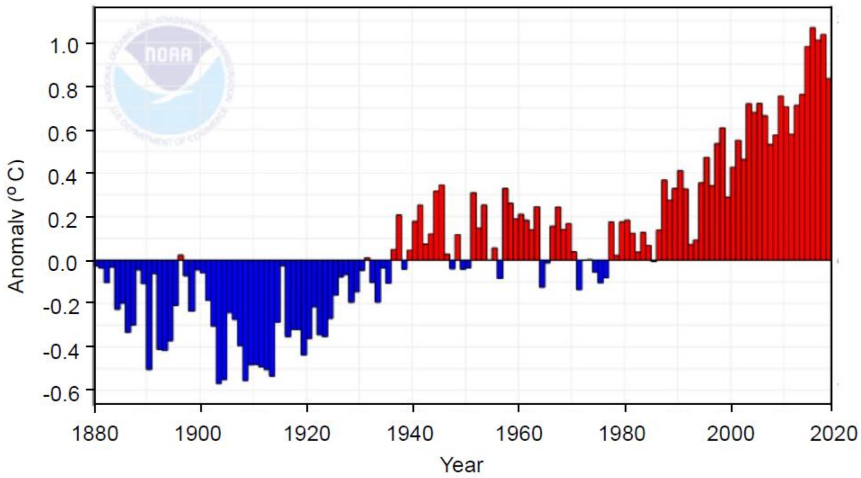

Figure 11.9 Temperature anomalies (deviations from long-term mean) of ocean temperatures in the Northern Hemisphere in August. Source: NOAA (https://www.ncdc.noaa.gov/cag/global/time-series/nhem/ocean/1/8/1880-2019)

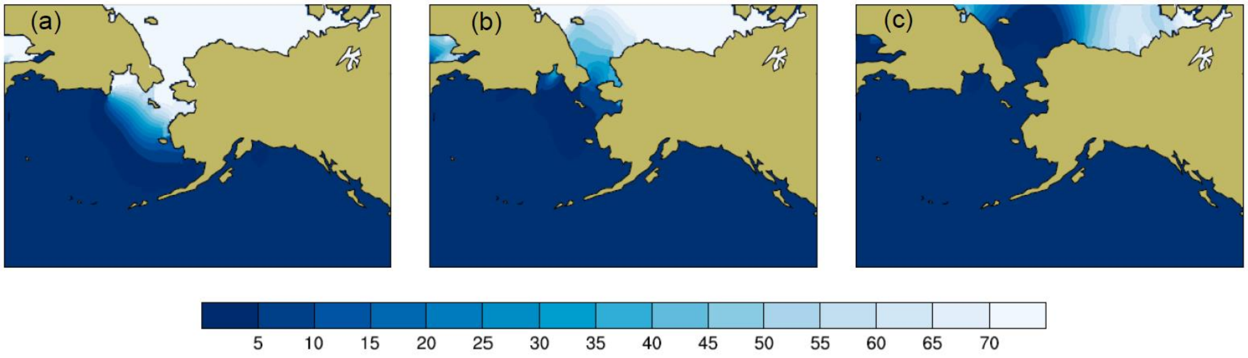

Figure 11.10 Projections of extent of average daily sea-ice concentration (%) in March for (a) 2011–20140, (b) 2041–2070, and (c) 2071–2100. From Lader *et al*. (2017)

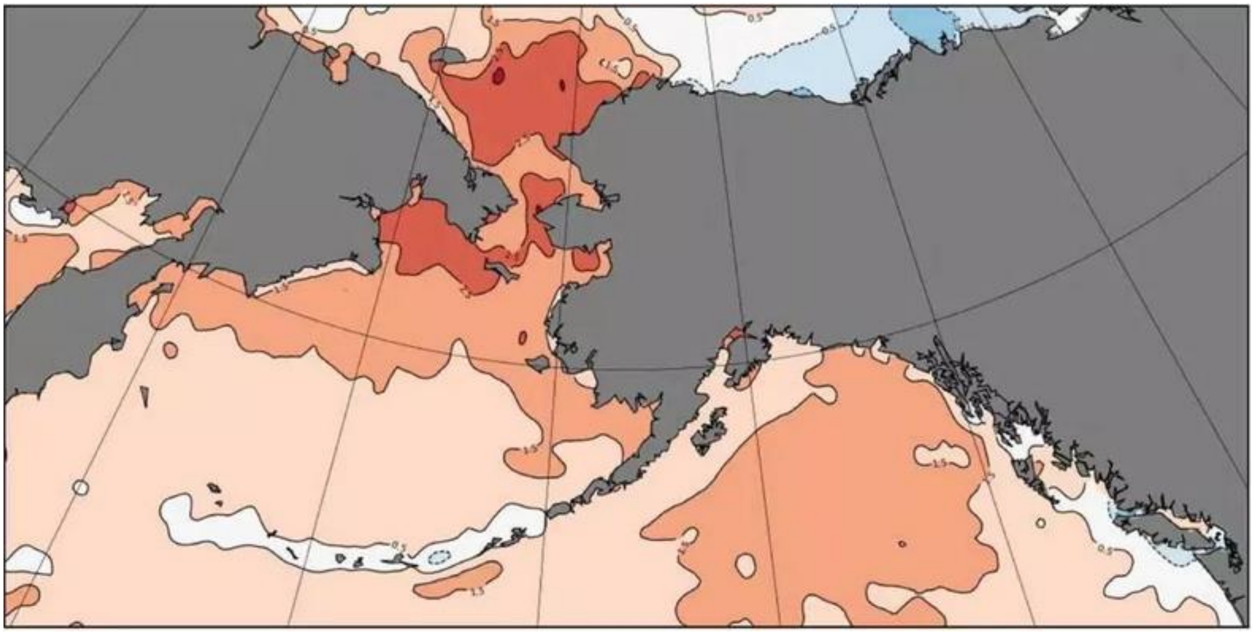

Figure 11.11 Chart showing distributions of warmer-then-normal sea surface temperatures in the Gulf of Alaska and Bering Sea in autumn 2018. Shades of red indicate temperatures that are warmer than long-term averages and shades of blue indicate cooler temperatures. (International Arctic Research Center, University of Alaska Fairbanks, https://uaf-iarc.org/2019/04/11/bering-strait-sea-ice-conditions-winter-2019/ accessed July 2019)

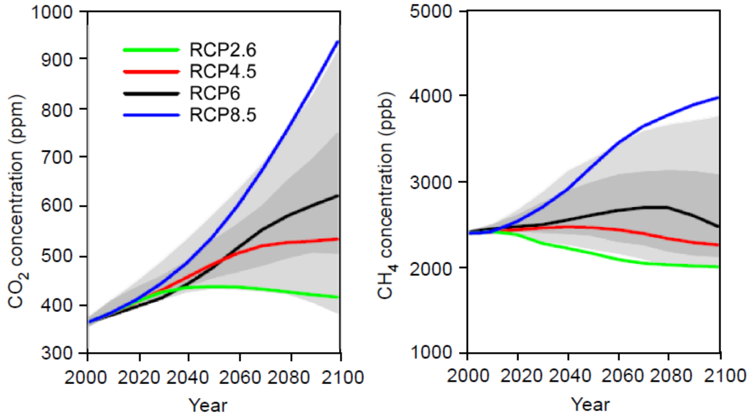

Figure 11.12 Projected CO_2 (left) and CH_4 (right) global atmospheric concentrations of two major greenhouse gases under four Representative Concentration Pathways (RCP) and comparison with projections of Clark *et al*. (2010) (grey shading: 90% & 98% percential projections), who used EMF22 scenarios for model simulations.

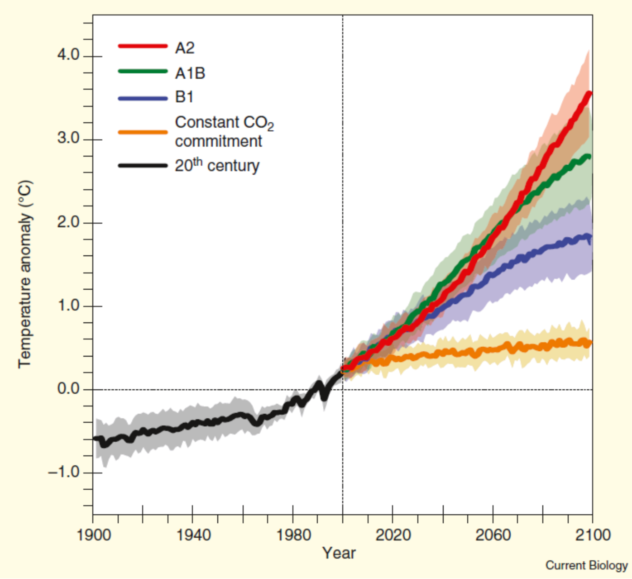

Figure 11.13 Mean global surface warming relative to 1980–1999 predicted by the SRES CO_2 emission scenarios. A2 (red), A1B (green) and B1 (purple) lines are averaged across models of climate projections with various outcomes of economic and social development, and energy production and usage. A constant CO_2 model (orange) indicates warming if increases in atmospheric CO_2 were immediately halted. Shadows represent ±1 standard deviation of simulated results. From Ridgwell & Valdes (2009) adapted from the IPCC (2007) report.

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Chapter 12: **General Conclusions and Future Directions**

The general goal of this project was to survey genetic variability in kelps in Alaskan waters. The use of three classes of molecular genetic markers provided insights into not only contemporary genetic structure, but, together with published DNA sequences, also into the evolutionary history of three kelps, *Saccharina latissima* (sugar kelp), *Hedophyllum nigripes* (split kelp), and *Alaria* spp. (winged or ribbon kelp). The different classes of molecular markers give different views of the same populations events because of differences in modes of inheritance and mutation rates. Mitochondrial and chloroplast DNAs are inherited from the female parent, but independently of each other. They have moderate mutation rates (10^{-8} to 10^{-7} per nucleotide site per generation) compared to nuclear encoding genes. The uniparental inheritance, and lack of recombination typical of nucelar genes, facilitates the reconstructions of gene genealogies that contain information about a species' deep history. Microsatellite DNA, on the other hand, is biparentally inherited and has much larger mutation rates (10^{-5} to 10^{-3}) that record contemporary population events. The contrast in mutation rates among markers opens a windows onto population events that occur on different time scales.

The pattern of organellar variability in all three species of kelp showed a mosaic genetic population structure of lineages that did not fall into regional regional groups. These species also did not show isolation by distance that is expected in continuously distributed species with limited dispersal ability. These chaotic patterns are best explained as legacies of historical isolations and colonizations during Pleistocene glaciations. The plants in several populations shared the same organellar haplotype so that within-population genetic diversities were generally small. Genetic homogeneity within populations may reflect 'winner-takes-all' dynamics during post-glacial colonizations, or may reflect the exclusion of propagules from plants not adapted to local environmental conditions. In either case, the sharp genetic discontinuities between collection sites indicates little connectivity between populations. Some adjoining populations were exceptions, indicating gene flow is occurring at least on small geographical scales between populations occupying similar habitats.

Organellar divergences between lineages were shallow in sugar and split kelps, but substantial *Alaria*. Five well separated lineages appeared in *Alaria* and this number exceeds the number of species described on the basis of morphology, which in kelps can vary even among plants with the same genotype. The distributions of microsatellite show that when organellar lineages occur at the same site, plants in the different lineages hybridize. This indicates that some of these subspecific taxa are not reproductively isolated from one another. More observational and experimental work is needed to better understand the genetic relationships between pairs of taxa and the extent of reproductive isolation, if any. Cursory observations suggest that plants in some lineages of *Alaria* sort by the effects of wave-action. Eco-genetic studies are needed to better understand the role of local adaptation in producing diversity in *Alaria*.

The genetic population structures of kelps contrast with those of marine fishes and invertebrates in Alaskan waters. Marine fishes and invertebrates tend to have relatively long lived larvae that can drift considerable differences and introduce new genes to distant populations. Seaweeds on the other hand, have spores that moves only a short distance from the parent before settling. Hence, the amount of genetic differention between populations is considerably different. Populations of marine fish and invertebrates, with few exceptions, tends to be genetically homogeneous over hundreds of kilometers. Whereas, seaweeds generally, and the kelps examined in this study specifically, show strong differences between populations on spatial scales as small as a few kilometers. The mosaic population structure of seaweeds reflects a fundamentally different reproductive and dispersal biologies. Unlike most marine fishes and invertebrates, seaweed populations have low levels of genetic diversity as measured by selectively neutral markers, but still appear to be adapted local habtiat conditions. Local adaptation may prevent non-adapted immigrants from becoming established in a population, thus limiting gene flow and re-inforcing sharp genetic differences between populations. These contrasts indicate that the genetic assumptions used to manage marine animals may not be appropriate for kelps.

The reduced levels of genetic diversity in most populations of the kelps that were examined brings into question the generally accepted dogma that genetic diversity is correlated with adaptive potential. Many highly successful plants reproduce reproduce asexually or clonally and are able to track environmental conditions with mininal stores of genetic variability. Low levels of genetic variation can arise as a results of founder events, population bottlenecks in size, natural selection or reproductive biology. For example, the giant kelp, *Macrocystis pyrifera*, can reproduce by self fertilization at the gametophytic stage. Since mieospores settle close parental sporophytes, gametophytes from the same plant are generally in close proximity and the chances of self fertilization are estimated to be as much as 40%. Little is know about the extent of selfing in the kelps examined in this study, other than that some plants had the same microsatellite genotypes, suggestive of self fertilization. Understanding the reproductive biologies of the kelps in Alaska’s waters is essential for developing an efficient program of kelp aquaculture. While self fertilization may be occurring in some populations of Alaskan kelps, the pervasive low levels of genetic diversity in the kelps examined in this study are more likely due to a genetic legacy of population turnover over the Pleistocene Epoch, and hence is unlikely to be an ongoing conservation concern.

It is therefore difficult to propose management guidelines not only because of kelp biology but also because of the sparsity of samples in our study relative to the small geographical scale of genetic differentiation. The extent that adaptive differences among populations are associated with differences detected with selectively neutral genetic markers is an area of on-going research in evolutionary biology. Adaptive and plastic responses to environmental variability in kelps can be assessed with common-garden and reciprocal-transplant experiments, which have been conducted on seaweeds in other regions. Alternatively, genomic studies of gene expression under different enviromental conditions may provide insights into the mechanisms underpining adaptative change.

The contrasts in genetic population structure between the three species examined in this study pose important evolutionary and eco-genetic questions. Why do these species show different degrees of intraspecific structure when they appear to have similar species' longevities of several million years? Can the deeper lineages in *Alaria* indicate a greater capcity for genetic adaption, or ecological plasticity, that leads to greater opportunities for isolation? In fact, both *Saccharina latissima* and *Hedophyllum nigripes*, which have shallow genealogies, inhabit a narrower range of environmental conditions than does *Alaria*, which can be found on both wave-exposed and wave-protected shores. In contrast, *S. latissima* is found only in quiet wave-protected bays, whereas *H. nigripes* inhabits areas of surf or fast currents. Also of interest is understanding the interaction between environmental variables, such as temperature, salinity and nutrient availability, on the physiology and synthesis of particular compounds in kelps. These areas of eco-genetic research have important implications for the development of kelp aquaculture in Alaska.

Alaska's populations of kelps will be greatly influenced by global climate warming in the future. Marine waters at high latitudes are changing not only from elevated temperatures, but also by ocean acidification as a result of the absorption of CO_2 by glogal seas. Increased concentrations of CO_2 in sea water can have both beneficial and detrimenal effects on algae. On one hand, greater concentrations of CO_2 can increase the rate of photosynthesis, but on the other they lead to lower levels of pH, which can alter nutrient uptake and the physiology of a plant. However, the greatest threats to kelps are from elevated temperatures which can prevent successful reproduction and recruitment. Alaskan waters have warmed considerably in the past few years, and continued warming will lead to poleward range extenions and local population extinctions.

The organellar DNA sequences generated in this project, together with published sequences from locations outside Alaska, provided an opportunity to visit broader phylogeographic issues. The greatest amounts of genetic diversity for each of the kelps examined occurs in the Gulf of Alaska. In split kelp, Alaskan populations had the highest levels of DNA diversity relative to those in Arctic and Northwestern Atlantic populations and in British Columbia. This pattern of diversity is supports a biogegraphic model of multiple northern ice-ace refugia rather than displacement into a refugium along unglaciated southern shores. Haplotypes closely related those in the Gulf of Alaska are found in the Arctic Ocean and Northwestern Atlantic, indicating recent dispersals across the Arctic from Alaska.

Alaska's populations of sugar kelp in the Gulf of Alaska and western Aleutians are part of a global complex of four well-differentiated regional groups located in the Northeastern and Northwestern Atlantic and in the Northwestern Pacific. As with split kelp, the large amount of genetic diversity in Gulf of Alaska populations argues for the existence of northern refugia during glacial maxima. Comparisons with sequences available in Genbank showed that the common *COI* haplotype in Alaska is also found in the Arctic populations as far as western Greenland. As with split kelp, the close genetic relationship between these widely separated populations indicates recent transArctic dispersals. For both split kelp and sugar kelp, a warmer Arctic Ocean will facilitate further transArctic dispersals.

The phylogeographic pattern for the winged kelp *Alaria* differed from those for the other two kelps. As noted above, five genetically distinctive lineages are present in the Gulf of Alaska, some of which have been descript as species. The addition of sequences from Genbank provided a broader view of phylogeography in *Alaria*. Unlike the two other kelps in this study, the distribution of the single North Atlantic species of *Alaria* extends across the Arctic and into the Bering Sea. The combined dataset also showed several well-differentiated species in the Northwestern Pacific, one of which appears to be part of the Northeastern Pacific subspecies complex. In the Gulf of Alaska, the distributions of the various subspecies of *Alaria* appear to be influenced by wave-action among other variables.

This NPRB project is only the beginning of a larger research program that is needed to understand the eco-genetics of Alaska's rich kelp resouces. Future research on Alaskan seaweeds has to be multidisciplinary, addressing questions not only in genetics but also in ecology. The apparently small spatial scale of adaptation along Alaska's shores points to the need for a much greater sampling effort to resolve the details of the genetic population structures and adaptive seascapes of these kelps. Such information is vital to the sustainable, environment-friendly development of industrial aquaculture in Alaska.

The rich diversity and abundance of marine species can support much needed economic development in Alaska through the growth of aquaculture and sea ranching. The development of an eco-friendly, but commerically viable, kelp industry requires not only an understanding the biologies, ecologies and genetics of kelps, but also the construction of fast-growing strains with value-added traits through selective breeding or other forms of genetic manipulation. Without high-performing strains, Alaska's kelp incipient kelp industry will unlikely be able to participate competitively in the global seawed market. High-quality, organic products are required to produce high-value items for human consumption, and fast-growing, large biomass strains with high polysaccharide content are needed for the production of biofuels.

13 Publications

Relevant peer-reviewed publications

Grant WS, Árnason E & Eldon B. 2016. New DNA coalescent models and old population genetics software. *ICES Journal of Marine Science*. 73(9): 2178–2180.

Grant WS, Jasper J, Bekkevold D & Adkison M. 2017. Responsible genetic approach to stock enhancements, stock restorations and sea ranching of marine fishes and invertebrates. *Reviews in Fish Biology and Fisheries* 27: 615–649.

Bowen, BW, Gaither MR, DiBattista JD, Iacchei M, Andrews KR, Grant WS, Toonen RJ, Briggs JC. 2017. Comparative phylogeography of the ocean planet. *Proceedings of the National Academy of Science* 113(29): 7962–7969. Also: Pp. 5–21 *In* Avise JC, Ayala FJ, Eds. *In the Light of Evolution, Volume X: Comparative Phylogeography*. Washington, DC: The National Academies Press. doi: 10.17226/23542.

Other peer-reviewed publications

Jasper J, Short M, Shelden C & Grant WS. 2018. Hierarchical Bayesian estimation of unobserved salmon passage through weirs. *Canadian Journal of Fisheries and Aquatic Sciences* 75: 1151–1159.

Grant WS, Baldwin A & Radenbaugh T. 2018. Invertebrates of Bristol Bay, Alaska. Chapter 8. In: Biological resources of Bristol Bay, Edited by C.-A. Woody. Ross Publishing, FL

Montes I, Zarraonaindia I, Iriondo M, Grant WS, Manzano C, Cotano U, Conklin D, Irigoien X & Estonba A. 2016. Transcriptome analysis deciphers evolutionary mechanisms underlying genetic differentiation between coastal and offshore anchovy populations in the Bay of Biscay. *Marine Biology* 163(10): e205.

Rugger P, Spendani A, Occhipinti G, Fioravanti T, Santojanni A, Leonori I, DeFelice A, Arneri, E, Procaccini G, Catanese G., Tičina V, Bonanno A, Cerioni PN, Giovannotti M, Grant WS & Barucchi VC. 2016. Biocomplexity in populations of European anchovy in the Adriatic Sea. *PLoS One* 11(4): e0153061 (21 pp).

Grant WS. 2016. Paradigm shifts in the phylogeographic analysis of seaweeds. Pp. 23–62. *In*: Zi-Min Hu & Ceridwen Fraser (eds) Seaweed Phylogeography: Adaptation and evolution of seaweeds under climate and environment changes. Springer Verlag.

Chapter 14: **Outreach Activities**

Articles

- Cordova Times Newspaper, September 2016 “Would like seaweed with that?” S. Grant (See below)
- AFS Newsletter, August 2019 “What’s up with sugar kelp” S. Grant (See below)
- Prince William Sound Science Center Newsletter, scheduled for late 2019

Talks

- Seminar: College of Fisheries and Ocean Sciences, Juneau, January 2019, “Marine Phylogeography of the North Pacific” S. Grant
- AFS Alaska annual meeting, March 2019 “Phylogeography of kelps in the NE Pacific” S. Grant & W. Cheng
- Seminar: Institute of Marine Science, University of Alaska Fairbanks, September 2019, S. Grant
- Plenary talk(?): Alaska Marine Science Symposium, January 2020, S. Grant
- Alaska Shellfish Growers Association, Annual Conference, January 2020, S. Grant

Poster

- AMSS January 2018, “Defining genetic population management units of kelps in Alaska” E. Chenoweth *et al*. (see below)

The Cordova Times
PRINCE WILLIAM SOUND'S OLDEST NEWSPAPER. ESTABLISHED 1914. TRIBALLY OWNED.

September 2016

Would you like seaweed with that?

Seaweed harvests are a $5-billion food industry with products including dried plants, dietary supplements, thickening agents and even sweeteners. Researchers are studying the potential for seaweed farming in Alaska

Cordova breakwater-green seaweed
Ulva on mussels Mytilus. Courtesy photo

By Stewart Grant
Alaska Department of Fish & Game

Alaska's rocky shores are covered with seaweeds of all kinds, and at low tide, they can be seen lining the shore in bands. Brown rockweed grows on upper intertidal rocks, then green and red seaweeds farther down and finally kelps at the waters edge on a minus low tide.

Harvests of seaweeds are a major industry with a value of about $5 billion worldwide. Asian countries, chiefly China, Korea and Japan, lead the way in seaweed production.

Sugar kelps can be as much as three to six times more productive than sugarcane, which is a fast growing plant itself.

Seaweeds are eaten as dried plants in many parts of the world.

Seaweeds have high contents of iodine and potassium and are used in dietary supplements and as food when dried, especially in Asia. The use of seaweed has grown in recent years and now many local stores sell packets of dried green seaweed for snack time.

The largest demand for seaweeds is for compounds that are used in many common products. Compounds in kelps (alginates) and some red seaweeds (carageenen and agar) are used in a variety of products. For example, alginates are used as thickeners in ice cream, jelly, salad dressing and tooth paste. Surprisingly, these compounds are also used to make long-lasting foam on beer.

There is now interest in developing seaweed farms in Alaska, because Alaska's waters are highly productive and relatively pristine compared to shorelines in many other areas.

"While the State encourages the development of its natural resources, that development must maintain the sustainability of the natural system," said Bill Templin in the Gene Conservation Laboratory at the Alaska Department of Fish & Game.

He points out that the State of Alaska is unique among the 50 states in having a clause in its constitution stating that ‘natural resources should be developed under the sustained yield principle.’ No other state has this starting point for managing their natural resources.

“Protecting wild populations is a fundamental responsibility when developing farming in Alaska,” Templin said.

The reason is that wild populations are adapted to local environments and may harbor stores of genetic diversity that support the ability of these species to change and adapt as the environment changes.

The first step in protecting wild species is to understand how populations are structured along the coast. The ADF&G genetics laboratory specializes in using molecular markers to help detect where one population ends and another starts. This information provides the scale at which local adaptations may exist.

The North Pacific Research Board has provided funds to study of the genetic population structures of seaweeds, and volunteers across the state are helping to collect seaweeds for the study.

“The major outcome of this study will help to establish guidelines for developing a sustainable seaweed industry in Alaska,” Templin said.

One concern is to avoid the mixing of different genetic strains of seaweeds by transplanting plants from distant populations. Adaptation to local environmental conditions is important for maintaining healthy populations.

The next time you eat a bowl of ice cream or put pour dressing over your salad, think seaweed. At the next low tide take a closer look at the rocks along the Cordova breakwater and see how many different seaweeds you can identify.

Stewart ‘Stew’ Grant is a fisheries biologist at the Alaska Department of Fish and Game in Anchorage, who is associated with the genetics laboratory. He was in Cordova to collect samples of seaweeds for a project to better understand the genetic population structure of seaweeds in Alaska.

ONCORHYNCHUS
Newsletter of the Alaska Chapter, American Fisheries Society
Vol. XXXIX Summer 2019 No. 3

In this issue:

Photographs of sugar kelp, ***Saccharina latissima.*** *An entire plant representing growth in one season is about 1.5 meters long (right), and plants at low tide (left). Photos from Stewart Grant.*

What's up with Sugar Kelp?

Stewart Grant

The DNA ancestry business is booming. For roughly $100 you can send a cheek swab to a lab that will determine your genotypes for several diagnostic genes and tell you about the origins of your family. We are all curious about where we came from. It seems to be an innate drive to belong to a group. We are social animals, after all.

My wife had herself genotyped by one of these labs and, to her surprise, was told not only that she was German, which she is, but that she most closely matched people from the Black Forest in southern Germany. The signal came from the mitochondrial DNA she had inherited from her mother, whose ancestors had been farmers in the rolling hills between the Black Forest and the Rhine River.

Such DNA genotyping can also be a great tool in the management of Alaska's natural resources. Conserving genetic diversity is fundamental for devising biologically and evolutionarily sound fishery management strategies to achieve long-term sustainability. As an example of a genetic application, salmon returning to the many rivers and streams in Alaska often mix as they funnel toward the mouth of a large river. While high fish densities promote successful harvesting, fishing gear cannot identify fish belonging to endangered spawning populations. However, DNA markers can be used to discriminate fish returning to particular streams so that fishery management can better protect weak populations.

In the past few years, the kelp industry has turned its attention to Alaska, and several kelp 'farmers' have begun operations in the state. In the past decade, the number of permitted seaweed farms has grown from 1 in 2008 to 13 in 2018. The first requirement for developing sustainable mariculture is to create a database of genetic diversity among populations. Based on the interests of an increasing number of seaweed farmers, we opted to focus on sugar kelp (*Saccharina latissima*). Along Alaska's coast this kelp typically inhabits calm bays and coves at depths from the sublittoral fringe down to 30 m. Living from 2 to 4 years, this plant exhibits a single, undivided blade that can grow to 5 m (16 ft) long and 20 cm (8 in) wide.

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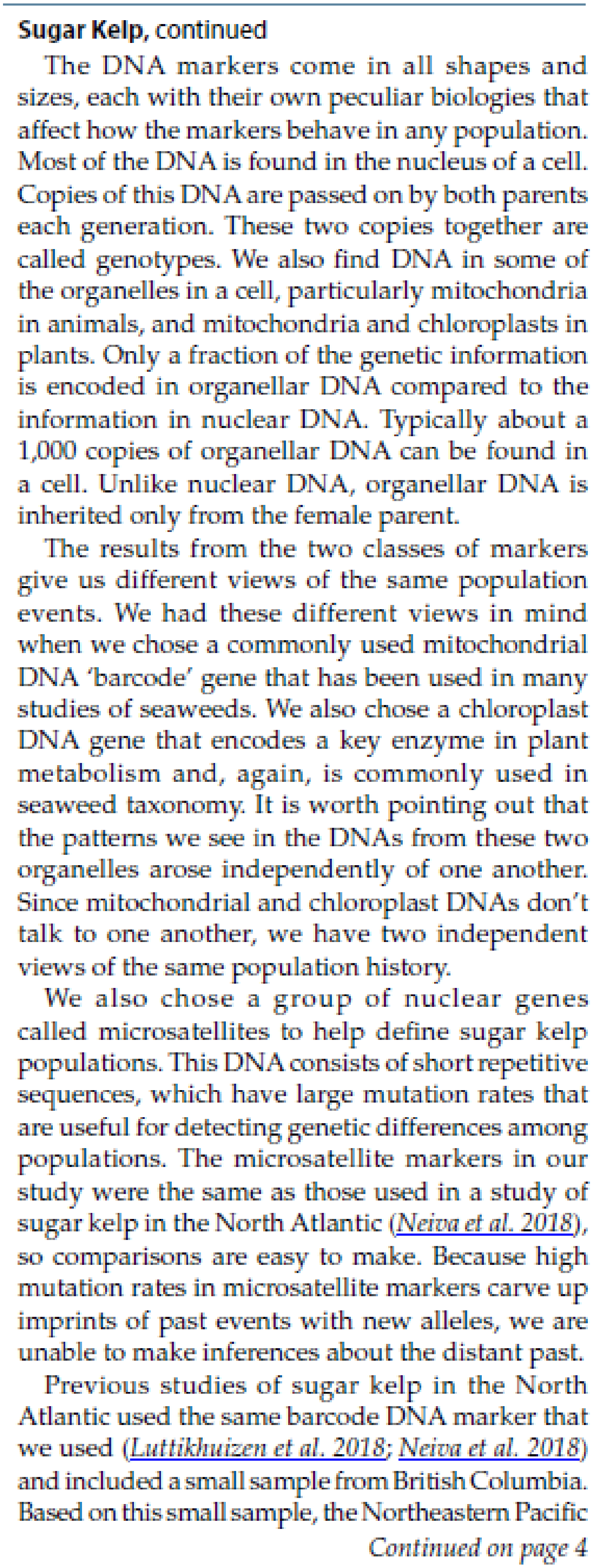

Sugar Kelp, continued

The DNA markers come in all shapes and sizes, each with their own peculiar biologies that affect how the markers behave in any population. Most of the DNA is found in the nucleus of a cell. Copies of this DNA are passed on by both parents each generation. These two copies together are called genotypes. We also find DNA in some of the organelles in a cell, particularly mitochondria in animals, and mitochondria and chloroplasts in plants. Only a fraction of the genetic information is encoded in organellar DNA compared to the information in nuclear DNA. Typically about a 1,000 copies of organellar DNA can be found in a cell. Unlike nuclear DNA, organellar DNA is inherited only from the female parent.

The results from the two classes of markers give us different views of the same population events. We had these different views in mind when we chose a commonly used mitochondrial DNA 'barcode' gene that has been used in many studies of seaweeds. We also chose a chloroplast DNA gene that encodes a key enzyme in plant metabolism and, again, is commonly used in seaweed taxonomy. It is worth pointing out that the patterns we see in the DNAs from these two organelles arose independently of one another. Since mitochondrial and chloroplast DNAs don't talk to one another, we have two independent views of the same population history.

We also chose a group of nuclear genes called microsatellites to help define sugar kelp populations. This DNA consists of short repetitive sequences, which have large mutation rates that are useful for detecting genetic differences among populations. The microsatellite markers in our study were the same as those used in a study of sugar kelp in the North Atlantic (*Neiva et al. 2018*), so comparisons are easy to make. Because high mutation rates in microsatellite markers carve up imprints of past events with new alleles, we are unable to make inferences about the distant past.

Previous studies of sugar kelp in the North Atlantic used the same barcode DNA marker that we used (*Luttikhuizen et al. 2018*; *Neiva et al. 2018*) and included a small sample from British Columbia. Based on this small sample, the Northeastern Pacific

Continued on page 4

Sugar Kelp, continued

sugar kelp populations appeared to lack genetic variability. In email correspondence with a European colleague, the first question he asked was whether I had found a high frequency of the 'A' genotype that they had found in a small sample from British Columbia. The answer was 'yes.'

A comparison of our sequences with those from British Columbia showed that nearly all the plants in the Gulf of Alaska bore the 'A' haplotype, except for two locations. One was Port Moller, where the plants carried a different genotype, and another at Auke Bay, where plants carried yet another genotype. Based only on data from this one marker, we could be reasonably certain that all the populations in the Gulf of Alaska had gone through a profound bottleneck in population size and had lost a tremendous amount of genetic diversity.

But wait, we also had results for chloroplast DNA. As expected, this gene had seen population history from a different perspective and gave us a more detailed view of population structure. We then glued the DNA sequences together for each plant and reanalyzed the longer, more informative sequences. These results showed more genetic variation among populations. Interestingly, many populations were still fixed for a single haplotype.

The story continues, because we still had yet to consider the microsatellite data. We measured the differences between populations with a standard statistic for the two classes of markers. Microsatellites showed about half of the amount of differentiation between populations overall (F_{ST} = 0.360) compared to the organellar DNA (F_{ST} = 0.777). The reason for lower microsatellite divergence lay in the ability of these highly polymorphic markers to capture recent dispersals. There were no 100% differences between populations among microsatellite alleles as there were for some organellar genes.

When plants were grouped by organellar

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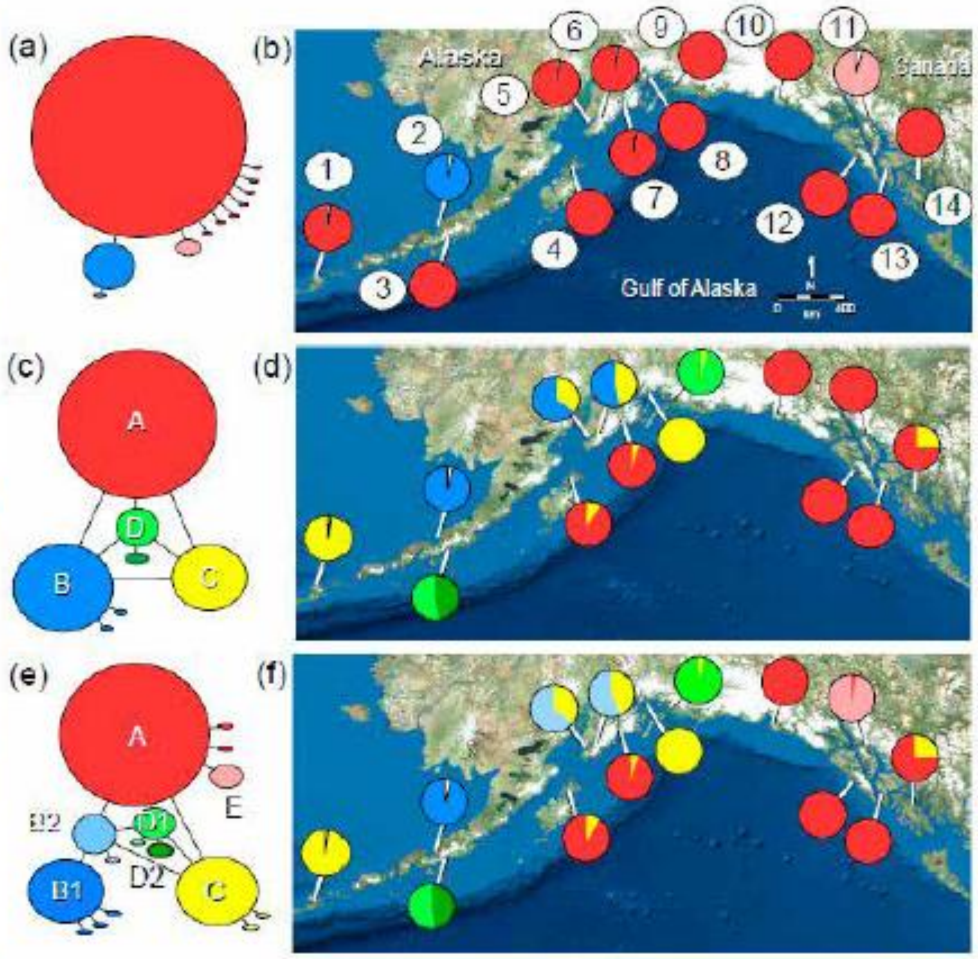

Haplotype networks and haplotype frequency distributions among samples of sugar kelp (a, b) Mitochondrial DNA cytochrome oxidase I (COI) (624 base pairs). (c, d) Chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) (735 base pairs). (e, f) Concatenated COI and rbcL sequences (1,359 base pairs). Figures from Stewart Grant.

Sugar Kelp, continued

lineages and projected onto microsatellite principal component analysis space, our samples clustered according to organellar lineage and not according to geographical location. The microsatellite and organellar DNA datasets gave different answers, because they had recorded events on different time scales. The slower mutation rates of organellar DNA do not obscure historical population events with new mutations as occurs for microsatellite loci.

How do we unravel these tangled data to make sense of them? The science philosopher Karl Popper encouraged scientists to make 'bold hypotheses.' Here, we use ideas from paleoclimatology, biogeography, ecology, and kelp reproductive biology to arrive at an explanation.

During the most recent ice-age cycle, which lasted a little over 100,000 years, lobes of terrestrial glaciers covered coastal areas in several places and drove many local sugar kelp populations to extinction. However, some populations survived in isolated northern refugia and began to diverge genetically from one another. We see the results of this divergence in contemporary populations as separate organellar lineages.

Microsatellite variability is superimposed on these lineages because of higher mutation rates among loci. The challenge is to account for the microsatellite similarity between widely separated populations in the same organellar lineage. This pattern can only be explained by recent dispersals of the various ancient lineages so that not enough time has elapsed for microsatellite allelic frequencies to diverge from one another.

The chaotic distributions of organellar lineages around the Gulf of Alaska imply post-glacial dispersals over 100s of kilometers in some cases. Long-distance dispersals are unusual events because most spores settle within a few meters of a parent plant.

The occurrence of a single genotype in many populations is a reflection of sugar kelp reproductive biology. Ecologists have tediously determined that individual plants can release as many as 10^8 or more spores in a season, but only one spore per square meter will geminate into a microscopic plant and survive to produce a large kelp plant. The high mortality of developing plants and sweepstakes recruitment leads to reproductive skew, in which only a few plants contribute offspring to the next generation. We see the result of this as low within-population genetic variability, but considerable among-population differences.

Sample identifier, site location, and sample size for this study of the genetics of sugar kelp.
Table from Stewart Grant.

Sample	Location	*N*
1	Nateen Bay, Unalaska	30
2	Port Moller, Alaska Peninsula	90
3	Kuiuk Bay, Alaska Peninsula	31
4	Malina Bay, Kodiak Island	32
5	Homer Spit, Kachemak Bay	31
6	Humpy Creek, Kachemak Bay	31
7	Lowell Point, Resurrection Bay	27
8	Whittier, Prince William Sound	6
9	Cordova, Prince William Sound	23
10	Boat Harbor, Yakutat	31
11	Homer Spit, Kachemak Bay	31
12	Harris Island, Sitka	26
13	Tokeen Bay, Scott Island	32
14	Kaguk Bay, Prince of Wales Island	42

Our genetic survey of sugar kelp populations will have to be augmented with samples from other localities to provide a more detailed description of population structure. This genetic baseline will aid in the transport and development of broodstock for the culture of this species. Coastal ecosystems and fishery populations have been over-exploited in most regions around the globe, and Alaskan fishery managers are in a unique position to conserve Alaska's bountiful marine resources.

Thanks to the many people who helped collect samples of kelp across Alaska. Several individuals at the Alaska Department of Fish and Game Gene Conservation Laboratory were instrumental in helping see this project through to completion. Wei Cheng oversaw the laboratory analyses of the kelp samples, Zac Grauvogel produced an excellent microsatellite dataset, and Eric Chenoweth, Paul Kuriscak, Marial Terry, Zach Pechacek,

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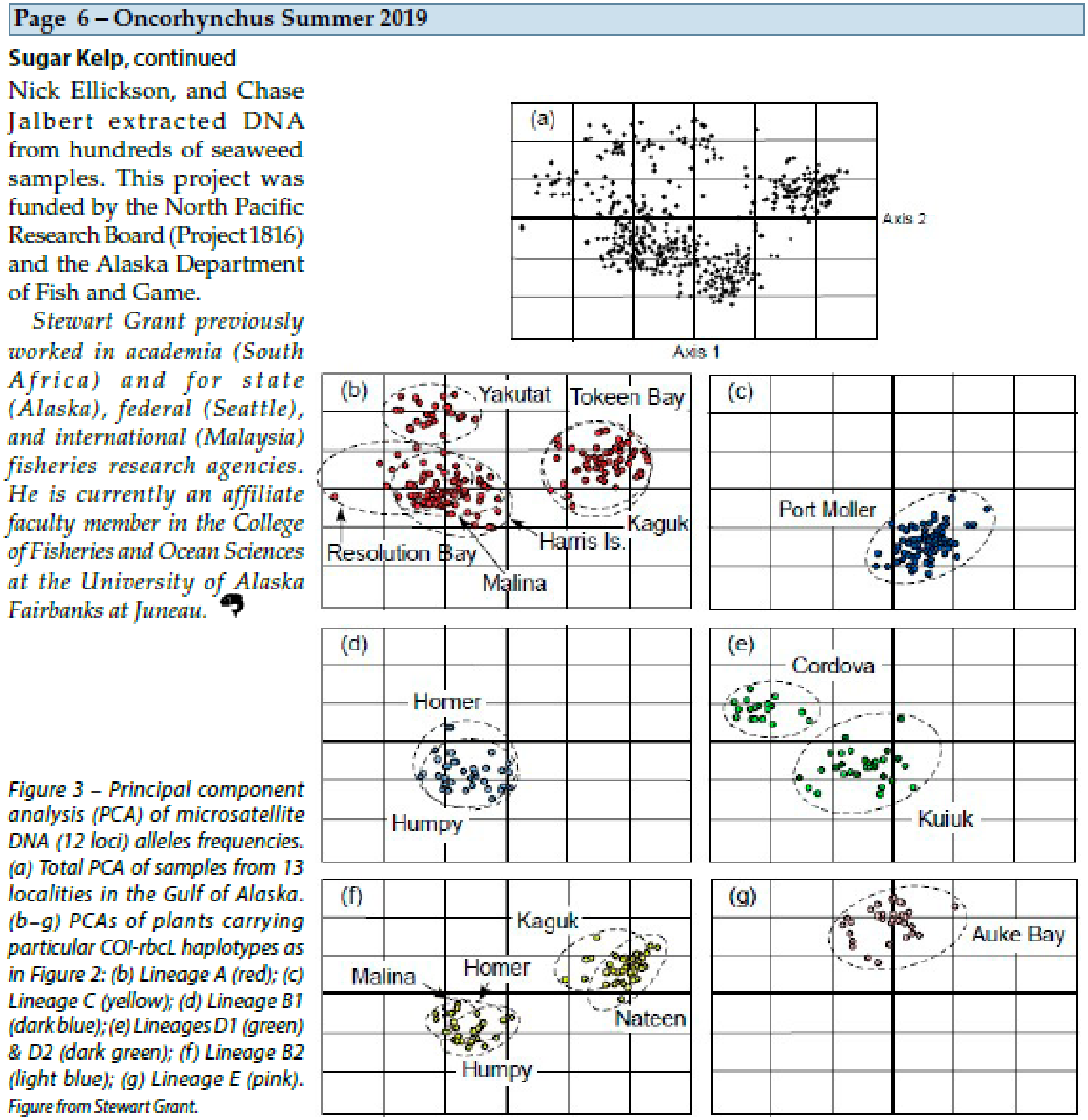


Page 6 – Oncorhynchus Summer 2019

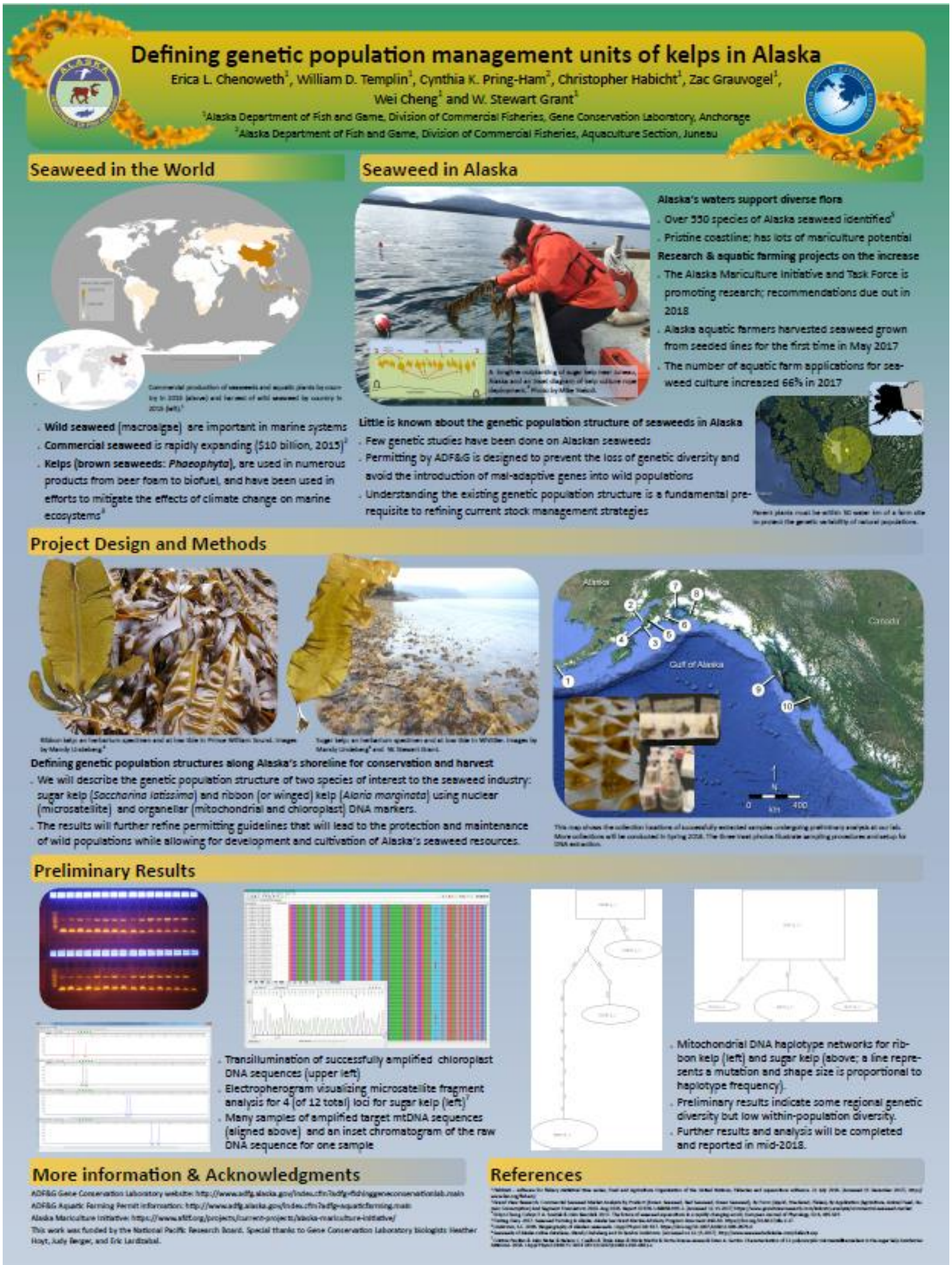
Sugar Kelp, continued

Nick Ellickson, and Chase Jalbert extracted DNA from hundreds of seaweed samples. This project was funded by the North Pacific Research Board (Project 1816) and the Alaska Department of Fish and Game.

Stewart Grant previously worked in academia (South Africa) and for state (Alaska), federal (Seattle), and international (Malaysia) fisheries research agencies. He is currently an affiliate faculty member in the College of Fisheries and Ocean Sciences at the University of Alaska Fairbanks at Juneau.

Figure 3 – Principal component analysis (PCA) of microsatellite DNA (12 loci) alleles frequencies. (a) Total PCA of samples from 13 localities in the Gulf of Alaska. (b–g) PCAs of plants carrying particular COI-rbcL haplotypes as in Figure 2: (b) Lineage A (red); (c) Lineage C (yellow); (d) Lineage B1 (dark blue); (e) Lineages D1 (green) & D2 (dark green); (f) Lineage B2 (light blue); (g) Lineage E (pink). Figure from Stewart Grant.

Poster presented at AMSS 2018.



Defining genetic population management units of kelps in Alaska

Erica L. Chenoweth[1], William D. Templin[1], Cynthia K. Pring-Ham[2], Christopher Habicht[1], Zac Grauvogel[1],
Wei Cheng[1] and W. Stewart Grant[1]
[1]Alaska Department of Fish and Game, Division of Commercial Fisheries, Gene Conservation Laboratory, Anchorage
[2]Alaska Department of Fish and Game, Division of Commercial Fisheries, Aquaculture Section, Juneau

Seaweed in the World

Commercial production of seaweeds and aquatic plants by country in 2015 (above) and harvest of wild seaweed by country in 2015 (left)[1]

- **Wild seaweed** (macroalgae) are important in marine systems
- **Commercial seaweed** is rapidly expanding ($10 billion, 2015)[2]
- **Kelps (brown seaweeds: *Phaeophyta*)**, are used in numerous products from beer foam to biofuel, and have been used in efforts to mitigate the effects of climate change on marine ecosystems[3]

Seaweed in Alaska

A longline outplanting of sugar kelp near Juneau, Alaska and an inset diagram of kelp culture rope deployment.[4] Photo by Mike Stekoll.

Alaska's waters support diverse flora

- Over 550 species of Alaska seaweed identified[5]
- Pristine coastline; has lots of mariculture potential

Research & aquatic farming projects on the increase

- The Alaska Mariculture Initiative and Task Force is promoting research; recommendations due out in 2018
- Alaska aquatic farmers harvested seaweed grown from seeded lines for the first time in May 2017
- The number of aquatic farm applications for seaweed culture increased 66% in 2017

Little is known about the genetic population structure of seaweeds in Alaska

- Few genetic studies have been done on Alaskan seaweeds
- Permitting by ADF&G is designed to prevent the loss of genetic diversity and avoid the introduction of mal-adaptive genes into wild populations
- Understanding the existing genetic population structure is a fundamental prerequisite to refining current stock management strategies

Parent plants must be within 50 water km of a farm site to protect the genetic variability of natural populations.

Project Design and Methods

Ribbon kelp: an herbarium specimen and at low tide in Prince William Sound. Images by Mandy Lindeberg[4]

Sugar kelp: an herbarium specimen and at low tide in Whittier. Images by Mandy Lindeberg[4] and W. Stewart Grant.

Defining genetic population structures along Alaska's shoreline for conservation and harvest

- We will describe the genetic population structure of two species of interest to the seaweed industry: sugar kelp (*Saccharina latissima*) and ribbon (or winged) kelp (*Alaria marginata*) using nuclear (microsatellite) and organellar (mitochondrial and chloroplast) DNA markers.
- The results will further refine permitting guidelines that will lead to the protection and maintenance of wild populations while allowing for development and cultivation of Alaska's seaweed resources.

This map shows the collection locations of successfully extracted samples undergoing preliminary analysis at our lab. More collections will be conducted in Spring 2018. The three inset photos illustrate sampling procedures and setup for DNA extraction.

Preliminary Results

- Transillumination of successfully amplified chloroplast DNA sequences (upper left)
- Electropherogram visualizing microsatellite fragment analysis for 4 (of 12 total) loci for sugar kelp (left)[7]
- Many samples of amplified target mtDNA sequences (aligned above) and an inset chromatogram of the raw DNA sequence for one sample

- Mitochondrial DNA haplotype networks for ribbon kelp (left) and sugar kelp (above; a line represents a mutation and shape size is proportional to haplotype frequency).
- Preliminary results indicate some regional genetic diversity but low within-population diversity.
- Further results and analysis will be completed and reported in mid-2018.

More information & Acknowledgments

ADF&G Gene Conservation Laboratory website: http://www.adfg.alaska.gov/index.cfm?adfg=fishinggeneconservationlab.main
ADF&G Aquatic Farming Permit information: http://www.adfg.alaska.gov/index.cfm?adfg=aquaticfarming.main
Alaska Mariculture Initiative: https://www.afdf.org/projects/current-projects/alaska-mariculture-initiative/

This work was funded by the National Pacific Research Board. Special thanks to Gene Conservation Laboratory biologists Heather Hoyt, Judy Berger, and Eric Lardizabal.

References

Chapter 15 **Acknowledgments**

Several individuals helped with the collection of samples throughout the State of Alaska, including Donna Aderhold, Robert Baer, Asia Breder, Jess Colthorp, Ben Daly, Lisa Fox, Heather Liller, Mandy Lindeberg, Mark Patterson, Sunny Rice, Philip Tscherisich, Scott Walker, Miranda Westphal, Carrier Worton, and Eric Wyatt. A warm thanks for these efforts, without which this study would have not been possible. Judy Berger catalogued the samples as they arrived and entered the specifics of the collections into a database. Eric Lardizabal helped store the data in the lab’s database and update the Latin names of species, some of which changed twice during the course of the study. Erica Chenoweth, Paul Kuriscak, Mariel Terry, Zach Pechacek, Nick Ellickson and Chase Jalbert slaved over a lab bench extracting DNA from the samples to amplify the genes of interest for this study with the polymerase chain reaction. Zac Grauvogel struggled with slimy seaweed extracts to produce the microsatellite DNA genotypes used in the study. Wei Cheng made a large effort to oversee the laboratory analyses and implement quality-control re-analyses of a portion of the samples. Barbara Dodson, Sara Gilk-Baumer, Chris Habicht, Heather Hoyt, and Bill Templin provided administative assistance.

Several algologist generously shared advice to help the project along and included Trevor Bringloe (University of New Brunswick, Canada), Ken Dunton (University of Texas, Austin), Zu-Min Hu (Ocean University, Qindao, China), Brenda Konar (University of Alaska Fairbanks), Scott Lindell (Woods Hole Oceanographic Insitute, Buzzard Bay), Mandy Lindeberg (NOAA, Juneau), Sandra Lindstrom and Sam Starko (University of British Columbia, Canada), João Neiva (University of the Algarve, Portugal), Ester Serrão (University of the Algarve, Portugal), and Mike Stekoll (University of Alaska Southeast). A special thanks to Pat Tester (NOAA, Beaufort, North Carolina) whose support was critical to the funding of this project.

This project was supported by the North Pacific Research Board Project 1618 and in part by the general funds of the Alaska Department of Fish & Game, Division of Commercial Fisheries.