AN ABSTRACT OF THE DISSERTATION OF

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Title: Genetic Patterns of Demography and Diversity in Eastern North Pacific Rockfishes (genus *Sebastes*).

Abstract approved:

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Michael A. Banks

The 100 North Pacific rockfish species in the genus *Sebastes* are highly diverse. Rockfishes fertilize their eggs internally and release swimming larvae. Complex courting behaviors may allow female rockfish to be selective about their mates and may promote and maintain speciation. In this study, I applied genetic techniques to survey the factors that structure rockfish populations and have potential to affect speciation. First, I used microsatellites to test the hypothesis that the rangewide signal of isolation-by-distance previously identified in copper rockfish is the result of localized habitat or oceanographic breaks. I found significant population divergence at both the whole-coast and Oregon-coast scales. I also identified a weak but significant barrier to larval migration south of Newport, Oregon. Second, I used a candidate gene approach to assess the possibility that olfactory receptor related to class A, type 2 (*Ora2*) genes could differentiate species at mating. I characterized sequence variation in *Ora2* genes between five distantly-related rockfishes and tested for evidence of positive selection. My results suggest that rockfishes possess a single, highly conserved *Ora2* gene. Although evidence of positive selection was found for nine amino acids, shared alleles were found across all five study species. This suggests

that the *Ora2* gene in isolation could not differentiate species during mating season. Finally, I tested the hypothesis that rockfishes mate non-randomly based on major histocompatibility complex (MHC) genotype, as has been seen in other fishes. I characterized MHC genotypes of copper and quillback rockfish in a captive-mating population, identified parents of fourteen broods, and drew inferences about mate choice based on known parentage. I found a significant proportion of multiple-fathered broods, a high degree of hybridization, and that these two species possess multiple, highly-variable MHC genes. I did not find strong evidence that females selected males based on MHC. This may be because larval survival is stochastic, which promotes a bet-hedging strategy, rather than a high degree of selectivity. This work complements our understanding of the population genetics of nearshore rockfishes, and offers a first look at two gene families that may function in mate choice to avoid hybridization and improve larval quality.

Genetic Patterns of Demography and Diversity in Eastern North Pacific Rockfishes (genus *Sebastes*)

by

Mattias L. Johansson

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APPROVED:

Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Mattias L. Johansson, Author

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CONTRIBUTION OF AUTHORS

Dr. Michael A. Banks, my major professor, closely advised the planning and execution of this research, and was the first reviewer and editor of the written results; he is therefore a coauthor on all three chapters of this dissertation. Dr. Vincent P. Buonaccorsi was central to planning, completing, and writing Chapter 1. Katie Glunt and Heather Hassel-Finnegan were involved in the data gathering for Chapter 1. Kevin Clifford and Brian Fodness assisted with animal husbandry, sample collections, and project design for Chapter 3.

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GENERAL INTRODUCTION

Rockfishes

The genus *Sebastes* (the rockfishes) is notable for being speciose (approximately 110 species worldwide) and extremely diverse, with members of the genus found in a variety of habitat types from the intertidal to depths over 1000 m (Love et al. 2002) in cool-temperate systems worldwide (Hyde and Vetter 2007). Rockfishes are most diverse in the north Pacific (~103 species; Kai et al. 2003; Kai and Nakabo 2008; Love et al. 2002), although four species are found in the north Atlantic (Johansen and Dahle 2004) and at least two species in the south Atlantic and south Pacific (Rocha-Olivares et al. 1999). Morphology varies widely, from streamlined, schooling, semi-pelagic species such as the shortbelly rockfish (*Sebastes jordani*) to deep-bodied, spiny, solitary, benthic forms, such as the tiger rockfish (*S. nigrocinctus*), and is correlated with life history and ecological role (Jordan and Evermann 1898). A full range of intermediate morphologies also exist in the genus (Hyde and Vetter 2007). The group is also particularly distinguished by the evolution of viviparity (Wourms 1991). With numerous examples of convergent evolution for similar lifestyles (Hyde and Vetter 2007), rockfish present unique opportunities to use genetic studies to examine demography, diversification, and the maintenance of species barriers. In this study, I used genetic approaches to assess the factors that structure rockfish populations and those that potentially affect speciation.

Population Genetics of Rockfishes

Large-scale studies of marine population structure have typically focused on broad-scale patterns, which has made it difficult to assess the isolating effect of particular oceanographic or habitat features on population genetic structure. In

addition, dispersal estimates may be drawn disproportionately from low-latitude regions and be dominated by low-dispersal species so may not accurately represent long-distance marine dispersal generally (Bradbury et al. 2008). A previous study on copper rockfish found significant population subdivision and a correlation between genetic and geographic distance among coastal samples spanning the species' range (Buonaccorsi et al. 2002). Estimates of mean larval dispersal in copper rockfish based on data from Buonaccorsi et al. (2002) and the Rousset analytical model (Rousset 1997) were low (under 40 km), even when accounting for four orders of magnitude of variation in possible effective population size (N_e; Buonaccorsi et al. 2004; Buonaccorsi et al. 2005). However, as noted in the Buonaccorsi et al. (2002) study, the extensive spacing between samples leaves the cause of population divergence essentially unresolved, due to the large number of confounding variables. Within Oregon, Cape Blanco may form a barrier to gene flow for larvae of marine species that are pelagic in the early upwelling season (such as copper rockfish). Offshore advection during the early upwelling season diminishes the opportunity for larvae to return to the continental margin and successfully recruit into adult populations (Barth and Smith 1998), thus possibly obstructing rockfish gene flow between the two areas north and south of Cape Blanco. Significantly higher recruitment rates of intertidal invertebrates have been documented north of Cape Blanco than anywhere south of the Cape. This indicates that the geographically narrow, but oceanographically strong offshore upwelling jet south of the Cape may limit the supply of larvae to nearshore benthic populations (Connolly et al. 2001). Another possibility is that areas of non-optimal habitat, such as sandy areas devoid of rocky reef habitat, may function as habitat breaks, where larvae are unlikely to persist in the water column long enough to cross the break and are unlikely to survive if they settle within the break. A 130-km stretch of mostly sandy habitat between Newport, OR and Coos Bay, OR may function as this kind of habitat break (Don Bodenmiller, Pers. Comm.), although the presence of an oceanographic break at Heceta Head within this area may also structure the

population. Finally, the live young extruded from rockfish may have an innate ability to enhance retention (Kingsford et al. 2002), limiting oceanographic influence on larval dispersal, consistent with an isolation-by-distance model of larval dispersal.

In chapter one, entitled "Influence of habitat discontinuity, geographical distance, and oceanography on fine-scale population genetic structure of copper rockfish (*Sebastes caurinus*)," I utilize genotype data from eleven microsatellite markers to assess fine-scale population genetic structure of copper rockfish in Oregon, and relate that structure to coastwide (broad-scale) genetic structure. I specifically set out to test two different hypotheses: 1. that populations are structured solely by geographic distance (isolation-by-distance), or 2. that the previously-published isolation-by-distance signal seen at the broad scale is actually the result of geographic or habitat breaks as the fine scale.

For chapter one, the copper rockfish (*Sebastes caurinus*) was chosen as a model organism to study larval dispersal for nearshore rockfishes that are strongly habitat associated. My goal was to test the alternative hypotheses that either 1. populations are structured solely by isolation-by-distance at fine scales or 2. that the published (Buonaccorsi et al. 2002) large-scale isolation-by-distance signal in copper rockfish results from oceanographic or habitat breaks at small scales. Adult copper rockfish are long-lived (greater than 50 years; Lea et al. 1999), late-maturing (at 7 years; Lea et al. 1999), and shallow-dwelling animals (commonly less than 130 m deep; Love 1996). They are found in nearshore kelp forests and rocky habitat from Kodiak Island, Alaska to southern California (Love et al. 2002; Mecklenburg et al. 2002). Adults, like many other nearshore rockfish, exhibit extremely limited migrations (a few kilometers) and are unlikely to leave the reef on which they have settled (Lea et al. 1999). Primary dispersal therefore would have to occur almost exclusively during the pelagic larval stage (Love et al. 2002) delineated by duration of the planktonic phase (Doherty et al. 1995; Waples and Rosenblatt 1987), oceanographic features (e.g. Wing et al. 1998), and behavior (Kingsford et al. 2002).

All rockfish are live-bearers and have pelagic larval and early juvenile stages that last from 2 to 6 months (Anderson 1983; Moser and Boehlert 1991), but the offshore distribution of copper rockfish larvae is unknown. In central California, peak parturition for copper rockfish occurs in February and March (Lea et al. 1999), somewhat before the onset of the upwelling season. Peak settlement occurs from April to May (Anderson 1983), suggesting a 2- to 3-month pelagic duration. During this time, larval advection into strong coastal currents could potentially homogenize widely spaced coastal populations. However, evidence for low realized dispersal is accumulating from genetic (Bentzen et al. 1996; Rocha-Olivares and Vetter 1999; Taylor and Hellberg 2003) and ecological studies (Marliave 1986; Miller and Shanks 2004; Swearer et al. 2002) for many marine species with a strong dispersal potential.

Olfactory receptors, Mate Selection, and the MHC

In contrast to the majority of teleost fishes, rockfishes fertilize their eggs internally and release live, swimming larvae. Observational evidence from a handful of species (*Sebastes inermis*, *S. mystinus*, *S. miniatus*, *S. melanops*, *S. emphaeus*) suggests that rockfishes undergo complex courting behaviors, which may include pheromones or sound cues, before mating can take place (Love et al. 2002). Changes in mate recognition processes may provide one mechanism for the formation of reproductive isolation in rockfishes. Although numerous closely related species live in close proximity, the mate recognition system in rockfishes is largely unknown. Mate recognition may involve visual, pheromonal, or sound cues (Love et al. 2002), and could conceivably be central to the development of the unusually high diversity seen in the genus. Mate pairing in rockfishes is discrete, because they engage in internal fertilization and require complex courtship rituals before copulation can take place (Love et al. 2002). Although specific behaviors may vary from species to species, based on the few species studied to date, male rockfish align themselves next to a female before swimming forward and placing their urogenital papilla near her snout

(Gingras et al. 1998; Hallacher 1974; Helvey 1982; Shinomiya and Ezaki 1991). It has been suggested that this behavior allows the male to release a courtship pheromone as near as possible to the olfactory rosette of the female (Love et al. 2002). Ultimately, the decision whether or not to mate appears to lie with the female (Hyde et al. 2008).

Although chance may play the primary role in reproductive success for bet-hedging marine fishes, such as rockfishes (Winemiller and Rose 1992), another potential benefit of mate choice and multiple paternity may be through avoidance of hybridization, potentially through olfactory receptor-mediated pheromone detection, or in improved pathogen or parasite resistance through optimization of major histocompatibility complex (MHC) genotypes. Multiple mating, with up to four different fathers, has been demonstrated in thirteen different rockfish species to date, and may be a common bet-hedging strategy in the genus (Gonzalez et al. 2009; Hyde et al. 2008; Sogard et al. 2008; Van Doornik et al. 2008). Van Doornik (et al. 2008) found that the majority (71.2%) of wild-caught Pacific ocean perch (*Sebastes alutus*) broods showed evidence of multiple paternity, although only 16.6% had more than two sires. Selective mate choice by females, combined with multiple paternity, may enhance genetic diversity and prevent the loss of rare alleles, may diminish the effect of inbreeding depression, and may increase the effective population size of organisms who experience sweepstakes recruitment (Hedgecock 1994).

In chapter two, entitled "Olfactory receptor related to class A, type 2 (*Ora*2) genes are conserved between distantly related rockfishes (genus *Sebastes*)," I utilized a candidate-gene approach to sequence and characterize partial sequences of a single *Ora2* gene from several individuals each of five different species of rockfishes, at varying evolutionary distances. The goal of this chapter was to assess the evolution of this gene, to test for a signal of selection acting on the gene, and to assess whether it could plausibly function to distinguish different species during the mating season.

The olfactory receptor related to class A (*Ora*) gene family, first described as V1r genes in rodents (Buck and Axel 1991), is one of two families of G protein-coupled receptors used to detect pheromones in mammals (Grus et al. 2005). Although fishes lack a vomeronasal organ, for which the gene family was originally named, numerous species have been shown to possess and express complete *Ora* genes (Pfister and Rodriguez 2005; Pfister et al. 2007; Saraiva and Korsching 2007; Shi and Zhang 2007). In contrast to the mouse and the rat, however, which contain ~100-150 functional receptors, no fish species has yet been found to have more than a handful (5 to 6) (Saraiva and Korsching 2007). Additionally, in fishes, some research has shown evidence of positive Darwinian selection on these genes, suggesting that some neofunctionalization may have occurred (Pfister et al. 2007). Other researchers have suggested that these genes are highly conserved, finding no evidence for positive selection (Saraiva and Korsching 2007).

In chapter two, we test the hypothesis that the amino acid sequence, and hence, ligand binding properties, of *Ora* pheromone receptor genes function to distinguish species during the mating season and aid in avoidance of hybridization. We describe *Ora* genes for five species of rockfishes (genus *Sebastes*), representing a broad sampling of rockfish phylogeny and ecology. *S. caurinus* and *S. maliger* are separated by approximately 1.6MY, co-occur in rocky reef habitats in the Northeast Pacific, and have been shown to hybridize in Puget Sound, WA, USA (Love et al. 2002; Seeb 1998). *S. melanops* occurs around the same rocky habitats as *S. maliger* and *S. caurinus* but is separated by approximately 6.3MY of evolution (Hyde and Vetter 2007; Love et al. 2002). *S. ruberrimus* and *S. crameri* occupy a similar geographic range and similar habitats, but may be found somewhat deeper and are separated from the other species in our set by approximately 7.1MY and 7.8MY, respectively (Hyde and Vetter 2007; Love et al. 2002). Complete life history data is lacking for several of these species, but it is known that some portion of the larval release period overlaps between all five species, as all release larvae during the spring and early summer

(Love et al. 2002). Although evidence suggests that rockfish are capable of sperm storage, and that insemination may precede fertilization by up to six months (Moser 1967; Sogard et al. 2008), it is conceivable that the mating season overlaps for the sample set as well, and that they could be exposed to reproductive members of other species.

In chapter three, entitled "Mate selection and the major histocompatibility complex (MHC) in captive-breeding rockfishes (genus *Sebastes*)," I combined the use of microsatellite markers with a candidate-gene approach to assess mating and mate selection in captive-breeding populations of copper and quillback rockfishes at the Oregon Coast Aquarium. First, I characterized MHC diversity in all the adult copper and quillback rockfishes in a captive population at the Oregon Coast Aquarium. Then, I assessed parentage in fourteen broods of larvae using six microsatellite markers. Finally, I assessed the MHC genotypes of the identified parents to assess the influence of MHC on mate selection in rockfishes.

The major histocompatibility complex (MHC) is a highly- polymorphic multigene family involved in self-nonself recognition in the immune system of vertebrates. MHC genes encode receptors that bind fragments of local and foreign peptides, then present those fragments to T cells, which may initiate a series of immune responses (Bernatchez and Landry 2003; Ploegh and Watts 1998). Two main subgroups of immunologically active molecules are found within the MHC. Class I molecules, expressed by nearly all nucleated cells, help defend against intracellular pathogens, such as viruses. Class II molecules are expressed by antigen presenting cells such as macrophages, and defend against extracellular pathogens and parasites (Piertney and Oliver 2006). The amino-acid sequence of the MHC protein determines antigen binding, and thus, which foreign peptides can be recognized (Brown et al. 1988), therefore, greater MHC diversity would be expected to result in a greater sensitivity to pathogens and a more effective immune response (Doherty and Zinkernagel 1975). Because self-reactivity can lower the number of T-cells in species

that express several MHC loci, an intermediate number of alleles may result in better pathogen recognition in these cases (Woelfing et al. 2009). Given the high polymorphism and potential benefits associated with MHC-based mate preference, two main hypotheses have been proposed. First, given the high polymorphism of the family, they could function to discriminate close kin, and might help females to avoid inbreeding (Landry et al. 2001; Penn and Potts 1999). The other possibility is that negative assortative mating based on MHC genotype might function to improve the pathogen resistance of offspring (Doherty and Zinkernagel 1975; Landry et al. 2001). Mate selection based on MHC genotype has been demonstrated in several fish species to date, including three-spined sticklebacks (*Gasterosteus aculeatus*; Aeschlimann et al. 2003; Eizaguirre et al. 2009; Kalbe et al. 2009; Lenz et al. 2009; Milinski et al. 2005; Reusch et al. 2001), a variety of salmonids (Atlantic salmon, *Salmo salar* L.; Consuegra and de Leaniz 2008; Landry et al. 2001; brown trout, *Salmo trutta* L.; Forsberg et al. 2007; Chinook salmon, *Oncorhynchus tshawytscha*; Garner et al. 2010; Neff et al. 2008), and other freshwater fish species (e.g. rose bitterling, *Rhodeus occelatus*; Casalini et al. 2009). This research has suggested that MHC genes do influence mate choice decisions in fish (Landry et al. 2001), that both good genes (i.e. specific alleles that confer improved pathogen resistance; Eizaguirre et al. 2009) and overall MHC diversity (Consuegra and de Leaniz 2008; Eizaguirre et al. 2009; Kalbe et al. 2009; Landry et al. 2001; Reusch et al. 2001) may be considered by females, and that mating decisions may be complicated by other factors, such as body size and coloration (Neff et al. 2008) or aggression between individuals (Garner et al. 2010). Little research has been undertaken to study MHC-based mate selection in marine fishes, however. One reason for this scarcity of research may be the difficulty of performing laboratory-based experimental research on large, long-lived marine fishes. Another reason may be that marine fishes are much less likely to encounter close kin, which would make the hypothesis that MHC-based mate selection partially functions to avoid inbreeding much less applicable (Landry et al. 2001). However, since marine

fishes are exposed to numerous parasite threats, the potential improvement in immunocompetence associated with mate selection based on MHC genotype should apply equally to marine fishes as freshwater types (Penn et al. 2002).

For chapter three, copper (*Sebastes caurinus*) and quillback (*Sebastes maliger*) rockfishes were chosen as model organisms to study major histocompatibility complex (MHC) diversity and mate selection in nearshore rockfishes. The copper rockfish, as described above, is found from the northern Gulf of Alaska to central Baja California, in waters from the sub-tidal zone to depths of ~180 m. The quillback rockfish ranges from the Gulf of Alaska to the northern California Bight, and is found from sub-tidal depths to approximately 275 m (Love et al. 2002). Both species prefer areas of high- to medium- relief rocks, although they may also be found over low- relief rock habitat. Both are long-lived, with coppers aged to 50 years, and quillbacks aged to at least 95 years. Likewise, both mature around 7 years of age (Lea et al. 1999; Love et al. 2002). Larval release occurs between January and June in copper rockfish, and between March and June in quillbacks (Love et al. 2002). Both species probably only produce a single brood annually (Moser 1967). An MHC class II beta genotype has been described for a single copper rockfish (Aguilar and Garza 2005), but no data have yet been published on quillback. No research has been published on multiple paternity or mate selection for either species.

Significance of Research

The objectives of this research are threefold: 1. To investigate the fine-scale population genetics of copper rockfish and test the hypotheses that populations are structured by isolation by distance or are structured by habitat or oceanographic breaks, 2. to assess the evolutionary history of olfactory receptor related to class A, type 2 (*Ora2*) genes, which may be involved in pheromone detection, in a suite of rockfish species representing a broad spectrum of evolutionary relationships, and 3. to characterize MHC diversity in depth in two species of captive-breeding rockfishes, to

identify mated pairs through parentage analysis, and to assess whether MHC-mediated mate choice had occurred.

This research adds to and complements our current understanding of the population genetics of marine organisms. By assessing the fine-scale structure of Oregon copper rockfish, we examine potential underlying mechanisms that may shape many marine populations. The work also represents a first look at two gene families that may play a role in the little-understood mate recognition system of rockfishes. *Ora2* genes are known to function in pheromonal communication in mammals and are expressed in the olfactory epithelium of fishes, where they could function to receive pheromonal cues. We describe the first sequences for *Ora2* genes from five distantly related species of rockfishes and assess their evolution. Finally, we describe MHC genes from captive-breeding copper and quillback rockfishes at the Oregon Coast Aquarium and assess parentage for larvae produced. This work supports previous findings of multiple mating in rockfishes, and the breakdown of barriers to hybridization seen in captivity may provide clues to the cues that rockfishes use to recognize appropriate mates in nature.

References

Aeschlimann PB, Haberli MA, Reusch TBH, Boehm T, Milinski M, 2003. Female sticklebacks Gasterosteus aculeatus use self-reference to optimize MHC allele number during mate selection. Behavioral Ecology and Sociobiology. 54:119-126.

Aguilar A, Garza JC, 2005. Analysis of major histocompatibility complex class II Beta genes from rockfishes (genus Sebastes). Journal of Fish Biology. 67:1021-1028.

Anderson T, 1983. Identification and development of nearshore juvenile rockfishes (Genus *Sebastes*) in central California kelp forests. In. Fresno, California: California State University, Fresno.

Barth JA, Smith RL, 1998. Separation of a coastal upwelling jet at Cafe Blanco, Oregon, USA. South African Journal of Marine Science-Suid-Afrikaanse Tydskrif Vir Seewetenskap. 19:5-14.

Bentzen P, Taggart CT, Ruzzante DE, Cook D, 1996. Microsatellite polymorphism and the population structure of Atlantic cod (Gadus morhua) in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences. 53:2706-2721.

Bernatchez L, Landry C, 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? Journal of Evolutionary Biology. 16:363-377.

Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE, 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proceedings of the Royal Society B. 275:1803-1809.

Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC, 1988. A Hypothetical Model of the Foreign Antigen-Binding Site of Class-Ii Histocompatibility Molecules. Nature. 332:845-850.

Buck L, Axel R, 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell. 65:175-187.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD, 2002. Population structure of copper rockfish (Sebastes caurinus) reflects postglacial colonization and contemporary patterns of larval dispersal. Canadian Journal of Fisheries and Aquatic Sciences. 59:1374-1384.

Buonaccorsi VP, Westerman M, Stannard J, Kimbrell C, Lynn E, Vetter RD, 2004. Molecular genetic structure suggests limited larval dispersal in grass rockfish, Sebastes rastrelliger. Marine Biology. 145:779-788.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD, 2005. Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, Sebastes auriculatus. Conservation Genetics. 6:697-713.

Casalini M, Agbali M, Reichard M, Konecna M, Bryjova A, Smith C, 2009. Male Dominance, Female Mate Choice, and Intersexual Conflict in the Rose Bitterling (Rhodeus Ocellatus). Evolution. 63:366-376.

Connolly SR, Menge BA, Roughgarden J, 2001. A latitudinal gradient in recruitment of intertidal invertebrates in the northeast Pacific Ocean. Ecology. 82:1799-1813.

Consuegra S, de Leaniz CG, 2008. MHC-mediated mate choice increases parasite resistance in salmon. Proceedings of the Royal Society B-Biological Sciences. 275:1397-1403.

Cushing DH, 1990. Plankton Production and Year-Class Strength in Fish Populations - an Update of the Match Mismatch Hypothesis. Advances in Marine Biology. 26:249-293.

Doherty PC, Zinkernagel RM, 1975. Enhanced Immunological Surveillance in Mice Heterozygous at H-2 Gene Complex. Nature. 256:50-52.

Doherty PJ, Planes S, Mather P, 1995. Gene Flow and Larval Duration in 7 Species of Fish from the Great-Barrier-Reef. Ecology. 76:2373-2391.

Eizaguirre C, Yeates SE, Lenz TL, Kalbe M, Milinski M, 2009. MHC-based mate choice combines good genes and maintenance of MHC polymorphism. Molecular Ecology. 18:3316-3329.

Forsberg LA, Dannewitz J, Petersson E, Grahn M, 2007. Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout - females fishing for optimal MHC dissimilarity. Journal of Evolutionary Biology. 20:1859-1869.

Garner SR, Bortoluzzi RN, Heath DD, Neff BD, 2010. Sexual conflict inhibits female mate choice for major histocompatibility complex dissimilarity in Chinook salmon. Proceedings of the Royal Society B-Biological Sciences. 277:885-894.

Gingras ML, Ventresca DA, Donnellan MD, Fisher JL, 1998. First observations of vermillion rockfish courtship are from a harvest refuge. California Fish and Game. 84:176-179.

Gonzalez EB, Murakami T, Teshima Y, Yoshioka K, Jeong DS, Umino T, 2009. Paternity testing of wild black rockfish Sebastes inermis (brownish type) from the Seto Inland Sea of Japan. Ichthyological Research. 56:87-91.

Grus WE, Shi P, Zhang YP, Zhang JZ, 2005. Dramatic variation of the vomeronasal pheromone receptor gene repertoire among five orders of placental and marsupial mammals. Proceedings of the National Academy of Sciences of the United States of America. 102:5767-5772.

Hallacher LE, 1974. The comparative morphology of extrinsic gasbladder musculature in the scorpionfish genus *Sebastes* (Pisces: Scorpaenidae). Proceedings of the California Academy of Sciences. 40:59-86.

Hedgecock D, 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In Beaumont AR ed. Genetics and Evolution of Aquatic Organisms. London: Chapman and Hall, p. 122-134.

Helvey M, 1982. First observations of courtship behavior in rockfish, genus *Sebastes*. Copeia.763-770.

Hyde JR, Vetter RD, 2007. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). Molecular Phylogenetics and Evolution. 44:790-811.

Hyde JR, Kimbrell C, Robertson L, Clifford K, Lynn E, Vetter R, 2008. Multiple paternity and maintenance of genetic diversity in the live-bearing rockfishes Sebastes spp. Marine Ecology-Progress Series. 357:245-253.

Johansen T, Dahle G, 2004. Discrimination among species of the genus Sebastes in the North Atlantic by random amplified polymorphic DNA. Sarsia. 89:478-483.

Jordan DS, Evermann BW, 1898. The fishes of North and Middle America. Bulletin of the United States National Museum. 47.

Kai Y, Nakayama K, Nakabo T, 2003. Molecular phylogenetic perspective on speciation in the genus Sebastes (Scorpaenidae) from the Northwest Pacific and the position of Sebastes within the subfamily Sebastinae. Ichthyological Research. 50:239-244.

Kai Y, Nakabo T, 2008. Taxonomic review of the Sebastes inermis species complex (Scorpaeniformes : Scorpaenidae). Ichthyological Research. 55:238-259.

Kalbe M, Eizaguirre C, Dankert I, Reusch TBH, Sommerfeld RD, Wegner KM, Milinski M, 2009. Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. Proceedings of the Royal Society B-Biological Sciences. 276:925-934.

Kingsford MJ, Leis JM, Shanks A, Lindeman KC, Morgan SG, Pineda J, 2002. Sensory environments, larval abilities and local self-recruitment. Bulletin of Marine Science. 70:309-340.

Landry C, Garant D, Duchesne P, Bernatchez L, 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (Salmo salar). Proceedings of the Royal Society of London Series B-Biological Sciences. 268:1279-1285.

Lea R, McAllister R, Ventresca DA, 1999. Biological aspects of the nearshore rockfishes of the genus *Sebastes* from central California. California Department of Fish and Game, Fish Bulletin. 177:1-109.

Lenz TL, Eizaguirre C, Scharsack JP, Kalbe M, Milinski M, 2009. Disentangling the role of MHC-dependent 'good genes' and 'compatible genes' in mate-choice decisions of three-spined sticklebacks Gasterosteus aculeatus under semi-natural conditions. Journal of Fish Biology. 75:2122-2142.

Love M, 1996. Probably More Than You Want to Know About Fishes of the Pacific Coast. Santa Barbara, California: Really Big Press.

Love MS, Yoklavich M, Thorsteinson L, 2002. The Rockfishes of the Northeast Pacific. Berkeley and Los Angeles, CA: University of California Press.

Marliave JB, 1986. Lack of Planktonic Dispersal of Rocky Intertidal Fish Larvae. Transactions of the American Fisheries Society. 115:149-154.

Mecklenburg CW, Mecklenburg T, Thorsteinson L, 2002. Fishes of Alaska. Bethesda, Maryland: American Fisheries Society.

Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T, 2005. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. Proceedings of the National Academy of Sciences of the United States of America. 102:4414-4418.

Miller JA, Shanks AL, 2004. Ocean-estuary coupling in the Oregon upwelling region: abundance and transport of juvenile fish and of crab megalopae. Marine Ecology-Progress Series. 271:267-279.

Moser H, 1967. Reproduction and development of *Sebastodes paucispinis* and comparisons with other rockfishes off California. Copeia. 1967:773-779.

Moser HG, Boehlert GW, 1991. Ecology of Pelagic Larvae and Juveniles of the Genus Sebastes. Environmental Biology of Fishes. 30:203-224.

Neff BD, Garner SR, Heath JW, Heath D, 2008. The MHC and non-random mating in a captive population of Chinook salmon. Heredity. 101:175-185.

Penn DJ, Potts WK, 1999. The evolution of mating preferences and major histocompatibility complex genes. American Naturalist. 153:145-164.

Penn DJ, Damjanovich K, Potts WK, 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. Proceedings of the National Academy of Sciences of the United States of America. 99:11260-11264.

Pfister P, Rodriguez I, 2005. Olfactory expression of a single and highly variable V1r pheromone receptor-like gene in fish species. Proceedings of the National Academy of Sciences of the United States of America. 102:5489-5494.

Pfister P, Randall J, Montoya-Burgos JI, Rodriguez I, 2007. Divergent Evolution among Teleost V1r Receptor Genes. PLoS ONE. 2:e379.

Ploegh H, Watts C, 1998. Antigen recognition - Editorial overview. Current Opinion in Immunology. 10:57-58.

Reusch TBH, Haberli MA, Aeschlimann PB, Milinski M, 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature. 414:300-302.

Rocha-Olivares A, Rosenblatt RH, Vetter RD, 1999. Cryptic species of rockfishes (Sebastes : Scorpaenidae) in the Southern Hemisphere inferred from mitochondrial lineages. Journal of Heredity. 90:404-411.

Rocha-Olivares A, Vetter RD, 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (Sebastes helvomaculatus). Canadian Journal of Fisheries and Aquatic Sciences. 56:803-813.

Rousset F, 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics. 145:1219-1228.

Saraiva LR, Korsching SI, 2007. A novel olfactory receptor gene family in teleost fish. Genome Research. 17:1448-1457.

Seeb LW, 1998. Gene flow and introgression within and among three species of rockfishes, Sebastes auriculatus, S-caurinus, and S-maliger. Journal of Heredity. 89:393-403.

Shi P, Zhang JZ, 2007. Comparative genomic analysis identifies an evolutionary shift of vomeronasal receptor gene repertoires in the vertebrate transition from water to land. Genome Research. 17:166-174.

Shinomiya A, Ezaki O, 1991. Mating habits of the rockfish *Sebastes inermis*. Environmental Biology of Fishes. 30:15-22.

Sogard SM, Berkeley SA, Fisher R, 2008. Maternal effects in rockfishes Sebastes spp.: a comparison among species. Marine Ecology-Progress Series. 360:227-236.

Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR, 2002. Evidence of self-recruitment in demersal marine populations. Bulletin of Marine Science. 70:251-271.

Taylor MS, Hellberg ME, 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science. 299:107-109.

Van Doornik DM, Parker SJ, Millard SR, Berntson EA, Moran P, 2008. Multiple paternity is prevalent in Pacific ocean perch (Sebastes alutus) off the Oregon coast, and is correlated with female size and age. Environmental Biology of Fishes. 83:269-275.

Waples RS, Rosenblatt RH, 1987. Patterns of Larval Drift in Southern-California Marine Shore Fishes Inferred from Allozyme Data. Fishery Bulletin. 85:1-11.

Wing SR, Botsford LW, Ralston SV, Largier JL, 1998. Meroplanktonic distribution and circulation in a coastal retention zone of the northern California upwelling system. Limnology and Oceanography. 43:1710-1721.

Winemiller KO, Rose KA, 1992. Patterns of life-history diversification in North American fishes - implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences. 49:2196-2218.

Woelfing B, Traulsen A, Milinski M, Boehm T, 2009. Does intra-individual major histocompatibility complex diversity keep a golden mean? Royal Society Philosophical Transactions Biological Sciences. 364:117-128.

Wourms JP, 1991. Reproduction and Development of Sebastes in the Context of the Evolution of Piscine Viviparity. Environmental Biology of Fishes. 30:111-126.

INFLUENCE OF HABITAT DISCONTINUITY, GEOGRAPHICAL DISTANCE, AND OCEANOGRAPHY ON FINE-SCALE POPULATION GENETIC STRUCTURE OF COPPER ROCKFISH (*SEBASTES CAURINUS*)

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Abstract

The copper rockfish is a benthic, non-migratory, temperate rocky reef marine species with pelagic larvae and juveniles. A previous range-wide study of the population-genetic structure of copper rockfish revealed a pattern consistent with isolation-by-distance. This could arise from an intrinsically limited dispersal capability in the species or from regularly-spaced extrinsic barriers that restrict gene flow (offshore jets that advect larvae offshore and/or habitat patchiness). Tissue samples were collected along the West coast of the contiguous United States between Neah Bay, WA and San Diego, CA, with dense sampling along Oregon. At the whole-coast scale (~2200 km), significant population subdivision (F_{ST} = 0.0042), and a significant correlation between genetic and geographic distance were observed based on 11 microsatellite DNA loci. Population divergence was also significant among Oregon collections (~450 km, F_{ST} = 0.001). Hierarchical AMOVA identified a weak but significant 130-km habitat break as a possible barrier to gene flow within Oregon, across which we estimated that dispersal (N_em) is half that of the coast-wide average. However, individual-based Bayesian analyses failed to identify more than a single population along the Oregon coast. In addition, no correlation between pairwise population genetic and geographic distances was detected at this scale. The offshore jet at Cape Blanco was not a significant barrier to gene flow in this species. These findings are consistent with low larval dispersal distances calculated in previous studies on this species, support a mesoscale dispersal model, and highlight the importance of continuity of habitat and adult population size in maintaining gene flow.

Introduction

Pacific coast rockfishes comprise an extremely diverse and successful species complex found off the west coast of North America, from Alaska to Mexico, occupying habitat types ranging from the intertidal to depths over 1500 m. Intensive commercial and recreational fisheries since the 19th century and, more recently, a major live-fish fishery have depleted many species to a small fraction of their virgin stock biomass (Ralston et al. 1996; Love et al. 1998; Love et al. 2002; Federal Register 33037). The establishment of a network of Marine Protected Areas (MPAs) has been proposed as an alternative to traditional fisheries management to preserve biodiversity and increase fisheries yields. MPAs are expected to be most beneficial for species that spend a significant proportion of their adult lives in the MPA and presumably seed both the MPA and adjacent areas with recruits (Botsford et al. 2003). MPAs for both fisheries and biodiversity goals require an increased understanding of how far larvae disperse on average (Palumbi 2001), and whether larvae exist as large homogeneous gene pools or a series of gene pools experiencing limited exchange (Botsford et al. 2001).

The concordance between genetic structure and oceanographic or topographical features may support the hypothesis that barriers to gene flow structure populations into homogeneous gene pools (Rocha-Olivares and Vetter 1999). Alternatively, population relatedness may simply be associated with geographic distance between populations, as expected for populations at equilibrium with respect to genetic drift and migration under an isolation-by-distance model of dispersal where individuals disperse most frequently into neighboring populations (Slatkin 1993). In such a case, the slope of the relationship between geographic and genetic distance can also be used to infer the average dispersal distance at different population densities (Rousset 1997).

The copper rockfish was chosen as a model organism to study larval dispersal for nearshore rockfishes that are strongly habitat associated. Adult copper rockfish are long-lived (greater than 30 years; Lea et al. 1999), late-maturing (at 7 years; Lea et al. 1999), and shallow-dwelling animals (commonly less than 130 m deep; Love 1996). They are found in nearshore kelp forests and rocky habitat from Kodiak Island, Alaska to southern California (Love et al. 2002, Mecklenburg et al. 2002). Adults, like many other nearshore rockfish, exhibit extremely limited migrations (a few kilometers) and are unlikely to leave the reef on which they have settled (Lea et al. 1999). Primary dispersal therefore would have to occur almost exclusively during the pelagic larval stage (Love et al. 2002) delineated by duration of the planktonic phase (Waples and Rosenblatt 1987; Doherty et al. 1995), oceanographic features (e.g. Wing et al. 1998), and behavior (Kingsford et al. 2002). All rockfish are live-bearers and have pelagic larval and early juvenile stages that last from 2 to 6 months (Anderson 1983; Moser and Boehlert 1991), but the offshore distribution of copper rockfish larvae is unknown. In central California, peak parturition for copper rockfish occurs in February and March (Lea et al. 1999), somewhat before the onset of the upwelling season. Peak settlement occurs from April to May (Anderson 1983), suggesting a 2- to 3-month pelagic duration. During this time, larval advection into strong coastal currents could potentially homogenize widely spaced coastal populations. However, evidence for low realized dispersal is accumulating from genetic (Bentzen et al. 1996; Rocha-Olivares and Vetter 1999; Taylor and Hellberg 2003) and ecological studies (Marliave 1986; Swearer et al. 2002; Miller and Shanks 2004) for many marine species with a strong dispersal potential.

Previous large-scale studies of rockfish population structure have typically focused on broad-scale patterns, which has made it difficult to assess the effect of particular oceanographic or habitat features on population genetic structure. In a previous study on copper rockfish, Buonaccorsi et al. (2002) found significant population subdivision and a correlation between genetic and geographic distance

among coastal samples spanning the species' range. Estimates of mean larval dispersal in copper rockfish based on data from Buonaccorsi et al. (2002) and the Rousset (1997) analytical model were low (under 40 km), even when accounting for four orders of magnitude of variation in possible effective population size (N_e, Buonaccorsi et al. 2004, 2005). However, as noted in the Buonaccorsi et al. (2002) study, the extensive spacing between samples leaves the cause of population divergence essentially unresolved, due to the large number of confounding variables. Within Oregon, Cape Blanco may form a barrier to gene flow for larvae of marine species that are pelagic in the early upwelling season, such as copper rockfish. Offshore advection during the early upwelling season diminishes the opportunity for larvae to return to the continental margin and successfully recruit into adult populations (Barth and Smith 1998), thus possibly obstructing rockfish gene flow between the two areas north and south of Cape Blanco. Significantly higher recruitment rates of intertidal invertebrates have been documented north of Cape Blanco than anywhere south of the Cape. This indicates that the geographically narrow, but oceanographically strong offshore upwelling jet south of the Cape may limit the supply of larvae to nearshore benthic populations (Connolly et al. 2001). Another possibility is that areas of non-optimal habitat, such as sandy areas devoid of rocky reef habitat, may function as habitat breaks, where larvae are unlikely to persist in the water column long enough to cross the break and are unlikely to survive if they settle within the break. A 130-km stretch of sandy habitat between Newport, OR and Coos Bay, OR may function as this kind of habitat break (Don Bodenmiller, Pers. Comm.). Finally, the live young extruded from rockfish may have an innate ability to enhance retention (Kingsford et al. 2002), limiting oceanographic influence on larval dispersal, consistent with an isolation-by-distance model of larval dispersal.

This study addressed three specific questions. (1) Will the coast-wide pattern of isolation-by-distance in copper rockfish (*Sebastes caurinus*) previously described by Buonaccorsi (2002) persist when sample size, number of sites, and number of loci

are doubled? 2) At the scale of the Oregon coast, is population structure in copper rockfish homogeneous? (3) If genetic structure is apparent at the Oregon coast scale, is it influenced by geographic distance, habitat breaks, Cape Blanco, or some combination of the three? In the present study, coarse sampling was performed at the coast-wide scale to aid comparison to previous studies, and fine-scale sampling was performed along the Oregon (OR) coast to assess the possible effect of two potential barriers to gene flow in copper rockfish: the upwelling jet at Cape Blanco and a 130-km stretch of poor adult habitat between Newport, OR and Coos Bay, OR. We used twice the number of loci as Buonaccorsi et al. (2002) to increase the power of our analyses as we focus on an extremely fine geographic scale in a weakly structured species.

Materials and Methods

Sampling and Laboratory Analysis

A total of 749 individuals of *S. caurinus* were collected over four field seasons along the west coast of the United States from San Diego, CA to Neah Bay, WA (Figure 1, Table 1) by the Oregon Department of Fish and Wildlife, Port Orford Ocean Resource Team, Washington Department of Fish and Wildlife, and California Department of Fish and Game. Fin-clip tissues were collected over a four-year period from dockside surveys of commercial and recreational fisheries (hook and line) and scientific collections (hook and line, spear fishing, trawl net, and gill net sampling) and stored in 95% ethanol. In addition to many new samples, DNA samples from Buonaccorsi et al. (2002) were included to represent Crescent City, Big Creek, and San Miguel Island.

Total genomic DNA was extracted using standard phenol-chloroform, DNEasy© (Qiagen Inc., Valencia, CA) or quick Chelex® (Bio-Rad Laboratories, Hercules, CA; as described in Banks et al. 2000) extraction protocols. The extracted DNA was used as template in polymerase chain reaction (PCR) amplification of five

Westerman et al. (2005) microsatellite loci (*Sra 7-7*, *Sra 7-25*, *Sra 11-103*, *Sra 15-9*, *Sra 16-5*), three microsatellite loci from Wimberger et al. (1999) (*Sma 3*, *Sma 10*, *Sma 11*), and three loci from Gomez-Uchida et al. (2003) (*Spi 6*, *Spi 10*, *Spi 18*). PCR was performed using the BRL PCR Reagent System (Gibco BRL) or Promega PCR Reagent System (Promega) following manufacturers protocols, with approximately 150 ng template DNA in 15 uL total volume. Typical cycling conditions included an initial denaturation at 94C for 2 min, followed by 25-36 cycles of 94C for 1 min, 56C for 30 sec to 1 min, and 72C for 30 sec to 1 min. Final extension was carried out at 72C for 7 min. Annealing times and temperatures were adjusted to optimize PCR conditions. Forward PCR primers were fluorescently labeled (MWG Biotech AG; Perkin Elmer; ABI), and PCR products were electrophoresed on 36 cm, 6% Long-Ranger denaturing polyacrylamide gels (FMC BioProducts) and detected using an ABI Prism^{TM} 310+ fragment analyzer or were electrophoresed through a 50 cm capillary and detected using an ABI 3730XL DNA analyzer. Internal molecular weight standards were included with each sample, and products were sized using Genescan^{TM} (Perkin Elmer, ABI) and GeneMapper^{TM} (ABI) software.

Population-Based Analyses

We analyzed patterns of genetic diversity among years and collections using Weir and Cockerham's (1984) unbiased *F*-statistics as estimated in GENEPOP v.3.4 (Raymond & Rousset 1995). Significance levels were adjusted using a Bonferroni correction for multiple tests. We tested the probability of conformance to Hardy-Weinberg and linkage equilibria using exact-significance methods and assessed the homogeneity of spatial and temporal distributions of allele frequencies using GENEPOP (100 batches and 1000 iterations). Probabilities over loci were combined using Fisher's method (Sokal & Rohlf 1995). The hypothesis that deviations from Hardy-Weinberg equilibrium were the result of null alleles was evaluated using MICROCHECKER v.2.2.3 (van Oosterhaut et al. 2004). The significance of

association between pairwise genetic ([F_{ST}/(1-F_{ST})]; Rousset 1997) and geographic distances among populations was evaluated at both coast-wide (Neah Bay, WA to San Diego, CA) and Oregon-only (Tillamook, OR to Crescent City, CA) scales using a Mantel test as implemented in GENEPOP. Simulation studies have shown that isolation-by-distance slopes should be reasonably accurate if sampling scale is a few times larger or smaller than 10 times σ (square root of the average squared axial parent-offspring distance; Leblois et al. 2003). In copper rockfish, 10 σ would be about 177 km (assuming N_E of 100/km, Buonaccorsi et al. 2004) indicating that the 450 km Oregon-coast sampling scale is appropriate for detection of isolation-by-distance and that the 2000 km whole-coast sampling scale may underestimate the true isolation-by-distance slope (Bradbury and Bentzen 2007). Average great circle distances (e.g. shortest distances between points along the surface of the Earth) among geographic positions were used, with the adjustment that all distances between southern-California locations and points north of Point Conception were calculated by rounding Point Conception, rather than as the straight overland distance. Factorial correspondence analysis by populations, as implemented in GENETIX v.4.05.2 (Belkhir 2000), was used to visualize relationships among sampling locations.

Alternative divisions of the Oregon coastline with possible biological significance were tested. We divided the coast into northern (Tillamook/Garibaldi to Newport), central (Coos Bay and Bandon), and southern regions (Port Orford to Crescent City, CA) (Fig. 1). This arrangement allowed us to test significance of the putative oceanographic barrier at Cape Blanco and habitat barrier between Newport and Coos Bay. Significance of these two potential barriers was tested both individually and simultaneously by using a hierarchical AMOVA based on allele frequencies by using ARLEQUIN v.3.01 (Excoffier *et al.* 2005). Consistency of AMOVA results was assessed by dividing the data set into pre-2003 (n = 371) and 2003-and-later (n = 608) sets, as well as by markers (*Sra* vs. *Sma* & *Spi*). Pairwise F_{ST} and significance values

among pools of northern, central, and southern regions were obtained by using GENEPOP as above.

Within-Oregon Individual-based Analysis

The program TESS v.1.1 (Francois et al. 2006) was used to identify clusters defined by allele frequency discontinuities above and beyond the decay of allele frequency correlation expected as geographic distance between samples increases (Francois et al. 2006, Chen et al. 2007). By identifying these allele frequency discontinuities, TESS determines the number of populations in the sample, and then assigns individuals to these populations. TESS also incorporates a user-specified prior to define the strength of connectivity of an individual sample to its neighbors (psi-value). Individuals sampled from the same location were spread out to place each one in its own space. This approach increases the power of the TESS analysis (Chen 2006). While spreading individuals, we were careful to avoid overlapping the geographic positions of closely-spaced samples (e.g. Depoe Bay and Newport samples). Latitude and longitude coordinates were randomly adjusted using TESS. Duplicate runs were performed with individual XY coordinates adjusted by varying amounts (5, 10, and 20 kilometers) to ensure that the clustering outcome was not dependent on the particular method used to generate spatial coordinates. Initial trials were performed to determine run lengths that resulted in likelihood stabilization. Then, a collection of batch runs was performed to determine the appropriate psi value (strength of connectivity prior) and Kmax (maximum population number) for more extensive sets of runs. For batch runs, the total number of sweeps was 25,000 with a burn-in of 5000. Psi values of 0.2, 0.4..1 were used with Kmax values of 2, 3..8 for each degree of sample spacing. Runs were performed under both admixture and no-admixture models. Dummy individuals were inserted to break biologically-impossible overland inter-individual connections (Chen 2006). We interpreted the following as evidence of a single, coast-wide population: at low psi values, the estimated number of

populations increased with increasing Kmax, while at higher psi values (0.6 and above), TESS estimated a single population independent of Kmax. Appropriate run conditions were identified as those where the number of populations stabilized as Kmax increased (Chen 2006). For copper rockfish, this was one cluster at psi values between 0.6 and 1.0. Finally, a set of 50 runs was performed at Kmax of 3 and psi values of 0.6, 0.8 and 1.0, with other parameters as above. The highest likelihood run within a set of parameter values was considered the best clustering outcome.

Results

Population-Based Analyses

An average of approximately 12 alleles per locus was observed within populations, and expected heterozygosity averaged 0.72 (Table 1). There was no significant difference among populations in either mean expected heterozygosity (Friedman test, $p = 0.18$) or allelic richness (Friedman test, $p = 0.12$). Two collections (Neah Bay and Coos Bay) had significant positive F_{IS} values (Table 1). Microchecker results suggested that the significant F_{IS} in Neah Bay was due to the presence of null alleles. Allele frequencies were adjusted for this population in Microchecker using the Brookfield (1996) null allele estimator 1 model. However, results of subsequent allele frequency-based analyses (FCA and F_{ST} values) did not differ substantially between corrected and uncorrected data sets, so all analyses were performed using the uncorrected data set. Microchecker results did not detect significant null allele problems in Coos Bay (data not shown), which suggests that deviations from Hardy-Weinberg equilibrium for Coos Bay were possibly due to admixture of two cryptic populations. In fact, a significant F_{ST} of 0.017, ($p < 0.001$) was detected between nine specimens collected from within the Coos Bay estuary and 65 specimens collected outside the bay. Due to the low salinity, lack of appropriate habitat, and small distances involved, the most likely explanation of the high pairwise F_{ST} is that it resulted from non-representative sampling. However, it is possible that these nine

specimens represent a cryptic population within Coos Bay. Because the estuary was undersampled, the estuary samples were excluded from further analyses. Significant F_{IS} remained even after removal of the inner estuary samples (Table 1). However, results of FCA and F_{ST}-based tests did not differ significantly after allele frequencies were adjusted for this population using the Brookfield (1996) null allele estimator 1 model in Microchecker, so all analyses used the uncorrected data set. No microsatellite markers had individually significant F_{IS} overall (Table 2). Significant divergence was not detected among samples collected during different years at the same location (data not shown).

Overall *S. caurinus* population structure was consistent with population subdivision and isolation-by-distance at the coast-wide scale (Fig. 2a). Analyses at the broad scale identified a highly significant F_{ST} of 0.0042 ($p < 0.0001$, Table 2). Four microsatellite markers had individually significant overall F_{ST} values, *Sra 11-103*, *Spi 6*, *Spi 10*, and *Spi 18* (Table 2). There was strong support at the 1000 km scale for the correlation of genetic and geographic distance for the whole coast (Mantel $p = 0.0009$, slope F_{ST} / 1- $F_{ST} = 0.006$, $R^2 = 0.42$, Fig. 2a, Table 2). Four markers, *Sra 7-25*, *Sra 15-9*, *Sma 11*, and *Spi 6*, had individually significant correlations of genetic and geographic distances (Table 2). Factorial correspondence analysis likewise revealed a pattern broadly consistent with isolation-by-distance, with California samples scoring low on factor 1 scale (16.4% of total variance explained, Fig. 3), Oregon samples in the middle, and the single Washington sample scoring high. There was no obvious geographic pattern in factor 2 (11.9% of total variance explained, Fig. 3) or factor 3 (11.5% of total variance explained, data not shown).

Population-based allele frequency analyses within Oregon suggested that populations were weakly structured, did not follow equilibrium isolation-by-distance expectations, and that the sand barrier was important. The overall F_{ST} within Oregon was very small (0.001) but significant ($p = 0.0156$, Table 2). No microsatellite marker had an individually significant F_{ST} value at this scale (Table 2). There was also no

support for the correlation of genetic and geographic distance at the 1000 km scale within Oregon (Mantel $p = 0.43$, slope $F_{ST} / 1- F_{ST} = 0.001$, $R^2 = 0.0013$; Fig. 2b, Table 2). In order to determine whether a single outlier population or locus obscured a correlation of genetic and geographic distance, we analyzed alternative subsets of the data, wherein each locus and population was dropped in turn and the remaining loci and populations analyzed, but significance was not detected in any subset (data not shown). Also, no individual markers had a significant correlation between genetic and geographic distance (Table 2). Factorial correspondence analysis of Oregon collections revealed that samples within regions clustered together, but among regions there was an anomalous genetic intermediacy of geographically southern rather than central collections according to factor 1 (20.2% of variance explained, Fig. 4). No geographic pattern was obvious in factors 2 (Fig. 4) or 3 (data not shown). When both the sand barrier and Cape Blanco were simultaneously included in an AMOVA model, results indicated that heterogeneity was significant among groups ($F_{CT} = 0.00232$, $p = 0.002$) and that heterogeneity among populations within the three groups was not significant ($F_{SC} = 0.00056$, $p = 0.431$). When the putative barriers were considered individually, the AMOVA results showed that there was significant heterogeneity among the groups defined by the sand ($F_{CT} = 0.00142$, $p = 0.018$) with only marginally significant heterogeneity remaining among populations within groups ($F_{SC} = 0.00140$, $p = 0.079$). Differences among groups were also marginally significant when separated by Cape Blanco alone ($F_{CT} = 0.00107$, $p = 0.075$), with significant heterogeneity remaining among populations within groups ($F_{SC} = 0.00168$, $p = 0.038$). These results suggest the best fit of the data to be with both barriers included (least within-group heterogeneity, greatest among-group heterogeneity), although the evidence for sand habitat as a barrier to gene flow is stronger than for Cape Blanco (when divided by Blanco there was a smaller between-group heterogeneity and larger within-group heterogeneity across sand). Results of the divided data set AMOVA analyses were consistent with those of the full data set (Table 3). When both barriers

were included in the model, F_{CT} was significant and F_{SC} was non-significant at the 0.05 level in all cases. The sand barrier was significant when the data set was divided by markers, but not when the data set was divided by year. Cape Blanco was only significant in the *Sra*-marker data set. The effect of combining the divided data sets into the full data set was to increase the power of the analysis, generally leading to lower *P* values for both F_{CT} and F_{SC}. Pairwise analyses between regions further supports the hypothesis that the sand barrier is a stronger barrier than Cape Blanco. A significant F_{ST} of 0.0027 ($p = 0.004$) was detected between the northern and central region, with a less significant F_{ST} of 0.0020 ($p = 0.131$) between central and southern regions. A comparatively low divergence between northern and southern regions (F_{ST} 0.0011; $p = 0.086$) further illustrates the lack of a strict correlation of genetic and geographic distance at the Oregon-only scale using population-based analytical methods and the pattern seen in FCA and Mantel analyses.

Individual-Based Analyses

TESS clustering results did not detect discrete barriers to gene flow. Results did not vary when samples were spaced at 5km versus 10 or 20km. Runs that included admixture returned only biologically-improbable disjunct distributions with estimated number of population tracking Kmax. These results are consistent with a single, panmictic population, and will not be reported further. In initial runs under the no-admixture model, with psi set at 0.2, the estimated number of populations settled between three and four populations, but clusters were not geographically coherent. With psi set at 0.4, TESS estimated two populations in all but one run in which the estimate was one population. Again, clusters were not geographically coherent. For psi values of 0.6 or greater the population number stabilized at 1. Subsequent runs with Kmax set at 3, and psi values of 0.6, 0.8, and 1.0, returned only single cluster solutions. Notably, there were no discontinuities recovered by TESS between northern

and central, or central and southern Oregon, even though population-based analyses suggested weak but significant regional divergence.

Discussion

Isolation-by-distance findings at the coast-wide scale are similar to those of previous genetic analyses in copper, brown, and grass rockfish (Buonaccorsi et al. 2002, 2004, 2005). The whole-coast slope of isolation-by-distance in the present study and previous research on copper rockfish (Buonaccorsi et al. 2002) were similar when standardized to the 1000 km scale (present study F_{ST} = 0.006, previous study F_{ST} = 0.008), even after doubling the number of loci. At the Oregon-coast scale, our study suggests that the signal of isolation-by-distance in copper rockfish is the result of a series of discrete extrinsic barriers along the coast. However, the effect of the sand and/or Cape Blanco as contributors to genetic structure is not wholly convincing in that the TESS analyses did not provide evidence for any population discontinuities along the Oregon coast. Chen et al. (2007) suggest that TESS may have difficulty discerning clusters at F_{ST} values at or below 0.01, which may explain the lack of evidence for discontinuities in this case. The best fit of the data in the population-based AMOVA analysis, however, was with both barriers included, although statistical support was stronger for the sand habitat than for the upwelling jet at Cape Blanco. Within the sand barrier, where population densities are low, we estimate that F_{ST} accumulates at twice the coast-wide average rate. This estimate is based on the pairwise F_{ST} of 0.0027 detected over an average distance of 191 km between the northern and central regions, corresponding to F_{ST} = 0.014 at a scale of 1000 km. This implies that dispersal (N_em) within the sand habitat is half the coastwide average rate. Outside the sand habitat within Oregon dispersal appears large enough to result in panmixia within regions. These results suggest that the signal of isolation-by-distance at the coast-wide scale may be the result of a series of discrete habitat barriers.

Isolation-by-distance may result from entrainment in static water masses, from a limited dispersal capability in terms of behavior or survival, or from a series of discrete barriers. Fronts associated with geographic promontories or embayments are known to entrain rockfish larvae and may form discrete barriers to gene flow (Graham and Largier 1997, Morgan and Botsford 1998, Wing et al. 1998). Cape Blanco has been thought to be one such geographic promontory along the coast of Oregon. If larval dispersal capability is limited, small areas of non-optimal habitat may function as additional biogeographic barriers to gene flow. In demersal rocky-reef species, such as the copper rockfish, areas of sand, such as between Newport and Coos Bay, could be an example of this type of habitat barrier. This would hold if the sand area were large enough to preclude the likelihood of survival across such a distance during the larval period and larval production within this area was greatly reduced. Our analyses indicate that one or both of these barriers do serve to structure copper rockfish populations in Oregon. Evidence is strongest for genetic distance and the sand barrier, but we cannot exclude the effect of Cape Blanco in structuring Oregon coast copper rockfish populations.

The effect of oceanographic or habitat barriers would be expected to be most pronounced in species with a complete nearshore life history. Copper, grass, and brown rockfish are all members of the subgenus *Pteropodus* (Seeb 1986, Taylor 1998, Rocha-Olivares et al. 1999) and have nearshore, non-migratory, benthic adults. It is likely that they all extrude their larvae in the nearshore environment, and it is known that *Pteropodus* juveniles are associated with relatively shallow nearshore waters (Lenarz et al. 1995). As a result, their larvae and juveniles experience reduced advection or migration into offshore bulk flows (Buonaccorsi et al. 2002). It has been suggested that, in order to disperse alongshore, a rockfish larvae must first disperse offshore (Largier 2003). This is because a boundary layer of "sticky water" within 1-3 km of shore greatly reduces advection and favors diffusive dispersal and may entrain larvae for up to one month (Zeidberg and Hamner 2002). Field measurements and

modeling studies suggest that cross-shelf movement of water decreases as one approaches the coast (Austin and Barth 2002, Austin and Lentz 2002, Largier 2003). In fact, studies of current drifters released landward of the upwelling front (within a few km of shore), indicate that drifters are not swept offshore; instead, they drift toward the coast (Barth and Smith 1998, Barth et al. 2000). It is also possible that larval or juvenile behavior may prevent dispersal away from the nearshore (Marliave 1986). It has been suggested that rockfish larvae may undergo vertical migrations (Buonaccorsi et al. 2002) due to their distinct vertical distributions (Moser and Boehlert 1991). Shanks and Brink (2005) showed that very slow swimming (approximately 0.1 cm s^{-1}) bivalve larvae were able to avoid being swept offshore by upwelling or onshore by downwelling through a simple depth-keeping behavior. In addition, even if movement away from the nearshore environment were necessary for larval development and survival, diffusive mortality models suggest that few individuals may be expected to recruit to regions outside the local range (Cowen et al. 2000). As a result of some or all of these factors, members of the *Pteropodus* subgenus, including the copper rockfish, may undergo far less dispersal than their two- to three- month pelagic phase might suggest.

Gunderson and Vetter (2006) suggested four distinct models of larval dispersal that may characterize Pacific coast rocky reef fishes: broad, mesoscale, diffusive, and non-dispersing. We can eliminate broad dispersal, which posits a single range-wide stock because this is not consistent with previous or current population genetic findings in copper rockfish (Buonaccorsi et al. 2002) as well as non-dispersing, in which populations are essentially closed. Both these models are unrealistic for copper rockfish dispersal. At the coast-wide scale, mesoscale dispersal, in which populations are self-recruiting on a regional scale, and diffusive dispersal, in which populations are largely self-recruiting with limited external recruitment from adjacent habitats, both predict a very similar pattern of isolation-by-distance. Mesoscale dispersal would result in little or no isolation-by-distance signal at the fine scale, but with significant

breaks at habitat or oceanographic barriers. Diffusive dispersal would result in a signal of isolation-by-distance even at the fine scale, although habitat or oceanographic breaks may also play an important role. In our analyses, geographic distance was not found to be a significant predictor of genetic distance at the fine scale. Thus it follows that our Oregon copper rockfish findings match the mesoscale dispersal model best.

This study addressed three specific questions. (1) Will the coast-wide pattern of isolation-by-distance in copper rockfish (*Sebastes caurinus*) previously described by Buonaccorsi (2002) persist when sample size, number of sites, and number of loci are doubled? Genetic analysis provided evidence of genetic structure consistent with the previous finding of isolation-by-distance among samples collected along the Pacific coast from Neah Bay, WA to San Diego, CA. (2) At the scale of the Oregon coast, is population structure in copper rockfish homogeneous? Genetic analysis provided evidence for weak but significant genetic structure among sample collections along the Oregon coast. (3) If genetic structure is apparent at the Oregon coast scale, is it influenced by distance, habitat breaks, Cape Blanco, or some combination of the three? The Oregon-coast genetic structure was not consistent with a pattern of pure isolation-by-distance. Instead, the present analysis suggests that isolation is a function of restricted dispersal across habitat barriers, in particular the sand habitat between Newport and Coos Bay. Data show that Cape Blanco was not as strong a factor in structuring Oregon-coast copper rockfish as was the reduction in adult breeding population size due to poor adult habitat. In copper rockfish, it seems that dispersal in the species may best be characterized as Gunderson and Vetter's (2006) mesoscale dispersal model. In this case it appears that non-optimal habitat, with some possible influence from the oceanographic jet at Cape Blanco, serves to limit dispersal in Oregon copper rockfish.

Conclusion

Previous studies of dispersal distance on this species (Buonaccorsi et al. 2002, 2005) included geographic distance as a sole factor causing restricted gene flow from Canada to southern California. The present study has demonstrated that some of the overall genetic divergence is related to habitat patchiness, not necessarily a restricted dispersal distance alone. Consequently the prior studies may have somewhat underestimated within-region dispersal, while simultaneously overestimating dispersal across habitat barriers. These findings suggest that it is appropriate to divide management into regions. This further suggests that some nearshore rockfish populations may be more finely divided than rockfish fisheries management currently considers. Therefore, it will be important to take habitat barriers into account when designing a marine reserve network. It may not be sufficient to place a single marine reserve within a region, because areas without a reserve would be unlikely to receive meaningful numbers of recruits from across habitat barriers. It will be important to test the generality of the combination of geographic distance and habitat barriers in structuring populations in other species and geographic areas. Research incorporating population genetic approaches and other tools, such as otolith microchemistry and tetracycline tagging, is needed to assess the universality of any proposed barriers. However, from the perspective of fisheries management, if this finding persists across taxa, it will be important to align management boundaries with these habitat barriers.

References

Anderson TW (1983) Identification and development of nearshore juvenile rockfishes (Genus *Sebastes*) in central California kelp forests. Masters Thesis, California State University, Fresno.

Austin JA, Barth JA (2002) Drifter behavior on the Oregon-Washington shelf during downwelling-favorable winds. Journal of Physical Oceanography 32: 3132-3144.

Austin JA, Lentz SJ (2002) The inner shelf response to wind-driven upwelling and downwelling. Journal of Physical Oceanography 22: 2171-2193.

Banks MA, Rashbrook VK, Calavetta MJ, Dean CA, Hedgecock D (2000) Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's central valley. Canadian Journal of Fisheries and Aquatic Sciences 57: 915-927.

Barth JA, Smith RL (1998) Separation of a coastal upwelling jet at Cape Blanco, Oregon, USA. South African Journal of Marine Science 19: 5-14.

Barth J, Pierce S, Smith R (2000) A separating coastal upwelling jet at Cape Blanco, Oregon and its connection to the California Current System. Deep-Sea Research II 47: 783-810.

Belkhir K (2000) GENETIX 4.02, Windows TM program for population gentics. Available at http://www.univ-montp2.fr/~genetix/genetix.htm.

Bentzen P, Taggart CT, Ruzzante DE, Cook D (1996) Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 53: 2706-2721.

Botsford LW, Hastings A, Gaines SD (2001) Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. Ecology Letters 4: 144-150.

Botsford LW, Micheli F, Hastings A (2003) Principles for the design of marine reserves. Ecological Applications 13 Supplement: S25-S31.

Bradbury IR, Bentzen P (2007) Non-linear genetic isolation-by-distance: implications for dispersal estimation in anadromous and marine fish populations. Marine Ecology Progress Series 340: 245-257.

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology 5(3): 453-455.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD (2002) Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and

contemporary patterns of larval dispersal. Canadian Journal of Fisheries and Aquatic Sciences 59:1374-1384.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD (2005) Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. Conservation Genetics 6: 697-713.

Buonaccorsi VP, Westerman M, Stannard J, Kimbrell C, Lynn E, Vetter R (2004). Molecular genetic structure suggests limited larval dispersal within grass rockfish, *Sebastes rastrelliger*. Marine Biology 145:779-788.

Chen C (2006) TESS reference manual. Grenoble: Institut d'Informathique et Mathématiques Appliquées de Grenoble. Available: http://www-timc.imag.fr/Olivier.Francois/tess.html. Accessed Sept. 1, 2007.

Chen C, Durand E, Forbes F, Francois O (2007) Bayesian clustering algorithms ascertaining spatial population structure: A new computer program and comparison study. Molecular Ecology Notes 7: 747-756

Connolly SR, Menge BA, Roughgarden J (2001) A latitudinal gradient in recruitment of intertidal invertebrates in the northeast Pacific Ocean. Ecology 82:1799–1813

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: open or closed? Science 287: 857-859.

Doherty PJ, Planes S, Mather P (1995) Gene flow and larval duration in seven species of fish from the Great Barrier Reef. Ecology 76: 2373-2391.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Francois O, Ancelet S, Guillot G (2006) Bayesian clustering using hidden Markov random fields in spatial population genetics. Genetics 174: 805-816.

Gomez-Uchida DG, Hoffman EA, Ardren WR, Banks MA (2003) Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species. Molecular Ecology Notes 3:387-389.

Graham WM, Largier JL (1997) Upwelling shadows as nearshore retention sites: an example of northern Monterey Bay. Continental Shelf Research 17: 509-532.

Gunderson DR, Vetter RD (2006) Temperate rocky reef fishes. Chapter 3. Pages 69-117 *in* JP Kritzer, PF Sale (eds), Marine Metapopulations. Elsevier.

Kingsford MJ, Leis JM, Shanks A, Lindeman KC, Morgan SG, Pineda J (2002) Sensory environments, larval abilities and local self-recruitment. Bulletin of Marine Science 70: 309-340.

Largier J (2003) Considerations in estimating larval dispersal distances from oceanographic data. Ecological Applications 13 Supplement: S71-S89.

Lea RN, McAllister RD, Ventresca DA (1999) Biological aspects of the nearshore rockfishes of the genus *Sebastes* from central California. California Department of Fish and Game, Fish Bulletin 177:1-109.

Leblois R, Estoup A, Rousset F (2003) Influence of mutational and sampling factors on the estimation of demographic parameters in a "continuous" population under isolation-by-distance. Molecular Biology and Evolution 20: 491-502.

Lenarz WH, VenTresca DA, Graham WM, Schwing FB, Chavez F (1995) Explorations of El Niño events and associated biological population dynamics off central California. CalCOFI (California Cooperative Oceanic Fisheries Investigations) Reports 36: 106-119.

Love MS (1996) Probably More Than You Want to Know About Fishes of the Pacific Coast. Really Big Press, Santa Barbara, CA.

Love MS, Caselle JE, Buskirk WV (1998) A severe decline in the commercial passenger fishing vessel rockfish (*Sebastes spp.*) catch in the Southern California Bight, 1980-1996. CalCOFI (California Cooperative Oceanic Fisheries Investigations) Reports 39: 180-195.

Love MS, Yoklavich M, Thorsteinson L (2002) The Rockfishes of the Northeast Pacific. University of California Press, Berkeley, CA.

Lubchenco J, Palumbi SR, Gaines SD, Andelman S (2003) Plugging a hole in the ocean: the emerging science of marine reserves. Ecological Applications 13 Supplement: S3-S7.

Marliave JB (1986) Lack of planktonic dispersal of rocky intertidal fish larvae. Transactions of the American Fisheries Society 115: 149-154.

Mecklenburg CW, Mecklenburg TA, Thorsteinson LK (2002) Fishes of Alaska. American Fisheries Society, Bethesda, MD.

Miller JA, Shanks AL (2004) Ocean-estuary coupling in the Oregon upwelling region: abundance and transport of juvenile fish and crab megalopae. Marine Ecology Progress Series 271: 267-279.

Morgan LE, Botsford LW (1998) The influence of larval transport and retention zones on recruitment patterns and the design of harvest refugia for rockfish. NOAA Tech. Memo. 255: 41-49.

Moser HG, Boehlert GW (1991) Ecology of pelagic larvae and juveniles of the genus *Sebastes*. Environmental Biology of Fishes 30: 203-224.

Musick JA, Harbin MM, et al. (2000) Marine, estuarine, and diadromous fish stocks at risk of extinction in North America (exclusive of Pacific salmonids). Fisheries 25(11): 6-30.

Palumbi SR (2001) The ecology of marine protected areas. In: Bertness MD, Gaines SD, Hay M (eds) Marine Community Ecology. Sinauer Associates, Inc., Sunderland, MA, p 509-530.

Ralston S, Ianelli J, Miller R, Pearson D, Thomas D, Wilkins M (1996) Status of bocaccio in the Conception/Monterey/Eureka INPFC areas in 1996 and recommendations for management in 1997. Appendix volume 1 to the status of the Pacific coast groundfish fishery through 1996 and recommended acceptable biological catches for 1997. Pacific Fishery Management Council, Portland, Oregon.

Raymond M, Rousset F (1995) GENEPOP (Ver. 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Rocha-Olivares A, Vetter R (1999) Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Canadian Journal of Fisheries and Aquatic Science 56: 803-813.

Rocha-Olivares A, Kimbrell CA, Eitner BJ, Vetter RD (1999) Evolution of a mitochondrial cytochrome *b* gene sequence in the species-rich genus *Sebastes* (Teleostei, Scorpaenidae) and its utility in testing the monophyly of the subgenus *Sebastomus*. Molecular Phylogenetics and Evolution 11: 426-440.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation-by-distance. Genetics 145: 1219-1228.

Seeb LW (1986) Biochemical systematics and evolution of the scorpaenid genus *Sebastes*. PhD Thesis, University of Washington, Seattle.

Shanks AL, Brink L (2005) Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. Marine Ecology Progress Series 302: 1-12.

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

Sokal RR, Rohlf FJ (1995) Biometry. 3rd ed. W.H. Freeman and Co., New York.

Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR (2002) Evidence of self-recruitment in demersal marine populations. Bulletin of Marine Science 70: 251-271.

Taylor CA (1998) Phylogenetics and larval identification of rockfishes (genus *Sebastes*) using mitochondrial DNA techniques. Masters thesis, San Diego State University, San Diego.

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299: 107-109.

van Oosterhaut C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

Waples RS, Rosenblatt RH (1987) Patterns of larval drift in Southern California marine shore fishes inferred from allozyme data. U.S. National Marine Fisheries Service Fishery Bulletin 85: 1-12.

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

Westerman ME, Buonaccorsi VP, Galver L, Taylor C, Lynn EA, Kimbrell CA, Vetter RD (2005) Cloning and characterization of novel microsatellite DNA markers for the grass rockfish, *Sebastes rastrelliger*, and cross-species amplification in 10 related *Sebastes spp.* Molecular Ecology Notes 5: 74-76.

Wimberger P, Burr J, Gray A, Lopez A, Bentzen P (1999) Isolation and characterization of twelve microsatellite loci for rockfish (*Sebastes*). Marine Biotechnology 1:311-315.

Wing SR, BotsfordLW, Ralston SV, Largier JL (1998) Meroplanktonic distribution and circulation in a coastal retention zone of the northern California upwelling system. Limnology and Oceanography 43: 1710-1721.

Zeidberg LD, Hamner WM (2002) Distribution of squid paralarvae, *Loligo opalescens* (Cephalopoda, Myopsida), in the southern California Bight in the three years following the 1997-1998 El Niño. Marine Biology 141: 111-122.

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Tables

Table 1.1. Sample collection information and summary statistics.

Abbr.	Location	Lat.	Long.	N	*A*	H_E	H_O	F_{IS}	*P*
NB	Neah Bay, WA	48.38	124.61	49	11.45	0.73	0.68	0.071	0.003
TG	Tillamook/ Garibaldi, OR	45.54	124.00	107	13.91	0.72	0.7	0.038	0.038
DP	Depoe Bay, OR	44.81	124.07	46	10.27	0.7	0.69	0.022	0.553
NP	Newport, OR	44.64	124.07	135	13.91	0.73	0.72	0.016	0.037
CB	Coos Bay, OR	43.37	124.35	65	12.55	0.72	0.72	0.012	0.000
BD	Bandon, OR	43.13	124.45	32	10	0.71	0.69	0.045	0.153
OF	Port Orford, OR	42.74	124.49	78	12.73	0.72	0.7	0.046	0.268
RG	Rogue Reef, OR	42.46	124.48	49	12.18	0.72	0.71	0.013	0.032
CC	Crescent City, CA	41.75	124.25	63	11.91	0.72	0.7	0.033	0.455
BC	Big Creek, CA	35.44	121.19	30	10.73	0.74	0.72	0.042	0.122
SM	San Miguel, CA	34.01	120.37	53	11.82	0.72	0.7	0.039	0.089
SD	San Diego, CA	32.81	117.00	39	10.09	0.71	0.71	0.013	0.816
	Average				11.8	0.72	0.7	0.033	0.214

Note: Average number of alleles per locus (*A*), expected heterozygosity (H_E), observed heterozygosity (H_O), local inbreeding coefficient (F_{IS}), significance of F_{IS} (*P*). Abbreviations: WA, Washington; OR, CA, California. Oregon;

Table 1.2. Summary statistics by locus.

Locus	Overall F_{ST}	*P*	Oregon F_{ST}	*P*	F_{IS}	*P*	AR	Overall Mantel *P*	Oregon Mantel *P*
Sra 7-7	0.006	0.0268	0.003	0.1516	0.048	0.0080	6.787	0.1082	0.6396
Sra 7-25	0.002	0.0572	-0.002	0.6663	0.029	0.0675	10.015	0.0012	0.6432
Sra 11-103	0.012	0.0000	0.003	0.1693	0.034	0.0998	3.446	0.1146	0.1962
Sra 15-9	0.006	0.2023	0.002	0.6651	0.035	0.2016	19.686	0.0004	0.1014
Sra 16-5	0.000	0.0094	-0.001	0.2786	0.031	0.0203	12.043	0.0292	0.3922
Sma 3	-0.002	0.3074	-0.001	0.5767	0.037	0.2565	6.289	0.8684	0.7110
Sma 10	0.007	0.0073	0.004	0.0299	0.010	0.1770	11.357	0.2942	0.2142
Sma 11	0.004	0.0480	-0.001	0.2211	0.057	0.0097	5.535	0.0008	0.8656
Spi 6	0.003	0.0000	0.001	0.0247	0.025	0.0572	16.335	0.0010	0.0248
Spi 10	0.006	0.0005	0.000	0.1081	0.043	0.3243	3.856	0.0866	0.1912
Spi 18	0.007	0.0000	0.005	0.1270	0.009	0.0528	14.324	0.2108	0.8616
All	0.004	0.0000	0.001	0.0156				0.0009	0.4282

Note: Coastwide fixation index (Overall F_{ST}), significance of Overall F_{ST} (*P*), Oregon-scale fixation index (Oregon F_{ST}), significance of Oregon F_{ST} (*P*), inbreeding coefficient (F_{IS}), significance of F_{IS} (*P*), allelic richness (AR), significance of coastwide isolation-by-distance (Overall Mantel *P*), significance of Oregon isolation-by-distance (Oregon Mantel *P*). Adjusted nominal level (5%) for multiple comparisons is 0.0045.

Table 1.3. AMOVA results for the full data set, and data sets divided by year and markers.

	Test	F_{CT}	*P*	F_{SC}	*P*
Full Data Set	Sand	0.00142	0.018	0.00140	0.079
	Blanco	0.00107	0.075	0.00168	0.038
	Both	0.00232	0.002	0.00056	0.431
Pre-2003	Sand	0.00123	0.147	0.00650	0.004
	Blanco	0.00326	0.091	0.00515	0.034
	Both	0.00358	0.012	0.00464	0.074
2003-on	Sand	0.00121	0.094	0.00167	0.143
	Blanco	-0.00022	0.243	0.00251	0.026
	Both	0.00186	0.003	0.00108	0.320
Sra	Sand	0.00164	0.022	0.00189	0.105
	Blanco	0.00145	0.041	0.00210	0.068
	Both	0.00271	0.003	0.00091	0.367
Sma & Spi	Sand	0.00124	0.036	0.00100	0.221
	Blanco	0.00075	0.129	0.00134	0.146
	Both	0.00201	0.008	0.00028	0.491

Note: Among-group fixation index (F_{CT}), significance of F_{CT} (*P*), among-population within group fixation index (F_{SC}), significance of F_{SC} (P). Shaded cells indicate significance at the 0.05 level.

Figures

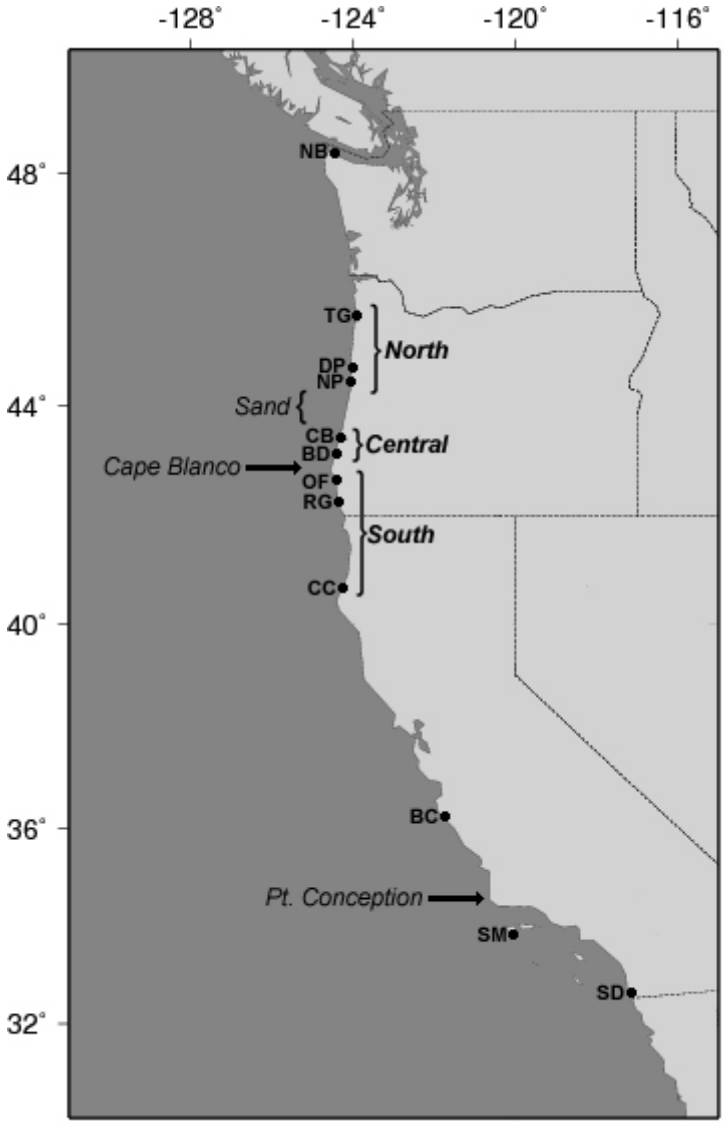

Figure 1.1. Collection locations along the Pacific coast of North America. Location abbreviations are as in Table 1. Locations of the sand habitat, Cape Blanco, and Point Conception are indicated. Regional groupings (north, central, south) for AMOVA analysis are also shown.

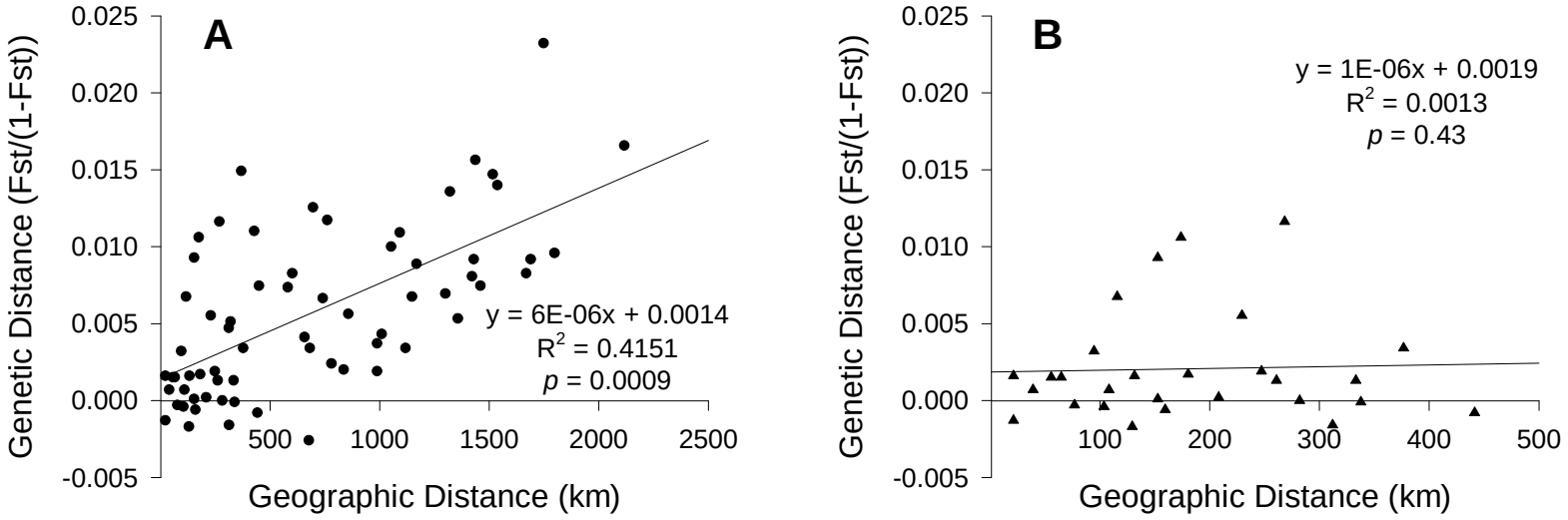

Figure 1.2. Pairwise comparison of genetic versus geographic distance in copper rockfish, *S. caurinus* at the coast-wide (A. Neah Bay to San Diego) and Oregon (B. Tillamook to Crescent City) scales. Genetic distance shown as linearized F_{ST}.

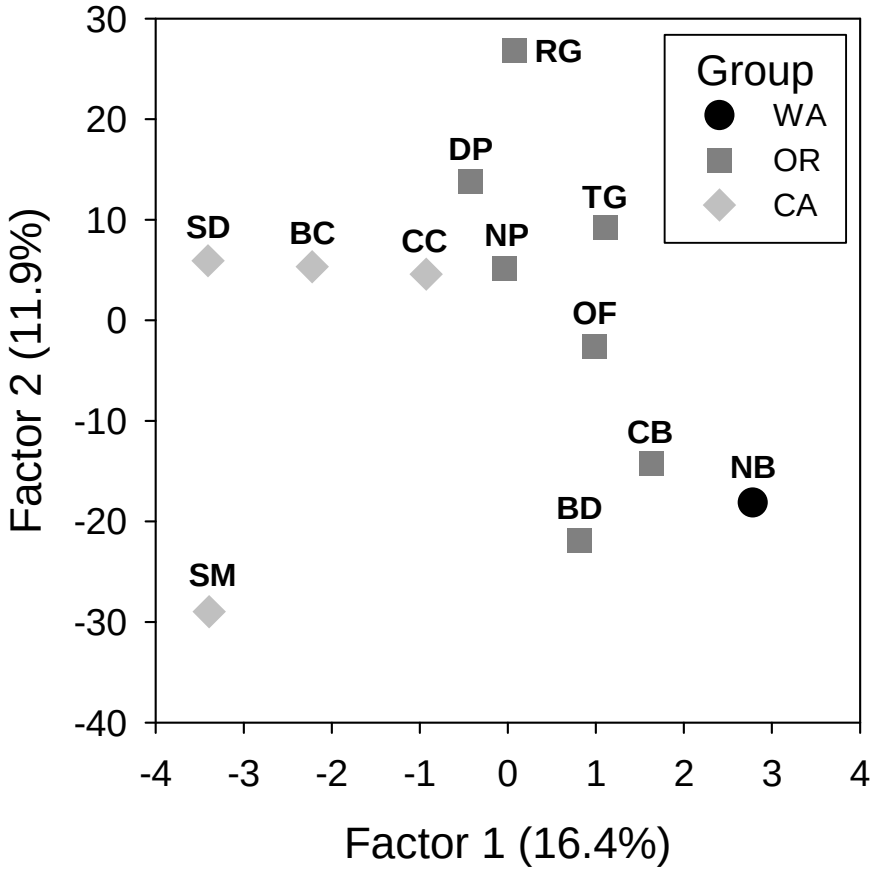

Figure 1.3. Two- factor factorial correspondence analysis results indicating similarity between collection locations at the whole-coast scale. Symbols indicate state (Washington, Oregon, California) of sample origin.

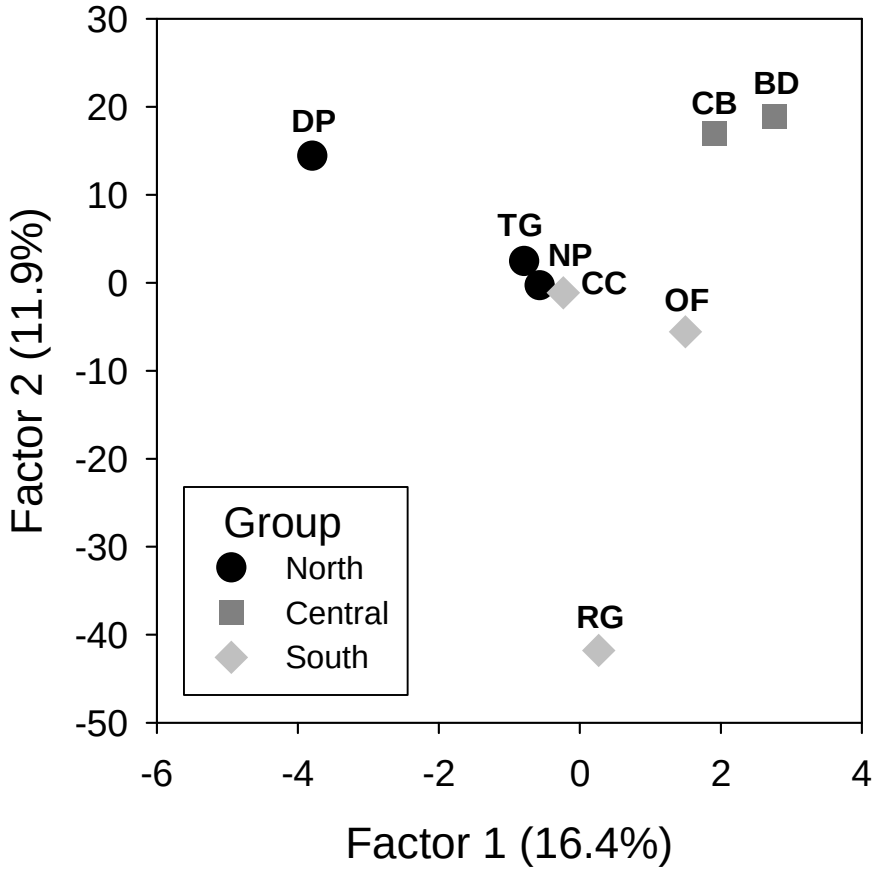

Figure 1.4. Two-factor factorial correspondence analysis results indicating similarity between collection locations at the Oregon scale. Symbols indicate region (north, central, south) of sample origin.

OLFACTORY RECEPTOR RELATED TO CLASS A, TYPE 2 (*ORA*2) GENES ARE CONSERVED AMONG DISTANTLY RELATED ROCKFISHES (GENUS *SEBASTES*)

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(In Review)

Abstract

*Ora*2 genes express putative chemoreceptors which may function as pheromone receptors in fishes. We used a candidate gene approach to test whether *Ora2* genes show evidence of positive selection that could suggest a role in mate recognition and the avoidance of hybridization between closely related rockfishes. We amplified a 492-bp fragment of a single *Ora2* gene from each of five species of rockfish. Despite separation of up to 7.8 million years, the sequence of *Ora2* is highly conserved. Genetic distances are small, and all of our study species shared at least one sequence with another species. Sequence comparisons suggested that, while most amino acids were subject to purifying selection, nine amino acids showed evidence of positive selection. Because these amino acids were not associated with the areas of the protein suggested to be involved in ligand binding based on structural similarity to other olfactory receptors, this signal may reflect an echo of the relaxation of selection associated with the speciation events that separate these species. Strong sequence conservation suggests that this gene is of functional significance. However, because of shared alleles among species, the *Ora2* gene, in isolation, would be unlikely to differentiate species during mating season.

Introduction

The vertebrate olfactory sense provides key information on the environment, predators and prey, and potential mates. In fishes, olfaction is mediated by four olfactory receptor families: olfactory receptors (ORs), trace amine-associated receptors, vomeronasal type 2 receptors (V2rs), and olfactory receptor class A-related receptors (Oras) (Pfister and Rodriguez 2005; Saraiva and Korsching 2007). Olfactory receptors are present in large numbers in fishes (143 genes in zebrafish) (Alioto and Ngai 2005), as are trace amine-associated receptors (57 genes in zebrafish) (Gloriam et al. 2005; Liberles and Buck 2006). Vomeronasal type 2 receptors (24 genes in zebrafish) sense amino acids in teleosts and, along with Oras (~6 genes in fishes) are thought to be involved in pheromone reception in mammals, although direct evidence is currently lacking for V2rs having such a role (Pfister and Rodriguez 2005; Saraiva and Korsching 2007; Speca et al. 1999).

The olfactory receptor related to class A (*Ora*) gene family, first described as V1r genes in rodents (Buck and Axel 1991), is one of two families of G protein-coupled receptors used to detect pheromones in mammals (Grus et al. 2005). Although fishes lack a vomeronasal organ, for which the gene family was originally named, numerous species have been shown to possess and express complete *Ora* genes (Pfister and Rodriguez 2005; Pfister et al. 2007; Saraiva and Korsching 2007; Shi and Zhang 2007). In contrast to the mouse and the rat, however, which contain ~100-150 functional receptors, no fish species has yet been found to have more than a handful (5 to 6) (Saraiva and Korsching 2007). Additionally, in fishes, some research has shown evidence of positive Darwinian selection on these genes, suggesting that some neofunctionalization may have occurred (Pfister et al. 2007). Other researchers have suggested that these genes are highly conserved, finding no evidence for positive selection (Saraiva and Korsching 2007). In this project, we examine one member of the Ora gene family in five species of the genus *Sebastes*, testing the hypothesis that

this gene may be experiencing positive selection, putatively due to a role in pheromonal communication and mate recognition.

Rockfishes of the genus *Sebastes* comprise an extremely diverse and successful species complex. With an estimated 110 species worldwide (Love et al. 2002), and numerous examples of convergent evolution for similar lifestyles (Hyde and Vetter 2007), rockfish present unique opportunities to use genetic studies to examine the process of speciation. Changes in mate recognition processes may provide one mechanism for the formation of reproductive isolation in rockfishes. Although numerous closely related species live in close proximity, the mate recognition system in rockfishes is largely unknown. Mate recognition may involve visual, pheromonal, or sound cues (Love et al. 2002), and could conceivably be central to the development of the unusually high diversity seen in the genus. Mate pairing in rockfishes is discrete, because they engage in internal fertilization and require complex courtship rituals before copulation can take place (Love et al. 2002). In general, male rockfish align themselves next to a female before swimming forward and placing their urogenital papilla near her snout (Gingras et al. 1998; Hallacher 1974; Helvey 1982; Shinomiya and Ezaki 1991). It has been suggested that this behavior allows the male to release a courtship pheromone as near as possible to the olfactory rosette of the female (Love et al. 2002).

In this study, we describe *Ora* genes for five species of rockfishes (genus *Sebastes*), representing a broad sampling of rockfish phylogeny and ecology. *S. caurinus* and *S. maliger* are separated by approximately 1.6MY, co-occur in rocky reef habitats in the Northeast Pacific, and have been shown to hybridize in Puget Sound, WA, USA (Love et al. 2002; Seeb 1998). *S. melanops* occurs around the same rocky habitats as *S. maliger* and *S. caurinus* but is separated by approximately 6.3MY of evolution (Hyde and Vetter 2007; Love et al. 2002). *S. ruberrimus* and *S. crameri* occupy a similar geographic range and similar habitats, but may be found somewhat deeper and are separated from the other species in our set by approximately 7.1MY

and 7.8MY, respectively (Hyde and Vetter 2007; Love et al. 2002). Complete life history data is lacking for several of these species, but it is known that some portion of the larval release period overlaps between all five species, as all release larvae during the spring and early summer (Love et al. 2002). Although evidence suggests that rockfish are capable of sperm storage, and that insemination may precede fertilization by up to six months (Moser 1967; Sogard et al. 2008), it is conceivable that the mating season overlaps for the sample set as well, and that they could be exposed to reproductive members of other species. We also test for a signal of positive and purifying selection on individual amino acids in our sequences, using a Bayesian approach to test for deviations of the rate of non-synonymous to synonymous nucleotide substitutions from neutral expectations (dN/dS = 1). If genes are involved in mate recognition or have played a role in speciation, we might expect to find a signal of positive selection at amino acids in the transmembrane domains of the protein, which are predicted to function in ligand binding based on structural analogy with related olfactory receptors. The transmembrane domains form a barrel-shaped heptahelical structure in the membrane (Palczewski et al. 2000), within which specific amino acids are predicted to interact with odorants. Additionally, we would anticipate finding evidence for divergence between species, particularly associated with these putatively functional domains.

Materials and Methods

To isolate and identify Ora genes from rockfishes, we first retrieved nucleotide sequences for Ora genes from *Botia macracantha*, *Danio albolineatus*, *D. frankei*, *D. malabaricus*, *D. rerio*, *Cyprinus carpio*, *Epalzeorhynchos frenatum*, and *Tetraodon nigroviridis* from Genbank. These sequences were aligned, and degenerate PCR primers designed using the program FastPCR (Kalendar 2009). Genomic DNA was extracted from fin-clip tissue samples using a glass fiber-plate protocol (Ivanova et al. 2006). Polymerase chain reaction (PCR) was performed on 8-24 individuals per species using primers 1F235 (5'-TGC-ATC-ACC-TGC-ATG-CTG-AGC-GT-3') and

R3BEM (5'-GGC-ACC-TGA-GGC-ACT-GTC-AGC-ATG-TAG-ATC-C-3') which amplify a 495-bp portion of the Ora type 2 gene. PCR was performed using the Promega GoTaq PCR reagent system (Promega) following the manufacturer's protocols and optimized for high fidelity (low $MgCl_2$ and low dNTP concentration, high annealing temperature, short extension time). Thermal cycling conditions were an initial denaturing step at 94 C for 2 min, followed by 30 cycles of 94 C for 1 min, 60 C for 1 min, 72 C for 1:30 and a final extension step of 72 C for 4 minutes. PCR products were separated via agarose gel electrophoresis, and purified using a QIAquick® gel extraction kit (Qiagen Inc., Valencia, CA). Purified PCR products were direct sequenced using the 1F235 and R3BEM PCR primers. To test for the existence of multiple Ora2 genes, or amplification of other members of the Ora gene family, PCR products were also cloned using the TOPO TA cloning kit (Invitrogen Inc., Grand Island, NY) and six colonies sequenced per individual. Clones were sequenced on an ABI 3730XL automated sequencer using M13 forward and reverse primers. Forward and reverse sequence reads were aligned and edited using Sequencher (Gene Codes Inc., Ann Arbor, MI). Unique sequences have been deposited in Genbank (Accession #s GU589587-GU589842).

We used jModelTest v. 0.1.1 (Guindon and Gascuel 2003; Posada 2008) to calculate the most likely model of evolution in our sequences. Based on these jModelTest results, pairwise genetic distances (HKY85+G) (Hasegawa et al. 1985) were calculated using PAUP* v4.0b10 (Sinauer Associates, Sunderland, MA). Bioedit v.6.0.7 (Hall 1999) was used to convert DNA to amino acid sequence. We used the WebLogo 3 (Crooks et al. 2004) online server (http://threeplusone.com/weblogo/) to generate an amino acid sequence logo. We then used the TMHMM server v.2.0 (Krogh et al. 2001); http://www.cbs.dtu.dk/TMHMM) and TMpred (Hofmann and Stoffel 1993) http://www.ch.embnet.org/software/TMPRED_form.html) to predict the transmembrane coding domains for the sequences.

To assess the hypothesis that positive selection may have caused the *Ora2* genes in different species to diverge in their functional domains, we used the SELECTON server v.2.4 (Stern et al. 2007); http://selecton.tau.ac.il/index.html) to identify amino acid sites under positive or purifying selection. In general, a dN/dS ratio (ω) > 1 at an amino acid site indicates positive selection on that site, whereas a ω < 1 suggests purifying selection at that site. We tested the significance of the selection estimates by calculating a 95% confidence interval for the ω value at each amino acid position and by performing a likelihood ratio test on the whole gene comparing the M8a null model (doesn't allow for positive selection) with the M8 model (does account for sites under positive selection).

Results

We successfully isolated a 492-base pair fragment of a single *Ora* type 2 gene from each of our five species of rockfish. None of our cloning work suggested the presence of additional *Ora*2 genes, or amplification of additional *Ora* genes; in no case did we find more than two sequences in a single (heterozygote) individual. We found 16 different sequences in 24 individual *S. caurinus*, 17 sequences in 24 *S. maliger*, 3 sequences in 8 *S. crameri*, 5 sequences in 8 *S. ruberrimus*, and 8 sequences in 8 *S. melanops*. Our sequences included most of transmembrane domain 3 and run through the first few amino acids of extracellular domain 3 (Figure 1). The overall picture of *Ora* type 2 diversity in our five rockfish species is that these genes are highly conserved, as evidenced by the strong amino acid conservation in our sequence logo (Figure 1) and by the short genetic distances (HKY85+G) we measured between species (Table 1). Additionally, we observed a number of shared sequences between species in our data set (Table 1).

Although most amino acids in the *Ora* sequences are characterized by purifying selection when tested with Selecton, we found significant evidence for strong positive selection in nine amino acids in the complete data set of *Ora* type 2 sequences (Figure 1 & 2). When we compared the M8 model, which allows positive

selection, with the M8a model, which does not allow positive selection, using a likelihood ratio test, the M8 model was a significantly better fit to the data (M8 Likelihood: -938.277, M8a likelihood: -948.199, Likelihood Ratio Test p = 0.001). These positively selected amino acids are not associated exclusively with the putatively ligand-binding transmembrane domains (Figure 1), however, at least one is found within each of the four transmembrane domains in our sequences.

Discussion

Overall, *Ora* type 2 genes seem to be highly conserved across the sample species, which represent a broad sampling of the *Sebastes* phylogeny. Several sequences were shared among species, most commonly in close relatives: *S. maliger* and *S. caurinus*, separated by approximately 1.6MY (Hyde and Vetter 2007), had 8 sequences in common. However, in a few cases sequences were shared between species that are separated by much greater lengths of time: *S. ruberrimus* shares sequences with *S. caurinus*, *S. maliger*, and *S. melanops*, although evidence suggests that ancestors of these species diverged from their most recent common ancestor by as much as 7.1MY ago (Hyde and Vetter 2007), and *S. crameri* and *S. melanops*, separated by 7.8MY (Hyde and Vetter 2007), share a sequence in our data set. Additionally, most of the amino acids in the sequence show evidence of moderate to strong purifying selection. As such, it seems unlikely that sequence differences alone function to differentiate species at mating time. Visual, auditory, or lateral line cues, along with differences in reproductive timing and location may also function to prevent hybridization in related rockfishes (Love et al. 2002).We found nine amino acid residues that showed a significant pattern of positive selection, based on testing with SELECTON, which might be interpreted as consistent with selection causing pheromone receptor genes to diverge in close relatives. Since the five species in our study are separated by numerous speciation events, another possibility is that the signal of positive selection reflects the relaxation of purifying selection or population bottleneck associated with speciation (Hughes 2007). These positively selected sites

are not isolated to the putatively functional transmembrane domains (Figure 1), which are associated with ligand binding in related olfactory receptors . However, at least one was found within each transmembrane domain, and those outside could conceivably be associated with conformational or other changes that affect ligand binding as well.

The overall high level of sequence conservation found in *Ora* type 2 genes in rockfishes likely reflects their strong functional significance. Pfister et al. (2007) found a similar pattern of overall purifying selection in V1r genes, with evidence of positive selection on selected amino acid residues across five related species of teleost fishes. In comparisons between orthologous *Ora* genes using a similar set of fish species, Saraiva and Korsching (2007) found low dn/ds ratios, indicating strong negative selection. The totality of evidence thus suggests that the *Ora* gene family is very ancient and slowly evolving, that orthologous *Ora* genes may have a common role across species, and that these receptors may be focused to recognize only a single or a very small number of molecules (Pfister and Rodriguez 2005; Pfister et al. 2007; Saraiva and Korsching 2007). The actual ligands recognized by fish *Ora* genes are currently unknown (Pfister et al. 2007; Saraiva and Korsching 2007), but if these genes recognize pheromones, as has been suggested for the related V1r genes in mammals (Boschat et al. 2002), then they would be expected to have little species-specificity, given their high level of sequence conservation (Saraiva and Korsching 2007). We hypothesize instead that these genes are involved in assessing mate condition, gender, or reproductive status, or other qualities which are important across species and regardless of species identity.

References

Alioto TS, Ngai J, 2005. The odorant receptor repertoire of teleost fish. Bmc Genomics. 6:173.

Boschat C, Pelofi C, Randin O, Roppolo D, Luscher C, Broillet MC, Rodriguez I, 2002. Pheromone detection mediated by a V1r vomeronasal receptor. Nature Neuroscience. 5:1261-1262.

Buck L, Axel R, 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell. 65:175-187.

Crooks GE, Hon G, Chandonia JM, Brenner SE, 2004. WebLogo: A sequence logo generator. Genome Research. 14:1188-1190.

Gingras ML, Ventresca DA, Donnellan MD, Fisher JL, 1998. First observations of vermillion rockfish courtship are from a harvest refuge. California Fish and Game. 84:176-179.

Gloriam D, Bjarnadottir T, Yan Y, Postlethwait J, Schioth H, Fredriksson R, 2005. The repertoire of trace amine G-protein-coupled receptors: large expansion in zebrafish. Molecular Phylogenetics and Evolution. 35:470-482.

Grus WE, Shi P, Zhang YP, Zhang JZ, 2005. Dramatic variation of the vomeronasal pheromone receptor gene repertoire among five orders of placental and marsupial mammals. Proceedings of the National Academy of Sciences of the United States of America. 102:5767-5772.

Guindon S, Gascuel O, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology. 52:696-704.

Hall T, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/NT. Nucleic Acids Symposium Series. 41:95-98.

Hallacher LE, 1974. The comparative morphology of extrinsic gasbladder musculature in the scorpionfish genus *Sebastes* (Pisces: Scorpaenidae). Proceedings of the California Academy of Sciences. 40:59-86.

Hasegawa M, Kishino K, Yano T, 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution. 22:160-174.

Helvey M, 1982. First observations of courtship behavior in rockfish, genus *Sebastes*. Copeia.763-770.

Hofmann K, Stoffel W, 1993. TMbase - a database of membrane-spanning protein segments. In Biol. Chem. Hoppe-Seyler.

Hughes AL, 2007. Looking for Darwin in all the wrong places: the misguided quest for positive selection at the nucleotide sequence level. Heredity 99:364-373.

Hyde JR, Vetter RD, 2007. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). Molecular Phylogenetics and Evolution.

Ivanova NV, Dewaard JR, Hebert PDN, 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes. 6:998-1002.

Kalendar R, 2009. FastPCR software for PCR primer and probe design and repeat search.

Krogh A, Larsson B, von Heijne G, Sonnhammer ELL, 2001. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. Journal of Molecular Biology. 305:567-580.

Liberles S, Buck L, 2006. A second class of chemosensory receptors in the olfactory epithelium. Nature. 442:645-650.

Love MS, Yoklavich M, Thorsteinson L, 2002. The Rockfishes of the Northeast Pacific. Berkeley and Los Angeles, CA: University of California Press.

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M, 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289:739-745.

Pfister P, Rodriguez I, 2005. Olfactory expression of a single and highly variable V1r pheromone receptor-like gene in fish species. Proceedings of the National Academy of Sciences of the United States of America. 102:5489-5494.

Pfister P, Randall J, Montoya-Burgos JI, Rodriguez I, 2007. Divergent Evolution among Teleost V1r Receptor Genes. PLoS ONE. 2:e379.

Posada D, 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution. 25:1253-1256.

Saraiva LR, Korsching SI, 2007. A novel olfactory receptor gene family in teleost fish. Genome Research. 17:1448-1457.

Seeb LW, 1998. Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. Journal of Heredity. 89:393-403.

Shi P, Zhang JZ, 2007. Comparative genomic analysis identifies an evolutionary shift of vomeronasal receptor gene repertoires in the vertebrate transition from water to land. Genome Research. 17:166-174.

Shinomiya A, Ezaki O, 1991. Mating habits of the rockfish *Sebastes inermis*. Environmental Biology of Fishes. 30:15-22.

Speca DJ, Lin DM, Sorensen PW, Isacoff EY, Ngai J, Dittman AH, 1999. Functional identification of a goldfish odorant receptor. Neuron. 23:487-498.

Stern A, Doron-Faigenboim A, Erez E, Martz E, Bacharach E, Pupko T, 2007. Selecton 2007: advanced models for detecting positive and purifying selection using a Bayesian inference approach. Nucleic Acids Research. 35:W506-W511.

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Table

Table 2.1. Minimum and maximum genetic distances (HKY85+G) between species (below the diagonal), maximum genetic distance within species (on the diagonal), and average dN/dS ratio for between-species sequence comparisons (above the diagonal).

	S. caurinus	*S. maliger*	*S. melanops*	*S. ruberrimus*	*S. crameri*
S. caurinus	0.01023	0.2905	0.2932	0.2751	0.2070
S. maliger	0 - 0.01025	0.01025	0.4232	0.4051	0.3054
S. melanops	0 - 0.01645	0 - 0.01648	0.01852	0.6027	0.2614
S. ruberrimus	0 - 0.01645	0 - 0.01645	0 - 0.01643	0.01643	0.5139
S. crameri	0.00203 - 0.01442	0.00203 - 0.01439	0 - 0.01025	0.00203 - 0.01439	0.00818

Figures

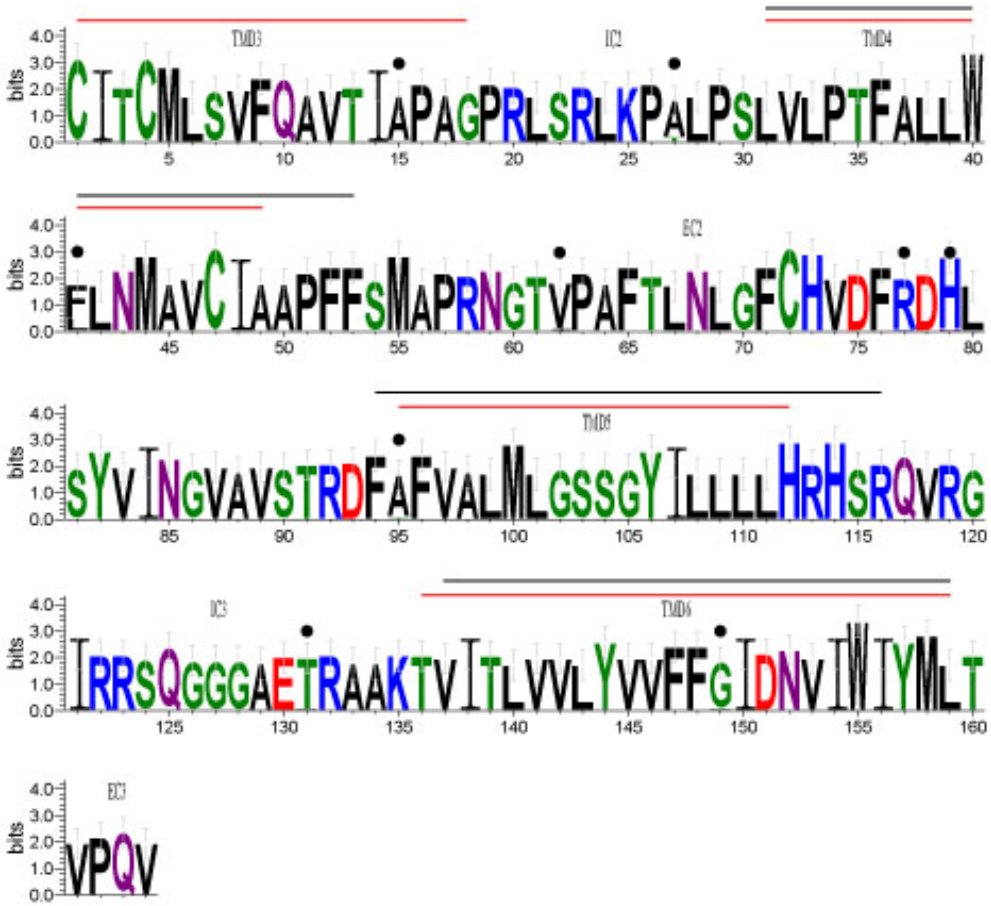

Figure 2.1. Amino acid sequence logo for *Ora*2 genes from five species of rockfish. Letter height indicates the relative frequency that a particular amino acid appears at that position in the five study species. Labeled features include transmembrane domains 3-6 (TMD) as predicted by TMpred (red bars) and TMHMM (black bars), intracellular loops (IC), extracellular loops (EC), and sites putatively under positive selection (●).

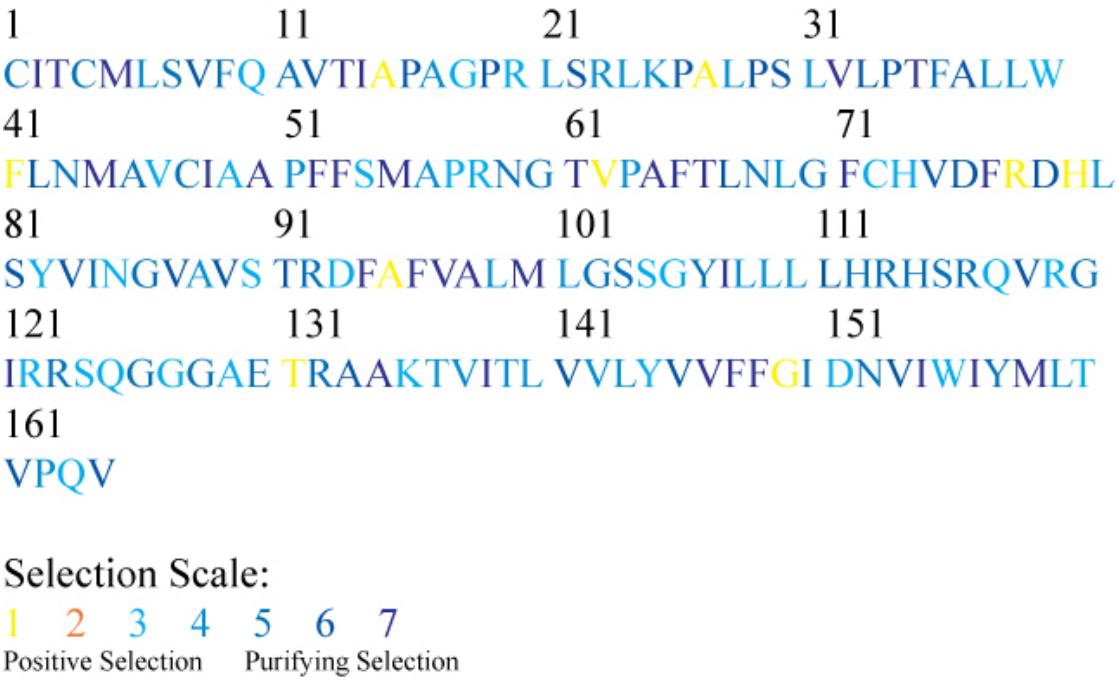

Figure 2.2. Site-specific positive and purifying selection results. Warm colors (colors 1 and 2) indicate a dN/dS ratio (ω) > 1 (positive selection), whereas cool colors (colors 3 through 7) indicate a ω < 1 (purifying selection).

MATE SELECTION AND THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) IN CAPTIVE-BREEDING ROCKFISHES (GENUS *SEBASTES*).

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Abstract

Rockfish species of the genus *Sebastes* are notable for being numerous and diverse. Rockfishes are unusual for fertilizing their eggs internally and releasing live, swimming larvae. They also undergo complex courting behaviors, which may allow females to be selective about their mates. The major histocompatibility complex (MHC) has been implicated as having an important role in mate selection in other fish species, especially in sticklebacks and salmonids. Research has suggested that females choose mates that optimize the MHC genotypes of their offspring. Previous research on rockfishes indicates that multiple functional MHC sequences may be found in each species, and that multiple mating is common in the genus, possibly as a bet-hedging strategy against stochasticity in larval survival. In this project, we characterized the MHC genotypes of copper and quillback rockfish parents, assessed parentage of fourteen larval broods, and assessed the MHC genotypes of the parents to determine if MHC-mediated mate choice was occurring. As in previous studies, we found evidence that rockfish possess multiple, highly-variable MHC genes, and that females may mate with multiple males. However, we did not find strong evidence of selection based on MHC genotype, finding that only maximum genetic distance deviated significantly from random expectations. Females were not selective based on relatedness, allele count, proportion of shared alleles, or mean genetic distance. This may be because survival through the larval phase is determined by stochastic environmental factors, with very poor survivorship in most years. This could promote a bet-hedging approach, with females preferring to mate with multiple males, rather than carefully selecting one.

Introduction

The genus *Sebastes* (the rockfishes) is notable for being speciose (approximately 110 species worldwide) and extremely diverse. Members of the species are found in a variety of habitat types, from the intertidal to depths over 1000 m (Love et al. 2002). Morphology varies widely, and is correlated with life history and ecological role. Types range from streamlined, semi-pelagic species such as the bocaccio (*Sebastes paucispinis*) to deep-bodied, spiny, benthic forms, such as the cowcod (*S. levis*). A full range of intermediate morphologies also exist in the genus (Hyde and Vetter 2007). Rockfishes are long-lived, with some species, such as the rougheye rockfish (*S. aleutianus*, 205 years) and shortraker rockfish (*S. borealis*, 156 years) estimated to be among the longest-lived fishes in the world (Love et al. 2002). The group is particularly distinguished by the evolution of internal fertilization (Wourms 1991), which may afford them a greater ability to be selective about their mates than is typical of marine fishes.

In contrast to the majority of teleost fishes, rockfishes fertilize their eggs internally and release live, swimming larvae. Larvae are released at the pre-flexion yolk-sac stage, which allows them to avoid the high mortality associated with the egg and early larval stages for a relatively small maternal investment (Boehlert and Yoklavich 1984). Rockfish are highly fecund, with broods containing thousands to millions of larvae per female (Love et al. 2002). In the unstable environmental conditions of the northeast Pacific, larval survival and recruitment are highly variable from year to year, and strong recruitment may occur only once in decades (Tolimieri and Levin 2005). Long lifespan coupled with high fecundity thus functions as a bet-hedging strategy to ensure population persistence between rare recruitment events in marine fishes such as rockfishes (Winemiller and Rose 1992).

Evidence suggests that rockfishes undergo complex courting behaviors, which may include pheromones, and sight or sound cues, before mating can take place (Love et al. 2002). Ultimately, the decision whether or not to mate appears to lie with the

female (Hyde et al. 2008). Multiple mating, with up to four different fathers, has been demonstrated in thirteen different rockfish species to date, and may be a common bet-hedging strategy in the genus (Gonzalez et al. 2009; Hyde et al. 2008; Sogard et al. 2008; Van Doornik et al. 2008). Selective mate choice by females, combined with multiple paternity, may enhance genetic diversity and prevent the loss of rare alleles, may diminish the effect of inbreeding depression, and may increase the effective population size of organisms who experience sweepstakes recruitment (Hedgecock 1994). Although chance may play the primary role in reproductive success for bet-hedging marine fishes, such as rockfishes (Winemiller and Rose 1992), another potential benefit of mate choice and multiple paternity may be in improved pathogen or parasite resistance through optimization of major histocompatibility complex (MHC) genotype.

The major histocompatibility complex is a highly- polymorphic multigene family involved in self-nonself recognition in the immune system of vertebrates. MHC genes encode receptors that bind fragments of local and foreign peptides, then present those fragments to T cells, which may initiate a series of immune responses (Bernatchez and Landry 2003; Ploegh and Watts 1998). The amino-acid sequence of the MHC protein determines antigen binding, and thus, which foreign peptides can be recognized (Brown et al. 1988), therefore, greater MHC diversity would be expected to result in a greater sensitivity to pathogens and a more effective immune response (Doherty and Zinkernagel 1975). Because self-reactivity can lower the number of T-cells in species that express several MHC loci, an intermediate number of alleles may result in better pathogen recognition in these cases (Woelfing et al. 2009). Given the high polymorphism and potential benefits associated with MHC-based mate preference, two main hypotheses have been proposed. First, given the high polymorphism of the gene family, they could function to discriminate close kin, and might help females to avoid inbreeding (Landry et al. 2001; Penn and Potts 1999). The other possibility is that negative assortative mating based on MHC genotype might

function to improve the pathogen resistance of offspring (Doherty and Zinkernagel 1975; Landry et al. 2001). Mate selection based on MHC genotype has been demonstrated in several fish species to date, including three-spined sticklebacks (*Gasterosteus aculeatus*; Aeschlimann et al. 2003; Eizaguirre et al. 2009; Kalbe et al. 2009; Lenz et al. 2009; Milinski et al. 2005; Reusch et al. 2001), a variety of salmonids (Atlantic salmon, *Salmo salar* L.; Consuegra and de Leaniz 2008; Landry et al. 2001; brown trout, *Salmo trutta* L.; Forsberg et al. 2007; Chinook salmon, *Oncorhynchus tshawytscha*; Garner et al. 2010; Neff et al. 2008), and other freshwater fish species (e.g. rose bitterling, *Rhodeus occelatus*; Casalini et al. 2009). This research has suggested that MHC genes do influence mate choice decisions in fish, that both good genes (i.e. specific alleles that confer improved pathogen resistance) and overall MHC diversity may be considered by females, and that mating decisions may be complicated by other factors, such as body size and coloration or aggression between individuals (Consuegra and de Leaniz 2008; Eizaguirre et al. 2009; Garner et al. 2010; Kalbe et al. 2009; Landry et al. 2001; Neff et al. 2008; Reusch et al. 2001). Little research has been undertaken to study MHC-based mate selection in marine fishes, however. One reason for this scarcity of research may be the difficulty of performing laboratory-based experimental research on large, long-lived marine fishes. Another reason may be that marine fishes are much less likely to encounter close kin, which would make the hypothesis that MHC-based mate selection partially functions to avoid inbreeding much less applicable (Landry et al. 2001). However, since marine fishes are exposed to numerous parasite threats, the potential improvement in immunocompetence associated with mate selection based on MHC genotype should apply equally to marine fishes as freshwater types (Penn et al. 2002).

Copper (*Sebastes caurinus*) and quillback (*Sebastes maliger*) rockfishes were chosen as model organisms to study MHC diversity and mate selection in nearshore rockfishes. The copper rockfish is found from the northern Gulf of Alaska to central Baja California, in waters from the subtidal zone to depths of ~180 m. The quillback

rockfish ranges from the Gulf of Alaska to the northern California Bight, and is found from subtidal depths to approximately 275 m (Love et al. 2002). Both species prefer areas of high- to medium- relief rocks, although they may also be found over low-relief rock habitat. Both are long-lived, with coppers aged to 50 years, and quillbacks aged to at least 95 years. Likewise, both mature around 7 years of age (Lea et al. 1999). Larval release occurs between January and June in copper rockfish, and between March and June in quillbacks (Love et al. 2002). Both species probably only produce a single brood annually (Moser 1967). Copper and quillback rockfish have been shown to hybridize in nature (Seeb 1998). An MHC class II beta genotype has been described for a single copper rockfish (Aguilar and Garza 2005), but no data have yet been published on quillback. No research has been published on multiple paternity or mate selection for either species.

Our goals in this study were threefold. First, we characterized MHC diversity in captive-breeding populations consisting of 69 copper and 89 quillback rockfishes at the Oregon Coast Aquarium (Newport, Oregon, USA). Then, we genotyped larvae of known mothers to assess parentage, including the prevalence of multiple paternity in these two previously unexamined species. Finally, we related the MHC genotypes of the parents of our realized matings to the suite of possible matings to draw inferences about the role of MHC genotype on mate choice in rockfishes.

Materials and Methods

Adult Tissue Collections

Copper and quillback rockfish were collected as newly settled juveniles or as adults from the Pacific Ocean near Newport, Oregon to populate the Passages of the Deep section of the Oregon Coast Aquarium between 1997 and 1999 (James Burke, Pers. Comm.). In September and October of 2009, all the adult copper and quillback rockfish were collected from the Orford Reef display tank in the Passages of the Deep section of the Oregon Coast Aquarium by divers using hand nets. Fish were placed

into an adjacent medical isolation tank until all individuals had been captured. Fin-clip tissue samples were collected from each individual and stored in 95% ethanol for DNA analysis, and each fish PIT tagged for future identification before release back into the display tank.

Larvae Collections

Gravid female copper and quillback rockfish were collected by SCUBA divers using hand nets from the Orford Reef tank, identified based on their PIT-tag number, and individually placed into 200-gallon plastic barrels with isolated inflow and filtered outflow until they released their larvae. Larval samples were collected by hand with dip nets and preserved in 50 ml Falcon tubes with 95% ethanol.

MHC Genotyping

Total genomic DNA was extracted from adult fin-clip tissue samples using a standard glass-fiber plate protocol (Ivanova et al. 2006). Polymerase chain reaction (PCR), with primers XIS and MRS (Cohen 2002), was used to amplify the majority of exon 2 of the MHC B1 unit, along with the preceding intron and a segment of the leader peptide for each sample. PCR reactions were performed with GoTaq Flexi polymerase (Promega, Madison, WI) and optimized for high fidelity. PCR cycling conditions were as follows: initial denaturation at 95 °C for 2 min, then 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. These PCR products were cloned using the TOPO TA cloning kit (Invitrogen, Inc., Grand Island, NY), and twelve colonies sequenced per individual. Clones were sequenced on an ABI 3730XL (Applied Biosystems, Foster City ,CA) capillary sequencer using M13 forward and reverse primers. All sequences were aligned and edited in Sequencher v4.7 (Gene Codes, Inc.), and trimmed to include only exon sequence (based on sequences from Aguilar and Garza 2005) for analysis. All exon sequences have been deposited in Genbank (Accession #s). Only unique exon sequences were used in MHC diversity analyses. Although the total number of

MHC genes in rockfishes is unknown, we hereafter refer to distinct sequences as alleles. jModeltest v.0.1.1 (Guindon and Gascuel 2003; Posada 2008) was used to estimate the most likely model of evolution in our sequences. Based upon these results, Paup* v4.0b10 (Sinauer Associates, Inc.) was used to calculate pairwise genetic distances between alleles according to a general time reversible model with invariable sites and rate variation among sites (GTR+I+G). Synonymous (d_s) and non-synonymous (d_n) substitution rates were calculated for the complete exon, and for putative antigen-binding site (ABS) and non-ABS codons (inferred from the human HLA-DRB1 locus; Brown et al. 1993; and following Aguilar and Garza 2005 and Cohen 2002) using DnaSP v.5.0 (Librado and Rozas 2009). Significance of d_n : d_s ratios was tested using a paired t-test in S-Plus (Insightful Corp.).

Parentage Analysis

Genomic DNA was extracted from adult fin-clip tissue samples or whole larvae using a standard glass-fiber-plate protocol (Ivanova et al. 2006). For adults, twenty-two previously published microsatellite markers (Table 1; Gomez-Uchida et al. 2003; Miller et al. 2000; Roques et al. 1999; Westerman et al. 2005; Wimberger et al. 1999) were tested for utility in parentage analysis. Locus-by-locus deviations from Hardy-Weinberg equilibrium and inbreeding coefficients were assessed using Genetix v.4.05.2 (Table 2; Belkhir et al. 2004). Larval genotypes utilizing six markers (Spi4, Spi6, Spi10; Gomez-Uchida et al. 2003; Sma3, Sma10, Sma11; Wimberger et al. 1999) were used to identify parents. Microsatellite markers were PCR amplified with fluorescently-labeled forward primers (MWG Biotech). PCR conditions were as follows: initial denaturation at 94 °C for 2 min, then 25 cycles of 94 °C for 1 min, 45-62 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. PCR products were visualized alongside internal molecular weight standards using an ABI 3730XL DNA analyzer and sized using Genemapper (ABI) software. Parentage was assessed using GERUD2.0 (Jones 2005) with known maternal genotypes. Putative

paternal genotypes reconstructed from larval genotypes in GERUD2.0 were matched to the data set of true parental genotypes to identify actual fathers.

Mate Selection

To assess whether females mated nonrandomly with the available males in our captive population, we conducted five different hypothesis tests. To test the hypothesis that females choose mates based on their level of relatedness, we calculated maximum likelihood estimates of relatedness (r) for all pairs of individuals in our dataset using ML-Relate (Kalinowski et al. 2006). We then compared the mean relatedness of our seventeen mate choice events with that of 1000 randomly-generated pairings drawn from the complete set of possible parents (all copper and quillback rockfish in Orford Reef) using a two-sample t-test in S-plus (Insightful Corp.). We also performed several different hypothesis tests to assess the association between MHC genotype and mate choice in rockfishes. To test the hypothesis that females choose mates by counting alleles, we calculated pairwise absolute differences between the numbers of distinct MHC alleles found in the mother versus number of alleles found in the father. We then compared the mean difference in the realized matings with the mean difference in 1000 randomly-generated pairings using a two-sample t-test. To test the hypothesis that females select based on shared alleles, we calculated the proportion of shared MHC alleles for the realized matings and for 1000 randomly-generated pairings, and compared those means using a two-sample t-test. The proportion of shared alleles is calculated as two times the number of alleles shared by the individuals in the pair, divided by the sum of the number of alleles found in each individual [$D = 2F_{ab}/(F_a + F_b)$] (Wetton et al. 1987). To test the hypothesis that females choose mates based on genetic distances between their own and potential mates' alleles, we calculated the mean and maximum GTR+I+G genetic distance between all the alleles found in a mating pair, as well as in 500 random groupings, and again compared the means using two-sample t-tests. In all MHC tests, we assumed that MHC genotypes were unbiased and were a random sampling of the loci present in

the individual, since the primers used (XIS & MRS; Cohen 2002) are highly degenerate.

Results

MHC Genotyping

Sequences between 400 and 800 base pairs (bp) in length were recovered from both study species. Genbank nucleotide BLAST (NCBI) searches utilizing these sequences as the query invariably returned previously published (Aguilar and Garza 2005) *Sebastes* MHC sequences as the highest-likelihood match. In accord with Aguilar and Garza's (2005) previous findings, sequences were composed of a long intron, containing a variably-repeated minisatellite sequence with no species-specific pattern, and a 255-bp exon sequence. We found between two and nine unique sequences per individual copper rockfish, and between two and ten sequences in quillback rockfish. The mean (± s.d.) number of unique sequences per individual (standardized to sequences per 10 clones) was 5.31 (± 1.62) for copper rockfish and 5.58 (± 1.63) for quillback rockfish (Figure 1). We identified a total of 166 alleles in 69 copper rockfish and 315 alleles in 89 quillback rockfish. Forty-one sequences were found in both species. Allele sequences varied widely, with genetic distances (GTR+I+G) between sequences ranging from a low of 0.00395 to a high of 0.38749 across both species. The ratio of non-synonymous (dn) to synonymous (ds) substitution rates was significantly greater than one in all comparisons (Table 4). This higher rate of non-synonymous substitutions was found in both species (combined, and considered separately), and irrespective of whether all codons were considered, solely putative ABS codons, or solely non-ABS codons.

Parentage Analysis

We identified paternity for a total of 1379 larvae in 14 broods (Table 3). Twelve different mothers and eight different fathers were represented in the sample set. Two mothers produced larvae in both 2009 and 2010. Three broods showed

evidence of multiple paternity (two sires), although the contribution of the second father was always a small fraction of the total (no more than 20%). One sire, #2592, was involved in producing eight of the fourteen broods in the sample set. This individual, a quillback rockfish, mated with females of both species and produced viable larvae with both (data not shown). Other hybrid matings were also observed in the sample set.

Mate Selection

We found no significant difference in mean relatedness between pairs of mates versus 1000 randomly paired fishes ($t = 1.76$, $df = 16$, $p = 0.0966$). There was also no significant difference between the mean difference in maternal MHC allele count and paternal allele count in our set of realized matings versus 1000 random pairings ($t = 0.4544$, $df = 16$, $p = 0.6554$). The mean proportion of shared MHC alleles was not significantly different between the mating set versus the random pairing data set ($t = 0.9734$, $df = 16$, $p = 0.3446$). The mean genetic distance between the mating group and the random set was also not significantly different ($t = -1.8113$, $df = 16$, $p = 0.0887$). Maximum genetic distance, that is, the single greatest GTR+I+G distance found between mates, was significantly lower in the mated pairs than in the random grouping ($t = -2.9068$, $df = 16$, $p = 0.01$).

Discussion

MHC Genotyping

Both copper and quillback rockfishes show evidence of multiple MHC class II B genes. Aguilar and Garza (2005) found between two and seven unique sequences in single individuals of twelve *Sebastes* species they considered, including copper rockfish. This compares closely with the average of 5.31 and 5.58 unique sequences per 10 clones sequenced for copper and quillback rockfish, respectively, in our sample set. Because intron sequences varied widely in repeat number and were thus unalignable, it was impossible to assess paralogous versus allelic status of our

different sequences. Both species were very diverse, both in terms of number of unique sequences, and in genetic distances between sequences. We also found strong evidence of balancing selection, both in antigen binding site and non-ABS codons. This agrees with previous findings, and likely indicates recent functionality for these genes. Our finding of a relatively high degree of trans-species allelism is also in line with previously published findings on MHC in rockfishes (Aguilar and Garza 2005; Garrigan and Hedrick 2003).

Parentage Analysis

We found evidence of multiple paternity in three of fourteen broods examined, all of which were produced by copper rockfish mothers. No evidence of multiple paternity was found in broods from quillback rockfish. Multiple paternity has been described from thirteen of twenty-one rockfish species examined to date (Gonzalez et al. 2009; Hyde et al. 2008; Sogard et al. 2008; Van Doornik et al. 2008; Yoshida et al. 2001), and seems to be common in the genus. Considering the close relationship of copper and quillback rockfish (Hyde and Vetter 2007), the absence of evidence of multiple paternity in quillback rockfish in the present study may be a consequence of low sample size, rather than evidence that it does not occur. Multiple paternity has been suggested to be a bet-hedging strategy for rockfishes which may improve the genetic diversity of offspring from a single female, thereby improving the odds that some fraction of a her larvae match environmental conditions and survive (Cushing 1990; Hyde et al. 2008; Van Doornik et al. 2008). Other potential benefits of multiple mating include a reduction in the probability of incomplete fertilization of eggs in a brood (Gunderson 1977; Sogard et al. 2008), reduced inbreeding depression, and an increase in the effective population size (N_E; Hyde et al. 2008). Evidence for hybrid matings was surprisingly common in our data (nine of fourteen broods). Although this may be related to effects from long-term holding or parents in captivity, hybridization has previously been described in wild populations of these two species (Seeb 1998), and has been conjectured to be a potential source of genetic diversity in the genus

Sebastes. Finally, a single male quillback rockfish sired part or all of eight of the fourteen broods in the sample set, including mating with both species of females. Although no previous data exists for mating preferences in rockfishes, results from another study of the related *Sebastiscus marmoratus* suggest that females may select the largest male available, when direct comparisons are possible (Ng et al. 2003). Thus, the dominance of one male in our samples may simply result from him being the largest available male. Without matched size and gender data for our samples, however, we can only conjecture.

Mate Selection

Our results suggest that rockfish females pay little attention to overall relatedness when selecting possible mates. We also found little evidence for mate selection based on MHC genotype. Only the maximum genetic distance between mates diverged significantly from random expectations. In this case, it appears that females may prefer males that are not too divergent in their MHC genotype. These findings are consistent with previous findings in sticklebacks, that females choose mates with intermediate MHC diversity (Lenz et al. 2009), in this case with respect to genetic distances. However, the overall pattern in our data is of random mating with respect to relatedness and MHC genotype.

Conclusion

Rockfish are characterized by low overall survivorship through the larval stage, coupled with a very high degree of stochasticity in larval survival between years (Love et al. 2002). In most years, few young fish may survive to recruit to the adult population, and strong year classes may only occur once in a decade (Love et al. 2002; Tolimieri and Levin 2005). The importance of chance in deciding early survivorship may severely discount the importance selecting mates to optimize larval MHC genotype. Instead, evidence from prior work, as well as the current study, suggests that

females mate with multiple males when possible, potentially as a bet hedging strategy against this randomness.

References

Aeschlimann PB, Haberli MA, Reusch TBH, Boehm T, Milinski M, 2003. Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize MHC allele number during mate selection. Behavioral Ecology and Sociobiology. 54:119-126.

Aguilar A, Garza JC, 2005. Analysis of major histocompatibility complex class II Beta genes from rockfishes (genus *Sebastes*). Journal of Fish Biology. 67:1021-1028.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F, 2004. Genetix 4.05, Windows program for population genetics. Available at http://www.University-montp2.fr/~genetix/genetix.htm.

Bernatchez L, Landry C, 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? Journal of Evolutionary Biology. 16:363-377.

Boehlert GW, Yoklavich MM, 1984. Reproduction, embryonic energetics, and the maternal fetal relationship in the viviparous genus *Sebastes* (Pisces, Scorpaenidae). Biological Bulletin 167:354-370.

Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC, 1988. A hypothetical model of the foreign antigen-binding site of class-II histocompatibility molecules. Nature. 332:845-850.

Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, Wiley DC, 1993. 3-Dimensional structure of the human class-II histocompatibility antigen Hla-Dr1. Nature. 364:33-39.

Casalini M, Agbali M, Reichard M, Konecna M, Bryjova A, Smith C, 2009. Male dominance, female mate choice, and intersexual conflict in the rose bitterling (*Rhodeus ocellatus*). Evolution. 63:366-376.

Cohen S, 2002. Strong positive selection and habitat-specific amino acid substitution patterns in Mhc from an estuarine fish under intense pollution stress. Molecular Biology and Evolution. 19:1870-1880.

Consuegra S, de Leaniz CG, 2008. MHC-mediated mate choice increases parasite resistance in salmon. Proceedings of the Royal Society B-Biological Sciences. 275:1397-1403.

Cushing DH, 1990. Plankton production and year-class strength in fish populations - an update of the match mismatch hypothesis. Advances in Marine Biology. 26:249-293.

Doherty PC, Zinkernagel RM, 1975. Enhanced immunological surveillance in mice heterozygous at H-2 gene complex. Nature. 256:50-52.

Eizaguirre C, Yeates SE, Lenz TL, Kalbe M, Milinski M, 2009. MHC-based mate choice combines good genes and maintenance of MHC polymorphism. Molecular Ecology. 18:3316-3329.

Forsberg LA, Dannewitz J, Petersson E, Grahn M, 2007. Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout - females fishing for optimal MHC dissimilarity. Journal of Evolutionary Biology. 20:1859-1869.

Garner SR, Bortoluzzi RN, Heath DD, Neff BD, 2010. Sexual conflict inhibits female mate choice for major histocompatibility complex dissimilarity in Chinook salmon. Proceedings of the Royal Society B-Biological Sciences. 277:885-894.

Garrigan D, Hedrick PW, 2003. Perspective: Detecting adaptive molecular polymorphism: Lessons from the MHC. Evolution. 57:1707-1722.

Gomez-Uchida D, Hoffman EA, Ardren WR, Banks MA, 2003. Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species. Molecular Ecology Notes. 3:387-389.

Gonzalez EB, Murakami T, Teshima Y, Yoshioka K, Jeong DS, Umino T, 2009. Paternity testing of wild black rockfish *Sebastes inermis* (brownish type) from the Seto Inland Sea of Japan. Ichthyological Research. 56:87-91.

Guindon S, Gascuel O, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology. 52:696-704.

Gunderson DR, 1977. Population Biology of Pacific Ocean Perch, Sebastes-Alutus, Stocks in Washington-Queen Charlotte Sound Region, and Their Response to Fishing. Fishery Bulletin. 75:369-403.

Hedgecock D, 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In Beaumont AR ed. Genetics and Evolution of Aquatic Organisms. London: Chapman and Hall, p. 122-134.

Hyde JR, Vetter RD, 2007. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). Molecular Phylogenetics and Evolution. 44:790-811.

Hyde JR, Kimbrell C, Robertson L, Clifford K, Lynn E, Vetter R, 2008. Multiple paternity and maintenance of genetic diversity in the live-bearing rockfishes *Sebastes* spp. Marine Ecology-Progress Series. 357:245-253.

Ivanova NV, Dewaard JR, Hebert PDN, 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes. 6:998-1002.

Johansen T, Dahle G, 2004. Discrimination among species of the genus *Sebastes* in the North Atlantic by random amplified polymorphic DNA. Sarsia. 89:478-483.

Jones AG, 2005. GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. Molecular Ecology Notes. 5:708-711.

Kai Y, Nakayama K, Nakabo T, 2003. Molecular phylogenetic perspective on speciation in the genus *Sebastes* (Scorpaenidae) from the Northwest Pacific and the position of *Sebastes* within the subfamily Sebastinae. Ichthyological Research. 50:239-244.

Kai Y, Nakabo T, 2008. Taxonomic review of the *Sebastes inermis* species complex (Scorpaeniformes : Scorpaenidae). Ichthyological Research. 55:238-259.

Kalbe M, Eizaguirre C, Dankert I, Reusch TBH, Sommerfeld RD, Wegner KM, Milinski M, 2009. Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. Proceedings of the Royal Society B-Biological Sciences. 276:925-934.

Kalinowski ST, Wagner AP, Taper ML, 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes. 6:576-579.

Landry C, Garant D, Duchesne P, Bernatchez L, 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). Proceedings of the Royal Society of London Series B-Biological Sciences. 268:1279-1285.

Lea R, McAllister R, Ventresca DA, 1999. Biological aspects of the nearshore rockfishes of the genus *Sebastes* from central California. California Department of Fish and Game, Fish Bulletin. 177:1-109.

Lenz TL, Eizaguirre C, Scharsack JP, Kalbe M, Milinski M, 2009. Disentangling the role of MHC-dependent 'good genes' and 'compatible genes' in mate-choice decisions of three-spined sticklebacks *Gasterosteus aculeatus* under semi-natural conditions. Journal of Fish Biology. 75:2122-2142.

Librado P, Rozas J, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25:1451-1452.

Love MS, Yoklavich M, Thorsteinson L, 2002. The Rockfishes of the Northeast Pacific. Berkeley and Los Angeles, CA: University of California Press.

Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T, 2005. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. Proceedings of the National Academy of Sciences of the United States of America. 102:4414-4418.

Miller KM, Schulze AD, Withler RE, 2000. Characterization of microsatellite loci in Sebastes alutus and their conservation in congeneric rockfish species. Molecular Ecology. 9:240-242.

Moser H, 1967. Reproduction and development of *Sebastodes paucispinis* and comparisons with other rockfishes off California. Copeia. 1967:773-779.

Neff BD, Garner SR, Heath JW, Heath D, 2008. The MHC and non-random mating in a captive population of Chinook salmon. Heredity. 101:175-185.

Ng WC, Sadovy Y, Leung FCC, 2003. Mating system of the rockfish, *Sebastiscus marmoratus* as revealed by DNA fingerprinting. Ichthyological Research. 50:339-348.

Penn DJ, Potts WK, 1999. The evolution of mating preferences and major histocompatibility complex genes. American Naturalist. 153:145-164.

Penn DJ, Damjanovich K, Potts WK, 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. Proceedings of the National Academy of Sciences of the United States of America. 99:11260-11264.

Ploegh H, Watts C, 1998. Antigen recognition - Editorial overview. Current Opinion in Immunology. 10:57-58.

Posada D, 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution. 25:1253-1256.

Reusch TBH, Haberli MA, Aeschlimann PB, Milinski M, 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature. 414:300-302.

Rocha-Olivares A, Rosenblatt RH, Vetter RD, 1999. Cryptic species of rockfishes (*Sebastes* : Scorpaenidae) in the Southern Hemisphere inferred from mitochondrial lineages. Journal of Heredity. 90:404-411.

Roques S, Pallotta D, Sevigny JM, Bernatchez L, 1999. Isolation and characterization of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei : Scorpaenidae, genus *Sebastes*). Molecular Ecology. 8:685-687.

Seeb LW, 1998. Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. Journal of Heredity. 89:393-403.

Sogard SM, Berkeley SA, Fisher R, 2008. Maternal effects in rockfishes *Sebastes* spp.: a comparison among species. Marine Ecology-Progress Series. 360:227-236.

Sogard SM, Gilbert-Horvath E, Anderson EC, Fishee R, Berkeley SA, Garza JC, 2008. Multiple paternity in viviparous kelp rockfish, *Sebastes atrovirens*. Environmental Biology of Fishes. 81:7-13.

Tolimieri N, Levin PS, 2005. The roles of fishing and climate in the population dynamics of bocaccio rockfish. Ecological Applications. 15:458-468.

Van Doornik DM, Parker SJ, Millard SR, Berntson EA, Moran P, 2008. Multiple paternity is prevalent in Pacific ocean perch (*Sebastes alutus*) off the Oregon coast, and is correlated with female size and age. Environmental Biology of Fishes. 83:269-275.

Westerman ME, Buonaccorsi VP, Stannard JA, Galver L, Taylor C, Lynn EA, Kimbrell CA, Vetter RD, 2005. Cloning and characterization of novel microsatellite DNA markers for the grass rockfish, *Sebastes rastrelliger*, and cross-species amplification in 10 related *Sebastes* spp. Molecular Ecology Notes. 5:74-76.

Wetton JH, Carter RE, Parkin DT, Walters D, 1987. Demographic-Study of a Wild House Sparrow Population by DNA Fingerprinting. Nature. 327:147-149.

Wimberger P, Burr J, Gray A, Lopez A, Bentzen P, 1999. Isolation and characterization of twelve microsatellite loci for rockfish (*Sebastes*). Marine Biotechnology. 1:311-315.

Winemiller KO, Rose KA, 1992. Patterns of life-history diversification in North American fishes - implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences. 49:2196-2218.

Woelfing B, Traulsen A, Milinski M, Boehm T, 2009. Does intra-individual major histocompatibility complex diversity keep a golden mean? Royal Society Philosophical Transactions Biological Sciences. 364:117-128.

Wourms JP, 1991. Reproduction and Development of *Sebastes* in the Context of the Evolution of Piscine Viviparity. Environmental Biology of Fishes. 30:111-126.

Yoshida K, Nakagawa M, Wada S, 2001. Pedigree tracing of a hatchery-reared stock used for aquaculture and stock enhancement based on DNA markers. Fish Genetics and Breeding Science. 30:27-35.

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Tables

Table 3.1. Genotyping primers for parentage.

Primer	Annealing Temperature (° C)	Citation
Sal 1	45	Miller et al. 2000
Sal 2	48	
Sal 3	48	
Sal 4	52	
Seb 9	62	Roques et al. 1999
Seb 31	62	
Sma 1	48	Wimberger et al. 1999
Sma 10	58	
Sma 11	58	
Sma 2	58	
Sma 3	58	
Sma 4	58	
Sma 5	58	
Spi 4	58	Gomez-Uchida et al. 2003
Spi 6	58	
Spi 10	58	
Spi 12	58	
Spi 18	56	
Sra 7-7	57	Westerman et al. 2005
Sra 7-25	57	
Sra 11-103	57	
Sra 15-8	52	
Sra 16-5	52	

Temperature Profile:
94 °C / 2 min, 25x(94 °C / 1 min, Anneal / 30 sec, 72 °C / 10 sec), 72 °C / 10 min, 10 °C hold

Table 3.2. Locus-by-locus number of alleles, expected (H_e) and observed (H_o) heterozygosities with significance (p), and inbreeding coefficient (F_{IS}) with significance (p), for all possible parents in Orford Reef. Markers used in parentage analysis are highlighted in grey.

Locus	Species	Number of Alleles	H_e	H_o	p	F_{IS}	p
Spi 4	Copper	15	0.88	0.84	0.342	0.042	0.187
	Quillback	16	0.88	0.88	0.739	0.010	0.472
Spi 6	Copper	20	0.90	0.88	0.340	0.022	0.321
	Quillback	19	0.88	0.88	0.359	0.007	0.481
Spi 10	Copper	5	0.64	0.75	0.980	-0.178	0.992
	Quillback	4	0.61	0.60	0.214	0.020	0.437
Spi 12	Copper	4	0.40	0.39	0.137	0.027	0.444
	Quillback	7	0.43	0.36	0.002	0.156	0.031
Spi 18	Copper	24	0.89	0.89	0.044	0.000	0.565
	Quillback	21	0.87	0.84	0.005	0.034	0.217
Sma 1	Copper	13	0.80	0.87	0.956	-0.093	0.988
	Quillback	12	0.67	0.63	0.077	0.061	0.171
Sma 3	Copper	7	0.58	0.61	0.728	-0.055	0.790
	Quillback	5	0.72	0.76	0.916	-0.067	0.899
Sma 10	Copper	16	0.70	0.64	0.153	0.084	0.123
	Quillback	15	0.81	0.72	0.001	0.116	0.013
Sma 11	Copper	6	0.66	0.73	0.856	-0.107	0.936
	Quillback	10	0.62	0.55	0.057	0.112	0.039
Seb 9	Copper	5	0.59	0.66	0.269	-0.117	0.900
	Quillback	5	0.67	0.66	0.280	0.017	0.443
Sal 1	Copper	11	0.81	0.71	0.069	0.122	0.033
	Quillback	8	0.77	0.72	0.125	0.076	0.115
Sal 3	Copper	10	0.68	0.64	0.241	0.054	0.255
	Quillback	8	0.63	0.43	<0.001	0.324	<0.001
Sra 7-7	Copper	11	0.70	0.77	0.865	-0.107	0.959
	Quillback	13	0.77	0.81	0.552	-0.045	0.834
Sra 7-25	Copper	13	0.83	0.83	0.507	0.001	0.548
	Quillback	11	0.81	0.73	0.018	0.098	0.040
Sra 11-103	Copper	3	0.43	0.40	0.277	0.068	0.338
	Quillback	3	0.28	0.25	0.181	0.129	0.169
Sra 15-8	Copper	12	0.81	0.79	0.162	0.033	0.311
	Quillback	7	0.80	0.79	0.364	0.013	0.476
Sra 16-5	Copper	30	0.95	0.97	0.666	-0.020	0.858
	Quillback	22	0.86	0.79	0.098	0.081	0.030
Sal 2	Copper	6	0.61	0.64	0.586	-0.052	0.794
	Quillback	9	0.72	0.67	0.037	0.065	0.143
Sal 4	Copper	10	0.82	0.81	0.085	0.011	0.475
	Quillback	8	0.79	0.77	0.468	0.022	0.401
Sma 2	Copper	6	0.32	0.31	0.021	0.012	0.510
	Quillback	5	0.47	0.38	0.047	0.182	0.030
Sma 4	Copper	7	0.60	0.54	0.017	0.109	0.129
	Quillback	6	0.71	0.66	0.001	0.078	0.135
Sma 5	Copper	3	0.12	0.10	0.004	0.186	0.149
	Quillback	4	0.44	0.46	0.615	-0.045	0.710

Table 3.3. Number of unique paternal alleles detected at each locus, identified sires, and the number of larvae fathered by each sire. *Indicates hybrid larvae.

Mother's ID	Species	No. of Larvae	Spi4	Spi6	Spi10	Sma3	Sma10	Sma11	ID of Sires (Number of Larvae)
2501 (2009 Brood)	*S. caurinus*	93	2	1	1	0	0	1	2592* (92), 2653* (1)
2501 (2010 Brood)	*S. caurinus*	91	2	1	1	0	0	1	2592* (91)
2502	*S. maliger*	94	2	1	0	1	0	2	2592 (94)
2504 (2009 Brood)	*S. caurinus*	92	2	3	2	0	2	2	2575 (87), 2529* (5)
2504 (2010 Brood)	*S. caurinus*	93	2	2	0	1	0	1	2592* (93)
2506	*S. maliger*	87	2	1	0	1	1	0	2523 (87)
2566	*S. maliger*	94	2	2	0	1	2	0	2585 (94)
2571	*S. caurinus*	93	1	1	1	0	1	0	2653* (93)
2574	*S. caurinus*	94	4	2	1	2	1	2	2638 (76), 2592* (18)
2590	*S. caurinus*	183	1	1	1	0	1	1	2653* (183)
2598	*S. caurinus*	86	2	1	0	1	0	2	2592* (86)
2608	*S. maliger*	93	1	2	0	1	0	1	2592 (93)
2628	*S. caurinus*	93	2	1	1	0	0	1	2582 (93)
2670	*S. caurinus*	93	1	2	0	1	0	1	2592* (93)

Table 3.4. Mean (± s.d.) non-synonymous (d_n) and synonymous (d_s) substitution rates for comparisons within and between species, d_n : d_s ratios, and results of paired t-tests testing the hypothesis that $d_n = d_s$.

Species	Gene Region	d_n	d_s	$d_n : d_s$	t	df	p
Both	ABS	0.348 (± 0.114)	0.146 (± 0.100)	2.381	926.0	144452	<0.001
	Non-ABS	0.156 (± 0.050)	0.078 (± 0.049)	2.008	635.1		<0.001
	Overall	0.209 (± 0.060)	0.096 (± 0.052)	2.174	705.6		<0.001
S. caurinus	ABS	0.351 (± 0.116)	0.153 (± 0.106)	2.289	278.9	29645	<0.001
	Non-ABS	0.153 (± 0.052)	0.078 (± 0.051)	1.971	299.9		<0.001
	Overall	0.208 (± 0.062)	0.098 (± 0.055)	2.120	392.0		<0.001
S. maliger	ABS	0.347(± 0.116)	0.143 (± 0.096)	2.427	381.8	50085	<0.001
	Non-ABS	0.159 (± 0.052)	0.078 (± 0.049)	2.038	434.1		<0.001
	Overall	0.211 (± 0.062)	0.096 (± 0.051)	2.204	561.9		<0.001

Figure

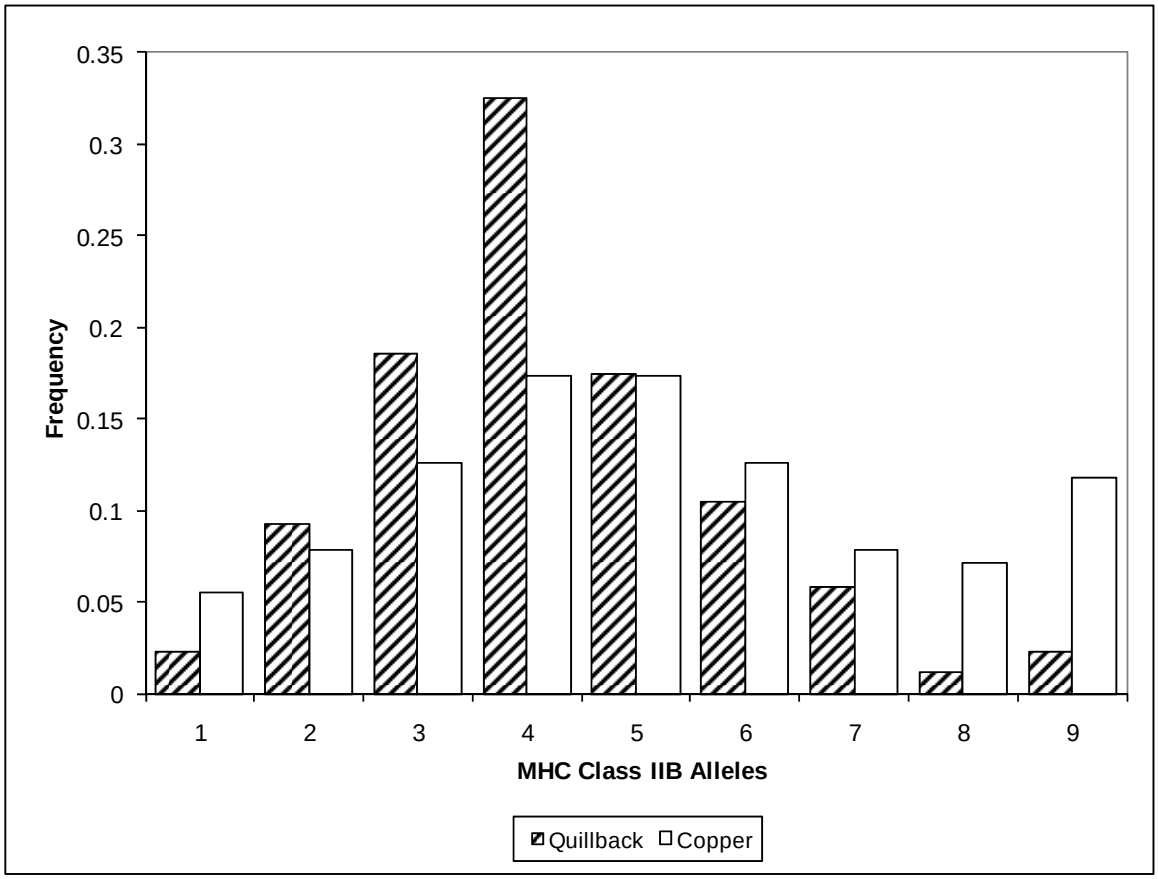

Figure 3.1. Frequency distribution of the number of MHC class-IIB alleles (exon 2 sequences) in 69 adult copper (striped bars) and 87 quillback (white bars) rockfishes from the Oregon Coast Aquarium. The mean number of MHC alleles per 10 clones sequenced (± s.d.) was 5.31 (± 1.62) for copper rockfish and 5.58 (± 1.63) for quillback rockfish.

GENERAL CONCLUSION

The overall objective of this research was to utilize microsatellite and candidate gene markers to assess the genetic basis for population- and species-level diversity in the genus *Sebastes*. In chapter one, I used genotype data to test for fine-scale population genetic structure in copper rockfish along the coast of Oregon, and to relate those findings to previously-published and current coastwide genetic structure. In chapter two, I applied a candidate gene approach to characterize *Ora2* genes in five species of rockfishes, to assess the evolutionary history of this gene and its role in mate discrimination. In chapter three, I characterized MHC diversity in a captive-breeding population of copper and quillback rockfishes, utilized microsatellite markers to identify parents of several broods produced by this population, and related actual mate choices to parental MHC genotypes to draw inferences about the role of MHC in rockfish mate selection.

In chapter one, I sought to address three specific questions: (1) Will the coast-wide pattern of isolation-by-distance in copper rockfish (*Sebastes caurinus*), previously described by Buonaccorsi (2002), persist when sample size, number of sites, and number of loci are doubled? Genetic analysis provided evidence of genetic structure consistent with the previous finding of isolation-by-distance among samples collected along the Pacific coast from Neah Bay, WA to San Diego, CA. (2) At the scale of the Oregon coast, is population structure in copper rockfish homogeneous? Genetic analysis provided evidence for weak but significant genetic structure among sample collections along the Oregon coast. (3) If genetic structure is apparent at the Oregon coast scale, is it influenced by distance, habitat breaks, Cape Blanco, or some combination of the three? The Oregon-coast genetic structure was not consistent with a pattern of pure isolation-by-distance. Instead, the present analysis suggests that isolation is a function of restricted dispersal across habitat barriers, in particular the sand habitat between Newport and Coos Bay. Data show that Cape Blanco was not as strong a factor in structuring Oregon-coast copper rockfish as was the reduction in

adult breeding population size due to poor adult habitat. In copper rockfish, it seems that dispersal in the species may best be characterized by a mesoscale dispersal model (Gunderson and Vetter 2006). In this case it appears that non-optimal habitat, with some possible influence from the oceanographic jet at Cape Blanco, serves to limit dispersal in Oregon copper rockfish.

Previous studies of population structure in copper rockfish (Buonaccorsi et al. 2002; Buonaccorsi et al. 2005) considered geographic distance as the sole factor restricting gene flow across the range of the species. This work demonstrated that some of the overall genetic divergence is related to habitat patchiness, not necessarily restricted dispersal distance alone. This suggests that nearshore rockfish populations may be more finely structured than has been previously suspected. However, it will be important to test the generality of these findings in other species and geographic areas.

In chapter two, my goal was to assess the possibility that *Ora2* genes could function in pheromone detection and mate selection in rockfishes. I determined that *Ora2* genes seem to be highly conserved across the five sampled species, with several sequences found in multiple species, separated by up to 7.8 million years of evolution (Hyde and Vetter 2007). Most of the amino acids in the sequence set showed evidence of moderate to strong purifying selection, however, nine amino acid residues showed a significant signal of positive selection, which might be consistent with selection causing pheromone receptor genes to diverge in close relatives. Overall, it seems unlikely that sequence differences alone function to differentiate species at mating time. Visual, auditory, or lateral line cues, along with differences in reproductive timing and location may also function to prevent hybridization in related rockfishes (Love et al. 2002).

The overall high level of sequence conservation found in *Ora* type 2 genes in rockfishes likely reflects their strong functional significance. The actual ligands recognized by fish *Ora* genes are currently unknown (Pfister et al. 2007; Saraiva and Korsching 2007), but if these genes recognize pheromones, as has been suggested for

the related V1r genes in mammals (Boschat et al. 2002), then they would be expected to have little species-specificity, given their high level of sequence conservation (Saraiva and Korsching 2007). I hypothesize instead that these genes are involved in assessing mate condition, gender, or reproductive status, or other qualities which are important across species and regardless of species identity.

In chapter three, I sought to characterize MHC diversity in a captive-breeding population of copper and quillback rockfishes, and then related MHC genotypes to realized matings to draw conclusions about the role of MHC on mate selection in rockfishes. Sequencing results suggest that both copper and quillback rockfishes possess multiple functional MHC class II B genes. I found an average of around 5.5 unique sequences per 10 clones sequenced for both copper and quillback rockfishes, which compares closely to the two to seven unique sequences previously described from twelve different species of rockfishes (Aguilar and Garza 2005). Both species were quite diverse, in terms of number of unique sequences, as well as in genetic distances. There was also evidence of balancing selection, which likely indicates recent functionality for these genes. A relatively high degree of trans-species allelism is also in line with previously published findings on MHC in rockfishes (Aguilar and Garza 2005; Garrigan and Hedrick 2003).

I found evidence of multiple paternity in several of the broods examined, although only from copper rockfish mothers. In light of the close relationship between the two study species, the absence of evidence of multiple paternity in quillback rockfish in the present study may be a consequence of low sample size, rather than evidence that it does not occur. Multiple paternity has been suggested to be a bet-hedging strategy for rockfishes which may improve the genetic diversity of offspring from a single female, thereby improving the odds that some fraction of a her larvae match environmental conditions and survive (Cushing 1990; Hyde et al. 2008; Van Doornik et al. 2008). Hybridization was also quite common in our sample set, and has been described in wild populations of these two species (Seeb 1998). Our mate

selection results suggest that female rockfishes pay little attention to either overall relatedness or MHC genotype when selecting mates. I hypothesize that this is related to the fact that rockfish are characterized by low overall survivorship through the larval stage, coupled with a very high degree of stochasticity in larval survival between years (Love et al. 2002). In most years, few young fish may survive to recruit to the adult population, and strong year classes may only occur once in a decade (Love et al. 2002; Tolimieri and Levin 2005). The importance of chance in deciding early survivorship may severely discount the importance of optimal larval MHC genotype. Instead, evidence from prior work, as well as the current study, suggests that females mate with multiple males when possible, potentially as a bet hedging strategy against this randomness.

References

Aguilar A, Garza JC, 2005. Analysis of major histocompatibility complex class II Beta genes from rockfishes (genus *Sebastes*). Journal of Fish Biology. 67:1021-1028.

Boschat C, Pelofi C, Randin O, Roppolo D, Luscher C, Broillet MC, Rodriguez I, 2002. Pheromone detection mediated by a V1r vomeronasal receptor. Nature Neuroscience. 5:1261-1262.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD, 2002. Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. Canadian Journal of Fisheries and Aquatic Sciences. 59:1374-1384.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD, 2005. Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. Conservation Genetics. 6:697-713.

Cushing DH, 1990. Plankton Production and Year-Class Strength in Fish Populations - an Update of the Match Mismatch Hypothesis. Advances in Marine Biology. 26:249-293.

Garrigan D, Hedrick PW, 2003. Perspective: Detecting adaptive molecular polymorphism: Lessons from the MHC. Evolution. 57:1707-1722.

Gunderson DR, Vetter RD, 2006. Temperate Rocky Reef Fishes. In Kritzer JP ed. Marine Metapopulations. Elsevier, p. 69-117.

Hyde JR, Vetter RD, 2007. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). Molecular Phylogenetics and Evolution. 44:790-811.

Hyde JR, Kimbrell C, Robertson L, Clifford K, Lynn E, Vetter R, 2008. Multiple paternity and maintenance of genetic diversity in the live-bearing rockfishes *Sebastes* spp. Marine Ecology-Progress Series. 357:245-253.

Love MS, Yoklavich M, Thorsteinson L, 2002. The Rockfishes of the Northeast Pacific. Berkeley and Los Angeles, CA: University of California Press.

Pfister P, Randall J, Montoya-Burgos JI, Rodriguez I, 2007. Divergent Evolution among Teleost V1r Receptor Genes. PLoS ONE. 2:e379.

Saraiva LR, Korsching SI, 2007. A novel olfactory receptor gene family in teleost fish. Genome Research. 17:1448-1457.

Seeb LW, 1998. Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. Journal of Heredity. 89:393-403.

Tolimieri N, Levin PS, 2005. The roles of fishing and climate in the population dynamics of bocaccio rockfish. Ecological Applications. 15:458-468.

Van Doornik DM, Parker SJ, Millard SR, Berntson EA, Moran P, 2008. Multiple paternity is prevalent in Pacific ocean perch (*Sebastes alutus*) off the Oregon coast, and is correlated with female size and age. Environmental Biology of Fishes. 83:269-275.

BIBLIOGRAPHY

Aeschlimann PB, Haberli MA, Reusch TBH, Boehm T, Milinski M, 2003. Female sticklebacks Gasterosteus aculeatus use self-reference to optimize MHC allele number during mate selection. Behavioral Ecology and Sociobiology. 54:119-126.

Aguilar A, Garza JC, 2005. Analysis of major histocompatibility complex class II Beta genes from rockfishes (genus *Sebastes*). Journal of Fish Biology. 67:1021-1028.

Alioto TS, Ngai J, 2005. The odorant receptor repertoire of teleost fish. Bmc Genomics. 6:173.

Anderson TW 1983. Identification and development of nearshore juvenile rockfishes (Genus *Sebastes*) in central California kelp forests. Masters Thesis, California State University, Fresno.

Austin JA, Barth JA 2002. Drifter behavior on the Oregon-Washington shelf during downwelling-favorable winds. Journal of Physical Oceanography 32: 3132-3144.

Austin JA, Lentz SJ 2002. The inner shelf response to wind-driven upwelling and downwelling. Journal of Physical Oceanography 22: 2171-2193.

Banks MA, Rashbrook VK, Calavetta MJ, Dean CA, Hedgecock D, 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's central valley. Canadian Journal of Fisheries and Aquatic Sciences 57: 915-927.

Barth J, Pierce S, Smith R 2000. A separating coastal upwelling jet at Cape Blanco, Oregon and its connection to the California Current System. Deep-Sea Research II 47: 783-810.

Barth JA, Smith RL, 1998. Separation of a coastal upwelling jet at Cafe Blanco, Oregon, USA. South African Journal of Marine Science-Suid-Afrikaanse Tydskrif Vir Seewetenskap. 19:5-14.

Belkhir K 2000. GENETIX 4.02, Windows TM program for population gentics. Available at http://www.univ-montp2.fr/~genetix/genetix.htm.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F, 2004. Genetix 4.05, Windows program for population genetics. Available at http://www.University-montp2.fr/~genetix/genetix.htm.

Bentzen P, Taggart CT, Ruzzante DE, Cook D, 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences. 53:2706-2721.

Bernatchez L, Landry C, 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? Journal of Evolutionary Biology. 16:363-377.

Boehlert GW, Yoklavich MM, 1984. Reproduction, embryonic energetics, and the maternal fetal relationship in the viviparous genus *Sebastes* (Pisces, Scorpaenidae). Biological Bulletin 167:354-370.

Boschat C, Pelofi C, Randin O, Roppolo D, Luscher C, Broillet MC, Rodriguez I, 2002. Pheromone detection mediated by a V1r vomeronasal receptor. Nature Neuroscience. 5:1261-1262.

Botsford LW, Hastings A, Gaines SD 2001. Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. Ecology Letters 4: 144-150.

Botsford LW, Micheli F, Hastings A 2003. Principles for the design of marine reserves. Ecological Applications 13 Supplement: S25-S31.

Bradbury IR, Bentzen P 2007. Non-linear genetic isolation-by-distance: implications for dispersal estimation in anadromous and marine fish populations. Marine Ecology Progress Series 340: 245-257.

Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE, 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proceedings of the Royal Society B. 275:1803-1809.

Brookfield JFY 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology 5(3): 453-455.

Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC, 1988. A hypothetical model of the foreign antigen-binding site of class-II histocompatibility molecules. Nature. 332:845-850.

Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, Wiley DC, 1993. 3-Dimensional structure of the human class-II histocompatibility antigen Hla-Dr1. Nature. 364:33-39.

Buck L, Axel R, 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell. 65:175-187.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD, 2002. Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. Canadian Journal of Fisheries and Aquatic Sciences. 59:1374-1384.

Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD, 2005. Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. Conservation Genetics. 6:697-713.

Buonaccorsi VP, Westerman M, Stannard J, Kimbrell C, Lynn E, Vetter RD, 2004. Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. Marine Biology. 145:779-788.

Casalini M, Agbali M, Reichard M, Konecna M, Bryjova A, Smith C, 2009. Male dominance, female mate choice, and intersexual conflict in the rose bitterling (*Rhodeus ocellatus*). Evolution. 63:366-376.

Chen C 2006. TESS reference manual. Grenoble: Institut d'Informathique et Mathématiques Appliquées de Grenoble. Available: http://www-timc.imag.fr/Olivier.Francois/tess.html. Accessed Sept. 1, 2007.

Chen C, Durand E, Forbes F, Francois O 2007. Bayesian clustering algorithms ascertaining spatial population structure: A new computer program and comparison study. Molecular Ecology Notes 7: 747-756

Cohen S, 2002. Strong positive selection and habitat-specific amino acid substitution patterns in Mhc from an estuarine fish under intense pollution stress. Molecular Biology and Evolution. 19:1870-1880.

Connolly SR, Menge BA, Roughgarden J, 2001. A latitudinal gradient in recruitment of intertidal invertebrates in the northeast Pacific Ocean. Ecology. 82:1799-1813.

Consuegra S, de Leaniz CG, 2008. MHC-mediated mate choice increases parasite resistance in salmon. Proceedings of the Royal Society B-Biological Sciences. 275:1397-1403.

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB 2000. Connectivity of marine populations: open or closed? Science 287: 857-859.

Crooks GE, Hon G, Chandonia JM, Brenner SE, 2004. WebLogo: A sequence logo generator. Genome Research. 14:1188-1190.

Cushing DH, 1990. Plankton production and year-class strength in fish populations - an update of the match mismatch hypothesis. Advances in Marine Biology. 26:249-293.

Doherty PC, Zinkernagel RM, 1975. Enhanced immunological surveillance in mice heterozygous at H-2 gene complex. Nature. 256:50-52.

Doherty PJ, Planes S, Mather P, 1995. Gene flow and larval duration in 7 species of fish from the Great Barrier Reef. Ecology. 76:2373-2391.

Eizaguirre C, Yeates SE, Lenz TL, Kalbe M, Milinski M, 2009. MHC-based mate choice combines good genes and maintenance of MHC polymorphism. Molecular Ecology. 18:3316-3329.

Excoffier L, Laval G, Schneider S 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Forsberg LA, Dannewitz J, Petersson E, Grahn M, 2007. Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout - females fishing for optimal MHC dissimilarity. Journal of Evolutionary Biology. 20:1859-1869.

Francois O, Ancelet S, Guillot G 2006. Bayesian clustering using hidden Markov random fields in spatial population genetics. Genetics 174: 805-816.

Garner SR, Bortoluzzi RN, Heath DD, Neff BD, 2010. Sexual conflict inhibits female mate choice for major histocompatibility complex dissimilarity in Chinook salmon. Proceedings of the Royal Society B-Biological Sciences. 277:885-894.

Garrigan D, Hedrick PW, 2003. Perspective: Detecting adaptive molecular polymorphism: Lessons from the MHC. Evolution. 57:1707-1722.

Gingras ML, Ventresca DA, Donnellan MD, Fisher JL, 1998. First observations of vermillion rockfish courtship are from a harvest refuge. California Fish and Game. 84:176-179.

Gloriam D, Bjarnadottir T, Yan Y, Postlethwait J, Schioth H, Fredriksson R, 2005. The repertoire of trace amine G-protein-coupled receptors: large expansion in zebrafish. Molecular Phylogenetics and Evolution. 35:470-482.

Gomez-Uchida D, Hoffman EA, Ardren WR, Banks MA, 2003. Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species. Molecular Ecology Notes. 3:387-389.

Gonzalez EB, Murakami T, Teshima Y, Yoshioka K, Jeong DS, Umino T, 2009. Paternity testing of wild black rockfish *Sebastes inermis* (brownish type) from the Seto Inland Sea of Japan. Ichthyological Research. 56:87-91.

Graham WM, Largier JL 1997. Upwelling shadows as nearshore retention sites: an example of northern Monterey Bay. Continental Shelf Research 17: 509-532.

Grus WE, Shi P, Zhang YP, Zhang JZ, 2005. Dramatic variation of the vomeronasal pheromone receptor gene repertoire among five orders of placental and marsupial mammals. Proceedings of the National Academy of Sciences of the United States of America. 102:5767-5772.

Guindon S, Gascuel O, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology. 52:696-704.

Gunderson DR, 1977. Population biology of Pacific ocean perch, *Sebastes alutus*, stocks in Washington-Queen Charlotte Sound region, and their response to fishing. Fishery Bulletin. 75:369-403.

Gunderson DR, Vetter RD 2006. Temperate rocky reef fishes. Chapter 3. Pages 69-117 in JP Kritzer, PF Sale (eds), Marine Metapopulations. Elsevier.

Hall T, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/NT. Nucleic Acids Symposium Series. 41:95-98.

Hallacher LE, 1974. The comparative morphology of extrinsic gasbladder musculature in the scorpionfish genus *Sebastes* (Pisces: Scorpaenidae). Proceedings of the California Academy of Sciences. 40:59-86.

Hasegawa M, Kishino K, Yano T, 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution. 22:160-174.

Hedgecock D, 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In Beaumont AR ed. Genetics and Evolution of Aquatic Organisms. London: Chapman and Hall, p. 122-134.

Helvey M, 1982. First observations of courtship behavior in rockfish, genus *Sebastes*. Copeia.763-770.

Hofmann K, Stoffel W, 1993. TMbase - a database of membrane-spanning protein segments. In Biol. Chem. Hoppe-Seyler.

Hughes AL, 2007. Looking for Darwin in all the wrong places: the misguided quest for positive selection at the nucleotide sequence level. Heredity 99:364-373.

Hyde JR, Kimbrell C, Robertson L, Clifford K, Lynn E, Vetter R, 2008. Multiple paternity and maintenance of genetic diversity in the live-bearing rockfishes *Sebastes* spp. Marine Ecology-Progress Series. 357:245-253.

Hyde JR, Vetter RD, 2007. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). Molecular Phylogenetics and Evolution. 44:790-811.

Ivanova NV, Dewaard JR, Hebert PDN, 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes. 6:998-1002.

Johansen T, Dahle G, 2004. Discrimination among species of the genus Sebastes in the North Atlantic by random amplified polymorphic DNA. Sarsia. 89:478-483.

Jones AG, 2005. GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. Molecular Ecology Notes. 5:708-711.

Kai Y, Nakabo T, 2008. Taxonomic review of the Sebastes inermis species complex (Scorpaeniformes : Scorpaenidae). Ichthyological Research. 55:238-259.

Kai Y, Nakayama K, Nakabo T, 2003. Molecular phylogenetic perspective on speciation in the genus *Sebastes* (Scorpaenidae) from the Northwest Pacific and the position of *Sebastes* within the subfamily Sebastinae. Ichthyological Research. 50:239-244.

Kalbe M, Eizaguirre C, Dankert I, Reusch TBH, Sommerfeld RD, Wegner KM, Milinski M, 2009. Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. Proceedings of the Royal Society B-Biological Sciences. 276:925-934.

Kalendar R, 2009. FastPCR software for PCR primer and probe design and repeat search.

Kalinowski ST, Wagner AP, Taper ML, 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes. 6:576-579.

Kingsford MJ, Leis JM, Shanks A, Lindeman KC, Morgan SG, Pineda J, 2002. Sensory environments, larval abilities and local self-recruitment. Bulletin of Marine Science. 70:309-340.

Krogh A, Larsson B, von Heijne G, Sonnhammer ELL, 2001. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. Journal of Molecular Biology. 305:567-580.

Landry C, Garant D, Duchesne P, Bernatchez L, 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). Proceedings of the Royal Society of London Series B-Biological Sciences. 268:1279-1285.

Largier J 2003. Considerations in estimating larval dispersal distances from oceanographic data. Ecological Applications 13 Supplement: S71-S89.

Lea R, McAllister R, Ventresca DA, 1999. Biological aspects of the nearshore rockfishes of the genus Sebastes from central California. California Department of Fish and Game, Fish Bulletin. 177:1-109.

Leblois R, Estoup A, Rousset F 2003. Influence of mutational and sampling factors on the estimation of demographic parameters in a “continuous” population under isolation-by-distance. Molecular Biology and Evolution 20: 491-502.

Lenarz WH, VenTresca DA, Graham WM, Schwing FB, Chavez F 1995. Explorations of El Niño events and associated biological population dynamics off central California. CalCOFI (California Cooperative Oceanic Fisheries Investigations) Reports 36: 106-119.

Lenz TL, Eizaguirre C, Scharsack JP, Kalbe M, Milinski M, 2009. Disentangling the role of MHC-dependent 'good genes' and 'compatible genes' in mate-choice

decisions of three-spined sticklebacks Gasterosteus aculeatus under semi-natural conditions. Journal of Fish Biology. 75:2122-2142.

Liberles S, Buck L, 2006. A second class of chemosensory receptors in the olfactory epithelium. Nature. 442:645-650.

Librado P, Rozas J, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25:1451-1452.

Love M, 1996. Probably More Than You Want to Know About Fishes of the Pacific Coast. Santa Barbara, California: Really Big Press.

Love MS, Caselle JE, Buskirk WV 1998. A severe decline in the commercial passenger fishing vessel rockfish (*Sebastes* spp.) catch in the Southern California Bight, 1980-1996. CalCOFI (California Cooperative Oceanic Fisheries Investigations) Reports 39: 180-195.

Love MS, Yoklavich M, Thorsteinson L, 2002. The Rockfishes of the Northeast Pacific. Berkeley and Los Angeles, CA: University of California Press.

Lubchenco J, Palumbi SR, Gaines SD, Andelman S 2003. Plugging a hole in the ocean: the emerging science of marine reserves. Ecological Applications 13 Supplement: S3-S7.

Marliave JB, 1986. Lack of planktonic dispersal of rocky intertidal fish larvae. Transactions of the American Fisheries Society. 115:149-154.

Mecklenburg CW, Mecklenburg T, Thorsteinson L, 2002. Fishes of Alaska. Bethesda, Maryland: American Fisheries Society.

Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T, 2005. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. Proceedings of the National Academy of Sciences of the United States of America. 102:4414-4418.

Miller JA, Shanks AL, 2004. Ocean-estuary coupling in the Oregon upwelling region: abundance and transport of juvenile fish and of crab megalopae. Marine Ecology-Progress Series. 271:267-279.

Miller KM, Schulze AD, Withler RE, 2000. Characterization of microsatellite loci in Sebastes alutus and their conservation in congeneric rockfish species. Molecular Ecology. 9:240-242.

Morgan LE, Botsford LW 1998. The influence of larval transport and retention zones on recruitment patterns and the design of harvest refugia for rockfish. NOAA Tech. Memo. 255: 41-49.

Moser H, 1967. Reproduction and development of *Sebastodes paucispinis* and comparisons with other rockfishes off California. Copeia. 1967:773-779.

Moser HG, Boehlert GW, 1991. Ecology of pelagic larvae and juveniles of the genus *Sebastes*. Environmental Biology of Fishes. 30:203-224.

Musick JA, Harbin MM, et al. 2000. Marine, estuarine, and diadromous fish stocks at risk of extinction in North America (exclusive of Pacific salmonids). Fisheries 25(11): 6-30.

Neff BD, Garner SR, Heath JW, Heath D, 2008. The MHC and non-random mating in a captive population of Chinook salmon. Heredity. 101:175-185.

Ng WC, Sadovy Y, Leung FCC, 2003. Mating system of the rockfish, Sebastiscus marmoratus as revealed by DNA fingerprinting. Ichthyological Research. 50:339-348.

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M, 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289:739-745.

Palumbi SR 2001. The ecology of marine protected areas. In: Bertness MD, Gaines SD, Hay M (eds) Marine Community Ecology. Sinauer Associates, Inc., Sunderland, MA, p 509-530.

Penn DJ, Damjanovich K, Potts WK, 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. Proceedings of the National Academy of Sciences of the United States of America. 99:11260-11264.

Penn DJ, Potts WK, 1999. The evolution of mating preferences and major histocompatibility complex genes. American Naturalist. 153:145-164.

Pfister P, Randall J, Montoya-Burgos JI, Rodriguez I, 2007. Divergent Evolution among Teleost V1r Receptor Genes. PLoS ONE. 2:e379.

Pfister P, Rodriguez I, 2005. Olfactory expression of a single and highly variable V1r pheromone receptor-like gene in fish species. Proceedings of the National Academy of Sciences of the United States of America. 102:5489-5494.

Ploegh H, Watts C, 1998. Antigen recognition - Editorial overview. Current Opinion in Immunology. 10:57-58.

Posada D, 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution. 25:1253-1256.

Ralston S, Ianelli J, Miller R, Pearson D, Thomas D, Wilkins M 1996. Status of bocaccio in the Conception/Monterey/Eureka INPFC areas in 1996 and recommendations for management in 1997. Appendix volume 1 to the status of the Pacific coast groundfish fishery through 1996 and recommended acceptable biological catches for 1997. Pacific Fishery Management Council, Portland, Oregon.

Raymond M, Rousset F (1995) GENEPOP (Ver. 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Reusch TBH, Haberli MA, Aeschlimann PB, Milinski M, 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature. 414:300-302.

Rocha-Olivares A, Kimbrell CA, Eitner BJ, Vetter RD 1999. Evolution of a mitochondrial cytochrome b gene sequence in the species-rich genus Sebastes (Teleostei, Scorpaenidae) and its utility in testing the monophyly of the subgenus Sebastomus. Molecular Phylogenetics and Evolution 11: 426-440.

Rocha-Olivares A, Rosenblatt RH, Vetter RD, 1999. Cryptic species of rockfishes (Sebastes : Scorpaenidae) in the Southern Hemisphere inferred from mitochondrial lineages. Journal of Heredity. 90:404-411.

Rocha-Olivares A, Vetter RD, 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Canadian Journal of Fisheries and Aquatic Sciences. 56:803-813.

Roques S, Pallotta D, Sevigny JM, Bernatchez L, 1999. Isolation and characterization of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei : Scorpaenidae, genus *Sebastes*). Molecular Ecology. 8:685-687.

Rousset F, 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics. 145:1219-1228.

Saraiva LR, Korsching SI, 2007. A novel olfactory receptor gene family in teleost fish. Genome Research. 17:1448-1457.

Seeb LW 1986. Biochemical systematics and evolution of the scorpaenid genus Sebastes. PhD Thesis, University of Washington, Seattle.

Seeb LW, 1998. Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. Journal of Heredity. 89:393-403.

Shanks AL, Brink L (2005) Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. Marine Ecology Progress Series 302: 1-12.

Shi P, Zhang JZ, 2007. Comparative genomic analysis identifies an evolutionary shift of vomeronasal receptor gene repertoires in the vertebrate transition from water to land. Genome Research. 17:166-174.

Shinomiya A, Ezaki O, 1991. Mating habits of the rockfish *Sebastes inermis*. Environmental Biology of Fishes. 30:15-22.

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

Sogard SM, Berkeley SA, Fisher R, 2008. Maternal effects in rockfishes *Sebastes* spp.: a comparison among species. Marine Ecology-Progress Series. 360:227-236.

Sogard SM, Gilbert-Horvath E, Anderson EC, Fishee R, Berkeley SA, Garza JC, 2008. Multiple paternity in viviparous kelp rockfish, *Sebastes atrovirens*. Environmental Biology of Fishes. 81:7-13.

Sokal RR, Rohlf FJ (1995) Biometry. 3rd ed. W.H. Freeman and Co., New York.

Speca DJ, Lin DM, Sorensen PW, Isacoff EY, Ngai J, Dittman AH, 1999. Functional identification of a goldfish odorant receptor. Neuron. 23:487-498.

Stern A, Doron-Faigenboim A, Erez E, Martz E, Bacharach E, Pupko T, 2007. Selecton 2007: advanced models for detecting positive and purifying selection using a Bayesian inference approach. Nucleic Acids Research. 35:W506-W511.

Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR, 2002. Evidence of self-recruitment in demersal marine populations. Bulletin of Marine Science. 70:251-271.

Taylor CA (1998) Phylogenetics and larval identification of rockfishes (genus Sebastes) using mitochondrial DNA techniques. Masters thesis, San Diego State University, San Diego.

Taylor MS, Hellberg ME, 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science. 299:107-109.

Tolimieri N, Levin PS, 2005. The roles of fishing and climate in the population dynamics of bocaccio rockfish. Ecological Applications. 15:458-468.

Van Doornik DM, Parker SJ, Millard SR, Berntson EA, Moran P, 2008. Multiple paternity is prevalent in Pacific ocean perch (*Sebastes alutus*) off the Oregon coast, and is correlated with female size and age. Environmental Biology of Fishes. 83:269-275.

van Oosterhaut C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

Waples RS, Rosenblatt RH, 1987. Patterns of larval drift in southern-California marine shore fishes inferred from allozyme data. Fishery Bulletin. 85:1-11.

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

Westerman ME, Buonaccorsi VP, Stannard JA, Galver L, Taylor C, Lynn EA, Kimbrell CA, Vetter RD, 2005. Cloning and characterization of novel microsatellite DNA markers for the grass rockfish, Sebastes rastrelliger, and

cross-species amplification in 10 related Sebastes spp. Molecular Ecology Notes. 5:74-76.

Wetton JH, Carter RE, Parkin DT, Walters D, 1987. Demographic-study of a wild house sparrow population by DNA fingerprinting. Nature. 327:147-149.

Wimberger P, Burr J, Gray A, Lopez A, Bentzen P 1999. Isolation and characterization of twelve microsatellite loci for rockfish (*Sebastes*). Marine Biotechnology 1:311-315.

Wing SR, Botsford LW, Ralston SV, Largier JL, 1998. Meroplanktonic distribution and circulation in a coastal retention zone of the northern California upwelling system. Limnology and Oceanography. 43:1710-1721.

Winemiller KO, Rose KA, 1992. Patterns of life-history diversification in North American fishes - implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences. 49:2196-2218.

Woelfing B, Traulsen A, Milinski M, Boehm T, 2009. Does intra-individual major histocompatibility complex diversity keep a golden mean? Royal Society Philosophical Transactions Biological Sciences. 364:117-128.

Wourms JP, 1991. Reproduction and development of *Sebastes* in the context of the evolution of piscine viviparity. Environmental Biology of Fishes. 30:111-126.

Yoshida K, Nakagawa M, Wada S, 2001. Pedigree tracing of a hatchery-reared stock used for aquaculture and stock enhancement based on DNA markers. Fish Genetics and Breeding Science. 30:27-35.

Zeidberg LD, Hamner WM 2002. Distribution of squid paralarvae, *Loligo opalescens* (Cephalopoda, Myopsida), in the southern California Bight in the three years following the 1997-1998 El Niño. Marine Biology 141: 111-122.