THE POPULATION GENETICS OF MORRO BAY EELGRASS (*ZOSTERA MARINA*)

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ABSTRACT

The Population Genetics of Morro Bay Eelgrass (*Zostera marina*)

Julia Gardner Harenčár

Seagrass populations are in decline worldwide. *Zostera marina* (eelgrass), one of California's native seagrasses, is no exception to this trend. In the last 8 years, Morro Bay, California has lost 95% of its eelgrass. Eelgrass is an ecosystem engineer, providing important ecosystem services such as sediment stabilization, nutrient cycling, and nursery habitats for fish. The failure of recent restoration efforts necessitates a better understanding of the causes of eelgrass decline in this estuary. Previous research on eelgrass in California has demonstrated a link between population genetic diversity and eelgrass bed health, ecosystem functioning, and resilience to disturbance and extreme climatic events. The genetic diversity and population structure of Morro Bay eelgrass populations has not been assessed until this study. Additionally, we compare Morro Bay eelgrass to Bodega Bay eelgrass in northern California. We conducted fragment length analysis of 9 microsatellite loci on 133 Morro Bay samples, and 20 Bodega Bay samples. We found no population differentiation within the bay, and no difference among samples growing at different tidal depths. Comparison with Bodega Bay in northern California revealed that Morro Bay eelgrass contains three first generation migrants from a northern eelgrass population, but remains considerably genetically differentiated. Despite the precipitous loss of eelgrass in Morro Bay between 2007 and 2017, genetic diversity remains comparable to other populations on the west coast.

Keywords: genetic diversity, bottleneck, population structure

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1. INTRODUCTION

Genetic diversity is the basis of evolution. Since the Modern Synthesis brought together genetics and evolution in the 30's and 40's, the broader ecological importance of biodiversity has been the topic of a large body of theoretical and empirical research (Loreau et al. 2002, Kinzig et al. 2002). Variation in traits is necessary for a population to be capable of adapting to environmental changes. If genetic diversity is low, the likelihood there are alleles that could be adaptive to changing environmental conditions will also be low. For this reason, genetic diversity is thought to be critical for the long-term persistence of populations. This is especially true under current anthropogenic climate change predictions. Populations with very little or no genetic diversity (as in very inbred populations or monocultures) are susceptible to extinction. In such cases, any cause of death for an individual, such as infection, herbivory, temperature changes, etc., could potentially wipe out the entire population.

Theory and mathematical models predict that sudden reductions in population size will result in a loss of genetic diversity (Wright, 1931; Nei et al., 1975; Chakraborty and Nei, 1977; Maruyama and Fuerstt, 1985). There is empirical support for this concept, including a study by England et al. (2003) in which replicate laboratory populations of *Drosophila* were subjected to controlled bottlenecks and the loss of heterozygosity was close to model predictions in both intense and diffuse (less intense bottleneck over a longer period) bottlenecks. Similar results of genetic diversity loss after a population bottleneck have been observed in wild populations. Research on the northern elephant seal, which experienced a population bottleneck in the late 19th century, found a loss of genetic diversity from pre- to post-bottleneck samples (Hoelzel et al., 2002).

When a population experiences a bottleneck, genetic diversity is lost in two main ways. First comes the immediate loss of alleles that were contained only in individuals that are removed. Typically, this involves the loss of rare alleles. Next there is loss of genetic diversity due to greater inbreeding and drift in the small, post-bottleneck population (Young et al., 1996; Amos and Harwood, 1998). In many studies, including that of the northern elephant seal (Hoelzel et al., 2002), much of the documented loss of genetic diversity is likely due to inbreeding within the remaining small population and drift post-bottleneck, rather than to the population decline itself (Amos and Harwood, 1998).

Despite the assertion that population declines will result in losses of genetic diversity, some studies have found little change in genetic diversity after a population bottleneck (Waldman et al., 1998; Waldick et al., 2002; Hailer et al., 2006). This retention of diversity has been attributed to factors such as rapid recovery and long generation time, which shorten the effective duration of a bottleneck by reducing the number of generations during which the population is small and more susceptible to drift and inbreeding (small populations lose genetic diversity more quickly than larger populations: Lacy, 1987; Lynch et al., 1995; Keller and Waller, 2002). This further indicates that the duration of time a population spends at a reduced size, in addition to the decline itself, is responsible for the loss of genetic diversity observed in some cases of bottlenecks.

Gene flow into a population can mitigate declines in genetic diversity following bottlenecks (eg. Lacy 1987; Ehrich & Jorde 2005; Jangjoo et al. 2016). Immigration bolsters genetic diversity by introducing new alleles, or reintroducing alleles that may have been present prior to population decline. On a local scale, connectivity, or a lack of

distinct subpopulations within a population can also reduce the loss of alleles in a severe bottleneck. When a population is structured, alleles are found only in geographically distinct portions of the range. Therefore, a population decline that affects one area more than another is likely to result in the loss of alleles, because all alleles found exclusively in that portion of the range will be lost.

Seagrasses, a group of marine flowering plants in the families Posidoniaceae, Zosteraceae, Hydrocharitaceae, and Cymodoceaceae, are critical ecosystem engineers along coastlines and in estuaries worldwide (Costanza et al., 1997; Hemminga and Duarte, 2000). Seagrass beds (dense underwater meadows) perform numerous important functions, including sediment stabilization, biogeochemical cycling, water quality improvement, and carbon sequestration (Short et al., 1996; Orr et al., 2005; McGlathery et al., 2007; Fourqurean et al., 2012; Lewis and Boyer, 2014). Seagrasses also form productive and particularly biodiverse marine ecosystems that provide food and/or habitat for a wide variety of invertebrate, fish, bird, and mammal species (Hemminga and Duarte, 2000; Beck et al., 2001; Williams and Heck, 2001; Holsman et al., 2006). In fact, the majority of commercially valuable marine species in the United States and elsewhere rely on seagrass beds during some stage of their life cycle (Hemminga and Duarte, 2000; Williams and Heck, 2001).

Seagrasses are experiencing dramatic population declines worldwide (Orth et al., 2006; Waycott et al., 2009). Since 1980, seagrasses have been declining globally at a rate of 110 km^2 per year, and the rate of loss has accelerated from 0. 9% per year before 1940, to 7% per year since 1990. These declines have resulted in the loss of 29% of the areal global coverage originally recorded in 1879 (Waycott et al., 2009). Numerous

anthropogenic and natural causes have been identified as contributing to seagrass decline. Waycott et al. (2009) identified the top two causes of global seagrass decline as the direct removal by coastal development and dredging, and indirect reduction caused by declining water quality. Other mechanisms of loss include massive die off due to a protist in the genus *Labyrinthula* that causes wasting disease (Tutin, 1942; Short et al., 1987), destructive fishing practices, oceanic storms, cascading effects from the loss of predators due to overfishing, overgrazing by native and invasive species, and algal blooms (Valiela et al., 1997; Jackson et al., 2001; Ruiz et al., 2001; Duffy et al., 2005; Orth et al., 2006; Myers et al., 2007; Williams, 2007). Climate change is predicted to have major negative impacts on seagrass populations, both by exacerbating already present challenges, and through other mechanisms such as changes in water temperature (Short and Neckles, 1999; Orth et al., 2006). The loss of seagrass species is causing the loss of ecosystems they construct, which will have cascading effects ranging from marine animal declines to further water quality degradation and sediment destabilization.

The decline of seagrass meadows will affect humans in several ways. The predicted effects of seagrass loss include: 1) declines in the nutrient cycling action of seagrass beds (Waycott et al., 2009), 2) major declines in the revenue and food produced by large, economically important fisheries (Duarte and Cebrián, 1996), 3) increased coastal erosion and shoreline regression, and 4) increased atmospheric carbon dioxide since seagrasses account for 12% of the total carbon storage in the ocean (Hemminga and Duarte, 2000). Additionally, this dramatic worldwide decline is likely to result in lowered genetic diversity in seagrass species over time, which lowers their evolutionary potential.

Higher genetic diversity within seagrass beds is correlated with greater health, resilience, and resistance to disturbance. Numerous studies on the effects of genetic diversity have been conducted on eelgrass (*Zostera marina* L.), a species of seagrass found worldwide in northern temperate coastal zones. Higher genetic diversity increases seed germination and results in more rapid increases in shoot density (Williams, 2001). Higher genetic diversity in eelgrass is also correlated with increased resilience and resistance to stress and disturbance such as algal blooms, biomass removal, and grazing (Hughes and Stachowicz, 2004, 2009, 2011). Research on Baltic Sea eelgrass revealed evidence of broader benefits of genetic diversity, whereby more genetically diverse beds experiencing near-lethal high temperatures had increased biomass production, plant density, and faunal abundance as compared to beds with lower genotypic diversity (Reusch et al., 2005). More genetically diverse eelgrass beds also retain higher shoot density (a measure of bed health) when exposed to high temperatures (Ehlers et al., 2008). This finding is particularly pertinent given the predicted challenges of climate change to coastal systems (Thorne et al., 2016). Genetic diversity has also long been understood as critical to the evolutionary potential of a population, as well as for the increased likelihood of preadaptation to processes that move faster than evolution.

Morro Bay, in central California, has been experiencing dramatic declines in its eelgrass population. The bay is located north of point Conception and is also the only commercial and recreational port for 100 miles in either direction, with Monterey being the closest bay to the north and Santa Barbara being the closest to the south (Morro Bay National Estuary Program, 2013). Morro Bay historically contained between 350 and 500 acres of eelgrass (Morro Bay National Estuary Program, 2010). Since the start of eelgrass

acreage estimation in Morro Bay in 1960, the population has experienced two major declines. In the most recent decline Morro Bay lost more than 95% of its eelgrass cover, dropping from over 300 acres to fewer than 20 (Morro Bay National Estuary Program, 2014, Figure 1 & 2). The cause of this decline is currently unclear but likely involves numerous combined stressors. Factors that could have contributed to the decline include: diminished water clarity due to winter storm runoff, substrate resuspension, and intertidal channel erosion (which was particularly pronounced in the 2010-2011 winter season); algal blooms; and prolonged dredging in the northern harbor portion of the bay (Morro Bay National Estuary Program, 2013). Starting in 2008, the decline progressed from the southern portion of the estuary, or back bay, north towards the mouth of the bay (Merkel & Associates, *2015*). In 2015, only seven beds remained in the bay, totaling about 13 acres. All seven are found along the channel in the northern part of the bay. In 2016, a few beds were found reappearing in the back of the bay (Figure 3).

Given the fluctuating population size of Morro Bay eelgrass in the past, and the recent dramatic decline, genetic diversity within the bay could be low. If there is low genetic diversity in Morro Bay eelgrass, the population will have a lower capacity to adapt in a changing environment. Low diversity would also mean greater likelihood of inbreeding depression. Aside from the direct fitness and evolutionary consequences, if the beds have low diversity they are likely to experience the lowered growth, health, ecosystem functioning, resistance to disturbance, and resilience to stressors associated with decreased diversity in other eelgrass studies (Williams, 2001; Hughes and Stachowicz, 2004, 2009, 2011; Reusch et al., 2005; Ehlers et al., 2008; Hughes et al., 2008; Kamel et al., 2012; Reynolds et al., 2016). If genetic diversity in Morro Bay is low,

future restoration efforts could consider introducing genotypes from the nearest southern or northern populations to facilitate genetic rescue.

Previous genetic research on Western North American eelgrass has largely not included central Californian populations. Morro Bay eelgrass was cursorily sampled in a broader study of seagrass hybridization in southern California (Olsen et al., 2014). The authors found strong genetic differentiation of Morro Bay from all other sampled populations, which are all to the south. This differentiation was attributed to the cold California current north of Point Conception, which likely acts as a barrier to gene flow between eelgrass populations. Morro Bay is isolated from the nearest eelgrass beds to the north by 100 miles, and from Bodega Bay by about 250 miles. It is therefore likely genetically distinct from northern populations, just as it is distinct from its nearest southern neighbor about 100 miles of coastline away (Olsen et al., 2014). If this is the case, there is the possibility of local adaptation that might be disrupted if restoration efforts try to introduce diversity by bringing in individuals from other bays. The detailed population genetics of Morro Bay's eelgrass have not been included in genetic research on Western North American populations. Some of these studies have found genetic structure within a bay. For example, there is significant genetic differentiation among populations within San Francisco, Bodega, and Tomales Bays, as well as significant differentiation among individuals growing at different tidal heights in both Bodega and Tomales Bays, and in at least one population in San Francisco Bay (Kamel et al., 2012; Ort et al., 2012).

Here, we investigate the population genetic structure of Morro Bay eelgrass for the first time. We ask how diverse Morro Bay eelgrass is, and whether it is genetically

distinct and geographically isolated from northern bays by comparing it with Bodega Bay eelgrass. We obtained 20 samples from Bodega Bay and collected tissue from 142 plants spanning all remaining eelgrass beds in Morro Bay. We utilized nine microsatellite markers to assess genetic diversity and population isolation and structure. Based on theory and past empirical research on the effects of population declines on genetic diversity, we predict that Morro Bay has low genetic diversity due to the recent population bottlenecks. Additionally, following the theory of isolation by distance, we predict that Morro Bay is genetically differentiated from Bodega Bay due to the large distance (about 250 miles) between the two bays, as well as the presence of the Monterey peninsula between Morro Bay and all northern populations. In terms of genetic differentiation within the bay, we predict that Morro Bay does not display population structure among beds due to the small size of the bay and the relatively short distances between beds. Similarly, we predict that there is no population structure among tidal heights due little differentiation in growing depth within beds.

Given the importance of eelgrass both to the natural systems and the humans of Morro Bay, the dramatic recent population decline, and the necessity of population genetic data in planning successful restoration, this research has immediate practical applications. Additionally, we add to a small, but growing body of empirical work demonstrating the various population genetic consequences of severe population declines.

2. MATERIALS AND METHODS

2.1 Collection

We collected tissue from 126 individuals of eelgrass (*Zostera marina)* from the seven remaining beds in Morro Bay, CA, USA between 13 July and 8 Aug 2016 (CDFW Scientific Collection Permit numbers SC-12412 and SC-13445, Table A1, Figure 4). We collected samples at intervals of roughly 10 m along a transect. Where beds were wider than two meters, we collected samples both at the shore edge and at the deeper edge. Where the beds were greater than five meters wide, we collected three samples, one at each edge and one in the center of the bed. When gaps occurred in an eelgrass bed, we would resume sampling immediately where the next closest bed began. We conducted additional sampling of the newly discovered back bay beds (Oyster Farm, Channel, and Mitchell) on January 27th, 2017, and added 16 samples for a total of 142 individuals.

For each sample, we collected roughly 2 cm pieces from the base of each leaf where tissue is softer and carries less epiphytic growth. We avoided leaves containing flowers or fruits. To remove epiphytic cover we gently wiped each sample and placed them in a 2 ml collection tube. We kept samples on ice until storage at 4°C.

We collected all Bodega Bay samples from individuals growing in tanks at the UC Davis Bodega Marine Laboratory (see Table A2 for sample details). Twelve of the 20 were originally collected by Jessica Abbott. They are a mix of individuals from four different sites and from plants growing at high, low, and subtidal zones in the bay. The other 8 samples are from plants originally sampled and place in tanks by Randall A. Hughes (for collection methods see Hughes et al. 2004).

2.2 Processing

We washed all collections in milliQ DI water to clean off mud, salt, and any remaining epiphytes present before isolating ~ 1 cm^2 of tissue. We extracted DNA with Qiagen DNeasy plant mini kits following the given protocol (QIAGEN 2013–2017). We extracted a total of 18 samples from each of the 7 beds in Northern Morro Bay, and 16 total from three newly discovered Southern beds (142 total individuals) and 20 samples from Bodega Bay.

2.3 Amplification and microsatellite scoring

We used 11 previously described primers to amplify microsatellite regions in eelgrass (Table 1). We also attempted to use ZosmarCT-19, but dropped this locus prior to fragment length analysis due to very poor amplification across our samples. ZosmarCT-12 was also dropped due to poor amplification after fragment length analysis (only 65/162 were scorable for fragment length). We used M13 tagged forward primers to incorporate florescent dyes with M13 tags (6-FAM, VIC, and PET) via PCR following the methods of Schuelke (2000). Our PCR reactions contained 6 µl 1X master mix (Promega: GoTaq Green), 0.25µM forward primer with M13 tail on the 5' end, 1 µM reverse primer, 1 µM dye-labeled M13 reverse primer, and 1-10 ng DNA in a final reaction volume of 12 µl. Thermocycler amplification parameters were as follows: 5 min denaturing at 94°C, followed by 30 cycles of 30 s at 94°C, 45 s annealing at 54-58°C (see Table 1 for primer specific annealing temperatures), and 45 s extension at 72°C, followed by 8 cycles of 30 s at 94°C, 45 s annealing at 53°C for better dye incorporation, and 45 s extension at 72°C, followed by a terminal extension step at 72°C for 10 min.

Following PCR, we multiplexed 0.9 µl each of PCR product for three samples tagged with different dyes into wells on a 96 well plate for fragment length analysis. Each well also contained 10 µl of HiDi Formamide (Applied Biosystems) and 0.5 µl of GeneScan 500 LIZ size standard (Applied Biosystems) for a total volume of 13.2 µl. We sent the products to the UC Berkeley DNA sequencing facility for fragment size analyses. We determined the size of each PCR product using the bioinformatics software geneious (2005-2016 Biomatters Ltd). If peaks could not clearly be called due to aberrant peaks, or peaks that were too small to confidently detect, we did not record a value for that individual at that locus. For details of how peaks were called, see figure A1. We duplicated the PCRs and fragment size analyses for 10% of our samples to check for inconsistency in sizing.

2.4 Data analysis

Of the 142 original Morro Bay samples, we eliminated five samples that were missing data for more than three of the 10 loci, and four individuals that were identified as clones. This left us with a total of 133 samples from Morro Bay and 20 from Bodega Bay (see Table A3 for full dataset). We used MICROCHECKER 2.2.3 to check our loci for the presence of null alleles, which are alleles that do not amplify consistently due to variation in in the microsatellite flanking region causing poor primer ligation (Van Oosterhout et al., 2004), and FSTAT to check for Hardy-Weinberg equilibrium (Goudet, 1995). To test for linkage disequilibrium we utilized both FSTAT and Arlequin (Goudet, 1995; Excoffier and Lischer, 2010). These linkage disequilibrium tests revealed linkage between ZMC19017 and CL172. For this reason, we removed ZMC19017 from all analysis.

2.4.1 Population structure

To investigate population structure and to compare Morro and Bodega Bay populations we used the Bayesian model-based clustering algorithm of the program STRUCTURE version 2.2.3 (Pritchard et al., 2000). To assess the number of genetic clusters K in our 10 Morro Bay sites we ran 10 Bayesian Markov chain Monte Carlo (MCMC) searches of 100,000 steps with a 50,000 step burn-in for values of K ranging from 1-11. We used the admixture model with allelic frequencies correlated among populations and ignoring prior population information. We then used the maximal values of ΔK based on the rate of change in the log probability of data between successive K values to find the best fit value of K (Evanno et al., 2005). We conducted analysis of molecular variance (AMOVA) to test for structure at the bed and depth level within Morro Bay, and Between Morro and Bodega Bays, using the R package "poppr". To visualize STRUCTURE results we generated three Bayesian clustering diagrams: one for Morro Bay with separation by collection site, a second for Morro Bay with separation by collection depth, and a third for the combination of Bodega Bay and Morro Bay. To generate a principal component analysis for further data visualization we utilized the R package "ade4".

To detect first generation migrants, we used GENECLASS v2.0 (Piry et al., 2004). We used the "detect migrant function" in GENECLASS, which calculates the likelihood of an individual being found in the population where it was collected (L_h), the greatest likelihood of the collection localities (L_{max}), and the ratio of these values (L_h/ L_{max}) to identify migrants. We selected the Rannala and Mountain criterion to distinguish

between true and statistical migrants (Rannala and Mountain, 1997). To determine the critical value of test statistics (L_h/ L_{max}), we used the Monte Carlo resampling algorithm of Paetkau et al. (2004) with n = 1000. We applied a threshold of 0.05 to the probability of being assigned to the reference population for determination of immigrants.

2.4.2 Richness and diversity

A single eelgrass individual often forms clonal patches. This can result in the collection of multiple shoots from a single individual. Identical genotypes may also be observed by the chance recombination of identical alleles during sexual reproduction. We called any samples with identical multilocus genotypes clones if they were collected within 500 meters of each other. By this criteria we identified four clones and excluded them from analyses.

We calculated allelic richness rarefied to the smallest sample size (Bodega Bay: n = 20), with the R software package "PopGenReport". We calculated observed and expected heterozygosity using the software package ARLEQUIN (Excoffier and Lischer, 2010). To calculate the inbreeding coefficient (F_{is}) for each population, we used the statistical program FSTAT (Goudet, 1995).

3. RESULTS

3.1 Summary

Analysis with MICROCHECKER revealed no evidence for null alleles, and in FSTAT the populations were found not to deviate significantly (at a 0.05 level) from HWE. Repeat sampling, in which we reran the PCR and fragment length analysis of 10% of sampled individuals, resulted a 0% error rate. As previously mentioned, tests for linkage disequilibrium revealed linkage between ZMC19017 and CL172, therefore ZMC19017 was removed from analysis.

3.2 Population structure

To determine the number of genetic clusters (K), we used the Evanno method, which is based on likelihood values calculated by STRUCTURE. We utilized the R package "Pophelper 2.1.0" to implement this method. The ΔK Evanno method graph contained multiple peaks, the largest of which was at K=8 (Figure A2). However, this is likely due to the fact that STRUCTURE cannot differentiate between a K of 2 and a K of 1. Bayesian clustering diagrams clearly show roughly equal distribution of genetic clusters at all K levels from 2 to 8 (Figures 5 and A3, respectively). This indicates that there is likely only one true genetic cluster in Morro Bay. The Evanno method clearly indicated a K of 3 for the combined dataset of Morro and Bodega Bays (Figure A4).

AMOVA and STRUCTURE output demonstrate no structure in Morro Bay eelgrass based on bed or depth (p-value = 0.304 and 0.296, respectively; Figure 5). Conversely, AMOVA, STRUCTURE, and principal component analysis show clear differentiation between Morro and Bodega Bays (p-value = 0.001; Figure 5; Figure 6).

Analysis with GENECLASS revealed three first generation migrants into the Morro Bay population that are more similar to Bodega Bay than Morro Bay (Table 2). Additionally, one individual was identified in the Bodega Bay population that is more similar to Morro Bay eelgrass than the other Bodega Bay samples. This gene flow is also indicated by some overlap of Morro and Bodega Bay populations in the principal component analysis (Figure 6).

3.3 Richness and diversity

Estimates of genetic diversity are summarized in Table 3. Rarefied allelic richness values for Morro and Bodega Bays are average and comparable, with Morro Bay slightly higher at 3.46 as compared with 3.10 in Bodega Bay. The heterozygosities of Morro and Bogeda Bays were both high (H_e = 0.503 and 0.515 respectively). Neither Morro Bay nor Bodega Bay had significant F_{is} values, indicating no significant inbreeding.

4. DISCUSSION

The lack of population structure we found within Morro Bay is in contrast to studies of numerous other bays. Namely, population structure has been found in Bodega Harbor, Tomales Bay, San Francisco Bay, the Port of Los Angeles, Alamitos Bay, Newport Bay, and San Diego Bay (Kamel et al., 2012; Ort et al., 2012; Olsen et al., 2014). Morro bay is considerably smaller than these other California bays at around 2,000 acres (U.S. Army engineer district, Los Angeles, CA, 1975). The high admixture in Morro Bay indicates high levels of gene flow within the bay, which could be due in part to its small size. Additionally, eelgrass seed and rhizomes are dispersed by water, and tidal fluctuations result in movement of water to and from the fore and back bay.

While a few studies have found some evidence for genetically based adaptation to depth (Dennison and Alberte, 1986; Procaccini and Mazzella, 1998; Procaccini and Piazzi, 2001; Ort et al., 2012), we did not find differentiation by depth of collections in Morro Bay. This is likely due to the lack of a large depth gradient in the beds. The difference between the shallowest and deepest eelgrass is about a meter. It is unknown whether eelgrass exists deeper in the channel, although none has been observed to date by the MBNEP, who conduct regular surveys of the bay. The lack of spatial genetic distinction indicates that there is high connectivity between populations within the bay, and that dispersal events can cover the length of the bay.

Morro Bay appears to be a relatively genetically isolated population on the Pacific coast. It is clearly genetically differentiated from Bodega Bay in the north, and Olsen et al. (2014) demonstrated that Morro Bay is genetically isolated from the eelgrass populations to the south (including the nearest southern population about 100 miles away

in Gaviota, CA). The differentiation between Morro Bay and the southern populations is attributed to the cold California current that begins at Point Conception and imposes a biogeographic barrier to eelgrass dispersal (Olsen et al., 2014). There is no obvious migration barrier to dispersal between Morro Bay and northern populations. In fact, some gene flow does occur between Morro Bay and northern bays as evidenced by the three first generation immigrants to Morro Bay from northern California revealed by our analysis. Given the north to south flow of the California current along the western coast of North America, dispersal events likely occur in a predominantly north to south direction. Future research should compare Morro Bay with the next closest northern population (Elkhorn Slough), and other central California populations to determine if there is a directionality of gene flow, and if Morro Bay is a unique population, or if there is a group of central California populations that are genetically similar to each other. Such information would clarify the patterns of gene flow along central coast eelgrass populations.

The lack of genetic differentiation by bed or sampling depth, and the marked differentiation of Morro Bay from other California bays, is of great relevance to restoration efforts. Propagules can be taken from anywhere within the bay, and no additional efforts, such as collecting at a variety of depths or locations, are necessary to capture the diversity of the bay in new plantings. The genetic differentiation of Morro Bay, along with the high diversity values, indicate that collecting from other bays for restoration is not necessary. Morro Bay eelgrass is not in apparent need of genetic rescue and is locally differentiated, such that local adaptation to conditions with Morro Bay may

have occurred. Therefore, any new introductions risk introducing maladapted genotypes, and could ultimately reduce the fitness of the local population.

Our estimates of genetic richness and diversity in Morro Bay eelgrass reveal a population with average diversity compared to other pacific populations (Kamel et al., 2012; Ort et al., 2012; Olsen et al., 2014). We can also directly compare our results for Morro Bay with Bodega Bay, which has not experienced any recorded dramatic population declines since the first survey of eelgrass in Bodega Bay in 1987 (Ramey, 2008). Therefore, Morro Bay demonstrating a marginally higher allelic richness than Bodega Bay, and a comparably high heterozygosity, indicates that this population contains a healthy level of genetic diversity relative to other populations that have not experienced severe declines.

The heterozygosity in Morro Bay is similarly high compared with that of eelgrass populations researched in other studies, and is notably higher than values published for the San Francisco Bay (Reusch et al., 2000; Kamel et al., 2012; Ort et al., 2012; Reynolds et al., 2016). The relatively high heterozygosity observed may be due to the excess heterozygosity relative to allele number observed post population bottleneck (Theoretical: Nei et al. 1975; Maruyama and Fuerstt 1985 Empirical: Leberg 1992). However, the comparable allelic richness of Morro Bay to other eelgrass populations that have not recorded any extreme bottlenecks indicates that the loss of genetic variation with population decline was not large (Kamel et al., 2012).

Research in other estuaries has shown that the degree of genetic variation within eelgrass beds is very important for the overall health and ecosystem functioning of the bed. Beds with high genetic diversity have higher growth rates, have greater resistance to

grazing disturbance, provide higher quality habitat, and are overall more resilient after disturbance, and more resistant to stressful conditions and extreme climate events (Ehlers et al. 2008; Hughes & Stachowicz 2004; Hughes & Stachowicz 2009; Hughes & Stachowicz 2011; Williams 2001). There are also community wide effects of high eelgrass diversity, which is correlated with higher biodiversity of the whole community (Reusch et al., 2005). In the face of climate change, the more general benefits of genetic variation are also critical, namely the positive relationship with evolutionary potential and greater long term population stability. The relatively high genetic variation present in Morro Bay eelgrass is therefore very positive for the future of this population. It is also surprising, considering the loss of over 95% of eelgrass cover from 2008 to 2013 (Morro Bay National Estuary Program, 2014). It is possible that Morro Bay had extremely high diversity prior to the bottleneck, and that some of that diversity was lost. It is also possible that there was very little loss of genetic diversity in part due to the high homogeneity throughout the bay. Either way, the high diversity seen in the bay now indicates that diversity was also relatively high after the previous population decline in the mid 90's. It could be that this high diversity is partially responsible for the ability of eelgrass to recover after the 90's decline. If so, the same may be true for recovery from the most recent decline.

Gene flow from other areas could increase diversity after a bottleneck, but this is not likely to be the case for Morro Bay's most recent decline. The decline in Morro Bay occurred between 2008 and 2013, leaving roughly four years between the end of the most dramatic years of the decline until sampling for this research. Eelgrass in Morro Bay is a long-lived perennial, and while there is variation in the frequency of sexual reproduction

between eelgrass populations, most populations in California experience little to no seedling establishment each year (Phillips et al., 1983; Olesen and Sand-Jensen, 1994). The cold current off Point Conception likely acts as a biogeographic barrier to the south of Morro Bay (Olsen et al., 2014), and the large distance to the nearest eelgrass population to the north (about 100 mi) likely limits gene flow from the north on short time scales. These factors combined make it unlikely that the three first generation migrants have considerably bolstered population genetic diversity since the decline. However, the identified recent migrants indicate that gene flow into Morro Bay is an important source of genetic diversity for the population. This gene flow may be partially responsible for the lack of dramatic declines in genetic diversity following the population decline in the 90's and any previous declines. There are also a few eelgrass beds that have been noticed in Estero Bay just outside of Morro Bay and in Avila Bay immediately to the south. Gene flow into Morro Bay from these beds is possible and more likely to occur on a shorter time scale. These nearby beds may be refugia for genetic diversity in the area.

Declines in population size are often connected to declines in genetic diversity, but this is not always the case (Galtier et al 2006, Hooft et al. 2001, Harper et al. 2003, Young et al. 1996). Eelgrass is a long-lived species. It is unlikely that a large portion of the population has grown from seed in the six years since the population reached its lowest recorded acreage. Therefore, this population has not existed at a small size for a long time relative to its generation time, and there has been little time for inbreeding depression or drift to cause a loss of genetic diversity. For this reason, it is important that

monitoring of the bay continue into the future, and that restoration efforts proceed quickly.

5. CONCLUSION

While the causes of the eelgrass decline in Morro bay are still unclear, low genetic diversity is unlikely to have an important role. As such, it is not necessary, and could be unadvisable given the genetic differentiation of Morro bay from northern bays, to bring material from other bays for restoration. Monitoring of eelgrass as well as restoration efforts are underway as part of a broad collaboration between the Morro Bay National Estuary Program, California Polytechnic University, and other partners. The restoration is using only materials from within the bay, and preliminary monitoring in the most recent year shows promise for the eelgrass population of Morro Bay.

6. TABLES

Table 1: **Microsatellite primer details**. Primers used for fragment length amplification. Note: the forward primer sequence had a 5' M13 tail (M13: 5′-CACGACGTTGTAAAACGAC-3′)

Primer	Motif	Source	Annealing Temp (°C)	Sequence (5'-3')
CL32 Contig2	AGG	Oetjen & Reusch 2007	54	F: AATCTGTTGCCACGAAGGAG R: TCACCTTCATCAAGCAGTCG
ZMC12075	CT or GT	Oetjen & Reusch 2007	54	F: CCTCTTTTTTCCTCTCTCTCTCTCT R: CTTCTGCGAATGATGCCATA
ZMC13053	CT	Oetjen & Reusch 2007	54	F: CCCCATCTTTTGAGTTTGGA R: TCATCATTTCTTGCAATTTGAATC
ZMC19017 (unused in analysis)	AAG	Oetjen & Reusch 2007	54	F: TCGTCGAGAAAGAGGAGGAA R: TGTTCTGATTCCGTTCTCCA
CL172 Contig 1	TGGC	Oetjen et al. 2010	56	F: CTCCTGGACGCAGAAATATG R: GACAAACGATTAATTCAGAAACAAAA
CL559 Contig1	AG	Oetjen et al. 2010	56	F: CCACTTCCGTAGTTGCTGTT R: CGATGAGGACGATGAGGAAT
ZMC19062	GAC	Oetjen et al. 2010	56	F: CACTCTCCTCTTTCCGTTCG R: CAGGGGCCTTCCTCTTACTC
Zosmar CT12 (unused in analysis)	CT	Reusch 2000	57	F: CGTTCATCTTGTCCTCGTCC R: TTTCATTTCCATTTCCCACC
Zosmar CT3	CT	Reusch et al. 1999	58	F: TGAAGAAATCCCAGAAATCCC R: AGACCCGTAAAGATACCACCG
Zosmar GA2	GA	Reusch et al. 1999	55	F: GGCAGCGATCTAATAACAATTAAGG R: ACGTCACATCTTTTCACGACC
Zosmar GA3	GA	Reusch et al. 1999	55	F: CGACGATAATCCATTGTTGC R: GCTTTTCATTTATCCAATAGTTTGC

Table 2: **GENECLASS first generation immigrant results**. GENECLASS output identifying first generation immigrants (bolded).

ID	Bay	-Log (L_h/ L_{max})	p-value	# of loci used	ID	Bay	-Log (L_h/ L_{max})	p-value	# of loci used
1	Morro	0	0.507	9	145	Morro	0	0.508	9
3	Morro	0	0.514	8	147	Morro	0	0.508	9
7	Morro	0	0.509	9	148	Morro	0	0.507	9
8	Morro	0	0.506	9	149	Morro	0	0.508	9
14	Morro	0	0.507	9	151	Morro	0	0.508	9
17	Morro	0	0.526	7	152	Morro	0	0.509	9
18	**Morro**	**0.193**	**0.013**	**9**	154	Morro	0	0.507	9
21	Morro	0	0.507	9	156	Morro	0	0.508	8
27	Morro	0	0.506	9	158	Morro	0	0.508	9
28	Morro	0	0.507	9	161	Morro	0	0.508	9
31	Morro	0	0.507	9	164	Morro	0	0.508	9
32	Morro	0	0.507	9	165	Morro	0	0.508	9
37	Morro	0	0.508	9	167	Morro	0	0.508	9
40	Morro	0	0.508	9	173	Morro	0	0.508	9
47	Morro	0	0.508	9	176	Morro	0	0.507	9
52	Morro	0	0.508	9	179	Morro	0	0.507	9
55	Morro	0	0.508	9	182	Morro	0	0.508	9
56	Morro	0	0.508	9	185	Morro	0	0.509	9
60	Morro	0	0.507	9	188	Morro	0	0.508	9
61	Morro	0	0.515	8	191	Morro	0	0.508	9
63	Morro	0	0.508	9	194	Morro	0	0.507	9
64	Morro	0	0.507	9	198	Morro	0	0.508	8
65	Morro	0	0.507	9	200	Morro	0	0.509	8
66	Morro	0	0.508	9	203	Morro	0	0.507	9
68	Morro	0	0.508	9	207	Morro	0	0.508	8
70	Morro	0	0.506	9	208	Morro	0	0.508	9
72	Morro	0	0.507	9	209	Morro	0	0.507	9
73	Morro	0	0.508	9	210	Morro	0	0.509	9
74	Morro	0	0.509	9	211	Morro	0	0.508	9
76	Morro	0	0.506	9	212	Morro	0	0.51	8
77	Morro	0	0.508	9	213	**Morro**	**2.08**	**0**	**9**
78	Morro	0	0.507	9	214	Morro	0	0.508	8
80	Morro	0	0.508	9	215	Morro	0	0.508	9
81	Morro	0	0.507	9	218	Morro	0	0.506	9
82	Morro	0	0.508	9	219	Morro	0	0.508	8
83	Morro	0	0.508	9	221	Morro	0	0.507	9
84	Morro	0	0.508	9	222	Morro	0	0.507	9
85	Morro	0	0.508	9	224	Morro	0	0.509	9
86	Morro	0	0.508	9	225	Morro	0	0.508	9

87	Morro	0	0.507	9
88	Morro	0	0.512	8
89	Morro	0	0.507	9
90	Morro	0	0.508	9
91	Morro	0	0.507	9
92	Morro	0	0.508	9
93	Morro	0	0.506	9
94	Morro	0	0.518	8
95	Morro	0	0.507	9
96	Morro	0	0.509	9
98	Morro	0	0.507	9
99	Morro	0	0.508	9
100	Morro	0	0.509	9
101	Morro	0	0.509	9
105	Morro	0	0.509	9
106	Morro	0	0.506	9
108	Morro	0	0.509	9
110	Morro	0	0.507	9
114	**Morro**	**0.706**	**0.005**	**9**
115	Morro	0	0.508	9
116	Morro	0	0.508	9
118	Morro	0	0.509	8
123	Morro	0	0.507	9
125	Morro	0	0.507	9
128	Morro	0	0.509	9
129	Morro	0	0.507	9
130	Morro	0	0.509	9
131	Morro	0	0.511	8
133	Morro	0	0.507	9
134	Morro	0	0.508	9
137	Morro	0	0.508	9
139	Morro	0	0.507	9
140	Morro	0	0.507	9
141	Morro	0	0.508	9
143	Morro	0	0.507	9
144	Morro	0	0.535	6

227	Morro	0	0.51	9
228	Morro	0	0.508	9
230	Morro	0	0.509	8
252	Morro	0	0.506	9
253	Morro	0	0.509	9
254	Morro	0	0.507	9
255	Morro	0	0.507	9
256	Morro	0	0.508	9
269	Morro	0	0.508	9
270	Morro	0	0.509	9
271	Morro	0	0.507	9
273	Morro	0	0.506	9
274	Morro	0	0.507	9
280	Morro	0	0.505	9
281	Morro	0	0.508	9
282	Morro	0	0.507	9
283	Morro	0	0.508	9
284	Morro	0	0.508	9
285	Morro	0	0.508	9
231	**Bodega**	**0.361**	**0.005**	**9**
232	Bodega	0	0.508	7
233	Bodega	0	0.507	9
234	Bodega	0	0.509	8
235	Bodega	0	0.507	9
236	Bodega	0	0.507	9
237	Bodega	0	0.511	9
238	Bodega	0	0.507	9
239	Bodega	0	0.509	9
240	Bodega	0	0.51	8
241	Bodega	0	0.507	9
242	Bodega	0	0.507	9
243	Bodega	0	0.507	9
244	Bodega	0	0.504	9
245	Bodega	0	0.503	9
246	Bodega	0	0.51	8
247	Bodega	0	0.511	8
248	Bodega	0	0.506	9
249	Bodega	0	0.506	9
250	Bodega	0	0.509	8

Table 3: **Summary of diversity values for Morro and Bodega Bays**. N - number of samples analyzed; G - number of genets (or the number of multilocus genotypes); AR - allelic richness rarefied to lowest sample size; H_o - Observed heterozygosity; H_e - expected heterozygosity under HWE; F_{is} - inbreeding coefficient (ns = not significant at a 0.05 level)

Location	Morro Bay	Bodega Bay
N	122	20
G	117	20
AR	3.46	3.10
H_o	0.519	0.494
H_e	0.503	0.515
F_{is}	-0.021^{ns}	0.073^{ns}

7. FIGURES

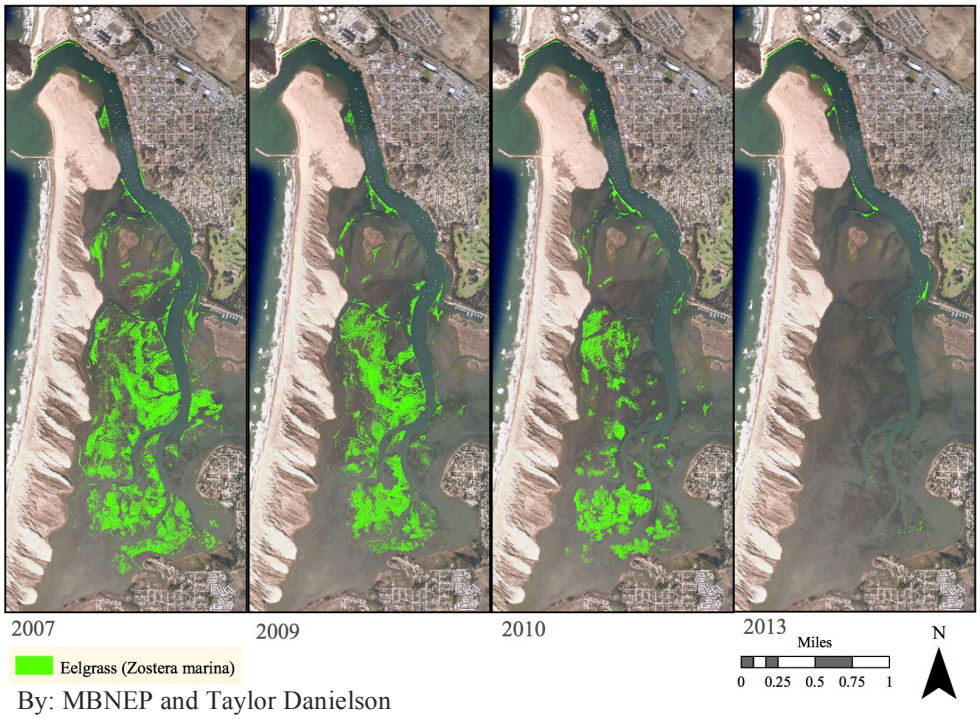

Figure 1: **Map of historic trends of eelgrass acreage in Morro Bay**. Eelgrass cover in Morro Bay for 2007, 2009, 2010, and 2013 created using data from interferometric sidescan sonar, (from the Morro Bay National Estuary Program and Taylor Danielson).

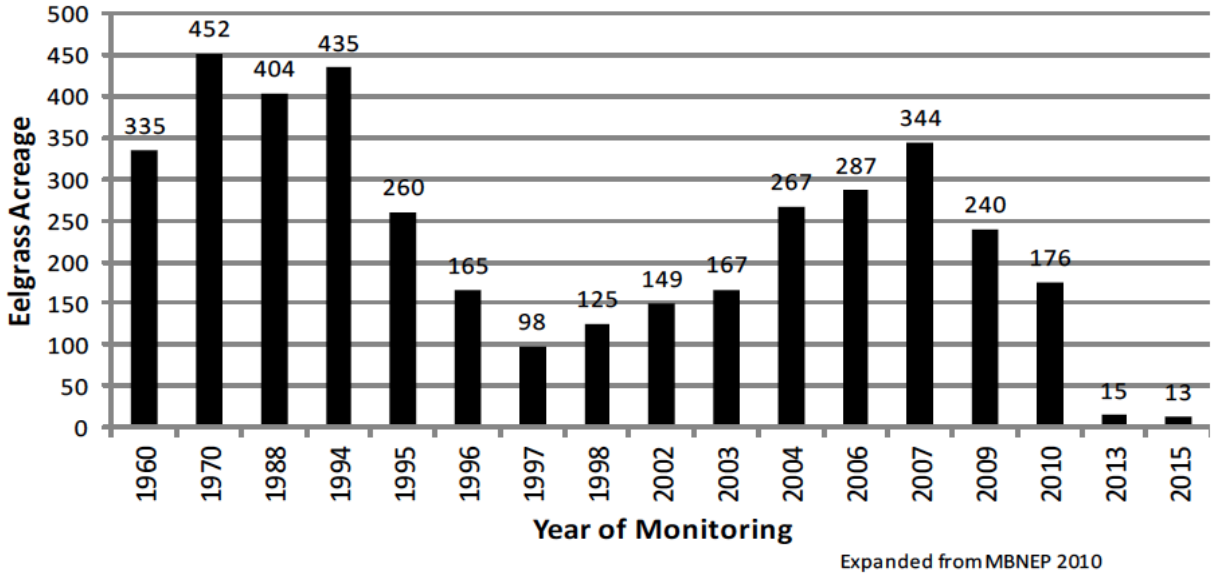

Figure 2: **Bar plot of historic trends of eelgrass acreage in Morro Bay.** Figure expanded from MBNEP state of the bay report (2010).

Figure 3: **Map of eelgrass beds in Morro Bay**. Rough location of the remaining eelgrass beds, drawn to include all areas where samples were collected. The Northernmost seven (Coleman, North Sandspit, Embarcadero, Tidelands, Reference Bed, Windy Point, and Marina) are the beds that remained in 2013, and the Southernmost three (Oyster Farm, Channel, and Mitchell) are beds that were found in 2016.

Figure 4: **Map of sample collection sites in Morro Bay**. Specifically a map of initial sampled beds and collection locations. Blue dots represent individuals that we identified as clones of other sample and removed from analysis. All displayed samples were collected between 13 July and 8 Aug 2016. The three back bay beds are excluded here (pictured in Figure 3) as only rough location data was recorded for these samples, which were collected on January 27th, 2017.

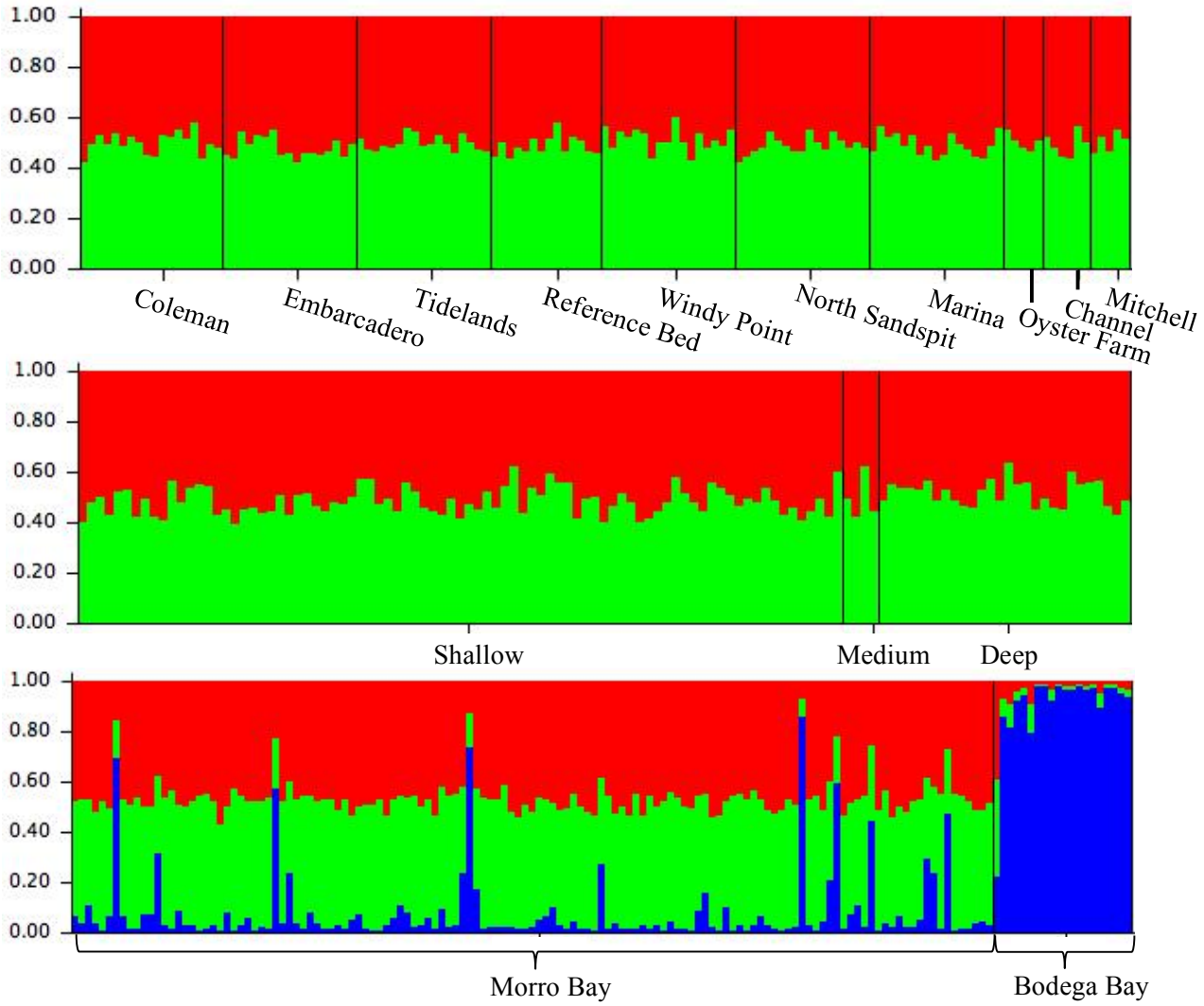

Figure 5: **Bayesian clustering diagram from STRUCTURE for Morro Bay by bed and depth, and for Morro Bay as compared to Bodega Bay**. By remaining bed (top), by depth (middle), and Morro Bay with Bodega Bay (bottom). Each bar represents an individual, each color represents a genetic cluster, and the y-axis represents the probability of assigning an individual to a given genetic cluster. The number of genetic clusters was determined the methods of Evanno et al. 2005.

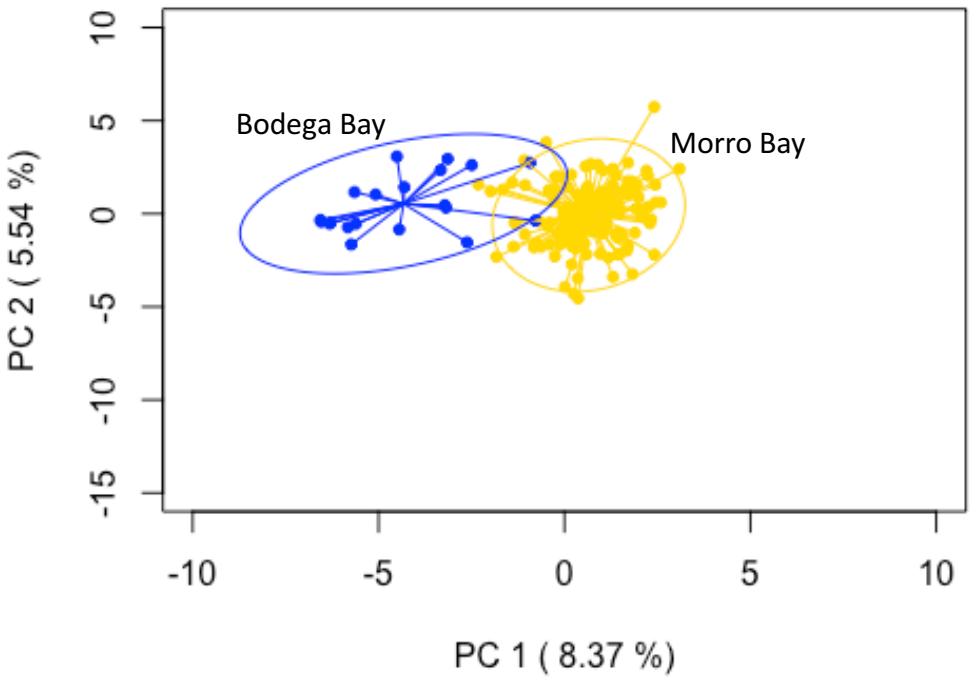

Figure 6: **Principal component analysis of Morro Bay as compared with Bodega Bay**. Generated in R with package "ade4". Bodega Bay samples indicated in Blue, Morro Bay samples indicated in yellow. Ellipses representing 95% confidence intervals.

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APPENDIX
Supplementary Tables and Figures

Table A1: **Morro Bay sample details.** Sample number and collection locations for all Morro Bay samples. Samples were collected in July and August of 2016.

Morro Bay Samples								
ID	Latitude	Longitude	ID	Latitude	Longitude	ID	Latitude	Longitude
1	35.36805789	-120.8652949	90	35.3592742	-120.8518049	161	35.36941668	-120.8603903
3	35.36921161	-120.864485	91	35.35903196	-120.8517465	164	35.3693062	-120.8600168
7	35.36951386	-120.8645153	92	35.35869819	-120.8515763	165	35.36885777	-120.8588598
8	35.36951386	-120.8645153	93	35.35850293	-120.8516264	167	35.36867463	-120.8585701
14	35.37038	-120.864183	94	35.35833136	-120.8515074	173	35.36843729	-120.8580056
17	35.37030868	-120.8642409	95	35.35791029	-120.8512718	176	35.3680122	-120.8570616
18	35.37049983	-120.8642177	96	35.35784508	-120.8508936	179	35.36747987	-120.856837
21	35.37078854	-120.8638937	98	35.3575111	-120.8502222	182	35.36679733	-120.8565517
27	35.37098162	-120.8633586	99	35.35735713	-120.8498638	185	35.36629023	-120.8562271
28	35.37098162	-120.8633586	100	35.35706774	-120.8494219	188	35.36531684	-120.8559468
31	35.37097299	-120.8633542	101	35.3588919	-120.8538865	191	35.36518549	-120.8559191
32	35.37118995	-120.8629648	105	35.35834921	-120.8537647	194	35.36405356	-120.8557614
37	35.37170129	-120.8619865	106	35.35775682	-120.8533534	198	35.36333837	-120.8554021
40	35.37182706	-120.861732	108	35.35747079	-120.8531624	200	35.3629746	-120.8553558
47	35.372279	-120.860537	110	35.35665929	-120.8524156	203	35.36223225	-120.8551464
52	35.3720286	-120.8597388	114	35.35596255	-120.8514633	207	35.36165868	-120.8550212
55	35.37172614	-120.859159	115	35.35571306	-120.8513638	208	35.36165868	-120.8550212
56	35.37166969	-120.8590584	116	35.35558871	-120.8509714	209	35.36165868	-120.8550212
60	35.37120676	-120.8583057	118	35.35873457	-120.854025	210	35.36165868	-120.8550212
61	35.37113748	-120.858081	123	35.35654844	-120.8522793	211	35.34828862	-120.8448991
63	35.37039794	-120.8569237	125	35.35615248	-120.8518504	212	35.34815937	-120.8448248
64	35.37009406	-120.8564665	128	35.35548285	-120.8509513	213	35.34792527	-120.8449966
65	35.37009406	-120.8564665	129	35.35560305	-120.8507662	214	35.34776492	-120.844805
66	35.37009406	-120.8564665	130	35.35555691	-120.8508089	215	35.34755189	-120.8449588
68	35.369913	-120.856111	131	35.35303869	-120.8456395	218	35.34724076	-120.8450261
70	35.3694989	-120.8552543	133	35.3528257	-120.8458055	219	35.34711528	-120.8450613
72	35.36922431	-120.8549603	134	35.35275949	-120.8456412	221	35.34709751	-120.8458123
73	35.369199	-120.85495	137	35.35247966	-120.845714	222	35.34688713	-120.8452623
74	35.36917532	-120.854779	139	35.35229408	-120.845567	224	35.34672359	-120.8458011

76	35.36882	-120.854606	140	35.35198328	-120.8454799	225	35.34674778	-120.8460459
77	35.368674	-120.85456	141	35.35176632	-120.8457559	227	35.34647821	-120.8459431
78	35.366797	-120.853975	143	35.35165183	-120.8463034	228	35.34746958	-120.8455445
80	35.36251045	-120.8525944	144	35.35169637	-120.8457265	230	35.34665046	-120.8447193
81	35.36220908	-120.8524759	145	35.35139869	-120.845411	252-256	35.3221	-120.8517
82	35.36220908	-120.8524759	147	35.35133574	-120.8451764	269-274	35.3358	-120.8445
83	35.36150064	-120.8523266	148	35.35116072	-120.844921	280-285	35.3286	-120.8492
84	35.36137579	-120.8521962	149	35.35105369	-120.8451168	**Clones**		
85	35.36099244	-120.8520596	151	35.35084908	-120.8448352	46	35.372233	-120.860513
86	35.36055206	-120.8519898	152	35.35068203	-120.8446319	69	35.36959114	-120.85555
87	35.35863948	-120.8514046	154	35.3503411	-120.8445491	111	35.35648583	-120.852191
88	35.36018719	-120.8520077	156	35.3498977	-120.8448042	136	35.3524807	-120.8456017
89	35.35944305	-120.8517932	158	35.3694989	-120.8607153	170	35.36856122	-120.8582397

Table A2: **Bodega Bay sample details.** Sample ID and original collector name, collection date, paper in which samples were used previously, and when available, collection site and depth.

Bodega Bay Samples					
ID	Original Collector	Collection Year	Reference Paper	Collection Site	Collection Depth
DPS14	Jessica Abbott	2012	Kamel et al. 2012	Doran Park	Subtidal
DPH04	Jessica Abbott	2012	Kamel et al. 2012	Doran Park	High
DPH06	Jessica Abbott	2012	Kamel et al. 2012	Doran Park	High
MML15	Jessica Abbott	2012	Kamel et al. 2012	Mason's Marina	Low
MMS08	Jessica Abbott	2012	Kamel et al. 2012	Mason's Marina	Subtidal
MMS17	Jessica Abbott	2012	Kamel et al. 2012	Mason's Marina	Subtidal
CCS09	Jessica Abbott	2012	Kamel et al. 2012	Campbell's Cove	Subtidal
WPH03	Jessica Abbott	2012	Kamel et al. 2012	Westside Park	High
WPL05	Jessica Abbott	2012	Kamel et al. 2012	Westside Park	Low
WPH12	Jessica Abbott	2012	Kamel et al. 2012	Westside Park	High
WPS20	Jessica Abbott	2012	Kamel et al. 2012	Westside Park	Subtidal
WPS03	Jessica Abbott	2012	Kamel et al. 2012	Westside Park	Subtidal
Red1	Randall Hughes	2004	Hughes et al. 2009		
Orange	Randall Hughes	2004	Hughes et al. 2009		
Green1	Randall Hughes	2004	Hughes et al. 2009		
Yellow1	Randall Hughes	2004	Hughes et al. 2009		
Blue1	Randall Hughes	2004	Hughes et al. 2009		
Gray	Randall Hughes	2004	Hughes et al. 2009		
Purple1	Randall Hughes	2004	Hughes et al. 2009		
White2	Randall Hughes	2004	Hughes et al. 2009		

Table A3: **Microsatellite allele length dataset.**

ID	CL32	CL32	ZMC 12075	ZMC 12075	ZMC 13053	ZMC 13053	ZCT3	ZCT3	ZGA2	ZGA2
1	112	112	121	121	102	117	128	130	180	182
3	112	112	-9	-9	102	117	128	136	180	180
7	112	112	121	121	102	119	128	132	182	182
8	112	112	121	125	102	102	128	132	180	182
14	112	112	118	125	102	102	128	132	180	182
17	112	112	-9	-9	117	117	128	130	182	184
18	112	112	118	121	102	117	128	136	182	182
21	112	115	121	121	102	117	130	132	176	182
27	112	112	121	125	102	102	126	130	180	182
28	112	112	121	121	102	117	126	128	180	180
31	112	112	118	121	102	102	126	130	176	182
32	112	112	118	121	102	102	126	130	176	182
37	112	112	118	121	102	102	130	130	176	180
40	112	112	121	121	102	117	123	130	180	182
47	112	112	121	121	113	121	128	140	180	180
52	112	112	121	121	102	117	130	130	180	182
55	112	112	121	121	102	117	128	136	180	182
56	112	112	121	121	102	117	130	130	180	180
60	112	115	121	121	102	102	126	128	180	182
61	-9	-9	121	121	102	117	126	126	180	182
63	112	112	118	121	117	121	126	130	176	180
64	112	112	125	125	102	102	128	130	182	182
65	112	112	125	125	102	117	128	128	176	180
66	112	112	121	121	102	117	126	134	178	180
68	112	115	125	125	102	102	126	128	180	180
70	112	112	121	121	102	102	126	128	176	182
72	112	112	121	125	117	117	126	130	180	182
73	112	112	121	121	102	102	126	128	182	182
74	112	112	121	121	102	117	123	128	182	182
76	112	112	121	121	102	117	128	130	180	182
77	112	112	121	121	102	117	126	128	176	180
78	112	115	121	121	102	102	128	128	180	182
80	112	112	118	121	102	117	126	128	176	180
81	112	112	121	121	102	117	128	132	180	182
82	112	112	121	121	102	117	128	132	180	180
83	112	112	121	125	102	102	126	130	174	176

84	112	115	121	121	102	117	128	130	180	180
85	112	112	121	125	102	117	126	128	180	182
86	112	112	121	121	102	102	128	132	176	182
87	112	112	118	121	102	117	126	128	180	180
88	112	112	-9	-9	102	117	128	130	176	180
89	112	112	121	125	102	102	128	128	176	182
90	112	112	121	121	117	121	128	132	176	180
91	112	112	125	125	102	102	128	128	180	180
92	112	112	121	121	102	102	123	128	182	182
93	112	112	125	125	102	117	128	130	176	180
94	112	112	114	121	102	102	126	130	180	182
95	112	112	121	125	102	117	128	130	180	182
96	112	112	121	125	102	117	126	128	176	180
98	112	112	114	121	102	117	128	128	174	180
99	112	112	118	121	102	102	128	132	180	182
100	112	112	118	121	102	102	128	130	180	182
101	112	112	121	125	102	102	128	130	180	182
105	112	115	121	121	102	102	130	136	180	180
106	112	112	121	121	102	102	128	128	180	180
108	112	112	121	121	102	117	126	136	180	182
110	112	112	121	121	102	102	128	128	176	182
114	112	115	121	121	102	117	128	136	180	182
115	112	112	121	121	117	117	128	130	176	180
116	112	112	121	129	102	117	128	128	178	182
118	112	112	121	121	117	117	132	136	174	182
123	112	115	121	125	102	117	128	130	182	182
125	112	112	121	121	102	102	128	136	178	180
128	112	112	121	125	102	117	130	136	182	182
129	112	112	121	121	102	117	128	128	180	182
130	112	112	118	121	102	102	128	128	180	182
131	112	115	-9	-9	117	119	128	130	182	182
133	112	115	121	121	102	102	128	136	182	182
134	112	115	121	129	102	102	128	130	180	182
137	112	112	121	125	102	102	130	136	182	182
139	112	112	121	121	102	102	130	132	180	182
140	112	112	114	121	102	119	128	132	180	180
141	112	115	121	121	102	117	128	130	182	182
143	112	112	121	125	102	102	126	128	176	182
144	-9	-9	121	125	102	102	128	132	-9	-9

145	112	112	121	125	102	121	126	134	180	184
147	112	112	121	121	102	117	128	130	176	176
148	112	112	121	121	102	102	126	128	180	182
149	112	112	118	125	102	117	128	130	180	184
151	112	112	118	125	102	102	128	130	180	180
152	112	112	121	125	102	102	128	132	180	182
154	112	112	121	125	102	117	126	128	170	182
156	112	112	125	125	102	117	128	128	176	184
158	112	112	121	121	102	117	126	128	180	182
161	112	112	121	125	102	117	128	130	180	182
164	112	112	121	125	102	117	126	128	182	182
165	112	112	121	121	117	117	128	136	180	182
167	112	112	115	121	102	117	130	140	180	182
173	112	112	114	125	102	102	130	132	180	180
176	112	112	125	125	102	117	128	132	180	180
179	112	112	121	125	102	117	128	128	180	180
182	112	112	121	125	102	102	128	130	182	182
185	112	112	125	125	102	117	128	128	176	184
188	112	112	121	125	102	117	128	130	180	182
191	112	112	121	121	102	102	128	130	180	180
194	112	115	121	121	102	102	126	134	180	180
198	112	115	118	121	102	117	-9	-9	180	180
200	112	112	121	125	102	117	126	128	180	182
203	112	115	121	121	102	117	126	136	180	180
207	112	112	121	121	102	117	-9	-9	180	182
208	112	112	121	125	102	102	128	130	180	182
209	112	112	125	125	117	119	130	134	180	182
210	112	112	121	121	102	117	130	132	180	180
211	112	112	121	121	102	117	130	132	180	180
212	112	112	114	121	102	117	128	130	182	182
213	112	112	121	121	102	117	128	130	180	182
214	112	112	121	121	102	117	128	132	180	182
215	112	115	121	121	102	117	126	126	182	182
218	112	112	121	125	102	117	128	128	182	182
219	112	112	121	121	102	117	-9	-9	180	182
221	112	112	121	125	102	117	128	130	176	180
222	112	112	121	125	102	121	130	130	182	182
224	112	112	121	121	102	102	128	136	182	182
225	112	112	121	121	102	117	128	128	180	182

227	112	112	121	121	102	117	128	128	182	182
228	112	118	121	121	102	102	126	128	176	180
230	112	112	115	121	102	119	-9	-9	180	182
252	112	112	121	121	102	117	128	136	180	180
253	112	112	121	125	117	117	128	130	176	182
254	112	112	121	125	102	117	128	128	180	182
255	112	115	125	125	102	102	128	130	176	180
256	112	112	118	121	102	117	126	132	176	180
269	112	112	121	125	102	117	128	136	180	180
270	112	112	121	125	102	102	128	130	180	182
271	112	112	121	125	102	102	128	130	176	182
273	112	112	121	121	102	102	126	132	180	180
274	112	112	121	121	102	102	130	130	180	180
280	112	112	121	121	117	117	123	126	180	180
281	112	112	114	121	102	102	126	126	180	182
282	112	112	121	121	102	117	128	128	182	182
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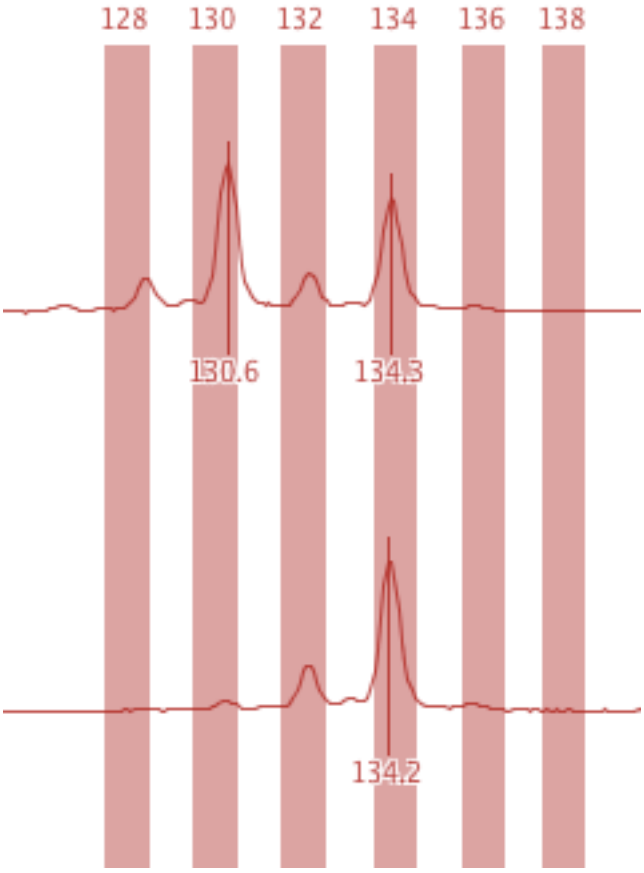

Figure A1: **Peak calling example**. Peaks in the dark read lines are called as fragment lengths when they have a number labeled vertical line. The red shaded bars represent the bins used by geneious to call peak lengths (in this case the 131 and 134 length bins would be utilized). The top individual is a heterozygote with an allele of rough length 131 and another of length 134. The bottom individual is a homozygote with alleles of length 134. Each microsatellite locus displayed a signature peak pattern that I used to accurately assess which peaks were representative of an allele length and which were simply stutter peaks. The pattern can be assessed by comparing clear homozygotes and heterozygotes. In the example above, the pattern is one small peak before a larger peak, with very small peaks preceding each. In looking at the homozygote on bottom alone, if the first peak were comparable in height to the second, one might assume a heterozygote, but by looking at a true heterozygote, we see that the first peak is not a true peak.

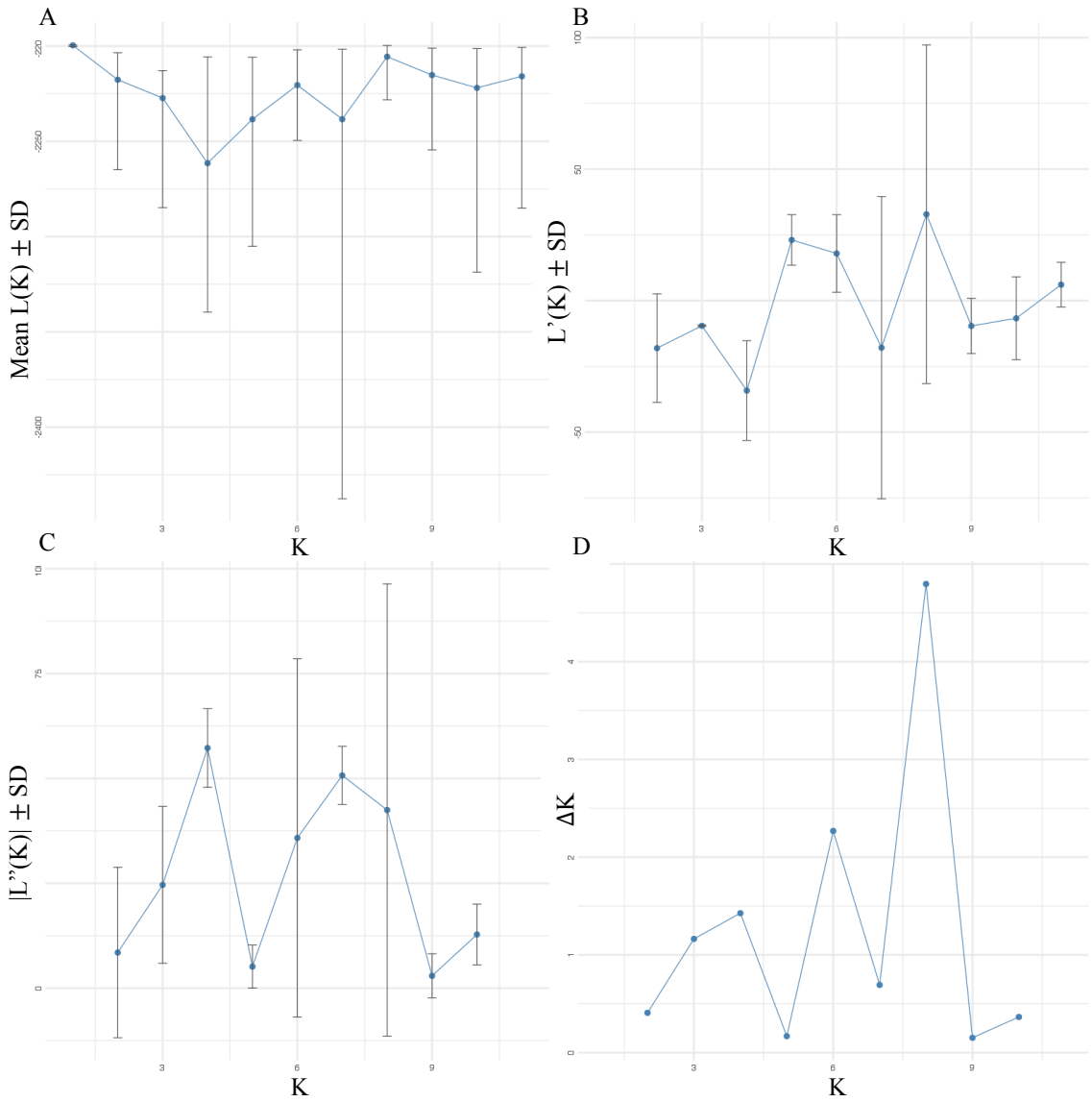

Figure A2: **K-value selection (Evanno method) graphs for Morro Bay**. Output of R package "Pophelper" showing the Evanno method for calculating the proper K from STRUCTURE output for Morro Bay.

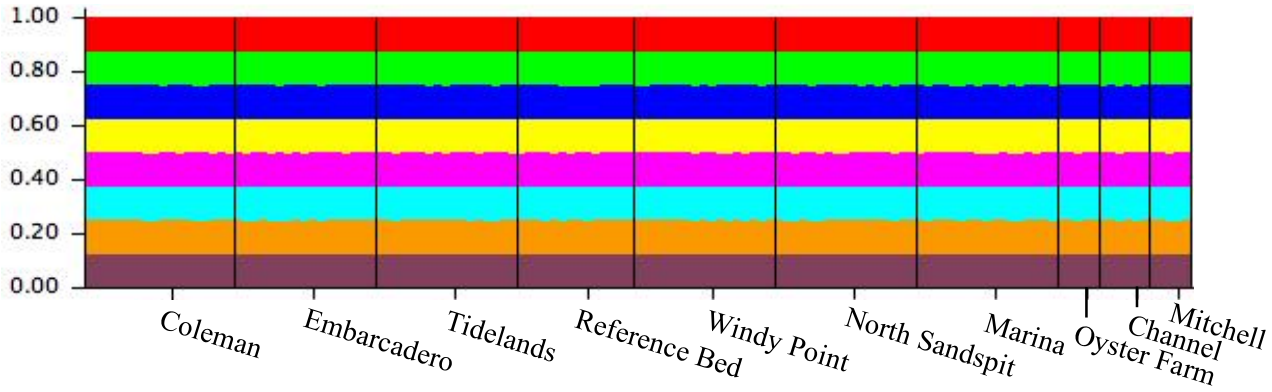

Figure A3: **Bayesian clustering diagram from STRUCTURE with a K=8 for Morro Bay by bed**.

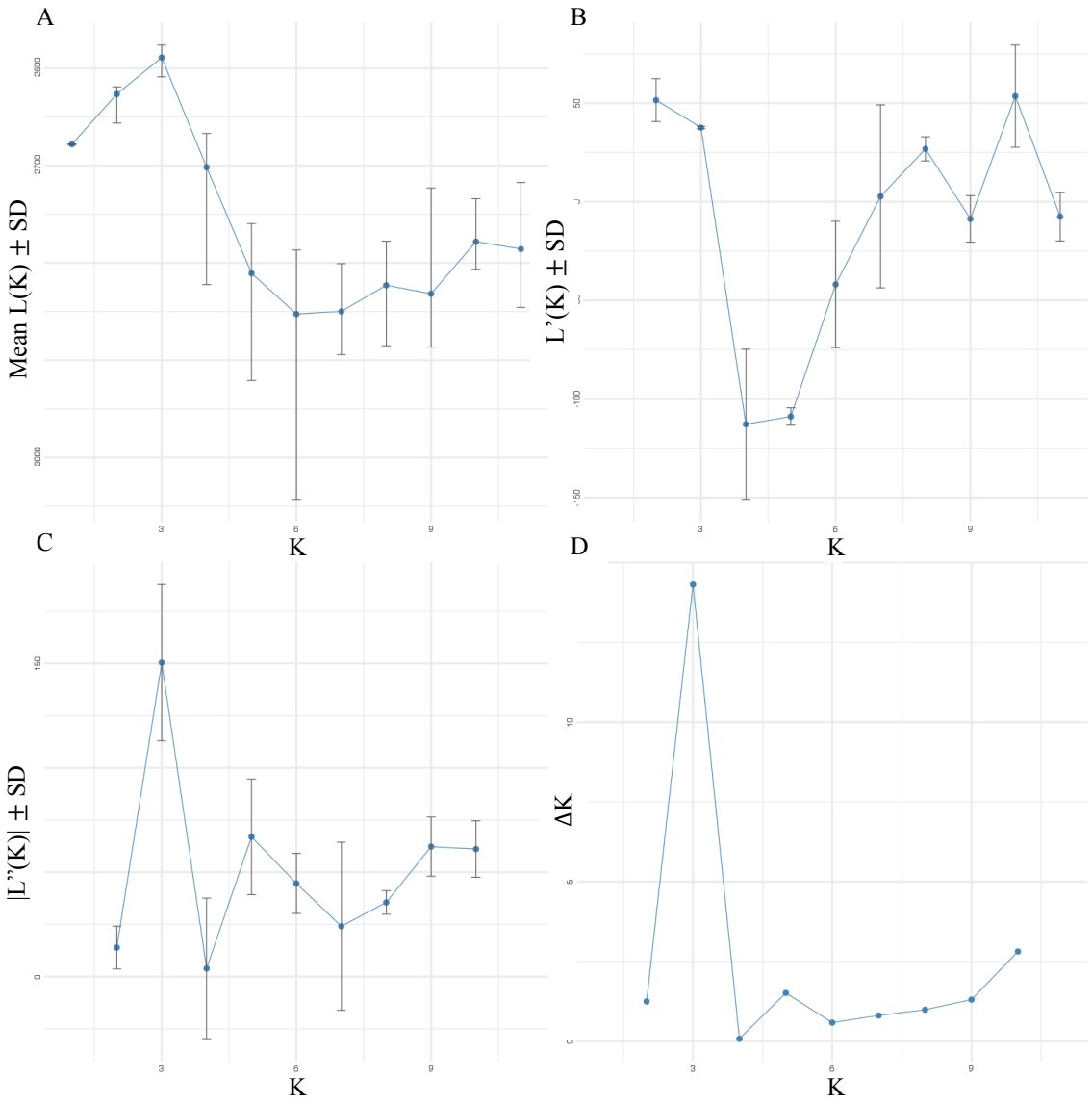

Figure A4: **K-value selection (Evanno method) graphs for Morro and Bodega Bays combined**. Output of R package "Pophelper" showing the Evanno method for calculating the proper K from STRUCTURE output for Morro Bay and Bodega Bay combined.