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# A Seascape Genetics Approach to Studying Genetic Differentiation in the Bull Kelp Nereocystis Luetkeana

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# A SEASCAPE GENETICS APPROACH TO STUDYING GENETIC DIFFERENTIATION IN THE BULL KELP *NEREOCYSTIS LUETKEANA*

by

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ABSTRACT

A SEASCAPE GENETICS APPROACH TO STUDYING GENETIC DIFFERENTIATION IN THE BULL KELP *NEREOCYSTIS LUETKEANA*

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The brown alga *Nereocystis luetkeana* is a foundation species found from Alaska to California. In the Salish Sea, *N. luetkeana* is declining, but little is known about its population structure. We explored *N. luetkeana* 1) allelic dissimilarity and richness using seven microsatellite markers, and 2) tested models of gene flow in the Salish Sea using a hydrodynamic transport model. Our results suggest that the *N. luetkeana* distribution is comprised of four genetic co-ancestry groups that are geographically coherent, apart the from separation of the Strait of Georgia/Puget Sound by the Strait of Juan de Fuca. Our model supported that environmental variables and oceanographic currents affect gene flow and population connectivity in the Salish Sea. Removal of geography and similarity of allelic identity and richness revealed that northern and southern sites were members of one cluster, supporting northern and southern refugia served as ancestral sources of modern-day genetic diversity.

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## I. Introduction

Ecosystems and their associated organisms are at risk of degradation and species loss due to the effects of climate change. Habitat destruction may lead to declines in the size of populations, reductions in species range (Smale and Wernberg 2013), loss of genetic diversity (Pauls et al. 2013), and even localized extinction (Rogers-Bennett 2007). Maintenance of high genetic diversity can confer resilience to these changing habitats and provide variation necessary for an evolutionary response to changing conditions (Reusch et al. 2005, Sgrò et al. 2011, Bernhardt and Leslie 2013). Sufficient gene flow between populations can potentially maintain or even increase genetic diversity within populations, and therefore is an important factor in population resilience and persistence (Palumbi, 2003). Population connectivity, or lack thereof, can be measured as genetic differentiation - a measure of how gene flow across long temporal scales shapes genetic structure (Pritchard et al. 2000, Waples and Gaggiotti 2006). This genetic structure can be a result of historical barriers to gene flow, range shifts or expansions from glacial refugia (Maggs et al. 2008, Assis et al. 2014).

The most apparent feature controlling gene flow is geographic distance between populations; dispersal is typically limited by distance, and thus, individuals that are geographically closer are often more genetically similar than individuals further away (Wright 1943). Geographic distance and genetic differentiation share a positive linear relationship under the isolation by distance (IBD) or the stepping stone model of IBD (Wright 1943, Kimura and Weiss 1964). In complex systems, however, geography alone often does not predict patterns of genetic structure, and IBD models may have poor fit to empirical data. This can be

due to low genetic differentiation from increased dispersal and resulting high connectivity or a recent recolonization event disturbing normal dispersal patterns (White et al. 2010).

Ocean currents can extend the gene flow of a species beyond geography and are an important means of dispersal for many marine species with pelagic larval stages (Kimberly et al. 2008, Selkoe et al. 2016). Oceanographic currents often disperse individuals so well that patterns of genetic structure in marine systems are weak or non-existent (Mitarai et al. 2009, White et al. 2010). A strong ocean current can connect two distant populations or separate two adjacent populations as a barrier to gene flow (Mitarai et al. 2009). The model of isolation by oceanographic distance (IBOD) describes a pattern in which ocean currents influence gene flow more than spatial distance alone (Kimberly et al. 2008, White et al. 2010, Alberto et al. 2011, Riginos and Liggins 2013)Therefore, modeling dispersal capabilities of these ocean currents can result in a better understanding of population structure and connectivity in marine species. The use of ocean current dispersal modeling is particularly useful in species that generally have limited dispersal mechanisms due to constraining characteristics of their biology.

Although ocean currents may extend dispersal of gametes, heterogeneity between source and destination environments can affect the establishment of migrants and decrease gene flow between different environments (Sexton et al. 2014). This pattern of isolation by environment (IBE) is characterized by an increase in genetic differences with environmental distance independent of geographic distance (Wang and Bradburd 2014). IBE alone does not explain the forces that drive this pattern, but IBE can be used to describe the pattern. Under certain conditions IBE may lead to isolation by adaptation where fitness differences prevent some genotypes from persisting under a set of environmental variables (Nosil et al. 2008). In

sessile organisms (e.g., plants and benthic algae), this difference is more pronounced because of their inability to migrate to a suitable environment.

*Nereocystis luetkeana,* or bull kelp, is a brown alga in the order Laminariales with distribution limited to the Northeast Pacific Ocean. Like other brown algae, it has a haplodiplontic life cycle in which adult diploid sporophytes release haploid spores that produce unisexual, diploid gametophytes. The gametophyte is an ephemeral stage that only exists between settlement on the benthos, the development of embryos, and growth into the mature sporophyte stage. *N. luetkeana* differs from other kelps in that spore-filled tissues, called sori, abscise completely from the blade and fall to the benthos releasing haploid spores (Fig. 1) (Walker 1980). Mature sori abscission happens between June and November, with the species reproducing annually. Spore dispersal from sori begins shortly before abscission and continues up to an hour post-abscission (Amsler and Neushul 1989). Although spores are flagellated, mobility is minimal and thus negligible in terms of dispersal (Norton 1992). Sorus abscission may reduce the proportion of spores released in the water column above the seafloor level and limit their dispersal. However, other kelp species demonstrate that long-distance dispersal is likely mediated by ocean currents transporting drifting sporophytes released from the substrate during storms. This is known as 'rafting' due to pneumatocysts, or gas-filled sacs that afford positive buoyancy and facilitate floating. For example, in giant kelp, *Macrocystis pyrifera*, adult sporophytes were observed with viable sori after traveling over long distances (Reed 1987, Hernández-Carmona et al. 2006) and sporophylls were found to maintain buoyancy for up to 21 days (Macaya et al. 2005). Although recruitment via spores from rafts may be a rare event, a

mean migration rate of one migrant per generation is required for gene flow to sufficiently prevent genetic differentiation at ecologically relevant time scales (Mills and Allendorf 1996).

As canopy-forming kelp (Maxell and Miller 1996, Pfister et al. 2017), *N. luetkeana* provides three-dimensional structure for complex habitat niches (Siddon et al. 2008). Due to their extensive growth rate, the resulting forests have high productivity and play a key role in nutrient cycling in coastal marine ecosystems (Foster and Schiel 1985, Graham et al. 2008). Recent declines in bed density have caused concern for some populations, specifically in northern California (Rogers-Bennett, Catton 2019), the Puget Sound (Berry 2017) and more recently Oregon. Multigenerational loss of kelp is particularly concerning, as *N. luetkeana* is an annual and yearly successful recruitment is paramount to persistence of beds. Understanding the dispersal biology and genetic diversity for this species are crucial for its conservation, as they are important factors to consider when planning restoration efforts.

The Pacific Northwest range of *N. luetkeana* has a dynamic geographic history that makes it of interest for studies of biogeographic patterns (Jacobs et al. 2004). During the last glacial maximum (LGM) in the Pleistocene approximately 25,000 years ago, areas of North American coast remained unglaciated and served as glacial refugia for many species. As the glaciers retreated, wide-spread coastal recolonization occurred across many taxa of species (Blanchette et al. 2008, Kelly and Palumbi 2010, Lindstrom et al. 2011). Modern patterns of genetic structure and diversity are shaped by the influence of this glacial period (Marko 2004).

Our study had two main goals: 1) describe *N. luetkeana* genetic structure across its entire range (Alaska to California) using microsatellite markers, and 2) test three hypotheses

regarding gene flow within the Salish Sea (IBE, IBD, IOD) to inform restoration efforts. If rafting is a viable means of gene flow, we will see that oceanographic currents affect genetic differentiation. We hypothesize that glaciation of the Pacific Northwest during the LGM will play a role in modern biogeographic patterns of *N. luetkeana* distribution in this area. Furthermore, *N. luetkeana* will demonstrate genetic structure throughout its distribution due to the limited dispersal predicted by its life-history traits. Life-history traits affecting dispersal, importance as an ecosystem engineer, and the geological history of the range it inhabits all make *N. luetkeana* an ideal species for this study.

## II. Methods

### *i. Sample collections & DNA extraction*

We sampled fifty-nine sites across the geographic range of *N. luetkeana*, ranging from Herring Island, Alaska (59.65°N, 151.59°W) to Cambria Bay, California (35.53°N, 121.09°W), from May 2016 to August 2017. We collected a higher sampling site density inside the Salish Sea, the inner water body composed of the Strait of Georgia in British Columbia, Canada, and Puget Sound in Washington, USA (Fig. 2, Table 1). Within each site, the average number of specimens collected was 40 and ranged from 7 to 51, resulting in a total of 2,188 individuals. We sampled specimens haphazardly, but separated by at least 2 meters, by cutting 2-4cm pieces of blade tissue in a non-destructive manner. We wiped the sampled blade tissue to remove epiphytes before storing the tissue in silica gel desiccant for preservation until DNA extraction. We used a Tissue Lyser II (Qiagen, Valencia, CA) to homogenize the silica dried tissue to a fine powder

before extracting DNA using the DNeasy Nucleospin 96 Plant Kit II (Machery-Nagel, Duren, Germany) following the kit protocol.

*ii. Microsatellite loci genotyping*

We characterized microsatellite regions for *N. luetkeana* and used seven of the resulting microsatellite loci (Ner-2, Ner-4, Ner-6, Ner-9, Ner-11, Ner-13, and Ner-14). We prepared PCRs in a total reaction volume of 15 µL comprised of 10 µM primer, 10 mM dNTP's per base (Promega, Madison, WI), 25 mM $MgCl_2$, 3.0 µl 5X PCR buffer, and 0.5 U GoTaq Polymerase. Thermocycler conditions consisted of a 5 minute denaturation step at 95°C, followed by 33 cycles of 20 seconds each at 95°C, 20 seconds at annealing temperature of 57°C -61°C , 30 seconds at 72°C followed by a final elongation step of 20 minutes at 72°C using a GeneAmp 9700 thermocycler (Primer Note). We sized microsatellite PCR fragments using fragment analysis on a 96-capillary DNA sequencer ABI 3730xl at the Madison Biotechnologies Center. We scored the resulting microsatellite fragments with STRand (https://www.vgl.ucdavis.edu/informatics/strand.php) and binned them into integer allele codes with the R (R Core Team, 2016) package MsatAllele (Alberto 2009).

*iii. Population genetics summary statistics*

Genepop version 4.2 (Rousset 2008) was used to estimate linkage disequilibrium, test for Hardy Weinberg Equilibrium in all populations, and estimate genetic differentiation measured as $F_{ST}$ . An additional genetic differentiation metric was estimated, Jost's $D_{EST}$, using the R package diversity (Keenan et al. 2013). Jost's $D_{EST}$ is included because it is not affected by differences of within-population heterozygosity like $F_{ST}$ (Jost 2008). We estimated allelic

richness standardized for samples of size seven individuals (the minimum among sampled sites) using the R package STANDARICH (Alberto 2006). Observed, unbiased, expected heterozygosity's and the inbreeding coefficient ($F_{IS}$) were calculated in Genetix (v. 4.05) 95% confidence intervals were calculated using 100,000 bootstraps.

*iv. Range-wide genetic differentiation*

We used the program STRUCTURE to characterize large-scale patterns of genetic differentiation across the entire distribution of *N. luetkeana* (Pritchard et al. 2000). We used the admixture model for all runs with allele frequencies correlated among populations and an initial burn in period of 250,000 with 250,000 MCMC (Monte Carlo Markov Chain) repetitions. We ran a total of 10 runs per *k* (number of genetic co-ancestry groups) and let *k* range from 1 to 8. We then used the Puechmaille method in STRUCTURESELECTOR to determine *k* (Puechmaille 2016, Li and Liu 2018). This method accounts for uneven sample size between populations. STRUCTURE outputs were submitted to CLUMPAK to align runs for each *k* value (Kopelman et al. 2015).

Additionally, to test for an isolation by environment (Wang and Bradburd 2014) pattern that could explain the genetic clusters estimated by STRUCTURE analysis, we ran a canonical correspondence analysis (CCA), in R package CCA (González et al. 2008). The response variables were the assignment probabilities (Q-values) for each site to belong to each of four genetic co-ancestry groups detected by STRUCTURE (see results). The environmental predictor variables were site-specific values obtained from NASA's MODIS AQUIS satellite for photosynthetically active radiation (PAR), particulate organic carbon (POC), summer sea surface temperature

(SST), spring SST, fall SST, winter SST, light attenuation (measured as kd490) and chlorophyll-a (Chl *a*). We obtained monthly environmental data, from 2002 to 2016, collected by NASA's MODIS satellite and accessed through ERDDAP (Simons, 2019). We used long-term average and seasonal variation (for SST) conditions, putatively acting on the physiology of bull kelp. We included in the predictor set the latitude and longitude of each site to control for spatial autocorrelation. We used a stepwise model-building approach in the R package vegan (Oksanen et al. 2015) to select the best model for the data and used variance partitioning to quantify the variation explained by the tested variables (Borcard et al. 1992, Økland and Eilertsen 1994). Principal components analysis was also performed in order to visualize the ordination of each sampling site with respect to the environmental variables (Appendix A).

*v. Seascape Genetics in the Salish Sea*

Seascape genetics analysis detailed below was only performed for the Salish Sea and adjacent North East Pacific coast sites where we had a higher sampling density and an available hydrodynamic transport model (Yang and Khangaonkar 2010).

*a. Defining an environmental distance between sites*

Environmental data were obtained from NASA's MODIS satellite and accessed through ERDDAP (Simons, 2019). We characterized pairwise environmental distances using the absolute difference between sites for each of eight variables: photosynthetically active radiation (PAR), particulate organic carbon (POC), particulate inorganic carbon (PIC), summer sea surface temperature (SST), spring SST, fall SST, winter SST, light attenuation (kd490) and chlorophyll-a (Chl *a*).

*b. Characterizing an oceanographic distance*

We used the Salish Sea Model (Yang and Khangaonkar 2010) to simulate particle transport times in the General NOAA Operational Modeling Environment, GNOME (Zelenke et al. 2012) with the intent of estimating oceanographic distance. Often the average distance between sampled sites exceeds the species dispersal in a single generation. This constitutes a challenge when using hydrodynamic models to estimate single generation dispersal between sampling sites; most pairwise site connectivity would be zero and thus not informative on the genetic differentiation between such sites. One way to tackle this limitation is to consider multiple-generation stepping stone gene flow to appropriately estimate connectivity, such that two sites may be connected through intermediate site(s) (Yang et al. 2016). This approach requires simulating transport from additional kelps beds than just those sampled, hereafter referred to as stepping stone connectivity sites (SSC-sites). We selected SSC-sites using historical kelp bed maps provided by the British Columbia Coastal Resource Information Management System (https://forms.gov.bc.ca/databc-data-request/). Using QGIS (QGIS Development Team, 2017), we overlaid a 25 km grid over the map of historical kelp bed cover and sampling sites. We selected SSC-sites from the center of grid cells that contained kelp, but where no sample was collected in our study. A total of 42 SSC-sites and 33 sample sites were therefore considered. Using the GNOME modelling platform, simulations one thousand particles were released from each of these sites and tracked for 14 days, assuming the same spore longevity as *Macrocystis pyrifera* (Macaya et al. 2005). Spore releases were done separately for 13 time periods (each 14 days long) from June to November to cover the season when sori are observed in *N. luetkeana* (Maxell and Miller 1996). We estimated the probability

of transport between pairs of sites as the proportion of particles released from the source site *i* that made it to the destination site *j*.

From these transport probabilities, we estimated stepping stone connectivity between sampling sites, using a directed network modeled in R package igraph (Csardi and Nepusz 2006). Network nodes were composed by sample and SSC-sites, while edges (the links between nodes) had weights representing the log probability of transport between sites derived from the GNOME transport simulations. The shortest path along the network between all pairs of sample sites was found using a Dijkstra's algorithm (Dijsktra 1959) in igraph. Our oceanographic distance was the stepping stone connectivity between two sampling sites, measured by summing the log-transformed probabilities of transport along the shortest path (Hock and Mumby 2015).

*c. Modeling genetic differentiation using multiple regression*

We fitted a multiple linear regression model to estimate how pairwise genetic differentiation, as measured by $F_{ST}$ and Jost's D, could be explained by over the water Euclidean distance (isolation by distance, IBD), environmental distance (isolation by environment, IBE) and oceanographic distance (isolation by oceanographic distance, IBOD). Multiple linear regression models were run in R and Akaike information criterion (AIC) was used to select the best model by eliminating all predictors that had poor fit with genetic differentiation. We added variables to the final optimized model in a stepwise fashion based on their significance with the threshold set at $p<0.05$, so the resulting model had only significant predictors. Correlation

among predictor variables was estimated using the variance inflation factor (VIF) and all variables in the final optimized model were not significantly correlated (VIF<3).

III. Results

*i. Population genetics analysis*

At each of the loci sampled, none of the populations showed evidence of linkage disequilibrium or deviation from Hardy-Weinberg Equilibrium. Mean allelic richness (AR), standardized to 7 individuals per sample, ranged between 1.6 and 7.0 alleles·locus$^{-1}$ with the highest values observed in central California at the southern limit of the species distribution. Lower values were observed in the Strait of Georgia and Puget Sound with the lowest value of 1.6 found in Squaxin island (HBSI), the site farthest from the ocean, within the Puget Sound. We found intermediate allelic richness for the oceanic coast of British Columbia and Washington and these values increased as site latitude decreased (Fig. 3). Allelic richness values also increased north of the outer Salish Sea to sites sampled in Alaska at the northern extent of the distribution. Genetic differentiation for all populations, as measured by global $F_{ST}$, was 0.1567. The inbreeding coefficient, as measured by $F_{IS}$, had a range of -0.059 to 0.512 with the values belonging to Koitlah Point, Washington (CPKP) and the northern tip of Vancouver Island, God's Pocket (LDGP) respectively. $F_{IS}$ 95% confidence interval represented <0.1 change in value for all sites. Observed heterozygosity varied from 0.1319 to 0.7899, while expected heterozygosity varied from 0.2310 to 0.8618 (Table 1).

*ii. Range-wide genetic differentiation*

STRUCTURE analysis estimated four total clusters (*k*) of genetic co-ancestry distributed in geographically coherent groups along the entire sampling distribution (Fig. 4). An exception to the continuous distributions of these groups was a genetic break observed between the Strait of Georgia and Puget Sound. STRUCTURE assigned populations in these bodies of water to the same co-ancestry group, but they were separated by the Strait of Juan de Fuca populations with co-ancestry dominated by an adjacent northeast Pacific coastal group (Fig. 5). There were two other clusters, one made up of sites in Alaska and another with sites from Oregon and California (Fig. 6). Sites from this southernmost cluster span over 1,000 km across areas of discontinuous habitat. An admixture of genetic co-ancestry was apparent from Haida Gwaii Island to northern Vancouver Island (Fig. 5).

To test the isolation by environment model, we used CCA to explore associations between environmental predictors and membership to genetic co-ancestry groups as determined by STRUCTURE analysis for *k*=4 co-ancestry groups (Fig. 7). Model selection resulted in latitude, longitude, PAR, light attenuation, and Chl *a* as significant predictors associated with cluster groups. Variance partitioning showed that 70% of the variance was explained by environmental predictors and geography. Of this explained variance, 3% is explained by geography, 19% from environmental variables and 75% jointly explained by both. When geography was controlled for, POC, PAR, light attenuation, and Chl *a* remained as significant predictors of population assignment to the different clusters. Sites that belong to the Alaska and Oregon/California co-ancestry groups were more closely associated with one another while the inner Salish Sea and outer coastal Washington/British Columbia remained

separated. Additionally, PCA revealed light attenuation, particulate organic carbon, and particulate inorganic carbon were associate with differences in the Salish Sea (Appendix A).

*iii. Seascape Genetics in the Salish Sea*

Multiple regression analysis identified ocean currents and environmental variables as predictors of genetic differentiation within the Salish Sea. Temporal variability in stepping stone connectivity, as measured with single regression models, revealed the second half of July as the best predictor of genetic differentiation Jost's $D_{EST}$ ($R^2$= 0.35) and was the only period considered in the final multiple regression model. All time periods considered had a higher association, as measured by adjusted $R^2$, with Jost's D than $F_{ST}$, and Jost's D is the response variable considered in the remainder of the study.

The final optimized multiple regression model included oceanic distance from July 15$^{th}$ to the 29$^{th}$, as measured from the hydrodynamic transport model network, light attenuation, Chl-*a*, and summer SST as environmental predictors of genetic differentiation (Fig. 8). Each one of these variables had an individual p-value >0.001, and the overall model had an adjusted $R^2$ of 0.38 p= $2.2x10^{-16}$ (Table 2). Of the variables included in this optimized model, oceanic distance had the highest percentage of independent effects meaning most of the variation explained by the model was associated with our measure of oceanic distance (Fig. 8).

IV. Discussion

*i. Range-wide genetic structure and diversity*

Our analysis of biogeography in *Nereocystis luetkeana* revealed four distinct genetic co-ancestry groups with coherent geographic distribution across the species range with the

exception of disjunct population structure within the Salish Sea (Fig. 5). Maximum observed genetic diversity was at the southern range of the distribution in central California. High diversity was also evident at the northern range in Alaska and Haida Gwaii (Fig. 3). These patterns of genetic structure and diversity support the hypothesis that the last glacial maximum influenced historic distribution of *N. luetkeana*. During the LGM (~25,000 years ago), much of the coastal Pacific Northwest was glaciated (Mann and Hamilton 1995, Jacobs et al. 2004) aside from small areas of glacial refugia where species were able to persist (Blanchette et al. 2008). These refugia maintained genetic diversity and when the glaciers retreated (~12,000 years ago) recolonization of suitable habitat from these refugia occurred. Interestingly, when *k* was constrained to three clusters in the structure analyses, sites in Alaska, Oregon and California all belonged to a single genetic co-ancestry group and were therefore more genetically similar to one another than they were to members of other clusters. This may be evidence that these sites in Alaska and California belong to glacial refugia and served as an ancestral gene pool while sites in the outer and inner Salish Sea came from a more recent colonization event post-glaciation and thus only represent a subset of the historical diversity (a founder's effect).

Allelic richness values provide further support for the influence of the LGM on present population structure and diversity. The populations at the northern limit of *N. luetkeana* shows high allelic richness, which supports northern sites serving as glacial refugium; this pattern has been widely recorded in other species (Jacobs et al. 2004, Carrara et al. 2007, Lindstrom 2009, Shafer et al. 2010). The admixture of other co-ancestry groups observed in the Haida Gwaii Island populations support the idea that the island remained unglaciated during the LGM, allowing it to serve as a refugium for many species (Byun et al. 1997, Shafer et al. 2010). This

hypothesis is supported by the likelihood of ice-free conditions (Mann and Hamilton 1995) and animal and plant morphological characteristics distinct from those of mainland counterparts (Clarke et al. 2001). Vancouver Island is also a site of admixture, suggesting that the continental shelf between the two islands remained ice-free and terrestrial (Barrie et al. 2003) up until the late Pleistocene when glaciers retreated, and sea levels rise filled the area between Haida Gwaii and Vancouver Island. The documented ice-free conditions (Mann and Hamilton 1995) and similar genetic admixture found in our study between these two islands suggested historic connectivity and a potential recolonization pathway originating in Haida Gwaii and extending into the Salish Sea. A stepwise decrease in allelic richness from Haida Gwaii into the Salish Sea and further into the Puget Sound also supports this pathway of recolonization from refugia.

The highest allelic richness values observed in central California just north of Point Conception, at the southern limit of the species distribution are likely due to a second glacial refugium that allowed this population to persist during cool periods in geologic history (Fig. 6). This pattern of maximum genetic diversity at the southern ranges of species distribution is well documented in the Northern Hemisphere in other seaweed species, such as *Fucus serrat*us (Hoarau et al. 2007), *Macrocysts pyrifera* (Johansson et al. 2015), and across taxa that maintained southern refugia during the LGM (Maggs et al. 2008), as southern waters were likely the only areas with habitable temperatures within the species distribution. The shared co-ancestry group for Alaska, Oregon and California sites when cluster groups were constrained to three, along with comparatively high genetic diversity across the distribution also support the persistence of both northern and southern refugia.

The giant kelp *Macrocystis pyrifera* exhibits a similar pattern of genetic structure – a species with similar life history characteristics (e.g. haplodiplontic with limited spore dispersal) and northern hemisphere distribution. *M. pyrifera* is distributed along the coast of the Pacific Northwest but extends further south to Baja California. Genetic structure analysis of *M. pyrifera* showed sites sampled in central and northern California belong to one genetic co-ancestry group and have the maximum genetic diversity observed (Johansson et al. 2015). This extensive genetic co-ancestry group spans over 1000 km of discontinuous habitat, suggesting dispersal distances much farther than the few kilometers spores alone can travel (Reed et al. 1988). This extended dispersal capability may be due to rafting of adult sporophytes – a mechanism that could also occur in *N. luetkeana*. Population genetic structure differs between the two kelps in the northern sites as *M. pyrifera* has a single northern co-ancestry group made up of sites from Alaska and Vancouver Island with the lowest observed genetic diversity. These sites belong to distinct co-ancestry groups in *N. luetkeana* with relatively high genetic diversity in comparison to sites within the Salish Sea. The presence of a single genetic co-ancestry group and low genetic diversity at the northern limit of *M. pyrifera* may be due to stepwise recolonization from a single southern glacial refugium. Additionally, *N. luetkeana* may be a more cold-adapted species than *M. pyrifera* (Provan and Bennett 2008) and therefore more ecologically successful at northern latitudes. Further, Dieck (1993) demonstrated that kelp gametophytes vary in their tolerance to lower temperature limits and this may constrain species northern ranges. The difference in present relative abundance of both *N. luetkeana* and *M. pyrifera* at higher latitudes suggests different relative success in colder regions, which increases the chances of

northern refugia serving as a significant source of recolonization for *N. luetkeana*, in comparison to *M. pyrifera.*

It is also interesting to note that the maximum genetic diversity for *N. luetkeana* persists in northern California despite the recent dramatic declines in population numbers (Rogers-Bennett and Catton 2019). Samples for this genetic study were taken in 2016, two years after the documented declines. This suggests that despite near-complete or complete canopy loss, genetic diversity is still comparable to maximal values recorded elsewhere in CA. This diversity could potentially be within life stages other than the conspicuous sporophyte; it is unknown how long gametophytes and juvenile sporophytes persist, and these cryptic life stages may be preserving genetic diversity when conditions are less favorable for adult sporophytes, such as following a strong storm event or nutrient-poor El Niño conditions. For example, Ladah and Zertuche-González (2007) showed that *M. pyrifera* juvenile sporophytes were the only life stage able to recover from simulated El Niño conditions. The resiliency of different life stages is likely to be a key factor in the persistence of kelp beds as trends shift to an increasingly warming ocean.

Small scale genetic structure analysis of the sea palm *Postelsia palmaeformis,* a brown algal with distribution similar to *N. luetkeana*, revealed strong genetic differentiation and limited gene flow between populations, even at a scale of 5 km (Kusumo et al. 2006). However, *P. palmaeformis* differs distinctly in its ecology from kelp – it is found higher up in the intertidal zone and experiences extended periods of complete exposure. It releases most spores during low tide (Kusumo et al. 2006) and thus has lower dispersal potential in comparison to *N. luetkeana* and *M. pyrifera*, which likely drives the patterns of high genetic structure.

Conversely, marine animals with Pacific Northeastern distributions and pelagic larvae often show little to no genetic structure across the coast of North America (Hedgecock 1994, Lee and Boulding 2009). These animals can have larval durations of up to four or five weeks, allowing them to survive prolonged periods in the water column, and therefore long distances, and can be dispersed via oceanographic currents. However, larval duration and dispersal mechanism are species specific, and therefore result in significantly different patterns of genetic structure (Kelly and Palumbi 2010). In general, species with longer larval duration (e.g. animals) have less spatial genetic structure, while species with limited dispersal capabilities often show strong genetic structure at small spatial scales (e.g. *P. palmaeformis*). *N. luetkeana* and *M. pyrifera* represent an intermediate dispersal potential with distinct genetic breaks that are driven by limited spore dispersal but exhibit some potential for gene flow over long distances via rafting.

In general, patterns of genetic structure for intertidal species in the Pacific Northeast are explained by environmental and oceanographic drivers. Modeling by Fenberg et al. (2015) identified biogeographic structure for rocky intertidal species including a major biogeographic break where the North Pacific Current diverges into the California Current and Alaska current just south of Haida Gwaii Island, which is consistent with data for the California sea cucumber (Xuereb et al. 2018). This diverging current does not appear to be a barrier to gene flow in *N. luetkeana,* as Haida Gwaii and Vancouver Island share similar genetic co-ancestry and are centers of admixture where there is successful gene flow from multiple different populations. Particle transport models by Robinson et al. (2005) found that sea surface currents would allow drifting individuals to be carried between the two islands. This provides an explanation for

connectivity in *N. luetkeana,* which floats on the sea surface current. Admixed genetic structure is also observed in *M. pyrifera* populations that border two regions of different co-ancestry – areas experiencing genetic admixture in California separate regions of distinct co-ancestry to the north and south (Johansson et al. 2015).

The CCA showed that the four STRUCTURE cluster groups were associated with latitude, longitude, PAR, light attenuation, and chl *a*. Light attenuation was not associated with latitude and longitude, while PAR was; when geography (latitude and longitude) was controlled for, light attenuation, PAR and POC were important variables separating the two STRUCTURE clusters from the Salish Sea and outer coastal area. Under the same model in which geography was excluded as a factor, Alaska and Oregon/California co-ancestry groups clustered closer together, despite being separated by the greatest oceanographic distance. This further supports the hypothesis of a northern and southern refugia.

*ii. Patterns of genetic differentiation within Salish Sea*

In the Salish Sea, disjunct population structure was evident where the Puget Sound and Strait of Georgia sites were significantly different from those in the outer coast and Strait of Juan de Fuca. This pattern does not follow the IBD model for explaining genetic differentiation because sites directly adjacent to one another belong to separate co-ancestry groups. This disjunctive pattern within the Salish Sea is inconsistent with similar studies on fish species that have mobile larval stages such as the Pacific hake, *Merluccius productus* (Iwamoto et al. 2015) and copper rock fish, *Sebastes caurinus* (Buonaccorsi et al. 2002). These studies support the hypothesis that currents in the Strait of Juan de Fuca serve as a barrier to gene flow between

these two inner waterways. The Strait of Juan de Fuca is a highly dynamic environment with large fluxes of water movement each day (Khangaonkar et al. 2017) that could prevent gene flow between the Strait of Georgia and Puget Sound.

One explanation for the inconsistency between fish models and *N. luetkeana* is that populations of *N. luetkeana* within the Puget Sound and Strait of Georgia originated from only a few colonizing individuals post-glaciation, resulting in a founder's effect and reduction of genetic diversity. This hypothesis is supported by allelic richness values that show a stepwise reduction from Alaska, southward, and into the Salish Sea. There is further reduction of diversity with proximity to the outer coast moving inward within the Salish Sea and the lowest value belongs to the southernmost site sampled within the Puget Sound, Squaxin Island. This reduction of genetic diversity with proximity to the outer coast is indicative of a founder's effect and seen in other species with distributions that span the outer coast of Washington and the Salish Sea, such as the pacific hake *M. productus* (Iwamoto et al. 2015). Founding populations are likely small and as drift acts on the already reduced genetic diversity, these two sites could converge to the same genetic co-ancestry group simply due to chance of fixing the same dominant alleles in the common ancestor population, or similarity of environmental constraints. Any further divergence between Strait of Georgia and Puget Sound might go undetectable if populations are highly homozygous or may require scanning a larger region of the genome (e.g., SNP analysis).

*iii. Seascape Genetics in the Salish Sea*

As suggested by the disjunct population structure, sea surface spatial distance was not a significant predictor of genetic differentiation in the optimized multiple regression model. Both the multiple regression model as well as the CCA supported the IBE hypothesis as an explanation of genetic differentiation within the Salish Sea. These analyses revealed latitude, longitude, light attenuation, chl *a*, summer SST and POC as important predictors of genetic patterns in this localized region. Both the Puget Sound and Strait of Georgia environments have strong influence from anthropogenic forces (Khangaonkar et al. 2017, Sobocinski et al. 2018), which could affect the survival of spores and thus recruitment in these locations. The major cities of Seattle and Vancouver provide a high influx of pollutants, such as sewage and other runoff, and are often in the form of suspended sediment in the water column. Sedimentation reduces penetration of light in the water column and negatively affects recruitment of *N. luetkeana* spores as well as juvenile sporophytes (Carney 2003).

Furthermore, The Puget Sound and even more so the Strait of Georgia have days with above average sea surface temperature during summer months (Simons 2019). High anomaly temperatures negatively affect kelp forest size (Assis et al. 2018, Wernberg et al. 2018, Cavanaugh et al. 2019) and shift ecosystem dynamics (Connell and Russell 2010). Additionally, temperatures above 19 °C inhibit the formation of germ tubes in *N. luetkeana* spores, effectively halting the life cycle before gametophyte development (B. Schiltroth pers comms). Therefore, days with above average SST may negatively affect the ecology and persistence of *N. luetkeana* within the Salish Sea.

Oceanographic distance provides the strongest explanation of genetic differentiation in the Salish Sea. This body of water is influenced by coastal oceanographic currents predominantly through the Strait of Juan de Fuca and less so the northern limit of the Strait of Georgia. Khangaonkar et al. (2017) showed that less than 12% of the water that flows from the Pacific into the Strait of Juan de Fuca makes it into the Puget Sound in comparison, up to 42% reaches the Strait of Georgia and the remaining 46% is returned into the Pacific Ocean. This suggests that ocean currents do affect the Salish Sea, but more directly in the Juan de Fuca and Georgia Straits than the Puget Sound. The importance of oceanographic currents as a driver of gene flow is consistent with studies on the sea snail *Kelletia kelletii* (White et al. 2010) and on *M. pyrifera* (Alberto et al. 2011). In *M. pyrifera,* oceanographic distance was also found to be an important predictor of genetic differentiation, despite differences in methodology. To estimate oceanographic distance, they used a Lagrangian particle transport model, thus incorporating data from the whole water column, effectively capturing the potential spore dispersal on an individualized scale. Conversely, our study used sea surface current data in order to better capture multigenerational long-distance dispersal via rafting sporophytes, which is more important in *N. luetkeana* given the unique characteristic of sorus abscission and its effect on spore dispersal. Our study supports the hypothesis that isolation by oceanographic distance is an important driver of gene flow and may extend connectivity for kelps.

## V. Conclusions/Restoration Applications

The distinct patterns of genetic structure and diversity in *N. luetkeana* might be shaped by the LGM, with potential recolonization from either northern or southern glacial refugia. Subsequent recolonization of outer-coastal British Columbia/Washington and the Salish Sea

from these refugia produced patterns of lower diversity with membership primarily limited to one genetic co-ancestry group. In order to determine if patterns of allelic richness and genetic structure are consistent with glacial refugia, a haplotype study should be done to rebuild the biogeographic history of the species and estimate relative times for large population expansions.

Minimal genetic diversity and membership to a single co-ancestry group provides evidence that populations within the Puget Sound are isolated. The reduction in genetic diversity within the Puget Sound and Strait of Georgia combined with environmental stressors due to proximity to metropolitan areas raises concerns for the future of kelp beds in this region. Low genetic diversity decreases resilience to increasing temperatures in other species of kelps (Wernberg et al 2018). Additionally, the complex fjord-like geography of the Puget Sound increases the likelihood that kelp beds located within the Sound are at risk for further isolation due to the inability of ocean currents to facilitate spore dispersal to and from these sites, which completes the cycle of their inability to increase genetic diversity and lowers their adaptive potential to environmental fluxes. Connectivity estimates for individual sites based on oceanographic currents should be calculated in order to identify *N. luetkeana* beds that are more isolated within the Puget Sound that should be prioritized for restoration.

Our study found support for an IBE model of genetic differentiation within the Salish Sea, which may be due to the harsh environmental conditions imposed by humans in this area. The reduced diversity found within the Puget Sound and Strait of Georgia may be a signature of strong selection for a few adaptive alleles. This would be more accurately described as isolation by adaptation where environmental conditions alone do not explain genetic differentiation (as

in IBE). Local adaptation to these stressful environments may be allowing kelp beds to persist in this region; however, experimental work in the form of reciprocal transplants is required to test the localized adaptation hypothesis. Restoration efforts should be planned with this in mind as the addition of foreign genotypes may swamp out the few adaptive alleles. This study was done on neutral genetic loci markers, and future studies should target the whole genome in order to explore areas of the genome that natural selection may be acting. This would allow broader conclusions to be drawn on how the environment affects the distribution of genotypes within the Salish Sea.

The maximum observed genetic diversity was also found to be in populations that have been in dramatic decline in northern California (Rogers-Bennett and Catton 2019). As temperatures warm, and El Niño oscillations become more frequent the importance of other life stages for preserving genetic diversity increases. Experiments are needed to better understand what conditions various life-stages can persist under.

Our study shows that distinct patterns of genetic structure are present throughout the distribution of *N. luetkeana*. These patterns can largely be explained by range contractions due to extensive glaciation and then recolonizations from northern and southern refugia. The disjunct population structure in the Salish Sea is associated with differences in the environment as well as oceanographic currents. Genetic diversity in northern California sites is still comparable to maximum observed values despite recent declines and diversity of kelps may be preserved by the cryptic gametophyte life stage. Restoration of declining kelp beds may be necessary to ensure the biodiversity preservation of these ecosystems. This study provides

genetic insight of *N. luetkeana* to highlight populations of particularly low genetic diversity and connectivity that can be used to guide future restoration plans.

## VI. Acknowledgements

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Table 1. Population genetics summary statistics for sampled sites of *N. luetkeana.* Summary of all sampled locations. *n*, sample size. AR, standardized allelic richness, based on 7 samples; HE unbiased expected heterozygosity; HO, observed heterozygosity; FIS, inbreeding coefficient (95% confidence interval represented <0.1 change in value)

| Population (Code) | Pop. Label | Abbrev. | Latitude | Longitude | *n* | AR | Fis | He | Ho |
|---|---|---|---|---|---|---|---|---|---|
| Herring Island, Alaska (BKHI) | 1 | BKHI | 59° 39' 9.72"N | 151° 35' 40.92"W | 51 | 4.617 | 0.129 | 0.694 | 0.611 |
| Cohen Island, Alaska (BKCI) | 2 | BKCI | 59° 32' 30.00"N | 151° 32' 30.00"W | 50 | 5.54 | 0.302 | 0.784 | 0.554 |
| Hezketh Island, Alaska (BKHK) | 3 | BKHK | 59° 30' 21.60"N | 151° 30' 15.60"W | 50 | 5.846 | 0.277 | 0.746 | 0.546 |
| Outside Beach, Alaska (BKOB) | 4 | BKOB | 59° 27' 51.00"N | 151° 42' 33.60"W | 50 | 5.743 | 0.154 | 0.718 | 0.614 |
| Port Graham, Alaska (BKPG) | 5 | BKPG | 59° 22' 14.400"N | 151° 53' 23.40"W | 51 | 5.54 | 0.159 | 0.726 | 0.617 |
| Dasani Island, Alaska (TSDI) | 6 | TSDI | 55° 45' 39.21"N | 133° 16' 45.96"W | 40 | 5.643 | 0.236 | 0.741 | 0.575 |
| Wiah Point/Cape Edenshaw, Haida Gwaii (GSWC) | 7 | GSWC | 54° 6' 25.16"N | 132° 21' 59.08"W | 33 | 6.474 | 0.186 | 0.783 | 0.649 |
| Gudal and Tana's Bay, Haida Gwaii (GSGT) | 8 | GSGT | 53° 11' 40.27"N | 132° 35' 31.88"W | 32 | 6.197 | 0.134 | 0.780 | 0.688 |
| Cumshewa Island, Haida Gwaii (GSCI) | 9 | GSCI | 53° 1' 50.41"N | 131° 36' 9.65"W | 26 | 5.837 | 0.202 | 0.760 | 0.621 |
| McMullin Islands NE | 10 | JBMI | 52° 4' 14.52"N | 128° 24' 23.40"W | 50 | 5.943 | 0.105 | 0.720 | 0.651 |
| Starfish Island (JBSI) | 11 | JBSI | 51° 40' 45.90"N | 128° 7' 33.90"W | 50 | 5.009 | 0.327 | 0.641 | 0.439 |
| West Beach, Alaska (JBWB) | 12 | JBWB | 51° 38' 57.48"N | 128° 9' 15.90"W | 51 | 5.777 | 0.239 | 0.719 | 0.554 |
| South Calvert Island Thrasher (JBTH) | 13 | JBTH | 51° 24' 41.76"N | 127° 55' 6.90"W | 50 | 5.551 | 0.285 | 0.716 | 0.519 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| God's Pocket (LDGB) | 14 | LDGP | 50° 52' 39.79"N | 127° 38' 53.18"W | 40 | 4.871 | 0.513 | 0.700 | 0.347 |
| Campbell River, British Columbia (WHQI) | 15 | WHQI | 50° 2' 15.12"N | 125° 14' 31.02"W | 40 | 3.591 | 0.119 | 0.391 | 0.350 |
| Cape Lazo Shoal (CLAZ) | 16 | CLAZ | 49° 41' 56.70"N | 124° 50' 39.09"W | 33 | 3.74 | 0.281 | 0.478 | 0.351 |
| Maude Reef, British Columbia (RZMR) | 17 | RZMR | 49° 29' 57.00"N | 124° 40' 54.60"W | 40 | 2.146 | 0.057 | 0.344 | 0.329 |
| Hornby Island, British Columbia (BHHI) | 18 | BHHI | 49° 29' 57.00" | 124° 40' 54.60"W | 40 | 2.534 | 0.162 | 0.449 | 0.382 |
| Stanley Park, British Columbia (BSSP) | 19 | BSSP | 49° 18' 10.44"N | 123° 6' 45.61"W | 42 | 3.457 | 0.150 | 0.442 | 0.381 |
| Tofino (Vancouver Island) (JETF) | 20 | JETF | 49° 6' 7.77"N | 125° 56' 53.95"W | 45 | 5.103 | 0.214 | 0.713 | 0.568 |
| Aguilar Point, British Columbia (LDAP) | 21 | LDAP | 48° 48' 54.59"N | 125° 10' 32.92"W | 20 | 4.871 | 0.142 | 0.631 | 0.557 |
| Cape Beale, British Columbia (LDCB) | 22 | LDCB | 48° 47' 30.00"N | 125° 12' 47.99"W | 20 | 4.969 | 0.027 | 0.608 | 0.607 |
| Dodd's Narrows, British Columbia (RFDN) | 23 | RFDN | 49° 8' 9.66"N | 123° 49' 6.60"W | 45 | 4.529 | 0.281 | 0.566 | 0.413 |
| Sansum Narrows (WHSN) | 24 | WHSN | 48° 46' 51.82"N | 123° 33' 25.52"W | 40 | 4.169 | 0.363 | 0.568 | 0.373 |
| Lumni Island (EHLI) | 25 | EHLI | 48° 39' 0.52"N | 122° 37' 31.60"W | 38 | 4.634 | 0.312 | 0.688 | 0.481 |
| Turn Rock, San Juan Channel (TMTR) | 26 | TMTR | 48° 32' 5.97"N | 122° 57' 51.94"W | 40 | 4.251 | 0.150 | 0.613 | 0.529 |
| Ben Uhre Island (DPBU) | 27 | DPBU | 48° 24' 18.36"N | 122° 37' 39.50"W | 40 | 4.366 | 0.379 | 0.649 | 0.410 |
| Koitlah Point, Washington (CPKP) | 28 | CPKP | 48° 23' 31.56"N | 124° 38' 40.56"W | 40 | 5.06 | -0.059 | 0.637 | 0.682 |
| Tatoosh Island, Washington (CPTI) | 29 | CPTI | 48° 23' 28.32"N | 124° 44' 15.38"W | 40 | 5.117 | 0.237 | 0.682 | 0.529 |
| Snow Creek Resort, Washington (FASR) | 30 | FASR | 48° 21' 12.32"N | 124° 32' 41.83"W | 39 | 4.537 | 0.127 | 0.600 | 0.531 |
| Callam Bay, Washington (FACB) | 31 | FACB | 48° 15' 24.17"N | 124° 16' 31.31"W | 40 | 4.56 | 0.143 | 0.611 | 0.531 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Partridge Point (Whibdey Island), (DPPP) | 32 | DPPP | 48° 13' 55.92"N | 122° 46' 10.49"W | 40 | 4.166 | 0.117 | 0.582 | 0.521 |
| Cape Alava, Washington (CPCA) | 33 | CPCA | 48° 10' 22.22"N | 124° 45' 2.95"W | 26 | 4.943 | 0.093 | 0.641 | 0.593 |
| Salt Creek, Washington (FASC) | 34 | FASC | 48° 9' 57.29"N | 123° 41' 49.51"W | 39 | 4.471 | 0.066 | 0.588 | 0.557 |
| North Beach Port Townsend, Washington (LHPT) | 35 | LHPT | 48° 8' 33.38"N | 122° 46' 57.03"W | 31 | 4.323 | 0.186 | 0.589 | 0.489 |
| Scatchet Head/Whidbey Island, Washington (JGSH) | 36 | JGSH | 47° 54' 36.04"N | 122° 24' 50.06"W | 40 | 3.557 | 0.121 | 0.473 | 0.421 |
| Richmond Beach (BARB) | 37 | BARB | 47° 46' 16.07"N | 122° 23' 39.26"W | 40 | 2.363 | 0.402 | 0.301 | 0.183 |
| Shilshole Bay (BASB) | 38 | BASB | 47° 40' 54.66"N | 122° 24' 32.87"W | 41 | 2.823 | 0.209 | 0.414 | 0.332 |
| Magnolia Bluff (BAMB) | 39 | BAMB | 47° 40' 50.70"N | 122° 23' 55.07"W | 40 | 2.669 | 0.374 | 0.392 | 0.250 |
| Destruction Island, Washington (LADI) | 40 | LADI | 47° 40' 29.53"N | 124° 28' 51.53"W | 44 | 4.434 | 0.113 | 0.629 | 0.565 |
| Vashon Island, Washington (FAVI) | 41 | FAVI | 47° 22' 49.48"N | 122° 31' 3.54"W | 7 | 2.429 | 0.279 | 0.283 | 0.225 |
| Tacoma Narrows, Washington (FATN) | 42 | FATN | 47° 15' 30.49"N | 122° 32' 58.50"W | 47 | 2.291 | 0.159 | 0.252 | 0.214 |
| Squaxin Island (Tucksel Point), Washington (HBSI) | 43 | HBSI | 47° 10' 1.75"N | 122° 53' 41.67"W | 40 | 1.631 | 0.440 | 0.231 | 0.132 |
| Otter Rock, Oregon (JWOR) | 44 | JWOR | 44° 45' 51.08"N | 124° 4' 51.72"W | 49 | 5.763 | 0.184 | 0.735 | 0.607 |
| Port Orford, Oregon (JWPO) | 45 | JWPO | 42° 44' 17.83"N | 124° 29' 58.03"W | 50 | 5.96 | 0.171 | 0.746 | 0.626 |
| Crescent City, California (RACC) | 46 | RACC | 41° 44' 53.63"N | 124° 12' 33.74"W | 50 | 6.054 | 0.132 | 0.734 | 0.644 |
| Trinidad Harbor (RATH) | 47 | RATH | 41° 3' 20.14"N | 124° 8' 48.02"W | 50 | 4.297 | 0.223 | 0.585 | 0.461 |
| Van Damm Patch1 | 48 | CCVDA | 39° 16' 21.47"N | 123° 47' 31.92"W | 50 | 5.654 | 0.406 | 0.736 | 0.444 |
| Van Damm Patch2 | 48 | CCVDB | 39° 16' 5.77"N | 123° 48' 2.16"W | 50 | 6.014 | 0.291 | 0.751 | 0.541 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Van Damm Patch3 | 48 | CCVDC | 39° 16' 9.77"N | 123° 47' 36.24"W | 50 | 6.526 | 0.147 | 0.797 | 0.688 |
| Ocean Cove, California (BSOC) | 49 | BSOC | 38° 33' 4.27"N | 123° 18' 26.52"W | 50 | 6.491 | 0.167 | 0.804 | 0.677 |
| Stillwater Cove, California (BSSW) | 50 | BSSW | 36° 33' 34.83"N | 121° 57' 7.15"W | 50 | 6.231 | 0.358 | 0.835 | 0.543 |
| Big Creek, California (BSBC) | 51 | BSBC | 36° 33' 34.83"N | 121° 36' 0.61"W | 51 | 6.951 | 0.086 | 0.855 | 0.790 |
| Cambria, California (DCCM) | 52 | DCCM | 35° 31' 56.44"N | 121° 5' 5.93"W | 50 | 7 | 0.158 | 0.862 | 0.735 |

Table 2. Results from multiple regression of the final optimized model including oceanographic distance, light attenuation, Chl *a*, and summer SST as explanatory variables and genetic differentiation (Jost's D) as the response variable. Adjusted $R^2$ and Akaike information criterion (AIC) values reported.

| Variable in Multiple Regression | Slope | P-value | Adj $R^2$ | AIC |
|---|---|---|---|---|
| Oceanographic Distance July 15 | 0.011 | $2x10^{-16}$ | | |
| Light Attenuation | 0.046 | 0.0002 | | |
| Chl *a* | 0.0004 | 0.028 | | |
| Summer SST | 0.007 | 0.005175 | | |
| Optimized Model | | | | |
| Oceanographic Distance July 15 + Light Attenuation + Chl *a* + Summer SST | | $<2.2x10^{-16}$ | 0.389 | -1063 |

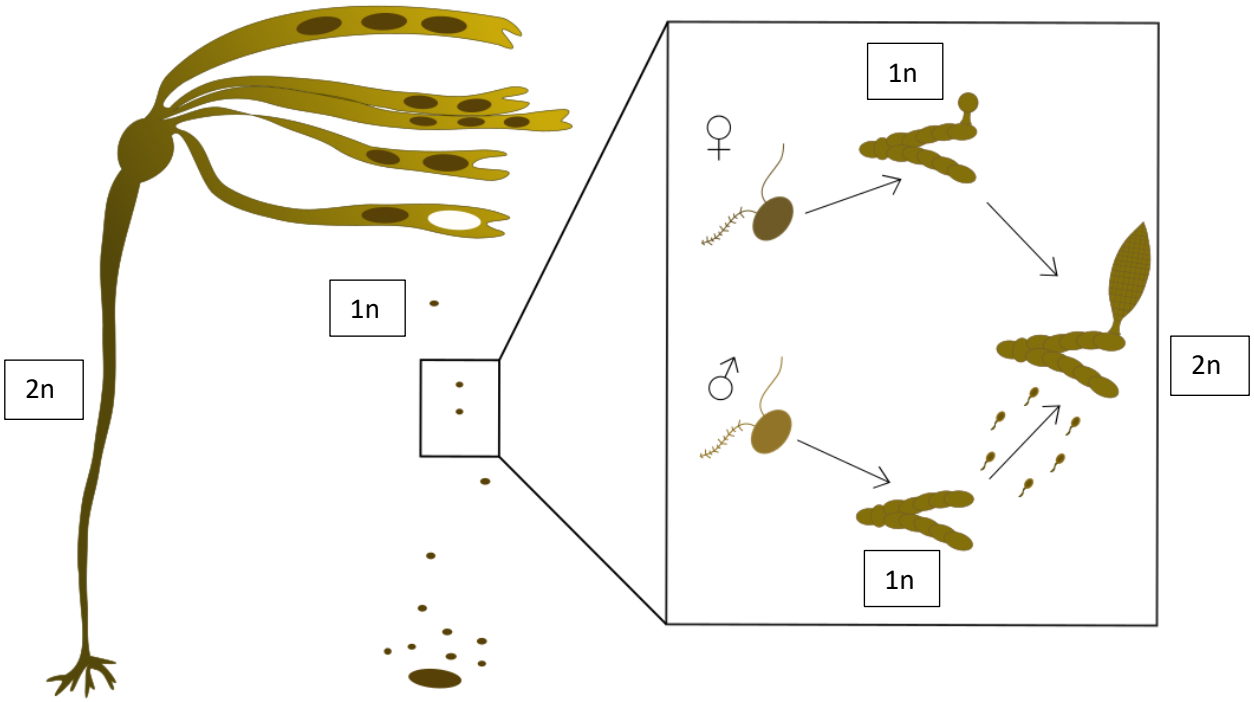


Figure 1. Haplodiplontic life cycle of *N. luetkeana* with sori abscission depicted as the darker colored sori tissue separating from the frond. Spores are shown releasing from the sori as it falls to the benthos.

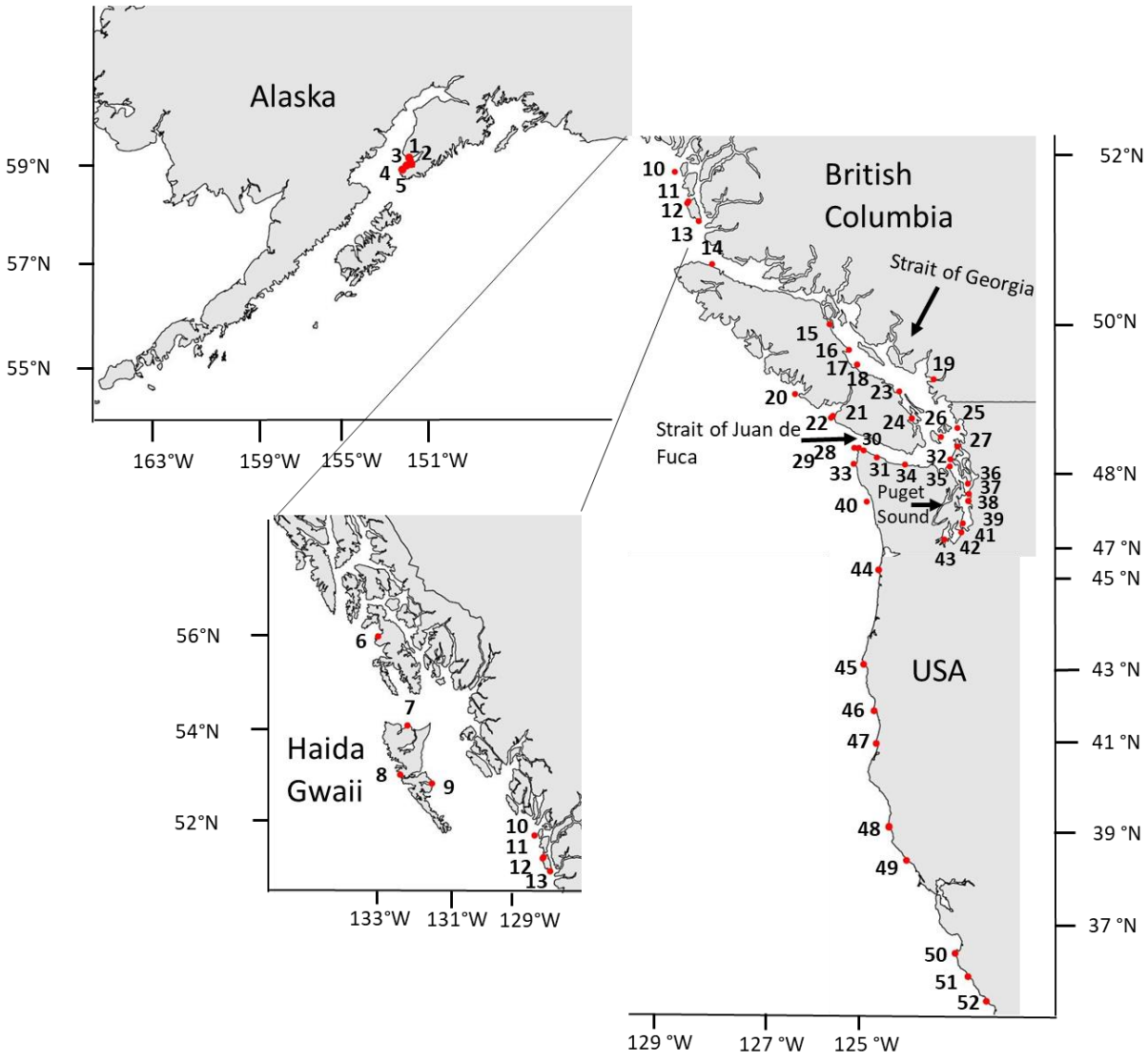


Figure 2. Sampling site locations of *Nereocystis luetkeana* across the Pacific coast of North America. Site numbers correspond to information in Table 1.

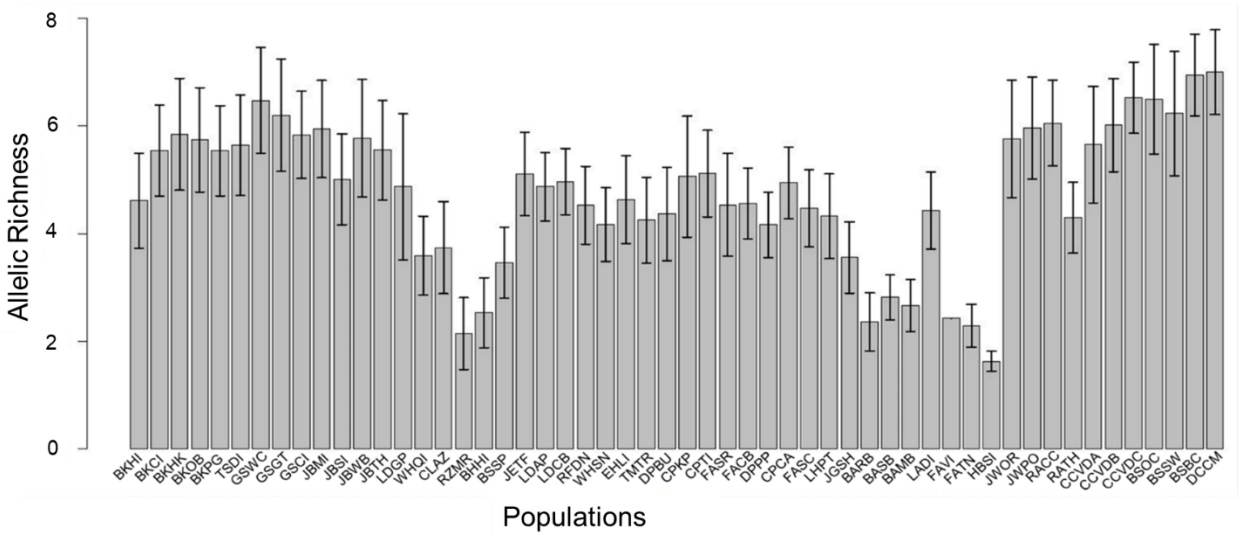


Figure 3. Allelic richness, across seven microsatellite markers, standardized to *n*=7 individuals per sample where error bars represent this standardization. *Nereocystis luetkeana* sampling sites are ordered left to right corresponds to approximate sampling location north to south on the coast. Population abbreviations are found in Table 1.

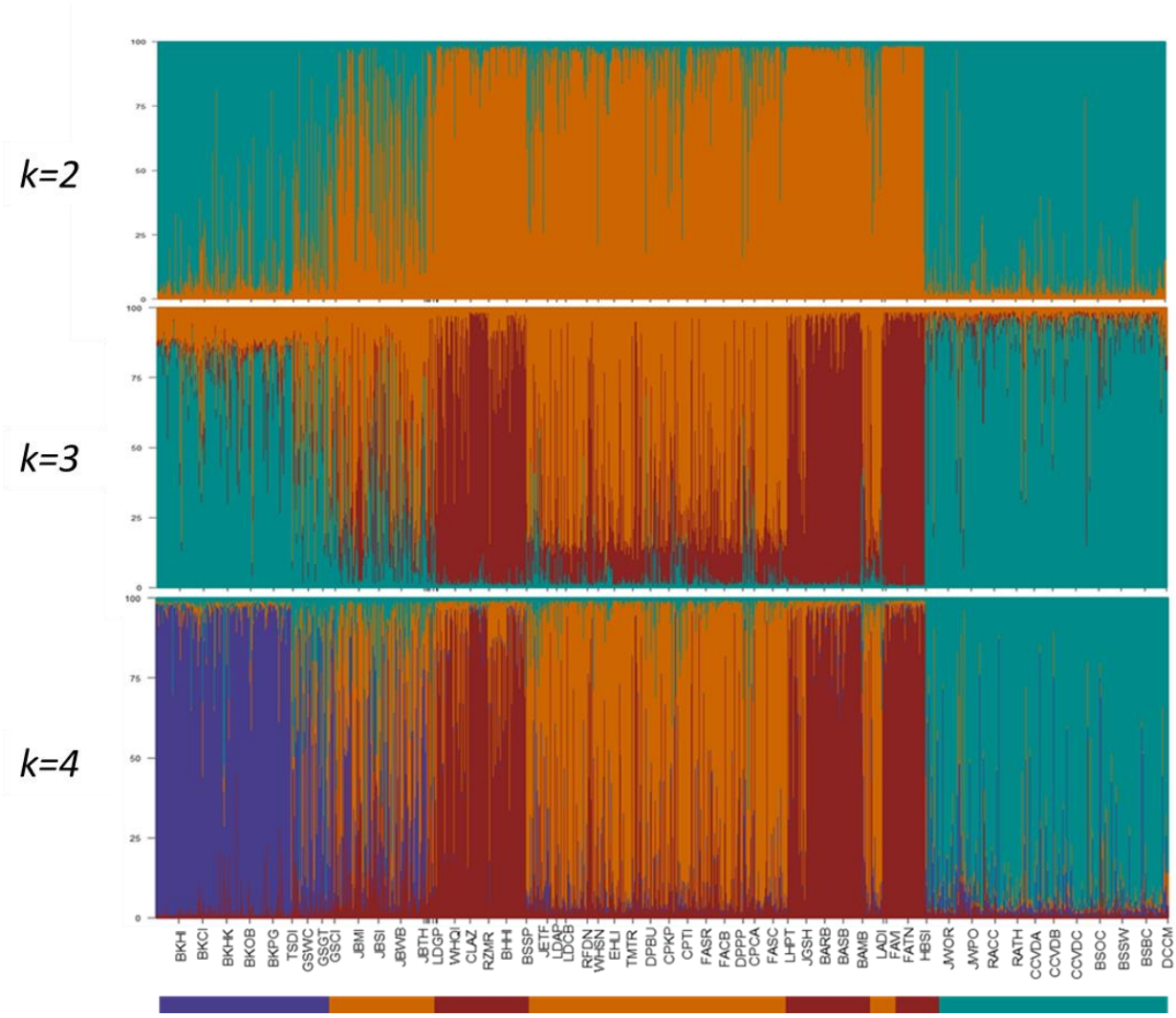


Figure 4. Stacked STRUCTURE bar plots for *k*=2 to *k*=4 where *k* is the number of co-ancestry groups. Population order left to right corresponds to approximate north to south location on the coast. The Puechmaille method provided support for *k*=4 co-ancestry groups. Each bar represents genetic co-ancestry membership for an individual and the solid bar at the bottom represent the color of the co-ancestry group that has a majority percent assignment.

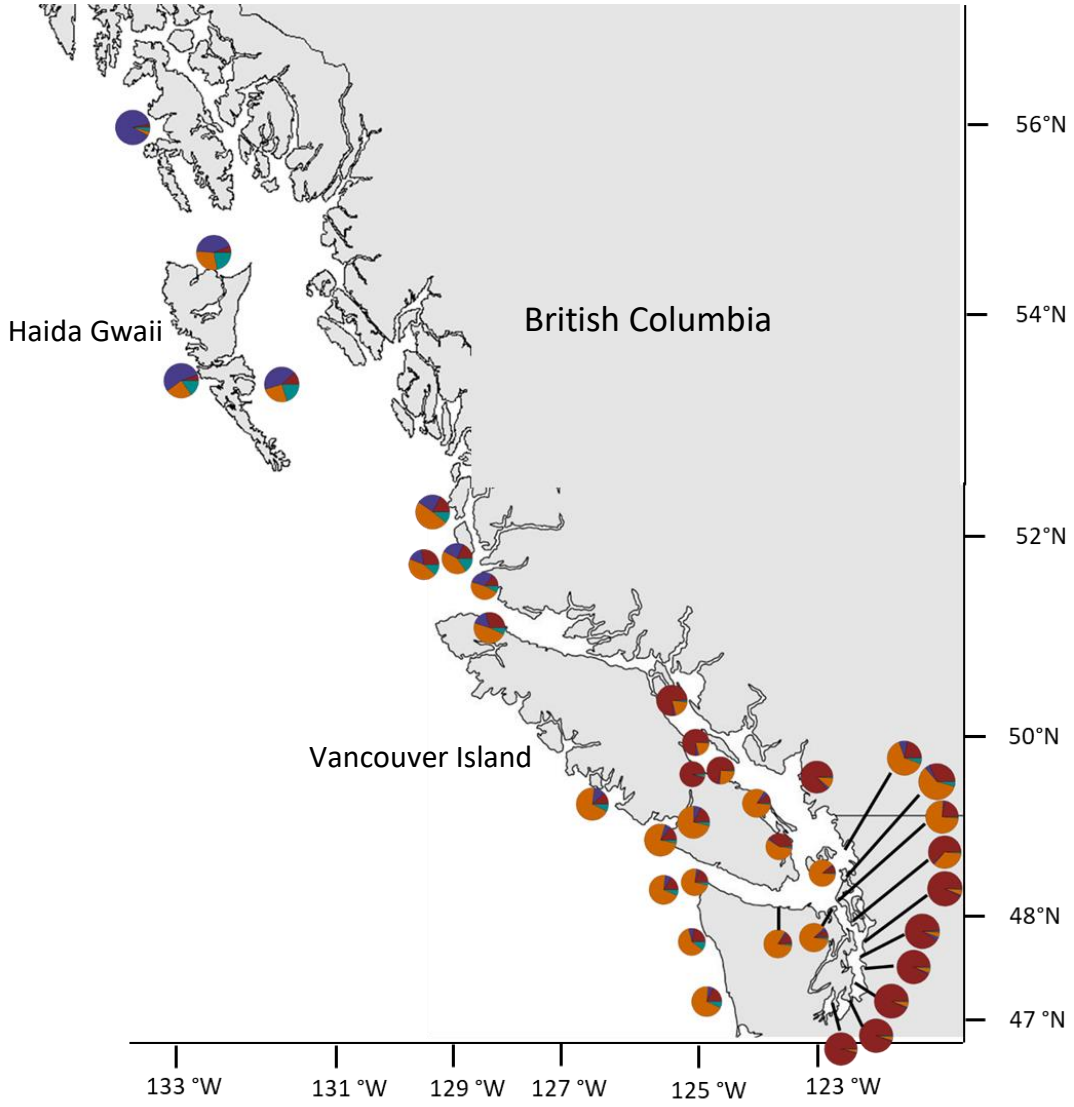


Figure 5. Map of northern sampling region including distribution of *k*=4 STRUCTURE clusters for *Nereocystis luetkeana* in the Salish Sea/Vancouver Island (bottom) and Haida Gwaii Island (top). Clusters are represented as the mean individual membership coefficient (proportion of an individual's genome inherited from ancestors in a particular population) for a given sampling location. The proportion of the populations membership to each of the *k*=4 clusters are represented in the pie chart by the following colors: Alaska (purple), outer coastal Washington/British Columbia/Juan de Fuca (orange), and Oregon/California (teal).

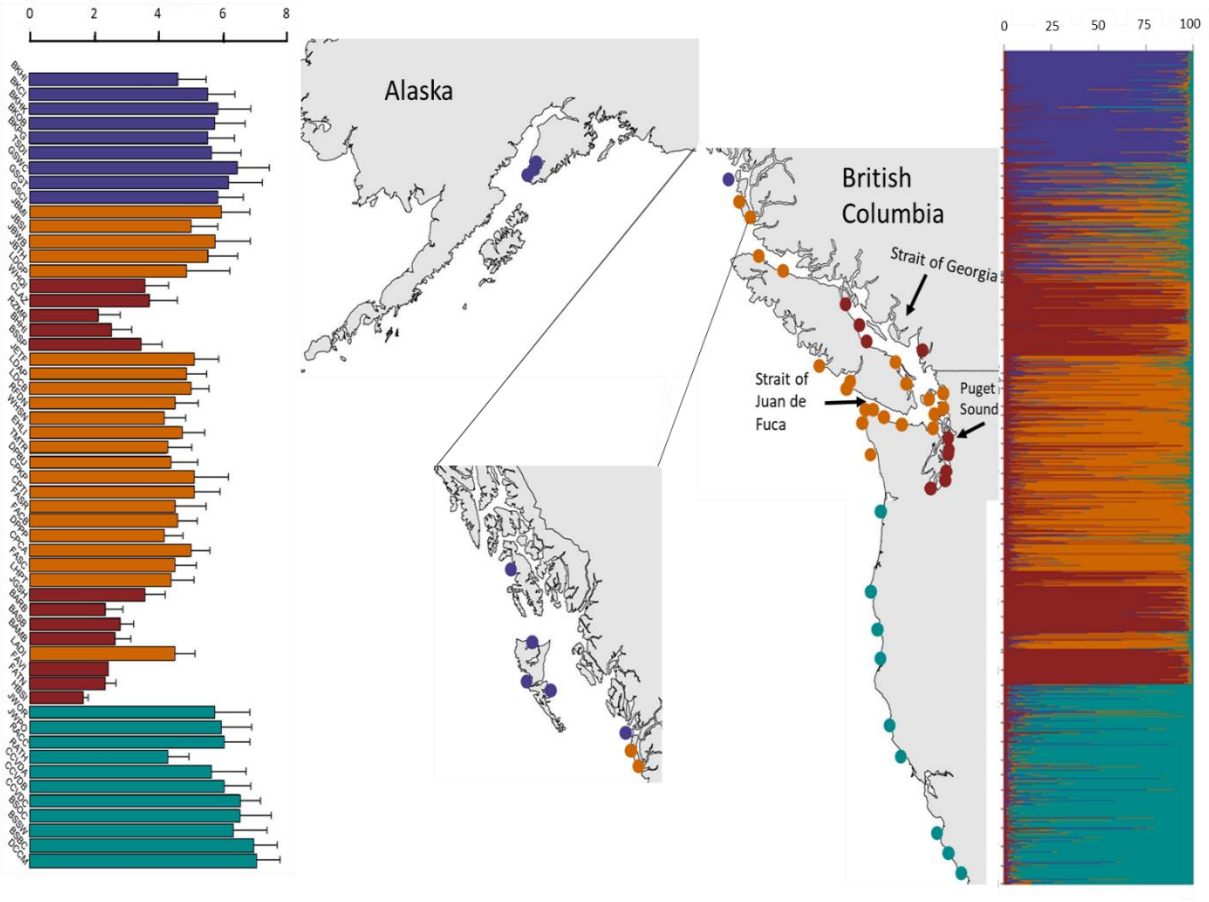


Figure 6. Left panel: Allelic richness values per site and color representing the major genetic co-ancestry group. Center panel: Map of sampling sites where the color of the site corresponds to the major genetic co-ancestry group. Right panel: Individuals percent assignment to *k*=4 genetic co-ancestry groups as measured from STRUTURE analysis.

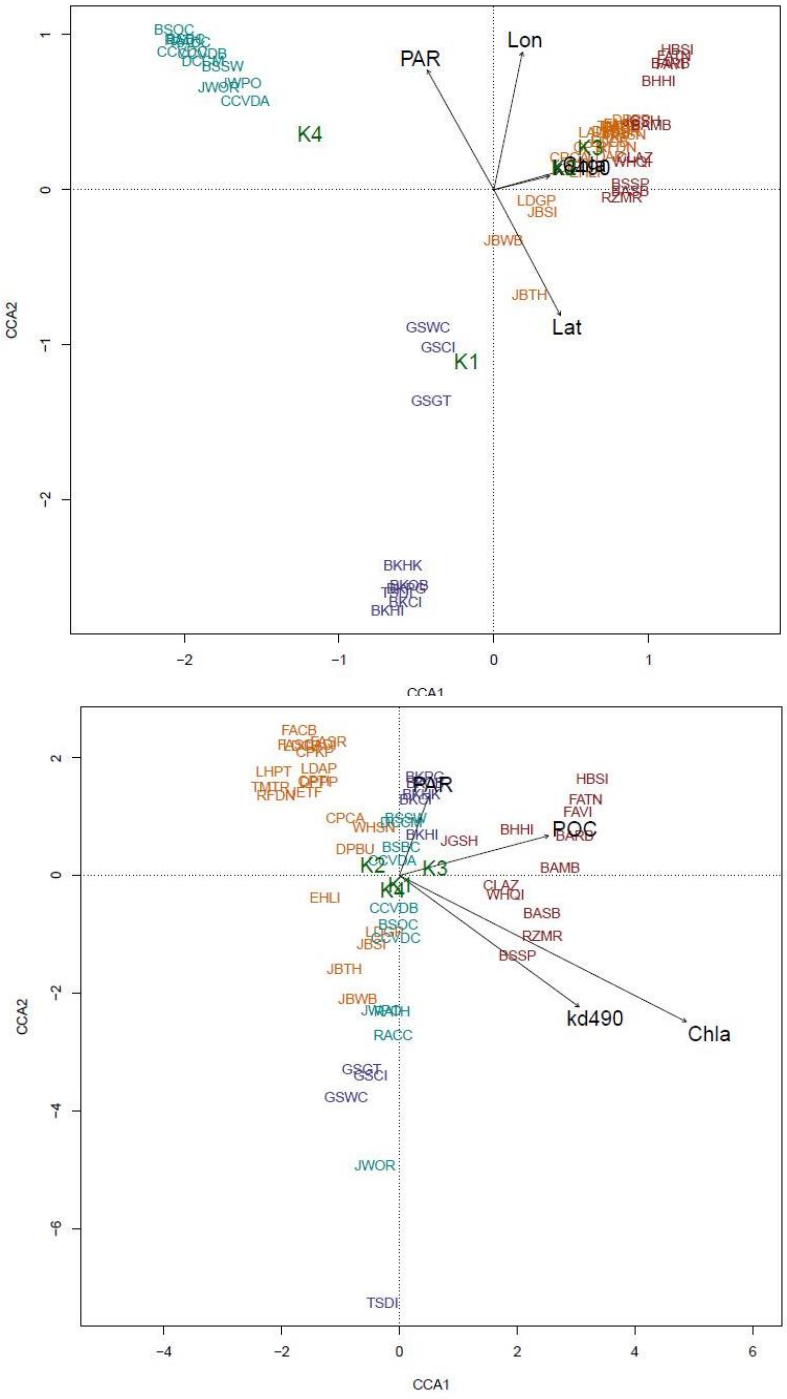


Figure 7. Canonical correspondence analysis with STRUCTURE analysis cluster groups and photosynthetically active radiation (PAR), particulate organic carbon (POC), summer sea surface temperature (SST), spring SST, fall SST, winter SST, light attenuation (kd490) and chlorophyll-a (Chl *a*). Clusters are Alaska (purple), outer coastal Washington/British Columbia/Strait of Juan de Fuca, Puget Sound/Strait of Georgia (red) and Oregon/California (teal). Population abbreviations found in Table 1 and information on variables found in Methods.

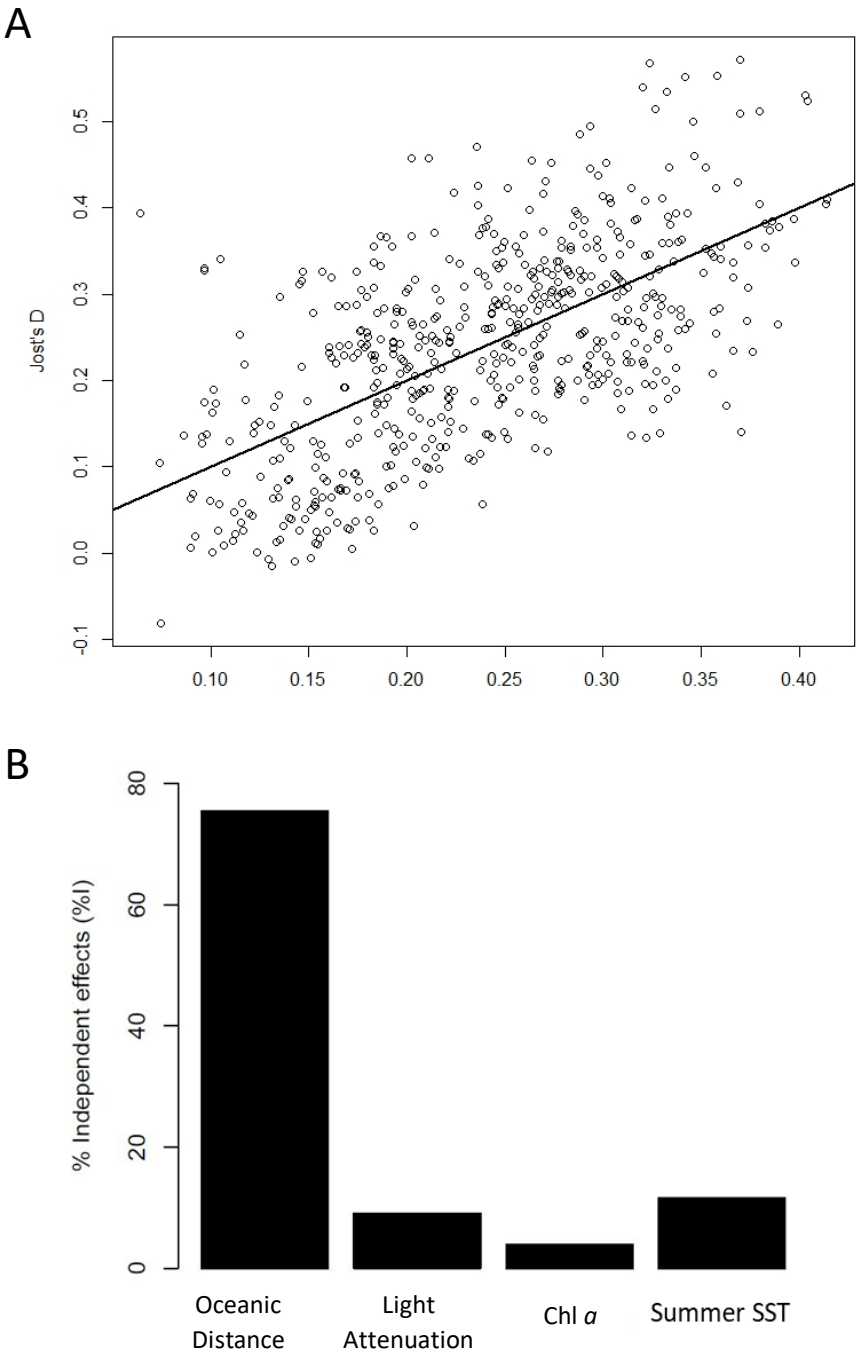


Figure 8. A: Optimized regression model with oceanographic distance, light attenuation, Chl *a*, and summer SST as predictor variables of genetic differentiation measured as Jost's D. ($R^2$=0.3883, p=< 2.2e-16). B: The percentage of independent effects each variable had on the optimized multiple regression.

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VIII. Appendix. Principle components analysis was done in R using the package MASS using the function princomp (Venables and Ripley 2002). Environmental variables were obtained from NASA's MODIS AQUIS satellite (Simons 2019).