Title
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SPECIATION AND GENETIC STRUCTURE IN A MARINE FISH WITH AN EXTENDED PELAGIC LARVAL PHASE: AN ANALYSIS OF BOTH THE JUVENILE AND ADULT POPULATIONS OF BLUE ROCKFISH (SEBASTES MYSTINUS)

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ABSTRACT
In long-lived species the reproductive population contains many generations, and the genetic structure of that population integrates many year-classes produced under a variety of conditions that affect the mating of adults and dispersal of larvae. Analyzing the genetic structure of both the adult population and individual year-classes provides information on both evolutionary events that shaped the population genetic structure and contemporary events that may have limited or promoted dispersal. As settled juveniles and adults, blue rockfish (Sebastes mystinus) are non-migratory inhabitants of kelp and rocky reef habitats along the California coast, contain approximately 8 generations of reproductive adults, and possess a pelagic larval and juvenile stage lasting over three months. Given the extended pelagic larval phase of this species, there is a potential for long-distance dispersal. We analyzed the genetic structure of the adult population throughout the range and contrast this to two different juvenile year-classes by using microsatellite markers and mitochondrial DNA sequence data (D-Loop). Results of the population genetic analysis of the adults revealed an extended area of admixture between two genetically distinct types of blue rockfish. Despite a lack of apparent morphological differences, the results of the genetic analyses suggest that there are reproductive barriers between the two types of blue rockfish. In addition, we found significant genetic divergence between the two adult locations south of Point Conception compared to populations to the north. Results of the microsatellite analyses also revealed significant genetic structure among juvenile locations in both year-classes that was spatially and temporarily ephemeral within California. These results suggest that potential dispersal may not translate into realized dispersal in a given year and that dispersal ability of larvae or reproductive potential of the adults may vary annually. Therefore, the genetic structure of individual year-classes within that adult population provides additional information that may help to elucidate the evolutionary relationships among adult locations throughout the species’ range and reveal the mechanisms that promote reproductive isolation or barriers between the two types of blue rockfish.

INTRODUCTION
Rockfishes (Genus Sebastes) that inhabit California’s coastal waters have been and continue to be a cornerstone of the state’s commercial and recreational marine fisheries. Several species of rockfish have suffered precipitous population declines in California, leading to unprecedented shelf-wide closures of groundfish fisheries at enormous economic costs1,2. While these draconian measures markedly reduce further fishing mortality on offshore stocks, the consequences of displaced commercial and recreational fishing effort to inshore stocks is unclear. Inshore species have experienced heavy exploitation for decades and several measures, including a statewide network of marine protected areas (MPAs), are being considered to prevent overexploitation of these stocks. All aspects of fisheries management, including networks of MPAs and other ecosystem-based fisheries management tools, require knowledge of the spatial scale of genetic exchange or movement of individuals among populations and degree to which this renders the stock self-replenishing. For example, a network design of MPAs for rockfishes requires knowledge of the spatial scale of larval dispersal to determine spacing and size of individual self-replenishing MPAs, such that the network maximizes the area over which stocks are replenished3,4. Thus, understanding spatial patterns of gene flow and movement of individuals among populations is critical for
assessing impacts of fishing on genetic composition and diversity of a stock, identifying relative contributions of local populations to replenishment of others, and how such spatial patterns vary through time in a dynamic coastal ocean\textsuperscript{5-7}. One critical piece missing from our understanding of “open” marine populations such as rockfishes, is the degree of connectivity among populations throughout the species range. Research programs addressing connectivity in coastal marine ecosystems should be designed such that the results can be directly applied to the management and conservation of exploited resources. In addition, research on spatial and temporal patterns of gene flow in marine environments will clarify how evolutionary processes are linked to ecological processes, such as how genetic structure of stocks (i.e. differences in gene frequencies among populations) is linked to larval dispersal among “open” populations.

Many marine organisms, including most of the nearshore rockfish species, are sedentary as adults and have larvae that are pelagic for 2 to 6 months, allowing the potential for dispersal over long distances. Analyses of the genetic structure of such marine organisms measure the level of realized connectivity among populations of the species throughout its range. In long-lived species like rockfishes, the genetic composition of the adult population reflects many year-classes, produced under a variety of conditions that affect mating of adults and dispersal of larvae. In contrast, the genetic composition of an individual year-class is not integrated over multiple years and indicates the direct effects of factors acting at the time of year-class formation. Therefore, an analysis of the genetic structure of new year-classes is essential to understanding dispersal potential for a given adult population. Since young-of-the-year (<1 year olds) reflect the genetic output of adult populations at a given time, spatial or temporal variation in the genetic composition of settling juveniles may reveal oceanographic, geographic, behavioral, or life history factors that influence larval dispersal and survival and contribute to both the spatial and genetic structure of populations\textsuperscript{6,8}. Information on these processes will be important for understanding the persistence of marine populations and is critical for developing sound management strategies\textsuperscript{9,10}.

Our current understanding of the relationship between genetic structure and dispersal ability in marine species is unresolved due to several studies with contrasting results\textsuperscript{10-12}. The discrepancy among studies has stimulated several hypotheses to explain situations were genetic structure is observed despite a potential for long-distance dispersal\textsuperscript{13-15}. Genetic patchiness among locations could be caused by settlement of individuals that represent just a small portion of the genetic composition of the adult population (larval survival bottleneck or variation in reproductive output among adults), by natural selection that may occur either before or after settlement, or settlement of juveniles that are comprised of patches from different parts of a genetically divergent adult population. Several studies have found genetic variation in the composition of young-of-the-year among years and locations within a given region\textsuperscript{9,16-18}. If there is variation within a region and this variation changes between years, processes acting prior to settlement, such as genetic drift or natural selection, may have occurred (natural selection\textsuperscript{14}, genetic drift or “sweepstakes” recruitment\textsuperscript{13}). Alternatively, a pattern among years in the genetic variation observed after settlement may suggest the effects of post-settlement selection or local adaptation within the species range\textsuperscript{19}.

In this study we examined the genetic patterns and consequences of larval dispersal for the exploited rockfish, blue rockfish (\textit{Sebastes mystinus}), which inhabits nearshore rocky reefs and kelp forests along the California coast and is targeted by nearshore commercial live-fish and recreational fisheries\textsuperscript{20,21}. Blue rockfish exemplify early life-histories that characterize most nearshore rockfish species. Young of the blue rockfish are born in the winter and have an extended pelagic phase for up to 6 months\textsuperscript{21}. The pelagic larval duration for blue rockfish is longer than most other species within the genus. Since the adults move little (<1-2 km\textsuperscript{22}), larval dispersal during the pelagic phase is the mechanism maintaining population connectivity throughout its respective range. Our goal was to characterize the effect of pelagic duration reflected in extended duration (up to 6 months) on the genetic structure of adults and of settling juveniles, and to analyze whether juveniles from different year-classes have the same degree of genetic continuity as
that found in the adult population. In addition, we analyzed the phylogeography of blue rockfish throughout its range to understand both evolutionary relationships among locations and identify genetic breaks within the species range. Information on geographic locations of genetic structure will be critical for identifying stocks (or management units), spatial and temporal scales of larval dispersal, and population connectivity for the species.

SPECIFIC GOALS OF THE RESEARCH

For analyzing and comparing the level of connectivity for this nearshore rockfish, we characterized and compared the genetic structure of adults and three year-classes of juvenile blue rockfish. In doing so, we addressed five fundamental questions:

Q1) Given the extended pelagic larval duration for blue rockfish, is the adult population genetically homogeneous throughout its range? (If not, are there geographic-base population breaks, indications of range expansion or contraction (e.g. population bottlenecks)).

Q2) Is the genetic structure of recently settled young-of-the-year rockfish homogenous among locations, reflecting a well-mixed larval pool along the coast? (If not, what is the spatial and temporal pattern of this structure?)

Q3) What portion of the adult population contributes to a given year-class (effective population size) and does that portion vary regionally and between years?

Q4) Does the spatial genetic structure of individual year-classes of young-of-the-year correspond with the spatial structure of adult populations? (If so, this suggests spatial structure due to local adaptation or dispersal limitation (local retention)).

Q5) Do cohorts of young-of-the-year (individuals of similar birthdates) that arrive over the settlement season at a location exhibit similar genetic composition? (If not, replenishment of a population draws from different parts of the adult population over time or space).

Each of these questions addresses specific aspects of genetic connectivity in marine populations. Given the current lack of understanding of how observed genetic patchiness in marine organisms with high dispersal potential occurs, this study attempts to test several proposed hypotheses over larger spatial and temporal scales than most previous studies. By simultaneously describing genetic structure of both young-of-the-year and adult populations, this study will reveal much more about the movement of larvae and constraints on the reproductive output of adult populations than past studies that examined either larvae or adults alone. The expansive temporal and spatial scale of this project will enhance our understanding of the magnitude and patterns of genetic variation and level of connectivity among locations.

METHODS

Field sampling:

To address the specific research questions outlined above, we completed all sampling of both juvenile and adult blue rockfish over multiple years (2000 to 2002) and over multiple spatial scales (among vs. within regions). To examine among-location genetic structure and for comparisons to adult patterns, we sampled fin-clips from approximately 200 juveniles (young-of-the-year) at locations within and among regions along the coast of California (from Santa Barbara to Fort Bragg; see Map). To identify patterns of genetic structure in adult blue rockfish throughout their population center (San Miguel Island to Fort Bragg), we sampled...
approximately 50 individuals from 10 locations that were evenly distributed between upwelling centers. For the phylogeographic analysis, we sampled 50 adults at 16 locations throughout the range from Santa Barbara, California to Neah Bay Washington.

**Molecular Tools:**

Spatial variation in genetic structure within marine species has been analyzed using nuclear or mitochondrial sequence data, restriction site variation in mtDNA, and microsatellite DNA. Microsatellite analyses facilitated detection of small-scale temporal and spatial genetic structure in larval cod (*Gadus morhua*), which was undetected in earlier studies using allozyme, mitochondrial DNA, or nuclear DNA restriction fragment length polymorphisms. Microsatellite loci are believed to be selectively neutral and to show higher levels of allelic polymorphisms. Due to the high mutation rate (10^{-3} to 10^{-5} mutation/locus), microsatellite loci are ideal for identifying structure in populations with high gene flow especially if the rate of mutation is faster than the rate of genetic exchange. Therefore, microsatellite analysis is a sensitive tool for finding subtle differences within and among adult samples and temporal pulses of settling juveniles. From the extracted genomic DNA, I have amplified 7 microsatellite loci designed from grass rockfish (*S. rastrelliger*; GenBank submission numbers AF269052 – AF269061) using the polymerase chain reaction (PCR). Of the 7 polymorphic microsatellites, five were used in a previous project for my master’s thesis, which will allow comparisons of the 2000 juvenile blue rockfish with the 2001 and 2002 collections and to the two year-classes of kelp rockfish (2001 and 2002). I have optimized and tested assumptions for these 7 microsatellite loci with both the 2000 and 2001 collections of juvenile blue rockfish. For these loci, there is no indication of linkage disequilibrium, of violations of Hardy-Weinberg equilibrium, or of the presence of null alleles and the loci have been tested for Mendelian inheritance. The polymorphism of the loci ranges from high (46 alleles) to moderate (13 alleles). To date, I have completed all of the microsatellite analyses for juvenile blue rockfish (2000, 2001, 2002 year-classes). To ascertain whether the current sample sizes at a given location are appropriate to capture the variation found at individual loci, I computed 1000 permutation re-samples of each collection at the same sample size (2N=100) using the computer software Resampling Stat (see Figure 1). These results show that a sample size of 50 to 100 individuals (2N=100 to 150) is adequate to capture the variation present at individual populations.

For the phylogeographic analyses and to check patterns observed with the microsatellite analyses, we sequenced 310 base pairs (bp) of the 5’ end of the mitochondrial control region (D-Loop) and 850 bp of the nuclear exon Rag1. Sequence data provides information on the evolutionary relationships among the populations of blue rockfish and will confirm patterns found using nuclear-based microsatellite markers. We sequenced 25 adults from all 16 locations for both the D-Loop and Rag1 gene. In addition, we sequenced juveniles from three locations, Fort Bragg, Ocean Cove and Monterey, to check for indications of introgression or hybrids, or both.

**RESULTS AND DISCUSSION**

**Adult Analysis:**

We found two genetically divergent types of blue rockfish within the adult population range using both the microsatellite and the mitochondrial sequence analyses. The degree of genetic divergence was significant and at the level observed between species within the genus *Sebastes*. The microsatellite loci, revealed significant genetic divergence between the two types with a pairwise $F_{ST}$ value of 0.1 ($P < 0.001$), which is greater than the $F_{ST}$ value ($F_{ST} = 0.046$) found between gopher (*Sebastes carnatus*) and black and...
yellow (*Sebastes chrysomelas*) rockfish using microsatellite loci. We found a similar result with the sequence data ($F_{ST} = 0.2; P < 0.001$). Our results showed that there was a range of overlap between the two types that extended from the Sonoma coast of California (north of San Francisco Bay) to southern Oregon (Cape Arago). Therefore, we have one type that was more southerly and one that was more northerly distributed. However, we are in the process of confirming the frequency of the northern-type in the southern part of the range, and we may find that both types have a sympatric distribution throughout the range.

To understand if these two types are “true” species under the biological species concept (BSC), we are confirming the observed patterns found with the microsatellite loci and mitochondrial sequence data by sequencing the nuclear gene Rag1. A similar finding of divergence in sequence data from both a nuclear and mitochondrial gene will provide further evidence that these two types are different species. To further understand the degree of reproductive isolation between these two types, we are analyzing individuals from the range of overlap for any indications of hybrids or introgression between the two types. A similar pattern of divergence between the two types at multiple markers and with little or no introgression or hybridization will help to resolve whether these two types are divergent populations, subspecies, or species under the BSC.

Within the range and looking at each type individually, we found no regional genetic structure with either the microsatellite or sequence analyses (pairwise $F_{ST} = 0.001; P > 0.05$). Therefore, within a region, once we isolated individual types, there was adequate mixing among locations such that each region was genetically homogeneous. However, results from the microsatellite analysis showed significant divergence between two locations south of Point Conception in California (Santa Barbara mainland and Santa Rosa Island) compared to locations in the central coast of California (Avila Beach to Ocean Cove), but the degree of divergence was not at the level found between the two types (microsatellite loci $F_{ST} = 0.02; P < 0.05$).

We did not find significant genetic divergence between these locations and those to the north with the sequence data, which may indicate that this is a more recent divergence.

In the process of analyzing the divergence between the two types of blue rockfish, we discovered that closest sister taxa to blue rockfish, widow rockfish (*Sebastes entomelas*), was more closely related to samples in the northern part of the range (northern Oregon and Washington). Looking at pairwise $F_{ST}$ values for the sequence data, we found $F_{ST}$ values of 0.3 between the northern samples of blue rockfish (Oregon and Washington) and 0.5 between the central/southern samples of blue rockfish (Ocean Cove to Santa Barbara) and widow rockfish. We are now in the process of confirming these patterns using both the D-Loop and Rag1 sequence data and by analyzing closely related taxa to both blue and widow rockfish.

The results of the analysis of the adult blue rockfish were surprising given that the extended pelagic duration increases the probability of dispersal and genetic exchange, which would cause little or no genetic differentiation in the population. Blue rockfish are found over an extensive geographic and dynamic coastal area, have differences in population densities throughout the range, and are probably subjected to different ecological and population conditions that may cause genetic differentiation in parts of the range. Yet, we found a level of genetic divergence that corresponds to the genetic differences found between different species of rockfish. Within each of these types, there appears to be little genetic divergence with the exception of the differences between the southern samples (Santa Barbara and Santa Rosa Island) and the central coast samples. Therefore, it appears that we have two genetically homogenous types of blue rockfish that have different frequencies of abundance throughout the range. To confirm this pattern, we will conduct additional sampling within the population center to, (1) resolve the frequency of the two types in this area and any geographic differences in that frequency, and (2) conduct a morphological analysis of both types to see if there are functional differences in the area were we have adequate numbers of both types (Fort Bragg). Since there are no apparent morphological differences between the two types, we will also confirm the habitat type and usage (e.g., stomach content) for those
individuals we sample. Finally, using the genetic signature of multiple year-classes we can understand the reproductive success of both types within the population center in California.

**Juvenile Analysis:**

We analyzed juvenile blue rockfish within the population center, from the southern California bight, throughout the central coast, to the northern coast of California, over three different years (2000, 2001, and 2002). We found a temporally consistent pattern of genetic divergence between juveniles from the Sonoma and Mendocino (Fort Ross to the north; see Map) coasts compared to samples from the Monterey peninsula to southern California (Sandhill bluff to the south; see Map). The degree of genetic divergence was similar to that found in the adult population between the two types ($F_{ST} = 0.1; P < 0.001$). This pattern was consistent among years (2000, 2001, and 2002). However, within the northern region in 2001 and 2002 two individual samples (Fort Ross in 2001 and Albion River in 2002) appeared to be a mixture of both types. In the central coast region (Monterey Peninsula, Big Sur coast, and Avila Beach) individual year-classes appeared to be genetically homogeneous with a few exceptions. The juvenile location of Monterey in 2001 and 2002 was genetically distinct from neighboring locations of Terrace Point and Sandhill Bluff and genetically similar to locations to the south (Carmel, Point Lobos). In addition, the 2002 juvenile sample at Naples Reef had significant genetic differentiation from those samples to the north. Similar to what we observed in the adult population, the degree of genetic divergence within the central coast was lower than the between region difference (within the central coast $F_{ST} = 0.01; P < 0.05$).

The results from the juvenile genetic analysis provided three critical pieces of information: (1) it confirms the patterns we observed in the adult population of two morphologically similar yet genetically distinct groups of blue rockfish that have an overlapping distribution within the Sonoma and Mendocino coasts of California, (2) both juvenile and adult samples south of Point Conception were significantly divergent from locations to the north, and (3) within a region of high adult population densities, juveniles showed genetic patchiness that was temporally and spatially variable. For example, within the northern region, some sampling locations were different from adjacent locations one year and similar the following year (Fort Ross in 2001 and 2002) and the Monterey juveniles were genetically distinct from adjacent locations but not distant ones. However, the northern samples were probably admixtures of the two types of blue rockfish adults found in this area. The genetic patchiness in the Monterey region, which has high adult population densities and appears to be predominately made up the southerly-distributed type of adults, was probably the results of differential survival of offspring (e.g. small effective population size).

**SIGNIFICANCE TO CALIFORNIA’S COASTAL ENVIRONMENTAL QUALITY**

Several species of rockfish currently suffer from overfishing in California and remediation is required to replenish depleted stocks. Pelagic and benthic juvenile rockfishes are key forage species for a wide diversity of coastal marine species including salmon, seabirds and many commercially and recreationally important fishes (e.g., lingcod, adult rockfishes), therefore, depleted stocks will have effects throughout the entire coastal ecosystem\textsuperscript{32,33}. Due to precipitous declines of several species of rockfish and recent closures of the offshore commercial fishery, it is clear that both managers and research must focus on clarifying the population dynamics and spatial connectivity of rockfish populations. Critical to the design of ecosystem-based fisheries management (e.g. MPAs), is knowledge of the level of connectivity throughout a species’ range. Population genetics is one of few tools currently available that directly measures connectivity among marine populations. Using both microsatellite loci with high mutation rates and sequence data will reveal the magnitude of connectivity not only over evolutionary time-scales (e.g. whether there are historic population breaks) but will also elucidate the degree of connectivity over ecological time-scales (e.g. level of genetic patchiness of young-of-the-year). The spatial patterns of
genetic variation of individual year-classes are essential for inferring patterns of larval dispersal and population connectivity. Moreover, using molecular tools to identify rockfish populations that might have small effective population sizes is an important component of managing fishery populations to prevent losses in genetic diversity and extinctions. Similarly, variation in the adult contribution to a year-class is critical for determining the percent of the population that needs to be conserved to replenish depleted rockfish stocks. An understanding the effective population size, any genetic breaks throughout the species range, the spatio-temporal scale of genetic patchiness (i.e. connectivity), and whether different populations are reproductively isolated will facilitate better population modeling for stock assessments analyzed by the Pacific Fisheries Management Council. These stock assessments are used to establishing catch limits and seasonal closures. Given the above considerations, my proposed research applies directly to both the second and fourth objectives of the Fisheries and Marine Ecosystems goals identified by the CEQI.

LITERATURE CITED


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Burford, M.O. Population admixture between two differentiated populations of adult blue rockfish (*Sebastes mystinus*). To be submitted to *Canadian Journal of Fisheries and Aquatic Sciences*.
Burford, M.O. In prep. Genetic analysis of year-class formation in the blue rockfish (*Sebastes mystinus*): larval-pool vs. sweepstakes recruitment. To be submitted to *Canadian Journal of Fisheries and Aquatic Sciences*.

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Burford, M.O. 2006. Genetic structure in a marine fish with an extended pelagic larval phase: an analysis of both the juvenile and adult populations of blue rockfish (*Sebastes mystinus*). Invited speaker at the Center for Population Biology Departmental seminar, UC Davis, California, October.

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