NOAA Technical Memorandum NMFS-NWFSC-80

Conservation of North Pacific Rockfishes: Ecological Genetics and Stock Structure

Proceedings of the Workshop
March 2–3, 2004
Seattle, Washington

April 2007

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

NOAA Technical Memorandum
NMFS Series

The Northwest Fisheries Science Center of the National Marine Fisheries Service, NOAA, uses the NOAA Technical Memorandum NMFS series to issue informal scientific and technical publications when complete formal review and editorial processing are not appropriate or feasible due to time constraints. Documents published in this series may be referenced in the scientific and technical literature.

The NMFS-NWFSC Technical Memorandum series of the Northwest Fisheries Science Center continues the NMFS-F/NWC series established in 1970 by the Northwest & Alaska Fisheries Science Center, which has since been split into the Northwest Fisheries Science Center and the Alaska Fisheries Science Center. The NMFS-AFSC Technical Memorandum series is now being used by the Alaska Fisheries Science Center.

This document should be cited as follows:

Berntson, E.A., P.S. Levin, and P.C. Moran (editors). 2007. Conservation of North Pacific rockfishes: Ecological genetics and stock structure. Proceedings of the workshop, March 2-3, 2004, Seattle, Washington. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-80, 80 p.

NOAA Technical Memorandum NMFS-NWFSC-80

Conservation of North Pacific Rockfishes: Ecological Genetics and Stock Structure

Proceedings of the Workshop
March 2–3, 2004
Seattle, Washington

Edited by Ewann A. Berntson, Phil S. Levin,* and Paul Moran

Northwest Fisheries Science Center
Conservation Biology Division
* Fishery Resource Analysis and Monitoring Division
2725 Montlake Boulevard East
Seattle, Washington 98112

April 2007

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

Most NOAA Technical Memorandums NMFS-NWFSC are available online at the Northwest Fisheries Science Center web site (http://www.nwfsc.noaa.gov)

Copies are also available from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
phone orders (1-800-553-6847)
e-mail orders (orders@ntis.fedworld.gov)

Table of Contents

Executive Summary

On 2 and 3 March 2004, the Conservation Biology Division and Fishery Resource Analysis and Monitoring Division sponsored a workshop at NOAA's Northwest Fisheries Science Center (NWFSC) in Seattle. The specific goals of the workshop were as follows:

- To bring together researchers and managers to help define current conservation problems and to provide an understanding of current opportunities (and limitations) of technologies such as molecular genetic methods and stable isotope analysis;
- To foster interactions between ecologists and geneticists and to promote collaborations that address long-standing conservation and recovery problems; and
- To prioritize conservation research and provide suggestions for future research needs.

Approximately 50 scientists attended the workshop from a variety of institutions, including federal, state, tribal, and academic. The workshop was organized into two days of presentations with ample opportunity for open discussion. Presentations covered a range of topics including ecology, microchemistry, genetics, stock structure, policy, and conservation, as do the presentation summaries included here. Many of the presentation summaries cover measuring movement of individual rockfish over the short and long term, using elemental or isotopic analysis over short periods, and using molecular methods to study movements over evolutionary time. The summaries also address systematic relationships, many in the context of larval identification.

Speakers presented diverse management and research perspectives, and identified a number of research priorities as they highlighted their current work. Given the precipitous decline of multiple rockfish species in the last 15 years, there is great opportunity for the incorporation of techniques, both new and traditional, to further the assessment, conservation, and recovery of North Pacific rockfishes. The perspective gained in this workshop is likely to be of general interest in applying genetic methods to stock assessment and marine conservation.

Acknowledgments

Contemporary rockfish biologists owe a great debt of gratitude to the nineteenth and early twentieth century ichthyologists who first described these remarkably diverse and fascinating creatures. In 1829 George Cuvier coined the genus name *Sebastes*, "magnificent," in Greek. Other pioneers such as William O. Ayres, Charles Girard, Theodore Gill, and Julius B. Phillips described the systematics and ecology of key species and laid the foundation for current investigations of genetics, stock assessment, and general biology. Although the role of these historical personalities is central to rockfish research today, one contemporary rockfish biologist has done more than any single individual to foster admiration and interest in rockfishes. Milton Love has done for rockfishes what Ethelwyn Trawavas and Tony Ribbink did for East African cichlids. He made them accessible, interesting, and important to scientists and the lay public. Rockfishes are very cool indeed!

For their facilitation and encouragement of this workshop, we also offer thanks to Elizabeth Clarke, director of the Fishery Resource Analysis and Monitoring (FRAM) Division, and Michael J. Ford, director of the Conservation Biology Division. The FRAM Division and NOAA Fisheries Service's Protected Resources Division provided funding to host this meeting. Arlene Frazier of FRAM generously contributed to logistics and organization.

Themes of the Workshop Highlight Management Advances in the Application of Genetic Methods to Stock Assessment

Paul Moran
Northwest Fisheries Science Center
Conservation Biology Division
2725 Montlake Boulevard East
Seattle, Washington 98112
paul.moran@noaa.gov

Precipitous declines in abundance of multiple rockfish (*Sebastes* spp.) species over the past 15 years have motivated efforts to improve stock-specific management. The hope is that genetic methods, microchemistry, telemetry, and other techniques that track the movement of individuals can refine stock-specific management to allow harvest (and bycatch) of abundant stocks while protecting and restoring depleted species and stocks. It is not yet clear how recently developed genetic tools will benefit groundfish conservation in general and rockfish stock assessment in particular. Considerable potential exists; however, rockfish species often present significant challenges to sampling and study design for genetic research. Species ranges are geographically broad and typically lack obvious breeding aggregates. Although many rockfish species are sedentary, pelagic larvae provide substantial opportunity for dispersal. There is some expectation for isolation by distance, but the geographic scale of that isolation is often unclear. It is difficult to know how individual fish samples (e.g., trawls) should be grouped into putative populations or stocks.[1] Where data are available, some species show evidence of relatively fine-scale population structure, whereas others show widespread gene flow.

Significant recent progress has been made toward addressing goals for rockfish genetics research that Russ Vetter identified more than 5 years ago (SWFSC 2006). Although he presented these goals as being of specific interest to the California Marine Life Protection Act, they are equally relevant to rockfish research in general. Vetter identified the following priorities throughout the ranges of multiple rockfish species: 1) characterize species and subgeneric relationships within the genus *Sebastes*, 2) identify larvae, and 3) define unit stocks for rockfish species.

[1] Population is a biological term that denotes a group of freely interbreeding individuals partially or completely isolated from other such groups within a species. A stock is a management unit that typically identifies a group that is demographically linked. Stocks often coincide with populations or groups of populations within species but may include multiple species (see discussion of cryptic species, e.g., Genetic Studies of Rockfish: Identification, Relationships, and Population Structure, p. 61, and Conservation Genetics of the Rockfishes: The West Coast's Most Species-Rich and Imperiled Genus of Fishes, p. 67).

These goals are clearly interrelated and although Vetter, his students, and others have done much to address these issues, this workshop underscored specific areas that need additional work. Gharrett et al. (Genetic Studies of Rockfish: Identification, Relationships, and Population Structure, p. 61) propose almost the exact same research priorities for molecular genetic studies in rockfishes: 1) improved descriptive phylogenetics, 2) larval identification, and 3) elucidation of population structure. Gharrett et al. state that improved species identification, high throughput markers, and improved systematics of Asian species are high priorities, but that the most work is needed in analysis of population structure within species. Population genetic structure is directly related to the movement of individuals among locations along with their subsequent reproductive success. Although these demographic connections are central to biologically sound stock assessment, conservation, and recovery, managers generally have incomplete demographic information about their stocks. Another important and related application of population genetic studies is to evaluate the evolutionarily effective population size of rockfish populations to examine genetic diversity relative to census size (Microsatellites Reveal Regional Genetic Structure and Effective Population Size in Darkblotched Rockfish, p. 47).

The goals of this workshop were threefold. First, we sought to use presentations and discussion to define the central issues in rockfish conservation as related to stock structure. Second, we hoped to give attendees some sense of the capabilities and the limitations of current molecular (and other) methods. Finally, we hoped the workshop would lead to collaborations and partnerships among harvest managers, ecologists, and geneticists. Toward this last goal we cast a broad net of invitations with hopes of bringing together different agencies and interests including tribal, state, federal, and academic scientists, managers, and public policy advocates. We take the diversity of presentations and wide-ranging discussions as an indication of success.

The need for accurately estimating stock structure is understood and appreciated in the research and management community, and the level of interest in this topic was reflected in the workshop's substantial attendance. For example, sustainable harvest and effective bycatch management require some understanding of the biological populations that are impacted. Such an understanding allows direction of harvest toward abundant populations and away from depressed or sensitive groups. Likewise, risk assessment, conservation, and recovery planning require the same kind of biological foundation. A first step in conservation genetics is to identify the units that are being conserved. Rarely are entire species represented by a single population, and individual populations almost certainly have different demographic trajectories (some might say by definition, e.g., McElhany et al. 2000). A potential liability of stock-specific management might be further depression of coast-wide abundance, that is, each stock is fished closer to the limit of sustainability. However, this concern is more appropriately directed at the application of new population genetic data. Instead, the issue would seem to be what harvest levels are allowed on those individual stocks. Alternatively, to limit harvest on abundant populations due to ignorance about the biological underpinnings of management units seems socially unacceptable if such biological information could be obtained.

Similarly an understanding of stock structure and dispersal is essential for designing, monitoring, and evaluating marine protected areas (MPAs) (Botsford et al. 2001, Palumbi 2003, Sale et al. 2005). If MPAs are too widely dispersed, then populations are isolated and may suffer inbreeding depression (Carson and Hentschel 2006). If individual MPAs support large

populations anyway, then inbreeding is not an issue. However, vast differences exist among species with respect to dispersal distances and population size (including census size, genetic effective population size, and the ratio of those parameters). These differences in dispersal underscore the complexity of optimizing the design of MPAs to benefit many diverse species.

Perhaps most important in the future, a basic understanding of population genetics is essential for effective artificial propagation (see Tribal Fisheries: The Role of Stock Structure in Regional Management, p. 15). Although the specific issues differ, population structure is as important to conservation programs (e.g., supplementation and gene banking) and fishery enhancement as it is to harvest management. The point is that, depending on the scale of the program, artificial propagation can have profound impacts on gene flow among groups that might have been isolated over recent evolutionary time (relative to generation time and rates of adaptive divergence among populations—again widely different among species). Artificial propagation also can reduce genetic diversity by amplifying a relatively small slice of the total diversity in the population (Ryman and Laikre 1991). Broodstock collections must often balance the desire to fully capture the diversity within the population of interest, yet not combine distinct and adaptively diverged populations. In some cases it is unclear whether a captive breeding or supplementation program is at greater risk for inbreeding or outbreeding. Indeed some programs suffer both effects simultaneously. Management challenges in Pacific salmon (*Oncorhynchus* spp.) strongly suggest that it is better to plan ahead than to begin large artificial propagation programs without considering the genetic implications. Although experience in salmon may be of limited utility in rockfish genetics due to fundamental differences in biology and genetic structure, it is worth considering the concerns that have developed in salmon artificial propagation. From concern over altering the metapopulation structure and genetic connectivity among populations to domestication selection and reduction of life history diversity, the salmon experience provides an initial framework for developing risk assessment in rockfish artificial propagation.

Another lesson from salmon genetics related to logistics rather than biology is that multilaboratory standardization of genetic markers and genotyping conventions is essential for addressing large-scale, multijurisdictional conservation and fishery management problems (Moran et al. 2006). It is very inefficient for individual laboratories to forge ahead, each with their own suite of nonoverlapping markers. Laboratories are best served by early agreement on a common suite of markers and standardized allele designations. Markers should be appropriate to the task. Microsatellite loci are suitable for fine-scale descriptive population genetic work, whereas mitochondrial DNA or single-copy nuclear genes might be suited for systematic relationships or species identification. The markers need the right power of resolution, need to be portable among laboratories, and need to be cost effective. Genetic standardization requires some initial investment of time and effort, and experience has shown that little progress is typically made in the absence of specific management objectives. Multilaboratory standardization generally requires extensive sharing of tissue or DNA samples and sharing of unpublished data. Sample and data sharing can raise issues of stewardship and ownership of samples and unpublished data.

Selecting markers is a critical element of interlaboratory standardization. If no markers are shared, then only limited combined analyses are possible. An almost immediate question is whether to use neutral or nonneutral markers. All that is needed for many applications is

discrimination of particular groups of fish. For example, managing harvest of fish from different stocks taken in mixed fisheries simply requires differentiating among potential source populations. In many applications, it does not matter whether markers are neutral. Indeed nonneutral markers are sometimes substantially more powerful than neutral markers (Miller et al. 2001, 2002). For most other applications, however, neutrality is an important prerequisite, for example, effective population size estimates, dispersal distances and gene flow among semi-isolated populations, and other issues related directly and indirectly to stock assessment. Whether markers are neutral or nonneutral is less important than considering how the markers are to be employed and what limitations are present in particular applications.

The future of genetics in rockfish stock assessment appears full of opportunity. Results presented in this workshop demonstrate real utility in rockfish management. Substantial new biological insight has been obtained related to demographics and movement of individuals, a cornerstone of stock assessment. Ecological genetics is at a critical juncture with rockfishes and other nonmodel organisms for several reasons. Molecular genetic techniques have matured to the point that extremely powerful data can now be obtained on a wide range of spatial and temporal genetic relationships. Along with the maturation of molecular methods, computing power has increased exponentially. These new tools and resources have contributed to a rebirth and reapplication of some important statistical methods. In particular, Bayesian methods of inference are now widely used for parameter estimation in complex multivariate problems. A major challenge, and an area of considerable current insight and creativity, involves developing efficient sampling strategies and algorithms that rapidly and efficiently explore posterior distributions without becoming trapped in local optima. It seems likely that as computing and molecular methods become more powerful, population genetic analysis is likely to gravitate toward analysis of individual fish. A statistical analogy would be to move from analysis of discrete variables (allele frequencies in population segments) to continuous variables (genetic relatedness among individuals relative to various biotic and abiotic correlates). Multivariate analysis of genetic data in a broad ecological context will be desirable in order to make generalizations about species distributions and connectivity between stocks.

This proceedings volume is organized much like the workshop, with summaries representing each of the presentations. One exception is a literature review, by organizer and editor Ewann A. Berntson, that expands on some of the points introduced here related to recent developments in rockfish genetics. The presentation summaries reflect a diversity of management and research perspectives. Most are concerned with movement of individuals, whether measured over short periods using elemental or isotopic analysis, or whether considering the net effect of those movements over evolutionary time, as measured with genetic methods. Numerous authors also address systematic relationships, often in the context of larval identification. The topics presented closely reflect the research priorities identified above and significant progress is reported here and in recent peer-reviewed literature (see Literature Review of North Pacific Rockfish Genetic Structure, p. 6). Nevertheless, a great deal of potential remains for using genetic methods for stock assessment, conservation, and recovery. The presentation summaries included here bring some of that potential to fruition.

References

Botsford, L. W., A. Hastings, and S. D. Gaines. 2001. Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. Ecol. Lett. 4:144–150.

Carson, H. S., and B. T. Hentschel. 2006. Estimating the dispersal potential of polychaete species in the Southern California Bight: Implications for designing marine reserves. Mar. Ecol. Prog. Ser. 316:103–113.

McElhany, P., M. H. Ruckelshaus, M. J. Ford, T. C. Wainwright, and E. P. Bjorkstedt. 2000. Viable salmonid populations and the recovery of evolutionarily significant units. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-42.

Miller, K. M., T. D. Beacham, and R. E. Withler. 2002. Use of neutral and adaptive genetic variation in the management and conservation of Pacific salmon. ICES Rep. CM 2002/U:17.

Miller, K. M., K. H. Kaukinen, T. D. Beacham, and R. E. Withler. 2001. Geographic heterogeneity in natural selection on an MHC locus in sockeye salmon. Genetica 111:237–257.

Moran, P., D. J. Teel, E. S. LaHood, J. Drake, and S. Kalinowski. 2006. Standardizing multi-laboratory microsatellite data in Pacific salmon: An historical view of the future. Ecol. Freshw. Fish 15:597–605.

Palumbi, S. R. 2003. Population genetics, demographic connectivity, and the design of marine protected areas. Ecol. Appl. 13:S146–S158.

Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conserv. Biol. 8:888–890.

Sale, P. F., R. K. Cowen, B. S. Danilowicz, G. P. Jones, J. P. Kritzer, K. C. Lindeman, S. Planes, N. V. C. Polunin, G. R. Russ, Y. J. Sadovy, and R. S. Steneck. 2005. Critical science gaps impede use of no-take fishery reserves. Trends Ecol. Evol. 20(2):74–80.

SWFSC (Southwest Fisheries Science Center). 2006. Rockfish genetics—stock structure. Online at http://swfsc.noaa.gov/textblock.aspx?Division=FRD&id=1090&ParentMenuId=39 [accessed 28 November 2006].

Literature Review of Genetic Structure in North Pacific Rockfishes

Ewann A. Berntson
Northwest Fisheries Science Center
Conservation Biology Division
2725 Montlake Boulevard East
Seattle, Washington 98112
ewann.berntson@noaa.gov

Rockfishes (*Sebastes* spp.) are important members of the groundfish community in the Pacific and Atlantic oceans. Approximately 74 species of rockfish are found in the northeast Pacific, and several have been a documented component of commercial fisheries since the mid-nineteenth century (Love et al. 2002). A great deal of effort is devoted to the management of rockfish species. Nine of the 74 rockfish species have been thoroughly assessed through the Pacific Fisheries Management Council in previous years, and two additional rockfish species were assessed in 2005. In 2000 seven species were designated overfished by the National Marine Fisheries Service: bocaccio (*S. paucispinis*), Pacific ocean perch (*S. alutus*), canary rockfish (*S. pinniger*), cowcod (*S. levis*), widow rockfish (*S. entomelas*), yelloweye rockfish (*S. ruberrimus*), and darkblotched rockfish (*S. crameri*). Much of the information needed for completing these assessments for the remaining species simply is not available.

Effective fisheries management decisions must be based on a solid biological foundation. A thorough understanding of life history and ecology may be difficult to acquire for marine fish species, which often have populations that cover large territories and may differ in distribution with life stage. In the absence of such information, a species (and sometimes multiple species) will be managed as a single, homogenous unit. If that species is actually comprised of separate stocks, however, overfishing may deplete portions of the species with little chance of rebuilding from the remaining stocks. Current fishing practices, which also are a concern to fisheries managers and conservation biologists, may bring genetic changes that will have substantial consequences for fish populations, relating to overall size, size and age at maturity, and genetic diversity. Genetic changes may be slow to reverse and will not be on a time scale relevant to fisheries managers (Stokes and Law 2000).

Recent advances in the field of genetics have given fishery managers some important tools in the pursuit of more biologically relevant management practices. Two general categories of genetic techniques have been used to address questions relating to stock management: 1) protein-based allozyme electrophoresis, and 2) DNA sequence-based techniques, including the direct comparison of sequences, restriction fragment length polymorphisms (RFLPs), and microsatellite analyses (hypervariable, noncoding regions of nuclear DNA). DNA sequence-based techniques usually exhibit greater levels of variation than allozymes, and they have the added advantage of requiring minute amounts of tissue so individuals can be sampled

nondestructively. Studies utilizing sequence-based techniques can be found increasingly in the recent literature (Gharrett et al. 2004). Different techniques have different levels of resolution for population studies, but all can be useful for assessing the health of marine populations.

Genetics can lend insight into attributes such as population subdivisions, sex- and life-stage-specific migration patterns, and effective population size, which may be significantly different from the census size (Hauser et al. 2002). Genetics also can be useful in the basic identification of species, which can be difficult in species groups such as rockfish; adults of some rockfish species closely resemble each other and many larvae and juveniles are morphologically indistinguishable. A 1998 commercial catch report from a Northwest Fisheries Science Center Groundfish Research Plan showed 1,699 mt (12% of the overall rockfish caught in the Pacific Ocean) listed as "unspecified rockfishes" (NMFS 2000). The lack of species identification will be an impediment to acquiring the accurate ecological information required for designing proper management policies. Allozyme electrophoresis has been used successfully in species identification (Seeb 1986, Seeb and Kendall 1991, LeClair and Buckley 2001), as have DNA sequence-based techniques (Rocha-Olivares 1998, Rocha-Olivares et al. 2000, Gharrett et al. 2001, Gray et al. 2006, Li et al. 2006a). Descriptive studies of genetic variation among species also can provide information useful to fisheries managers by recounting the demographic history of a species, investigating species or subspecies differences (Gharrett et al. 2004), and identifying introgression or hybridization when it occurs between species. This information can help create a better ecological picture for management decisions.

Species-level and Phylogenetic Questions

Molecular techniques have proven useful at multiple levels in rockfish: broad-scale phylogenetics, species identification, finer-scale speciation, introgression, and population dynamics. Allozyme information generated by Seeb (1986) was combined with cytochrome *b* sequences to provide evidence that rockfish constitute one of the few marine examples of a species flock (Johns and Avise 1998)—a species-rich yet relatively similar grouping differentiating within a short amount of time. These similarities, in part, have created difficulties for taxonomists and systematists studying this group. Genetic analyses can help elucidate relationships among closely related species. Narum et al. (2004) used microsatellite markers to show clear evidence for differentiation between gopher rockfish (*S. carnatus*) and black-and-yellow rockfish (*S. chrysomelas*), a sympatric species pair that differ primarily in color. Comparisons of mitochondrial DNA (mtDNA) control region sequences for gopher rockfish and black-and-yellow rockfish have suggested that the speciation processes may be ongoing within rockfish (Alesandrini and Bernardi 1999). Without an understanding of current species groupings, managers will be unable to accurately model fisheries stocks.

Phylogenetic analyses will help elucidate relationships among closely related species. Molecular information, primarily mitochondrial sequence information and RFLPs, was used to create phylogenies for thornyheads (*Sebastolobus* spp.) (Stepien et al. 2000), the *Sebastes* subgenera *Sebastomus* (Rocha-Olivares et al. 1999a, 1999b), and *Pteropodus* (Li et al. 2006b). Nuclear sequences were used to examine the phylogeny of rockfish as a whole (Asahida et al. 2004), and similar levels of divergence were found compared to studies using mtDNA (Gharrett et al. 2001). Asahida et al. (2004) found many of the Asian species clustering together, divergent from most of the North American species.

Introgression may be an important factor in the population dynamics and genetic diversity for rockfish. Seeb (1998) used allozyme and RFLP analyses to detect striking changes in allele frequencies in the subgenus *Pteropodus* (gopher rockfish, quillback rockfish [*S. maliger*], and brown rockfish [*S. auriculatus*]) over very short (70 km) distances within Puget Sound in the absence of any obvious barriers to gene flow; however, when the Puget Sound populations were removed from the analyses, no measurable differentiation was detected from Washington to Alaska. Seeb suggested that introgression occurs among these three species within Puget Sound, and that it may contribute to overall genetic diversity within the genus as a whole. A microsatellite study of brown rockfish came to the same conclusions regarding potential introgression among these three species in Puget Sound (Buonaccorsi et al. 2005). The authors hypothesize that introgression may occur more readily in areas with low population numbers. As rockfish populations drop, introgression may play a larger role for other rockfish species in other areas. Introgression will need to be considered in stock assessments.

Population-level Questions

Genetic studies that may help identify population differentiation and stock structure within commercially important species are perhaps of most interest to fisheries managers. A handful of species have been examined with stock structure in mind. Allozyme studies have proven useful in some species: two stocks were identified for canary rockfish (*S. pinniger*) (Wishard et al. 1980), significant geographic variation was found within brown rockfish (Seeb 1998), and two groups of sympatric rougheye rockfish (*S. aleutianus*) also were delineated (Hawkins et al. 1997, 2005). An additional study using mtDNA sequences and microsatellite loci found the same two rougheye rockfish groups, and the authors suggested they are likely different species—a greater difference between mitochondrial haplotypes exists between the two groups than can be found between other pairs of rockfish species (Gharrett et al. 2005, 2006). Mitochondrial RFLP analysis of mtDNA (ND-3/ND-4 and 12S/16S ribosomal RNA [rRNA] genes) showed divergences within 8 of 15 rockfish species tested from the eastern Gulf of Alaska (Gharrett et al. 2001). Mitochondrial control region sequences have shown significant population differentiation in blue rockfish (*S. mystinus*) from Washington to California (Cope 2004), and in rosethorn rockfish (*S. helvomaculatus*) from California to the Gulf of Alaska (Rocha-Olivares and Vetter 1999). A distinct genetic break was identified for rosethorn rockfish at the divergence of the Alaska Current and the California Current. Control region sequences also demonstrated high levels of diversity within species in thornyhead populations (longspine thornyhead [*Sebastolobus altilevis*] and shortspine thornyhead [*S. alascanus*]) in the northern Pacific, with most individuals having unique haplotypes (Stepien et al. 2000). Mitochondrial sequences showed this diversity where earlier allozyme studies (Siebenaller 1978) did not. Each of these studies provides specific information that has immediate relevance to fishery management in general and stock assessment in particular.

Five rockfish species from the western Pacific were investigated using mtDNA sequences and amplified fragment length polymorphisms (AFLP) analyses—another sequence-based technique for measuring genetic diversity across the entire genome. Higuchi and Kato (2002) demonstrated variation in the nucleotide diversity of usu-mebaru (*S. thompsoni*), togotto-mebaru (*S. joyneri*), mebaru (*S. inermis*), kurosoi (*S. schlegeli*), and *S. owstoni*. The authors concluded that this technique might be suitable for stock structure analysis in future studies. A second study used mtDNA sequences and AFLPs to distinguish among three color morphotypes within

mebaru (Kai et al. 2002). Both methods indicated some gene flow among the types, however, which suggested to the authors that introgression may be occurring or that speciation of the three was very recent.

Microsatellite analyses currently are the most useful technique for measuring within-species genetic variation. The high levels of variability and relative ease and speed of analysis make microsatellites an obvious choice for efforts to delineate stock structure. In addition it is likely that microsatellite loci shown to be variable within one species also may be variable within other, closely related species. Several loci have been published recently for use in rockfish, and have potential for use in determining stock structure. A summary of currently published loci and the species for which they have proven to be variable, along with measured heterozygosities and references, is provided in Appendix A (p. 73).

Studies utilizing microsatellite loci to identify potential stock structure are becoming more prevalent in published literature. Genetic diversity correlating to geographic distance has been shown for grass rockfish (*S. rastrelliger*) (Buonaccorsi et al. 2004) and brown rockfish (Buonaccorsi et al. 2005) from the eastern Pacific, as well as usu-mebaru populations from Japan (Sekino et al. 2001). Previous mtDNA sequence analyses were unable to detect population subdivisions within usu-mebaru (Sekino et al. 2001). A recent microsatellite study detected spatial structure in darkblotched rockfish from Washington to California and also focused on a discussion of the effects of pooling small sample sizes (Gomez-Uchida and Banks 2005). Microsatellite analyses of quillback rockfish from Puget Sound to southeast Alaska indicated genetic structure also (Burr 1999). Quillback rockfish from Washington State are managed currently as a single stock, but Burr suggested this decision should be revisited if further sampling confirms these findings (Burr 1999). A study of bocaccio using microsatellites detected population structure consistent with the California Current flow regimes along the coastline and suggested restricted movement of juveniles and adults (Matala et al. 2004a). Bocaccio population numbers now are 2–3% of those measured in 1969 (Love et al. 2002). Given the rapidly declining bocaccio populations, any successful conservation efforts must consider the potential existence of genetic structure.

The literature contains several examples of studies using multiple techniques to analyze population structure, some of which have been mentioned above. In some cases, but not all, results from the different techniques agree. Copper rockfish (*S. caurinus*) and quillback rockfish were studied using allozymes and mtDNA (Seeb 1998) and microsatellites (Buonaccorsi et al. 2002). Seeb (1998) found significant genetic differentiation over the whole range with particularly strong shifts in allozyme frequencies in quillback rockfish from Puget Sound. Introgression was suggested as a possible explanation (as mentioned previously). Copper rockfish microsatellite analyses also showed evidence of significant population subdivision in fish sampled from southern California to British Columbia including the Puget Sound (Buonaccorsi et al. 2002). Another example of concordance among techniques is in the Mexican rockfish (*S. macdonaldi*, the southernmost rockfish from the northeast Pacific Ocean), which ranges from California south to Baja, Mexico, including the Gulf of California (Rocha-Olivares et al. 2003). Mitochondrial DNA sequence information (Bernardi et al. 2003) and microsatellites (Rocha-Olivares et al. 2003) showed no significant differentiation among sampling sites. Small amounts of genetic partitioning suggested the possibility of recent dispersal rather than current gene flow or an older founder effect (Rocha-Olivares et al. 2003). In a third example an

allozyme study looked at yellowtail rockfish (*S. flavidus*) collected from the northeast Pacific coastline and found no genetic differentiation (Wishard et al. 1980). This result was largely confirmed using mtDNA RFLPs (McGauley and Mulligan 1995).

The literature also holds examples of multiple genetic analyses differing in their conclusions for a given species. Microsatellite analyses of shortraker rockfish (*S. borealis*), found on the Pacific Rim from California to Japan showed population structure in Alaskan waters not addressed by current stock divisions (Matala et al. 2004b), where a second study using allozymes found no genetic differentiation (Hawkins et al. 2005). Two allozyme studies (Wishard et al. 1980, Seeb and Gunderson 1988) and one microsatellite study (Withler et al. 2001) examined populations of Pacific ocean perch. Wishard et al. (1980) distinguished three different stocks along the northeast Pacific coastline, whereas Seeb and Gunderson (1988) found only slight amounts of genetic variation across a similar area. The microsatellite study found evidence for three distinct populations from coastal sites in British Columbia (Withler et al. 2001). The higher levels of variation of microsatellite loci gave Withler et al. (2001) the ability to distinguish these populations on a much smaller geographic scale than the allozyme studies could. Below we discuss considerations of the characteristics and performance of different marker classes, and interpretation of analysis results.

Issues of Concern in Genetic Stock Assessments

Genetic techniques can be extremely useful, if not essential, in determining stock structure of marine fish species. There are some important caveats, however, for the understanding and appropriate use of genetic information in devising management policies. Techniques with lower variability, such as allozyme electrophoresis, may show a given population to be homogenous over a given range, where more-variable microsatellite loci may show finely detailed genetic structure. Therefore a result showing no detectable genetic variation does not necessarily mean that the populations in question are homogenous. Analyses using multiple techniques (genetic and otherwise) and sampling over time can help determine which populations may be truly homogeneous.

The interpretation of levels of genetic differentiation found also must be done carefully, particularly in species that have the potential for high levels of gene flow. One of the most commonly used methods for estimating the level of gene flow among populations is the calculation of the F_{ST} parameter, which compares the proportion of genetic variation found within populations to that found among populations. The interpretation of F_{ST} is based on several assumptions, however: the number of subpopulations is infinite, the effective population size is the same in every subpopulation, breeding is random within each subpopulation, migration rates are small and constant for every subpopulation, the genes chosen are selectively neutral, and no mutation occurs (Waples 1998). These assumptions will be violated for any biological organism studied, more severely for some than others. In species experiencing relatively high rates of gene flow, genetic differentiation and the calculated F_{ST} values will be low, and violations of the assumptions will become more significant. In addition F_{ST} values will vary among loci and over time, so better estimates of true rates of gene flow will come from the analysis of multiple loci, on populations sampled over multiple time periods.

The significance of F_{ST} values to the development of management policies should be examined carefully for each case. Very few migrants per generation are required to produce low F_{ST} values, seemingly indicating stocks experiencing near panmixia; however, the minimum level of migration necessary to produce near genetic homogenization would be insignificant for rebuilding stocks depleted through overfishing, at least on time scales of interest to fisheries managers. The calculations may be statistically significant but demographically irrelevant. Additional information on the ecology and life history of the species, specifically direct measurement of dispersal rates if possible, may be necessary to adequately determine the ability of neighboring populations to recover from depletion (Waples 1998).

With increasing fishing pressures worldwide, many species are experiencing dwindling numbers. Genetic techniques can be extremely useful in determining stock structure for marine fish species, a key component of successful management plans. The literature contains a few genetic studies of some rockfish species in the North Pacific, utilizing primarily allozyme electrophoresis, mitochondrial RFLP and direct sequence comparisons, and microsatellite analyses. While each of these analyses can provide important information for determining genetic patterns within species, microsatellite analyses hold much promise for delineating genetic differentiation on small geographic scales. Multiple microsatellite loci have now been developed, so we are poised for an explosion of new data for multiple species. With a solid understanding of the advantages and concerns regarding genetic information, fisheries management practices could benefit greatly from this additional information as it becomes available. Ultimately however, the utility of genetic information will rely on the willingness of managers to incorporate such information.

References

Alesandrini, S., and G. Bernardi. 1999. Ancient species flocks and recent speciation events: What can rockfish teach us about cichlids (and vice versa)? J. Mol. Evol. 49:814–818.

Asahida, T., A. K. Gray, and A. J. Gharrett. 2004. Use of microsatellite locus flanking regions for phylogenetic analysis? A preliminary study of *Sebastes* subgenera. Environ. Biol. Fishes 69:461–470.

Bernardi, G., L. Findley, and A. Rocha-Olivares. 2003. Vicariance and dispersal across Baja California in disjunct marine fish populations. Evolution 57:1599–1609.

Buonaccorsi, V. P., C. A. Kimbrell, E. A. Lynn, and R. D. Vetter. 2002. Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. Can. J. Fish. Aquat. Sci. 59:1374–1384.

Buonaccorsi, V. P., C. A. Kimbrell, E. A. Lynn, and R. D. Vetter. 2005. Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. Conserv. Genet. 6:697–713.

Buonaccorsi, V. P., M. Westerman, J. Stannard, C. Kimbrell, E. Lynn, and R. D. Vetter. 2004. Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. Mar. Biol. 145:779–788.

Burr, J. C. 1999. Microsatellite analysis of population structure of quillback rockfish (*Sebastes maliger*) from Puget Sound to Alaska. Thesis. Univ. Puget Sound, Tacoma, WA.

Cope, J. M. 2004. Population genetics and phylogeography of the blue rockfish (*Sebastes mystinus*) from Washington to California. Can. J. Fish. Aquat. Sci. 61:332–342.

Gharrett, A. J., A. K. Gray, and J. Heifetz. 2001. Identification of rockfish (*Sebastes* spp.) by restriction site analysis of the mitochondrial ND-3/ND-4 and 12S/16S rRNA gene regions. Fish. Bull. 99:49–62.

Gharrett, A. J., S. Keller, R. G. Gustafson, P. Johnson, J. L. Nielsen, J. E. Seeb, L. W. Seeb, W. W. Smoker, G. H. Thorgaard, and R. L. Wilmot. 2004. Introduction to genetics of subpolar fish and invertebrates. Environ. Biol. Fishes 69:1–5.

Gharrett, A. J., A. P. Matala, E. L. Peterson, A. K. Gray, Z. Li, and J. Heifetz. 2005. Two genetically distinct forms of rougheye rockfish are different species. Trans. Am. Fish. Soc. 134:242–260.

Gharrett, A., J., C. W. Mecklenburg, L. W. Seeb, Z. Li, A. P. Matala, A. K. Gray, and J. Heifetz. 2006. Do genetically distinct rougheye rockfish sibling species differ phenotypically? Trans. Am. Fish. Soc. 135:792–800.

Gomez-Uchida, D., and M. A. Banks. 2005. Microsatellite analyses of spatial genetic structure in darkblotched rockfish (*Sebastes crameri*): Is pooling samples safe? Can. J. Fish. Aquat. Sci. 62:1874–1886.

Gray, A. K., A. W. Kendall, Jr., B. L. Wing, M. G. Carls, J. Heifetz, Z. Li, and A. J. Gharrett. 2006. Identification and first documentation of larval rockfishes in southeast Alaskan waters was possible using mitochondrial markers but not pigmentation patterns. Trans. Am. Fish. Soc. 135:1–11.

Hauser, L., G. J. Adcock, P. J. Smith, J. H. Bernal Ramirez, and G. R. Carvalho. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proc. Natl. Acad. Sci. 99:11742–11747.

Hawkins, S. L., J. Heifetz, C. M. Kondzela, J. E. Pohl, R. L. Wilmot, O. N. Katugin, and V. N. Tuponogov. 2005. Genetic variation of rougheye rockfish (*Sebastes aleutianus*) and shortraker rockfish (*S. borealis*) inferred from allozymes. Fish. Bull. 103:524–535.

Hawkins, S., J. Heifetz, J. Pohl, and R. Wilmot. 1997. Genetic population structure of rougheye rockfish (*Sebastes aleutianus*) inferred from allozyme variation. Alaska Fish. Sci. Quart. Rep. July-August-September 1997:1–10.

Higuchi, M., and K. Kato. 2002. Sequence variability in the mitochondrial DNA control region of five *Sebastes* species. Fish. Sci. 68:643–650.

Johns, G. C., and J. C. Avise. 1998. Tests for ancient species flocks based on molecular phylogenetic appraisals of *Sebastes* rockfishes and other marine fishes. Evolution 52:1135–1146.

Kai, Y., K. Nakayama, and T. Nakabo. 2002. Genetic differences among three colour morphotypes of the black rockfish, *Sebastes inermis*, inferred from mtDNA and AFLP analyses. Mol. Ecol. 11:2591–2598.

LeClair, L. L., and R. M. Buckley. 2001. Electrophoretic identification of juvenile rockfish (genus *Sebastes*) recruiting to drifting algae and seagrass habitats off the Washington coast. Northwest Sci. 75:53–60.

Li, Z., A. K. Gray, M. S. Love, T. Asahida, and A. J. Gharrett. 2006a. Phylogeny of members of the rockfish (*Sebastes)* subgenus *Pteropodus* and their relatives. Can. J. Zool. 84:527–536.

Li, Z., A. K. Gray, M. S. Love, A. Goto, T. Asahida, and A. J. Gharrett. 2006b. A key to selected rockfishes (*Sebastes* spp.) based on mitochondrial DNA restriction fragment analysis. Fish. Bull. 104:182–196.

Love, M. S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the northeast Pacific. University of California Press, Berkeley.

Matala, A. P., A. K. Gray, A. J. Gharrett, and M. S. Love. 2004a. Microsatellite variation indicates population genetic structure of bocaccio. N. Am. J. Fish. Manag. 24:1189–1202.

Matala, A. P., A. K. Gray, J. Heifetz, and A. J. Gharrett. 2004b. Population structure of Alaskan shortraker rockfish *Sebastes borealis* inferred from microsatellite variation. Environ. Biol. Fishes 69:201–210.

McGauley, K., and T. J. Mulligan. 1995. Polymerase chain reaction-restriction fragment length polymorphism analysis of mitochondrial rRNA genes from yellowtail rockfish. J. Fish Biol. 47:744–747.

Narum, S. R., V. P. Buonaccorsi, C. A. Kimbrell, and R. D. Vetter. 2004. Genetic divergence between gopher rockfish (*Sebastes carnatus*) and black and yellow rockfish (*Sebastes chrysomelas*). Copeia 2004:926–931.

NMFS (National Marine Fisheries Service). 2000. West Coast groundfish research plan. Northwest Fisheries Science Center, Seattle. Online at http://www.nwfsc.noaa.gov/publications/researchplans/gfsp2002.pdf [accessed 29 November 2006].

Rocha-Olivares, A. 1998. Multiplex haplotype-specific PCR: A new approach for species identification of the early life stages of rockfishes of the species-rich genus *Sebastes Cuvier*. J. Exp. Mar. Biol. Ecol. 231:279–290.

Rocha-Olivares, A., C. A. Kimbrell, B. J. Eitner, and R. D. Vetter. 1999a. 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*. Mol. Phylogenet. Evol. 11:426–440.

Rocha-Olivares, A., R. H. Rosenblatt, and R. D. Vetter. 1999b. Molecular evolution, systematics, and zoogeography of the rockfish subgenus *Sebastomus* (*Sebastes*, Scorpaenidae) based on mitochondrial cytochrome *b* and control region sequences. Mol. Phylogenet. Evol. 11:441–458.

Rocha-Olivares, A., R. A. Leal-Navarro, C. Kimbrell, E. A. Lynn, and R. D. Vetter. 2003. Microsatellite variation in the Mexican rockfish *Sebastes macdonaldi*. Sci. Mar. 67:451–460.

Rocha-Olivares, A., H. G. Moser, and J. Stannard. 2000. Molecular identification and description of pelagic young of the rockfishes *Sebastes constellatus* and *Sebastes ensifer*. Fish. Bull. 98:353–363.

Rocha-Olivares, A., and R. D. Vetter. 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Can. J. Fish. Aquat. Sci. 56:803–813.

Seeb, L. W. 1986. Biochemical systematics and evolution of the scorpaenid genus *Sebastes*. Doctoral thesis. Univ. Washington, Seattle.

Seeb, L. W. 1998. Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. J. Hered. 89:393–403.

Seeb, L. W., and D. R. Gunderson. 1988. Genetic variation and population structure of Pacific ocean perch (*Sebastes alutus*). Can. J. Fish. Aquat. Sci. 45:78–88.

Seeb, L. W., and A. W. Kendall, Jr. 1991. Allozyme polymorphisms permit the identification of larval and juvenile rockfishes of the genus *Sebastes*. Environ. Biol. Fishes 30:191–201.

Sekino, M., N. Takagi, M. Hara, and H. Takahashi. 2001. Analysis of microsatellite DNA polymorphisms in rockfish *Sebastes thompsoni* and application to population genetic studies. Mar. Biotechnol. 3:45–52.

Siebenaller, J. F. 1978. Genetic variability in deep-sea fishes of the genus *Sebastolobus* (Scorpaenidae). *In* B. Battaglia and J. Beardmore (eds.), Marine organisms. Plenum, New York.

Stepien, C. A., A. K. Dillon, and A. K. Patterson. 2000. Population genetics, phylogeography, and systematics of the thornyhead rockfishes (*Sebastolobus*) along the deep continental slopes of the North Pacific Ocean. Can. J. Fish. Aquat. Sci. 57:1701–1717.

Stokes, K., and R. Law. 2000. Fishing as an evolutionary force. Mar. Ecol. Prog. Ser. 208:307–309.

Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. J. Hered. 89:438–450.

Wishard, L. N., F. M. Utter, and D. R. Gunderson. 1980. Stock separation of five rockfish species using naturally occurring biochemical genetic markers. Mar. Fish. Rev. 42:64–73.

Withler, R. E., T. D. Beacham, A. D. Schulze, L. J. Richards, and K. M. Miller. 2001. Co-existing populations of Pacific ocean perch, *Sebastes alutus*, in Queen Charlotte Sound, British Columbia. Mar. Biol. 139:1–12.

Tribal Fisheries: Role of Stock Structure in Regional Management

Rob Jones
Northwest Indian Fisheries Commission
Fishery Management and Planning Services
6730 Martin Way East
Olympia, Washington 98516
rjones@nwifc.org

Treaty fisheries are conducted in fixed areas known as usual and accustomed stations and grounds (U&As). The tribes have always managed each area-based fishery separately—concentrating their fishing in areas of highest abundance while ensuring that abundance remains relatively stable over time. The four coastal tribes (Makah, Quileute, Hoh, and Quinault) with treaty rights to groundfish conduct four major groundfish fisheries, all of which currently face constraints due to interactions with overfished rockfish (*Sebastes*) species. The primary fisheries are midwater trawling for Pacific whiting (hake [*Merluccius productus*]) and yellowtail rockfish (*S. flavidus*) plus setline fisheries for Pacific halibut (*Hippoglossus stenolepis* Schmidt) and sablefish (*Anoplopoma fimbria*). Midwater trawl fisheries are primarily constrained by bycatch of canary rockfish (*S. pinniger*) and widow rockfish (*S. entomelas*), while setline fisheries are constrained due to interactions with both species.

Presently there is little abundance data for most groundfish species in the Pacific Fishery Management Council's Groundfish Fishery Management Plan. Even less is known about the structure of most rockfish stocks. Thus the current management regime treats virtually all groundfish species as coast-wide stocks. Seven rockfish species are currently classified as overfished and are subject to rebuilding plans. These rebuilding plans are projected to take decades, in some cases close to 100 years. Other rockfishes could be designated as overfished as data become available. The restrictions faced under rebuilding affect most of the groundfish fisheries and even some of the nongroundfish fisheries along the coast.

Some relief has been realized by applying different management strategies to distinct stocks within a population. When a species shows biological differences within its overall range, it is beneficial to manage those different segments of the population separately in order to prevent localized depletion. This strategy also prevents restrictions in areas that are far removed from the regions where overfishing actually occurs or where fishing strategies are drastically different. Delineating and managing to stock differences provides local opportunities rather than coast-wide restrictions.

Collection of genetic, stable isotope, and trace element samples from fishery landings coupled with location data from logbooks could aid in answering important questions about stock structure. The Makah Tribe's proposed rockfish enhancement project also would help

delineate stock structure for some species. Understanding stock structure, competitive interactions within and between species, and mechanisms of genetic drift or isolation are critical first steps to effectively accelerating rebuilding while avoiding the mistakes of some salmon hatcheries in the past. While this approach is being proposed for a few of the most constraining overfished species, the resultant knowledge would have broad application in transitioning to more effective regional and ecosystem-based management.

As management evolves from single-species management to a management strategy that accounts for ecosystem interactions and essential fish habitat (EFH), it is of paramount importance that the necessary data are collected and incorporated into the management process. Legal mandates must be met whether or not the information to properly fulfill them is available. This evolution of management could exacerbate the current groundfish crisis if ecosystems are defined arbitrarily or EFH designation is not properly tied to production (i.e., regional depletion could worsen where stock structure is poorly understood or marine protected areas inappropriately sited).

Tribal fisheries have been managed regionally within the U&As for eons. Applying this approach all along the coast in concert with an ecosystem-based management regime, if properly implemented, could reduce the instances of regional stock depletions and allow for faster rebuilding times for overfished rockfish species. Comanagement of rockfish fisheries will benefit from increasing understanding of ecosystem dynamics, such as stock structure and the role of habitat in fish production through collaboration of federal, state, and tribal scientists. Better understanding of rockfish stock structure could provide all West Coast groundfish fisheries the benefits of opportunities afforded by regional management rather than constraints resulting from coast-wide restrictions.

Movement and Activity Patterns of Rockfish

Christopher G. Lowe
California State University, Long Beach
Department of Biological Sciences
1250 Bellflower Boulevard
Long Beach, California 90840
clowe@csulb.edu

The understanding of the movement and activity patterns of rockfish (*Sebastes* spp.) has increased considerably over the past 50 years due to advances in technology (Lowe and Bray 2006). Although technological advances have led to more effective harvest of many species, the lack of sufficient knowledge of fish movements has hampered management and conservation measures. Nevertheless quantifying movement patterns of fishes poses serious challenges because movements vary over spatial and temporal scales, and among and within species, populations, and even individuals.

Methods for Quantifying Movements of Rockfish

A variety of tools have been used to quantify and evaluate movements of rockfish over these various scales, but no one tool is adequate for resolving movements over various scales. Fishing catch data have been used to determine stock movement or distribution, but due to biases in fishing method or the fishery, this technique cannot adequately resolve movements, only distribution of a population or stock. Tag and recapture methods often rely on fishery participation and are useful in determining movement patterns of a population, yet these techniques cannot resolve fine-scale movements and their success is fishing dependent. In situ monitoring via diver, remotely operated vehicle, or submersible observations has evolved as a powerful tool in quantifying movements of small, reef-associated species and factors that may influence movements of fishes (i.e., habitat composition, social influences, diel changes); however, this technique cannot provide longer-term data sets because of the short duration over which observations can be made. Some of the greatest advancements in studying fish movements have been due to the advent of remote-sensing technologies (e.g., sonar, acoustic telemetry tracking, acoustic telemetry monitoring, and satellite telemetry). Remote-sensing methods are good for resolving movements of tagged individuals over varying spatial and temporal scales; however, these methods are expensive and often limited in sample size and the size of fish that can be instrumented with tags (Matthews et al. 1990, Lowe et al. 2003). Genetics and other types of "natural" tags (i.e., parasites) are powerful tools in resolving population-scale movements, but are also limited by sampling and collecting tissues (Lowe and Bray 2006) (Table 1).

Table 1. Advantages and disadvantages of methods used to quantify the spatial and temporal variability of movements of marine fish.

Method	Spatial resolution	Temporal resolution	Advantages	Disadvantages
Fishing/ catch data	Ocean wide	Long term (years, decades)	Population level, dispersal potential	Fishery dependent, sampling biased
Standard tag and recapture	Ocean wide	Short term (days) to long term (years)	Population level, inexpensive, can be used to gather growth data	Spatial and temporal poor resolution, fishery dependent, sampling biased
In situ observations	Fine scale (≈10–1,000 m^2)	Short term (<12 hours)	Individual level, direct observation of movements	Influence behaviors, too short in duration
Sonar	Fine scale (≈1–100s m^2)	Short term (<few hours)	Population or individual, can observe entire school and species composition	Expensive, time consuming, limited in duration, may influence schooling behavior
Acoustic telemetry tracking	Fine scale (≈10–10,000 m^2)	Short term (hours–days)	Individual level, can quantify habitat use, home range size, activity patterns	Expensive, labor intensive, limited in the size of fish that can be tagged
Acoustic telemetry monitoring	Larger scale (≈100 m^2–km^2)	Long term (<6 years)	Individual level, can observe conspecific interactions, site fidelity	Expensive, limited in size of fish that can be tagged
Satellite telemetry	Larger scale (100 km–ocean wide)	Long term (<1 year)	Individual level, can also record depth and temperature; best for wide-ranging, surface-oriented species	Expensive, limited in size of fish that can be tagged, tag retention duration, poor spatial resolution (>60 nmi)
Natural tags (genetics, parasites)	Large scale (100 km–ocean wide)	Long term (>1 year)	Population level, good for identifying populations and degree of population movement	Fishery dependent, expensive, usually lacking in spatial resolution

Factors Influencing Movements of Rockfish

Aside from larval dispersal and seasonal migrations, most juvenile and adult rockfish are thought to use home ranges, areas used on a daily basis (Love et al. 2002). Surprisingly very few studies actually have quantified home range behavior in rockfish (Larson 1980a, 1980b, 1980c, Matthews 1990, Matthews et al. 1990, Starr et al. 2000, 2002). In general it is thought that fish most closely associated with complex habitats tend to move less than those more loosely associated with habitat. Although resource availability largely influences this relationship, there also appears to be a large amount of variability in behavior between species and individuals

(Figure 1). Nevertheless the general differences in movements are likely associated with energetic trade-offs between finding food and mates, and avoiding predation or unsuitable abiotic conditions (Lowe and Bray 2006).

Depending on life history, it is also thought that many fish exhibit some sort of ontogenetic shift in home range or activity space size (Love et al. 2002, Lowe and Bray 2006). Early life history for most rockfish utilizes planktonic dispersal, after which larvae settle and recruit to juvenile or adult habitat. Two differing models of ontogenetic space use may be proposed based on whether the species exhibits territorial behavior as an adult, which has been demonstrated for some reef-associated rockfishes (Larson 1980b, 1980c). For nonterritorial species, newly settled individuals may generally have small home ranges based on their lower energy requirements and food availability, whereas larger individuals may increase their home ranges or activity spaces as they get larger to accommodate increased energy demand and reduced prey availability (Mace et al. 1983). The opposite trend is likely seen from territorial species, where newly settled individuals are frequently displaced from adult home ranges and territories. As a result, juveniles must continuously move to avoid conflicts or predation. As individuals get larger, they are better able to hold and defend territories, thereby reducing their home range or activity space requirements (Lowe and Bray 2006). In addition many of the same trade-offs have likely influenced the evolution of activity patterns seen in rockfish.

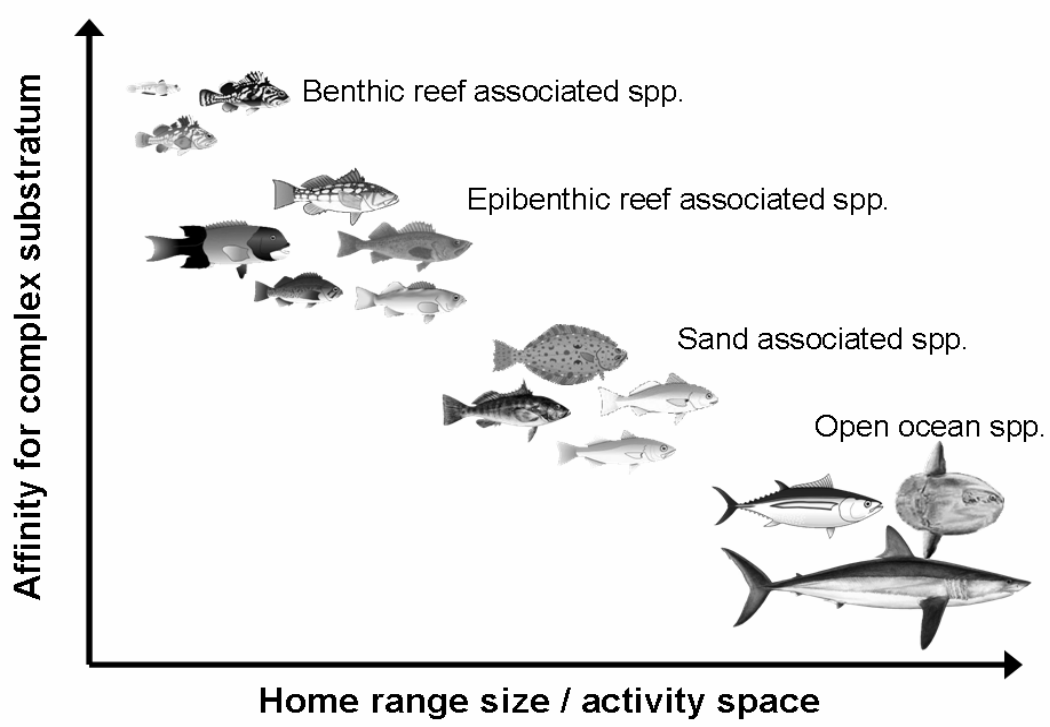

Figure 1. Theoretical model of home range or activity space size relative to affinity to complex substratum for various assemblages of fish. High complexity substratum would include habitat offering high rugosity and relief (e.g., rock reef), whereas low complexity substratum would have low rugosity and relief (e.g., sand and mud).

Conclusions

Development of new technology and techniques has significantly furthered knowledge of rockfish movement and activity, and it is likely these advances will continue. Because of the economic importance of many rockfish species, the need to understand fish behavior has increased, particularly for its application in resource and fisheries management. While we have a better understanding of the proximate mechanisms that influence movement and activity pattern in fish, we still do not thoroughly understand the ultimate mechanisms behind these behaviors. In addition, while research has been done to examine the movement patterns of larger fish, still relatively little is known about movements and space use by small fish. Further investigation of ontogenetic shifts in movement patterns, home range behavior, and the factors that influence shifts in home ranges of rockfish will provide a better understanding of stock structure. This information could be extremely valuable to resource managers in developing better fisheries management models and when establishing marine protected areas.

References

Larson, R. J. 1980a. Competition, habitat selection, and the bathymetric segregation of two rockfish (*Sebastes*) species. Ecol. Monogr. 50:221–239.

Larson, R. J. 1980b. Influence of territoriality on adult density in two rockfishes of the genus *Sebastes*. Mar. Biol. 58:123–132.

Larson, R. J. 1980c. Territorial behavior of the black-and-yellow rockfish and gopher rockfish (Scorpaenidae, *Sebastes*). Mar. Biol. 58:111–122.

Love, M. S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the northeast Pacific. University of California Press, Berkeley.

Lowe, C. G., and R. N. Bray. 2006. Fish movement and activity patterns. *In* L. G. Allen, D. J. Pondella II, and M. H. Horn (eds.), The ecology of California marine fishes: California and adjacent waters, p. 524–553. University of California Press, Berkeley.

Lowe, C. G., D. T. Topping, D. P. Cartamil, and Y. P. Papastamatiou. 2003. Movement patterns, home range, and habitat utilization of adult kelp bass (*Paralabrax clathratus*) in a temperate no-take marine reserve. Mar. Ecol. Prog. Ser. 256:205–216.

Mace, G. M., P. H. Harvey, and T. H. Clutton-Brock. 1983. Vertebrate home-range size and energetic requirements. *In* I. R. Swingland and P. J. Greenwood (eds.), The ecology of animal movement, p. 32–53. Oxford University Press, New York.

Matthews, K. R. 1990. A telemetric study of the home ranges and homing routes of copper and quillback rockfishes on shallow rocky reefs. Can. J. Zool. 68:2243–2250.

Matthews, K. R., T. P. Quinn, and B. S. Miller. 1990. Use of ultrasonic transmitters to track demersal rockfish movements on shallow rocky reefs. *In* N. C. Parker, R. C. Giorgi, D. B. Heidinger, D. B. Jester, Jr., E. D. Prince, Jr., and G. A. Winans (eds.), Fish-marking techniques, p. 375–379. American Fisheries Society, Bethesda, MD.

Starr, R. M., J. N. Heine, J. M. Felton, and G. M. Cailliet. 2002. Movements of bocaccio (*Sebastes paucispinis*) and greenspotted (*Sebastes chlorostictus*) rockfishes in a Monterey submarine canyon: Implications for the design of marine reserves. Fish. Bull. 100:324–337.

Starr, R. M., J. N. Heine, and K. A. Johnson. 2000. Techniques for tagging and tracking deepwater rockfishes. N. Am. J. Fish. Man. 20:597–609.

Toward Establishing Patterns of Connectivity for Kelp Rockfish: Geographic Structure in Elemental Signatures in Larval Otoliths and a Review of Potential Proxies for Natal Signatures

Jennifer E. Caselle and Robert R. Warner
University of California, Santa Barbara
Department of Ecology, Evolution, and Marine Biology
Santa Barbara, California 93106
caselle@lifesci.ucsb.edu

One of the most important yet least understood aspects of marine populations is the degree to which they are connected via larval dispersal. The pathways of larval transport and the extent to which populations are seeded from local or distant sources have important consequences for population replenishment, colonization of new habitats, and resiliency to harvest. In addition knowledge of the spatial patterns of connectivity can help in the design and placement of marine protected areas.

Knowledge of connectivity among marine populations lags behind that for populations of other animals such as mammals or birds, due mainly to the difficulty of artificially tagging and recapturing small marine larvae. Jones et al. (1999) studied the chemical tagging of coral reef fish egg masses on an island in the Great Barrier Reef. Recruited individuals were later captured, and from the number of returns it was estimated that 15–65% of the total recruitment to the island was from local production. Such tagging studies, while important, are necessarily limited in spatial scale.

We focus on the use of natural tags, specifically trace element signatures in the hard parts (e.g., otoliths and statoliths) of marine organisms. The idea behind using natural tracers in the ocean to track larvae is based on the presumption that the chemical composition of the water is extremely variable, especially near the coast (Bruland 1983, Donat and Bruland 1995). As larvae travel through water masses with differing chemical composition, elements are taken up into the otoliths. Our general approach is to attempt to characterize local signatures over a wide geographic area. We are investigating the spatial scales over which these local signatures vary, as well as their stability through time. If local signatures can be mapped in space and time, the next step is to collect recruits from target areas, analyze the signature in the natal portion of the otolith, and assign them to particular source populations.

Most published work on natal assignment has focused on species that migrate between relatively discrete habitat types such as estuaries, rivers, or enclosed bays and open coastal habitats (Thorrold et al. 1998, Gillanders 2002, Swearer et al. 2003). One would expect detectable geographical variation in otolith elemental composition in these systems due to

substantial gradients in salinity, temperature, and trace element inputs. Because it is possible that gradients in oceanographic conditions along open coasts may often be too subtle to register in a natural tag (but see Campana et al. 1994, Patterson et al. 2001), we focus on open coastal regions of central and southern California, where there are abrupt and distinctive spatial patterns in oceanographic conditions (Harms and Winant 1998).

Otoliths are calcium carbonate (mainly aragonite) structures in the inner ear canal of fish and are used for balance and hearing. Three important features of otoliths allow us to analyze chemical signatures in order to assess residency in different water masses (Campana and Thorrold 2001):

1. The time of deposition can be estimated by counting the daily increments found in many otoliths.
2. Because the aragonitic structure is metabolically inert and not reworked, there is likely to be no resorption or change in the elemental composition of an area of the otolith once it is deposited.
3. The calcium carbonate and trace elements are taken up from the ambient water, so the trace elemental concentration in the otolith should reflect the environmental characteristics of the water (Secor et al. 1995, Campana and Thorrold 2001).

We address two questions. What is the magnitude and scale of spatial variation in source signatures in otoliths? And, are there feasible methods for estimating this variation that do not rely on larval otolith collection?

We briefly review progress made in measuring source signatures directly using prepelagic larval otoliths of the kelp rockfish (*Sebastes atrovirens*) and indirectly using three potential proxies: seawater, resin-based elemental accumulators, and the outermost edges of otoliths of resident kelp rockfish adults.

Collection and analytical chemistry methods are given in detail in Warner et al. (2005).

Results

The most direct approach to assessing a local or source signature is to measure it directly from the otoliths of larvae still resident at the natal location. To do this we focused on species that are ovoviviparous, such as the rockfish, or those that lay benthic egg masses. Rockfish larvae begin to form otoliths prior to parturition, and larvae can be harvested from near-term pregnant females in the field.

Using laser ablation inductively coupled plasma mass spectrometry on kelp rockfish larval otoliths, we found significant spatial variation in multielement natal signatures between regions and sites separated by only tens of kilometers (Warner et al. 2005). For many but not all elements, variation was consistent between the 2 years of the study.

The obvious advantage of using larval otoliths to measure source signatures is that the signature is preserved throughout life and can later be directly measured in the core region of the otolith of older life stages. For the goal of assigning juveniles or adults onto maps of potential

source signatures, this technique provides the most unequivocal and direct measure; however, capturing pregnant female fish or locating egg masses in the field is difficult, labor intensive, and may be impossible in some systems (e.g., for deepwater rockfish). In addition the isolation and preparation of larval otoliths for laser-based inductively coupled plasma mass spectrometry is initially difficult (as protocols are developed) and will remain time consuming throughout any project. Thus we investigated the development of a suitable proxy—one that is more easily sampled than larval otoliths and captures the local trace element environment as recorded in the otolith.

We first investigated trace element incorporation into resin-based elemental accumulators—diffusive gradients in thin films or DGT (Zhang and Davison 2000). DGT consists of an open-pore hydrogel layer backed with a chelex binding agent and is used for the quantitative determination of metals in situ. Because of the nature of the binding agent in DGT as currently designed, not all elements detectable in larval otoliths were extractable from DGT samples. Of the four elements that were detectable in both DGT and larval otoliths, two showed significant regional variation in DGT, but only one (manganese, Mn) had a qualitatively similar spatial pattern as the larval otoliths (Warner et al. 2005).

DGT is an ideal field sampler because it can be placed in known locations and easily collected at predetermined frequencies. Although DGT eliminates the biological sampling problem and integrates elemental chemistry over time like an otolith, as currently designed it does not uptake many of the elements that are incorporated and easily detectable in otoliths. This issue may be overcome by designing a resin-based elemental accumulator with new properties. Analytically DGT must be run using solution-based techniques while larval otoliths can be analyzed using laser ablation. Direct comparison of concentrations will require calibration studies.

We also collected seawater samples at the same times and locations as DGT samples and larval otoliths. Seawater and larval otoliths had five detectable elements in common. Of those five, three varied significantly among regions but only one (Mn) showed a spatial pattern similar to larval otoliths (Warner et al. 2005). Seawater tended to show higher within-year temporal variability than larval otoliths or other proxies, likely because water samples are taken at specific points in time (every 2 weeks in this study). Increasing the frequency of sampling, even approaching continuous sampling, could reduce this variability and may still prove easier than collecting larvae. Analytically seawater is problematic because of extremely low elemental concentrations and the calibration issues that exist when comparing between solution and laser-based techniques also must be addressed for comparisons between seawater and otoliths.

Finally we collected adult kelp rockfish at the same locations as larvae in both years of the study. We analyzed the outermost edges of the adult otoliths and compared them to larval otolith. Concentrations of most trace elements at the edges of otoliths of adults were considerably lower than levels found in larvae. Only three elements were detectable in the edges of adult otoliths and all three also were found in larval otoliths. Of the three elements, two varied significantly among regions but only in one of the two years. In general there was little similarity in spatial patterns between adult otolith edges and larval otoliths (Warner et al. 2005).

For species with limited home ranges as adults, such as many rockfish (Love et al. 2002), the outer edge of the adult otolith could conceivably provide a measure of elemental concentrations similar to larval otoliths forming at the same time and location. This could provide some advantage because adults are often easier to collect than preparturition larvae or egg masses. However, physiological differences between adults and larvae appear to result in differences in elemental uptake, and this limits the utility of using adult data as proxies for larval signatures.

Conclusions

Microchemical composition of fish larvae otoliths collected at their natal sites (before pelagic dispersal) shows significant spatial variation at scales of tens of kilometers along an open coastline (Warner et al. 2005). This result opens the possibility of creating a spatial map of natal signatures, although more information is needed about the spatial and temporal scales of variability in natal signatures.

Drawing a spatial map would be considerably simplified if natal otolith signatures could be predicted from knowledge of local water chemistry or otolith chemistry of adults from the same area. Unfortunately, we found little correspondence between such potential proxies and the natal signatures, indicating that currently available proxy measures are inadequate to characterize natal otolith chemistry.

References

Bruland, K. W. 1983. Trace elements in sea water. *In* J. P. Riley and R. Chester (eds.), Chemical oceanography, vol. 8, p. 157–220. Academic Press, New York.

Campana, S. E., A. J. Fowler, and C. M. Jones. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser-ablation ICPMS. Can. J. Fish. Aquat. Sci. 51:1942–1950.

Campana, S. E., and S. R. Thorrold. 2001. Otoliths, increments, and elements: Keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. 58:30–38.

Donat, J. R., and K. W. Bruland. 1995. Trace elements in the oceans. *In* B. Salbu and E. Steinnes (eds.), Trace elements in natural waters, p. 247–281. CRC Press, Boca Raton, FL.

Gillanders, B. M. 2002. Connectivity between juvenile and adult fish populations: Do adults remain near their recruitment estuaries? Mar. Ecol. Prog. Ser. 240:215–223.

Harms, S., and C. D. Winant. 1998. Characteristic patterns of the circulation in the Santa Barbara Channel. J. Geophys. Res. 103:3041–3065.

Jones, G. P., M. J. Milicich, M. J. Emslie, and C. Lunow. 1999. Self-recruitment in a coral reef fish population. Nature 402:802–804.

Love, M. S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the northeast Pacific. University of California Press, Berkeley.

Patterson, W. F., III, H. J. Cowan, Jr., C. A. Wilson, and N. Julien. 2001. Discriminating between age-0 red snapper, *Lutjanus campechanus*, nursery areas in the northern Gulf of Mexico using otolith microchemistry. Proc. Gulf Caribb. Fish. Inst. 52:74–86.

Secor, D. H., A. Henderson-Arzapalo, and P. M. Piccoli. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol. Ecol. 192:15–34.

Swearer, S. E., G. E. Forrester, M. A. Steele, A. J. Brooks, and D. W. Lea. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. Estuar. Coast. Shelf Sci. 56:1111–1123.

Thorrold, S. R., C. M. Jones, P. K. Swart, and T. E. Targett. 1998. Accurate classification of juvenile weakfish (*Cynoscion regalis*) to estuarine nursery areas based on chemical signatures in otoliths. Mar. Ecol. Prog. Ser. 173:253–265.

Warner, R. R., S. E. Swearer, J. E. Caselle, M. Sheehy, and G. Paradis. 2005. Natal trace-elemental signatures in the otoliths of an open-coast fish. Limnol. Oceanogr. 50:1529–1542.

Zhang, H., and W. Davison. 2000. Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using diffusive gradients in thin films. Anal. Chem. 72:4447–4457.

Stable Isotope Analyses of Otoliths and the Stock Structure of Yelloweye Rockfish

Yongwen Gao
Makah Fisheries Management
PO Box 115
Neah Bay, Washington 98357
gaoy@olypen.com

Farron R. Wallace
Washington Department of Fish and Wildlife
48 Devonshire Road
Montesano, Washington 98563
wallafrw@dfw.wa.gov

Tien-Shui Tsou
Washington Department of Fish and Wildlife
600 Capitol Way North
Olympia, Washington 98501
tsoutt@dfw.wa.gov

A lack of genetic differentiation in marine fish populations is a common problem in stock structure studies (Grant et al. 1987). Possible reasons may include the large population size of marine fish reducing the importance of genetic drift in producing genetic differences between populations, the original low genetic differentiation of marine fish as compared to freshwater fish and salmon, and the migration of marine fish erasing the effects of genetic drift (Ward 2000). As an alternative, stable isotope ratio analysis ($^{18}O/^{16}O$ or $\delta^{18}O$ and $^{13}C/^{12}C$ or $\delta^{13}C$) in otoliths has proven informative and useful for determining discrete stock structure of marine fish along the Pacific West Coast (e.g., Gao et al. 2001, Gao and Beamish 2003, Gao et al. 2004). The basic principle is that otolith layers are deposited in or very close to oxygen isotopic equilibrium with the ambient seawater where a fish lived, and faithfully record the environmental fluctuations throughout the life history of the animal. Thus stable oxygen isotope ratios extracted from otoliths can provide information about habitat alteration, water temperature, migration, and climate regime shifts experienced by an individual fish (e.g., Devereux 1967, Kalish 1991, Gao 2002). Stable carbon isotope ratios in otoliths are generally precipitated in isotopic disequilibrium with the ambient seawater, but can record changes in metabolism of the fish and dietary shifts (e.g., Mulcahy et al. 1979, Fry 1988, Schwarcz et al. 1998). Therefore if fish spawn or live at different localities with different $\delta^{18}O$ and $\delta^{13}C$ values, these isotopic variations would be signatures that are characteristics of different areas or of different spawning stocks.

Yelloweye rockfish (*Sebastes ruberrimus*) are considered overfished (Methot et al. 2002), and this resulted in serious concerns and management restrictions (PFMC 2002). The life history

features of this species include extreme longevity (maximum age of 120 years) and strong association with high relief, hard bottom substrates. To examine their stock structure, 200 sagittal otoliths of yelloweye rockfish were collected off the Washington and Oregon coast (Figure 1) and were analyzed for stable oxygen and carbon isotope ratios. The affinity for hard bottom substrates and age of the fish led us to extract two aragonite powder samples from each otolith during microsampling. One sample was taken from the nucleus of yelloweye rockfish

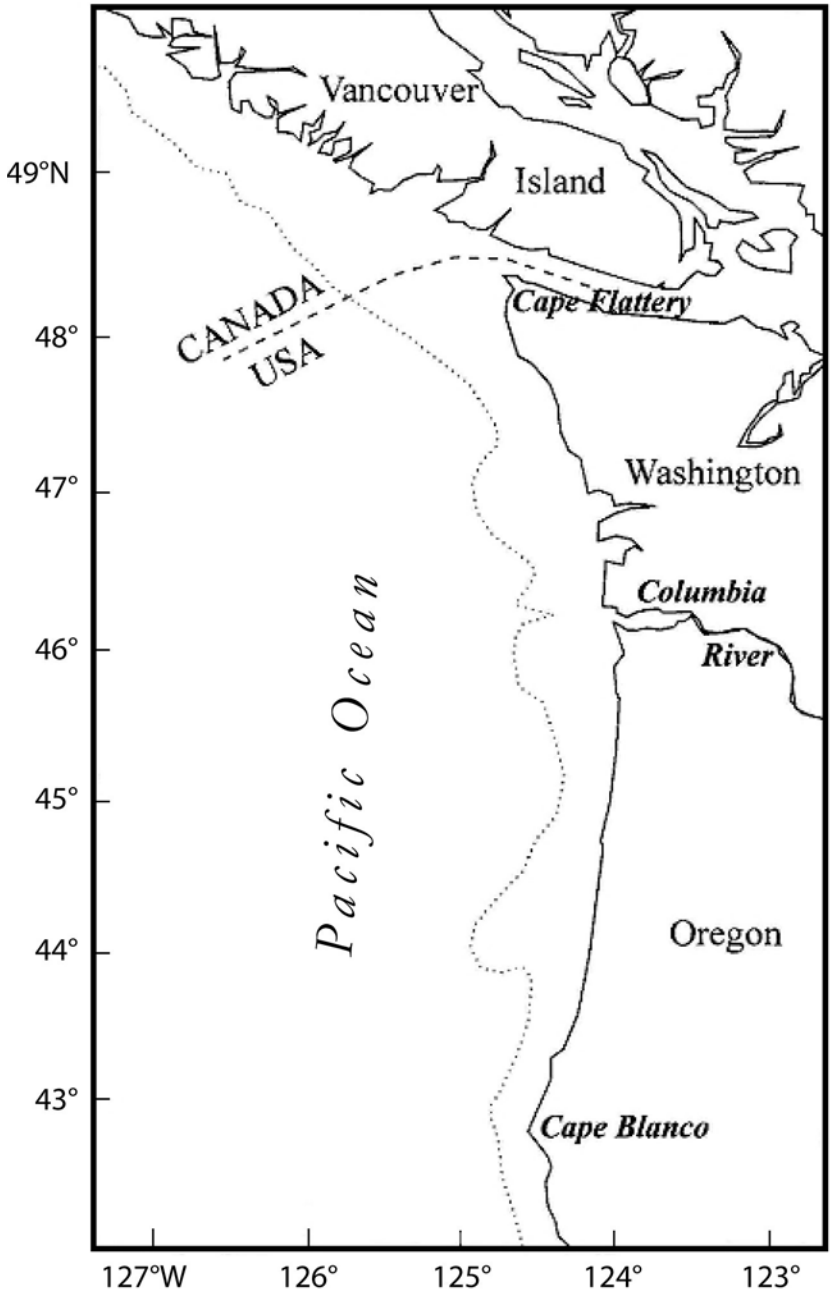

Figure 1. Location map showing the yelloweye rockfish otolith sampling along the Washington and Oregon coast. The dotted line is the depth range of 183 m.

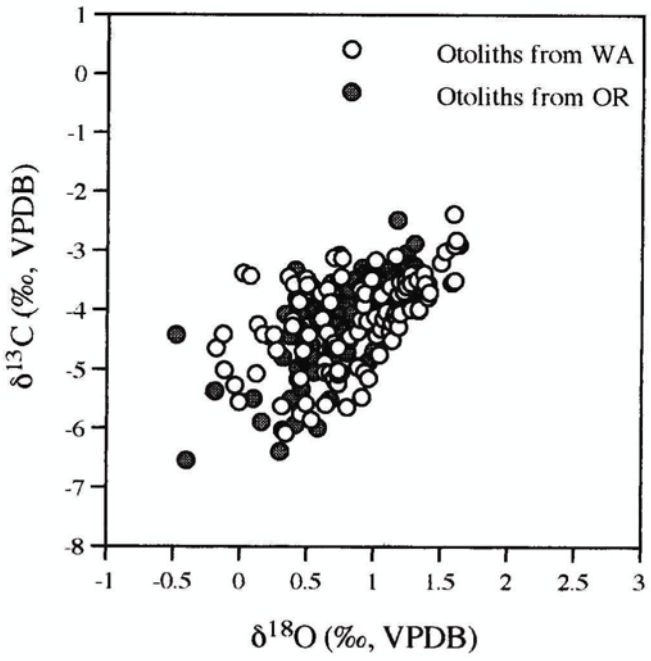

Figure 2. Stable isotopic composition of otolith nuclei of yelloweye rockfish from Washington (open circles) and Oregon (solid circles) coast.

otoliths (the starting time of the life history) and the other was taken from the fifth annual zone (assuming age-5 if validated). Data of otolith nuclei can provide information on the natal sources and spawning stock separation of the fish (Gao et al. 2001), whereas the isotopic signatures from age-1 to age-5 would indicate their behavior over the sampling period.

Among the 200 otoliths analyzed, $\delta^{18}O$ values of yelloweye rockfish ranged from −0.5 to 2.2‰ Vienna Peedee belemnite (VPDB), while $\delta^{13}C$ values of the same otoliths ranged from −6.5 to −0.1‰ VPDB. There were no isotopic differences in otolith nuclei (Figure 2), suggesting there might be a single spawning stock for yelloweye rockfish along the Washington and Oregon coast. However, the distinct isotopic differences between samples from otolith nuclei and the fifth annual zones (Figure 3) indicated that the fish might move to different habitats as they grow. Compared to the fifth annual zones, the isotopic values between Washington and Oregon samples were significantly different (t-test, $p < 0.0001$ for $\delta^{18}O$, and $p < 0.0001$ for $\delta^{13}C$). Interestingly, the difference appeared mainly in $\delta^{13}C$, not in $\delta^{18}O$, suggesting that the food sources or composition of the two areas might be slightly different.

In conclusion yelloweye rockfish might be a single coast-wide population as the Pacific Fishery Management Council currently manages it. From age-1 to age-5, the fish might change their habitat or associated bottom substrates for different levels of food. These isotopic interpretations and conclusions seem in agreement with genetic studies and observations of underwater video for this species,[2] but differ from results of sablefish (*Anoplopoma fimbria*) otoliths coast wide (Gao et al. 2004).

[2] J. Tagart, Washington Dept. of Fish and Wildlife, Olympia, WA. Pers. commun., 30 June 2003.

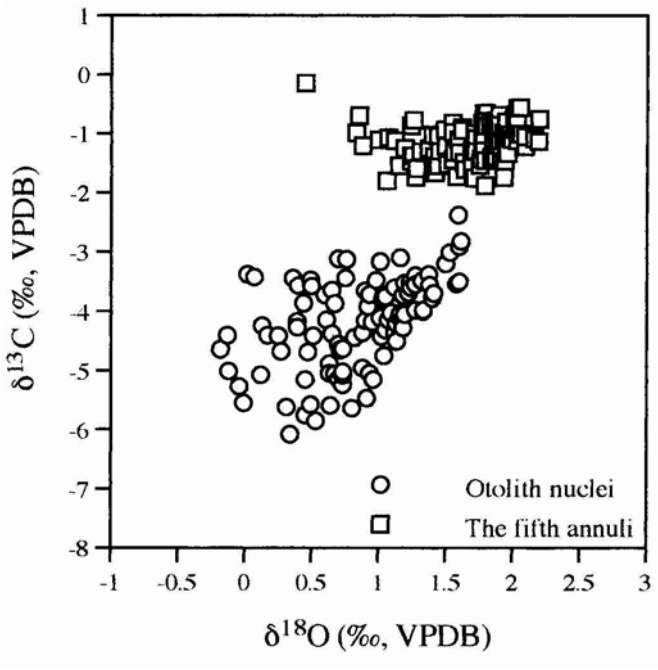

Figure 3. Isotopic comparison between samples from otolith nuclei (open circles) and the fifth annual zones (open squares) from the Washington coast.

References

Devereux, I. 1967. Temperature measurements from oxygen isotope ratios of fish otoliths. Science 155:1684–1685.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol. Oceanogr. 33:1182–1190.

Gao, Y. W. 2002. Regime shift signatures from stable oxygen isotopic records of otoliths of Atlantic cod (*Gadus morhua*). Isotopes Environ. Health Stud. 38:100–113.

Gao, Y. W., and R. J. Beamish. 2003. Stable isotopic composition of otoliths from tagged Pacific halibut, *Hippoglossus stenolepis*. Environ. Biol. Fishes 67:101–109.

Gao, Y. W., S. Joner, and G. Bargmann. 2001. Stable isotopic composition of otoliths in identification of spawning stocks of Pacific herring (*Clupea pallasi*) in Puget Sound. Can. J. Fish. Aquat. Sci. 58:2113–2120.

Gao, Y. W., S. Joner, R. Svec, and K. Weinberg. 2004. Stable isotopic comparison in otoliths of juvenile sablefish (*Anoplopoma fimbria*) from waters off the Washington and Oregon coast. Fish. Res. 68:351–360.

Grant, W. S., C. Zhang, T. Kobayashi, and G. Stahl. 1987. Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Can. J. Fish. Aquat. Sci. 44:490–498.

Kalish, J. M. 1991. Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). Mar. Biol. 110:37–47.

Methot, R., F. Wallace, and K. Piner. 2002. Status of yelloweye rockfish off the U.S. West Coast in 2002. Appendix: Status of the Pacific Coast groundfish fishery through 2002 and recommended acceptable catches for 2003. Stock assessment and fishery evaluation. (Available from Pacific Fishery Management Council, 7700 NE Ambassador Place, Suite 200, Portland, OR 97201.)

Mulcahy, S. A., J. Killingley, C. Phleger, and W. Berger. 1979. Isotopic composition of otoliths from a bentho-pelagic fish, *Coryphaenoides acrolepis*, Macrouridae: Gadiformes. Oceanol. Acta 2:423–427.

PFMC (Pacific Fishery Management Council). 2002. New information on West Coast rockfish may lead to significant cuts in several fisheries. Pacific Fishery Management Council, Portland, OR. Online at http://www.pcouncil.org/groundfish/gfnewinfo.html [accessed 24 October 2006].

Schwarcz, H. P., Y. Gao, S. Campana, D. Browne, M. Knyf, and U. Brand. 1998. Stable carbon isotope variations in otoliths of cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 55:1798–1806.

Ward, R. D. 2000. Genetics in fisheries management. *In* A. M. Sole-Cava, C. A. M. Russo, and J. P. Thorpe (eds.), Marine genetics: Developments in hydrobiology, p. 191–201. Kluwer Academic, Norwell, MA.

Population Structure in Black Rockfish: A Comparison of Otolith Microchemistry and DNA Microsatellites

Jessica A. Miller and Alan L. Shanks
Oregon State University
Hatfield Marine Science Center
Coastal Oregon Marine Experiment Station
2030 SE Marine Science Drive
Newport, Oregon 97365
jessica.miller@oregonstate.edu

Otolith microchemistry and DNA microsatellites are relatively recent technological advances that can provide information on individual movements and population structure in marine fishes. Although both techniques have been touted as being among the most powerful means to distinguish among fish populations in recent years, the two techniques are rarely combined in the same study. Otolith microchemistry has the potential to provide information on individual movements and exchange among groups of fish within a generation, whereas microsatellite data can provide information on population connectivity over longer periods.

The black rockfish (*Sebastes melanops*) is a long-lived, viviparous species with an extended pelagic larval duration (3–5 months) and a broad continental distribution along the northeast Pacific Ocean. Marine species with extended larval durations are assumed to have widespread dispersal and little population structure. We combined otolith microchemistry and DNA microsatellites to estimate the relative movements of juvenile and adult black rockfish and to test for evidence of genetic structure along the Oregon and Washington coast.

We examined otolith elemental composition in juvenile and adult black rockfish collected from locations 120 to 460 km apart, between Grays Harbor, Washington, and Brookings, Oregon. In 2001 and 2002 we examined elements in juvenile otoliths at three regions: core, edge, and midway between the core and edge. Each otolith region represents a distinct life history period, including 1) egg and early larval, 2) pelagic larval, and 3) late larval and early juvenile periods. In 2001 elements in adult otoliths were examined at the core (which represents on average the initial 4 months of life) and edge (which represents the collection year).

Significant differences in metal to calcium ratios (i.e., Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca, and Ba:Ca) were found among collection locations for both juveniles and adults at each otolith region sampled. Discriminant function analyses based on geochemical signatures consistently group greater than 85% (jackknifed = 74%) of juveniles and greater than 75% (jackknifed = 68%) of adults to collection location throughout their life history (Tables 1 and 2). For the juveniles we considered four alternatives to explain the relatively high classification accuracy of individuals to

Table 1. The percentage of juvenile black rockfish accurately classified to collection location based on discriminant function analyses (DFA) using otolith elemental composition. The collection site, year of collection, otolith region (i.e., early larval, larval, and juvenile), percent correctly assigned, and the jackknifed percent correctly assigned (in parentheses) are presented.

	DFA % correct (jackknifed % correct)			
Site	Early larval	Larval	Juvenile	Site average
2001				
Grays Harbor, WA	88 (59)	100 (76)	100 (94)	96 (76)
Cape Arago, OR	89 (81)	89 (75)	93 (89)	90 (82)
Lone Ranch, OR	80 (40)	60 (50)	70 (40)	70 (43)
Average	86 (60)	83 (67)	88 (74)	85 (67)
2002				
Tillamook Bay, OR	91 (87)	100 (91)	95 (86)	95 (88)
Cape Arago, OR	78 (78)	88 (88)	88 (83)	85 (83)
Lone Ranch, OR	83 (78)	74 (70)	83 (70)	80 (73)
Average	84 (81)	87 (83)	88 (80)	87 (81)

Table 2. Site classification success based on both otolith microchemistry and DNA microsatellites. Collection sites and sample size are presented. For otolith microchemistry, the percent correctly assigned, jackknifed percent correctly assigned (in parentheses), and average percent are presented for the otolith core (1st year) and the edge (collection year). For DNA microsatellites, site classification success was based on resampled allele frequencies (N = 1,000). Ten iterations per population were generated and averaged for 5 and 7 loci. The percent correct (±SD) and the total averages are presented.

		Percentage correct (jackknife % correct)			**Percentage correctly classified**	
Site	**Sample size**	1st year	Collection year	Average	5 loci	7 loci
Grays Harbor, WA	22	77 (59)	67 (62)	70 (61)	59.7 ± 2.1	68.9 ± 1.0
Cape Arago, OR	26	81 (73)	50 (46)	66 (60)	72.7 ± 1.4	86.2 ± 0.7
Gold Beach, OR	28	100 (89)	89 (89)	95 (89)	43.5 ± 1.2	68.4 ± 2.1
Brookings, OR	17	71 (65)	76 (53)	74 (59)	79.5 ± 1.5	N/A
Total average		82 (72)	71 (63)	77 (68)	63.1 ± 0.6	74.6 ± 0.6

collection location throughout the early life history based on otolith elemental data: 1) fish stayed together relative to other collection locations (i.e., did not mix), 2) fish did not move appreciable distances alongshore, 3) both 1 and 2, or 4) fish from the same location followed similar dispersal pathways. Parturition dates within each collection site, estimated from otolith increment analysis of juveniles, were spread over a 22–66 day period. Therefore larvae could not have remained together since parturition because they were not released at the same time. Furthermore the predominant alongshore surface current over the continental shelf undergoes a seasonal reversal within the study area, and there is a spring transition when conditions shift

from primarily downwelling to persistent upwelling along the coast. Given that black rockfish females usually release larvae during late winter and juveniles arrive in nearshore areas between May and August, it is difficult to envision a larval dispersal pathway that would persist over a 22–66 day period in this ocean regime. We suggest that a more parsimonious explanation is that the majority of juveniles collected at these geographic locations did not mix, even between locations 120 km apart. Similarly, the adult otolith microchemistry data also indicate limited mixing among individuals collected at these geographic locations.

Limited alongshore movements of larval, juvenile, and adult black rockfish may contribute to genetic divergence along the Oregon and Washington coast. Therefore we completed DNA microsatellite analysis on the same fish used in otolith chemical analysis.[3] We examined seven microsatellites and found significant genetic differences among adults collected 340–460 km apart ($F_{ST} = 0.018 \pm 0.007$) and no evidence for isolation by distance. There was no evidence of population structure in juveniles ($F_{ST} = 0.003 \pm 0.003$) and weak evidence for isolation by distance ($p = 0.05$). WHICHLOCI[4] was used to classify adults to collection location based on allele frequencies. On average 69% of adults were correctly classified to collection location (Table 2).

The relatively high site classification accuracy of juveniles and adults based on otolith microchemistry suggests minimal alongshore movement (i.e., <120 km) for the majority (≈70%) of black rockfish used in this study. Such limited movements may contribute to the genetic structure observed in black rockfish collected 340–460 km apart. This type of comparative approach can provide corroborative information on population structure as well as identify potential isolating mechanisms. Such information is important for population ecology and fisheries management and conservation.

The lack of information on the extent of exchange among populations hinders current management and conservation efforts. There is relatively little quantitative information on how far marine fish larvae disperse and the proportion of individuals that disperse widely. The maintenance of genetic diversity within a population may not be in jeopardy if greater than 10% of the new recruits come from external sources. The recovery of a depleted population, however, depends not only on the size and proximity of adjacent populations but also on the proportion of larvae from those populations that disperse widely enough to provide new recruits to the depleted population. Therefore the rate of recovery of such a population could be substantially slower in a species where typically 80%, compared with 20%, of the successful recruits are generated internally. Similarly, such principles apply in the design of marine reserves. Successful reserve design requires knowledge of the mean dispersal distance as well as the proportion of successful recruits generated within a reserve. Much of the debate surrounding marine reserve design (i.e., are single, larger reserves more successful than networks of smaller reserves) will remain largely theoretical until more specific information on larval dispersal is generated.

[3] This work was completed with Michael Banks of the Marine Fisheries Genetics Laboratory at Oregon State University's Hatfield Marine Science Center in Newport.

[4] WHICHLOCI is available online at http://marineresearch.oregonstate.edu/genetics/whichloci.htm [accessed 16 November 2006].

Fish and Chips: Genetic Identification of Rockfish Larvae and Juveniles

John R. Hyde
Scripps Institution of Oceanography
8604 La Jolla Shores Drive
La Jolla, California 92037
john.hyde@noaa.gov

Ichthyoplankton sampling can be an informative tool for understanding temporal and spatial dynamics of spawning events. These early life stages are useful as they can be utilized as measures of spawning biomass (Ralston et al. 2003) and reproductive output. Rockfish (*Sebastes* spp.) along the West Coast are an important commercial and recreational fishery, with several species currently identified as overfished. Of the approximately 60 species that are known to occur within the California Cooperative Oceanic Fisheries Investigations (CalCOFI) study area, only 6 are currently identified at the larval stage. This is due primarily to the interspecific overlap of meristic and morphologic characters and the intraspecific plasticity of these characters. For this reason the majority of rockfish larvae are lumped together as *Sebastes* species in the CalCOFI data series. This grouping represents the fourth most abundant fish group in this data set (Moser et al. 2001). Clearly current identification techniques are inadequate and perhaps through the use of genetic techniques we can resolve this difficulty.

The use of genetics for identification purposes requires a full understanding of interspecific divergence and intraspecific DNA variation. To this end it is necessary to have reference data for every species that occurs within and adjacent to the study area. Also it is necessary to have data from multiple individuals of each of the species to understand the intraspecific variation. It has been difficult to obtain DNA sequence data for several of the rockfish species because they are rarely found in fisheries and may be represented by only a few type specimens. For these species I have developed a protocol to obtain DNA from formalin preserved museum specimens. This protocol has allowed me to complete a DNA sequence database for all of the currently identified species in the eastern Pacific Ocean (Figure 1). Interspecific genetic divergence for these species ranges from 0.1% to 8.7%. Sequencing of the cytochrome *b* gene from multiple individuals of each of the species has allowed me to measure intraspecific variation. For eastern Pacific Ocean species the variation found ranges from 0 to 4.2% but is predominantly below 1%. Higher levels of intraspecific genetic variation can be viewed in several ways. First, genetically isolated populations can accumulate added variation by genetic drift, higher than normal variation at a gene being indicative of species with limited dispersal potential, and multiple genetically distinct populations. Second, high levels of variation can be expected when two species are mistakenly analyzed as one, a possibility when cryptic species exist but are unable to be separated by traditional meristic criteria. In my analysis of intraspecific variation I have come across two cases where an argument for the existence of cryptic species can be made.

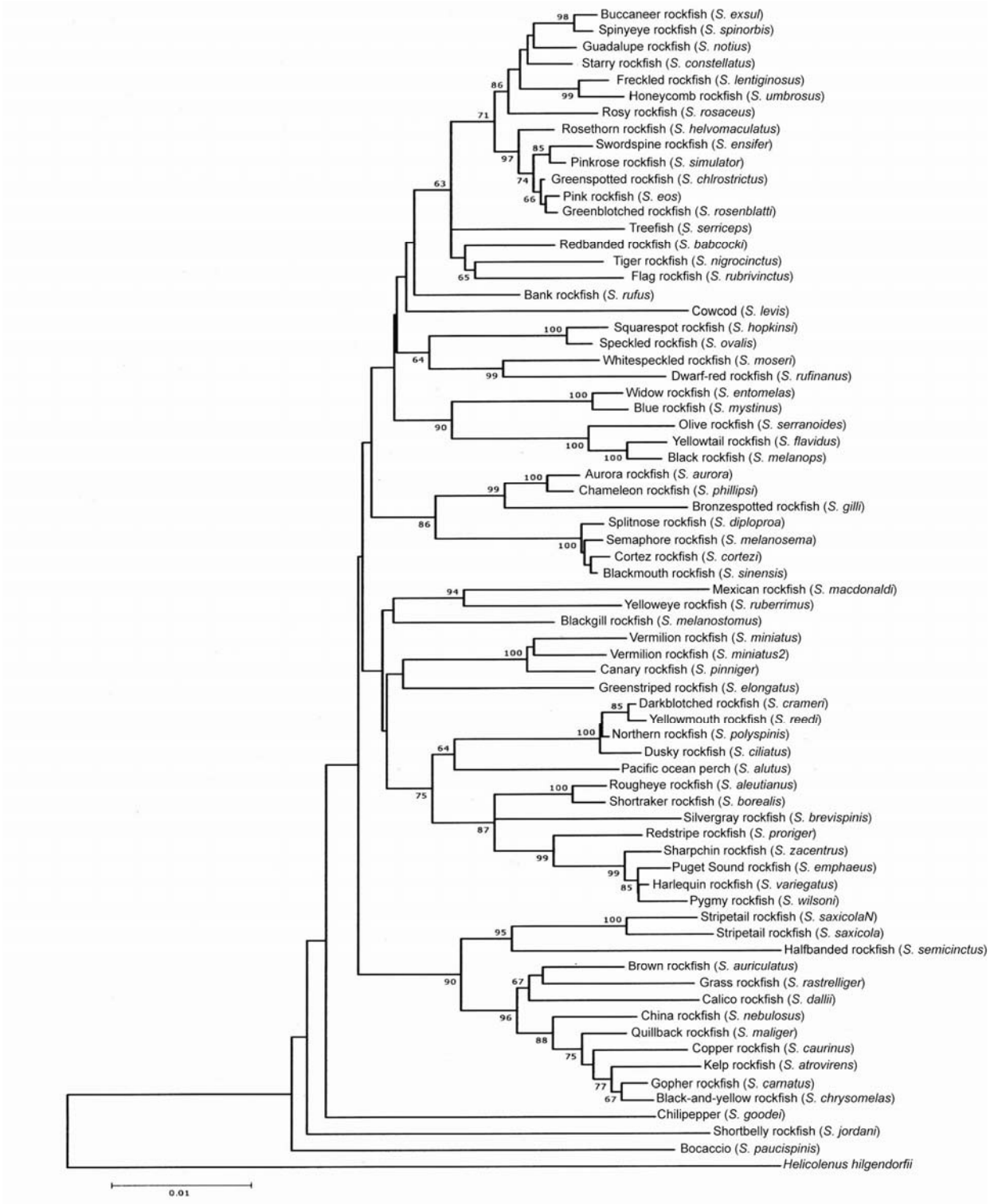

Figure 1. Minimum evolution phylogenetic tree for mitochondrial cytochrome *b* and 16S genes from rockfish found in the eastern Pacific Ocean generated using MEGA2 (v2.1, Kumar 2001), Kimura 2-parameter model, and 1,000 bootstrap replicates. Branch nodes with bootstrap support greater than 60% are labeled. *Helicolenus hilgendorfii* (GenBank accession number NC_003195) is used as the outgroup to root the tree.

The stripetail rockfish (*S. saxicola*), a small, fishery insignificant species, shows a fixed 1.7% divergence between sequence clades (cytochrome *b* data, Figure 1). Current sample collections seem to support a north-south barrier coincident with the northern Channel Islands in the Southern California Bight (SCB), close to the Oregonian-Californian biogeographic faunal break at Point Conception (Briggs 1974). Using microsatellite markers to test for reproductive isolation between these two mitochondrial clades, I found highly significant differences at all five loci examined (genic differentiation, Fisher's exact test as implemented in Genepop v3.4 [Raymond and Rousset no date]: locus SR7-2.2 [$p = 0.0000$], SR7-7.3 [$p = 0.0000$], SR7-25.4 [$p = 0.0000$], SR11-103.6 [$p = 0.0001$], SR16-5.8 [$p = 0.0025$] Chi2 = ∞, df = 10, highly significant). Significance was maintained between samples from both clades taken in a zone of co-occurrence in the Santa Barbara Channel, however, further sampling in this region is necessitated due to small sample size ($n = 17$). Maintenance of genetic heterogeneity within an area of sympatry would suggest that these two mitochondrial clades should be viewed as separate species.

The vermilion rockfish (*S. miniatus*) shows a split similar to that of the stripetail rockfish with two clades separated by a 1.5% fixed genetic divergence (cytochrome *b* data, Figure 1). It is of particular interest that both of these clades are only 1.2% different from the canary rockfish (*S. pinniger*). They are more different from each other than either is from the recognized sister species. Unlike the stripetail rockfish, the vermilion rockfish has no clear geographic separation yet between the clades. This could be an artifact of inadequate sample size both north and south of the SCB. Members of both clades are found in the SCB in nearshore kelp forests and offshore submarine ridges. As done with stripetail rockfish, microsatellites were used to examine reproductive isolation between these two mitochondrial clades. Again significant differences were found at all loci examined (genic differentiation, Fisher's exact test as implemented in Genepop v3.4 [Raymond and Rousset no date]: locus SR7-2.2 [$p = 0.0000$], SR7-7.3 [$p = 0.0000$], SR7-25.4 [$p = 0.0000$], SR16-5.8 [$p = 0.0000$], SR15-8.9 [$p = 0.0000$] Chi2 = ∞, df = 10, highly significant). Overall F_{ST} (Weir and Cockerham 1984) and R_{ST} (Rousset 1996) values are 0.0544 and 0.2384 respectively when comparing the two mitochondrial clades. A comparison with canary rockfish using the same microsatellites indicates similar genetic distance between either vermilion clade or canary rockfish (F_{ST} of 0.0673 and 0.0331, R_{ST} of 0.4614 and 0.3526). These results, plus the maintenance of genetic heterogeneity in sympatry, suggest that these clades should be viewed as distinct species despite the lack of traditional meristic and morphologic characters to separate them. Clearly this can cause difficulty in management of fishery important species such as the vermilion rockfish. Since both of the cryptic species mentioned are found primarily within the SCB, this region should be viewed and managed separately from the rest of the coast due to its complex and seasonably variable oceanography. Also the majority of rockfish species found in the eastern Pacific Ocean are found here, suggesting a center of speciation most likely caused to some degree by historic variability in oceanographic conditions and sea level.

With an adequate understanding of the genetic variation within and between species it becomes possible to assign species identification to larvae based on DNA sequence data alone. Traditionally this would involve sequencing a large portion of DNA and subsequent phylogenetic analysis of this sequence with a set of reference data. While robust, this method is time consuming and expensive. Though a useful tool, DNA sequencing is not applicable for the throughput that would be needed to analyze the material usually collected from a typical

ichthyoplankton survey. For this, autapomorphic genetic characters—nucleotide sites that are unique to single species—can be exploited to effectively minimize sequence analysis from hundreds of bases to one or two. Careful analysis of the reference data set of DNA sequences allows species-specific probes to be designed for a variety of assays. Ideally probes are unique for single species, but in some cases this is not possible and several probes may be used in concert with identity inferred through the use of a dichotomous key. Once species-specific probes have been designed, the format of the identification assay can take several forms depending on the number of taxa to be examined and budgetary constraints.

The first assay is the simplest and cheapest but can identify the least number of taxa. Through use of a universal forward primer in conjunction with a set of species-specific probes as reverse primers, a polymerase chain reaction (PCR) is performed to amplify a portion of DNA using the universal primer and the matching species-specific primer (Rocha-Olivares 1998, Hyde et al. 2005). Because only a single fragment is generated, the assay can be designed to produce a different size fragment for each taxon. The unique size of the resulting fragment is then diagnostic for the identity of the unknown. Amplicon size can be easily determined by electrophoresis on an agarose gel and subsequent comparison to a set of standards. Because size resolution is limited, a finite number of taxa can be assayed in this manner. In my experience, as many as 8–10 taxa can be reliably included in an assay of this type. Figure 2 depicts such an assay designed to identify members of the *Sebastes* subgenus *Pteropodus*. Assays of this manner are rapid (identification in 3 hours) and relatively inexpensive (less than $0.50 per sample). Following this design, an assay was designed for shipboard identification of billfish (family

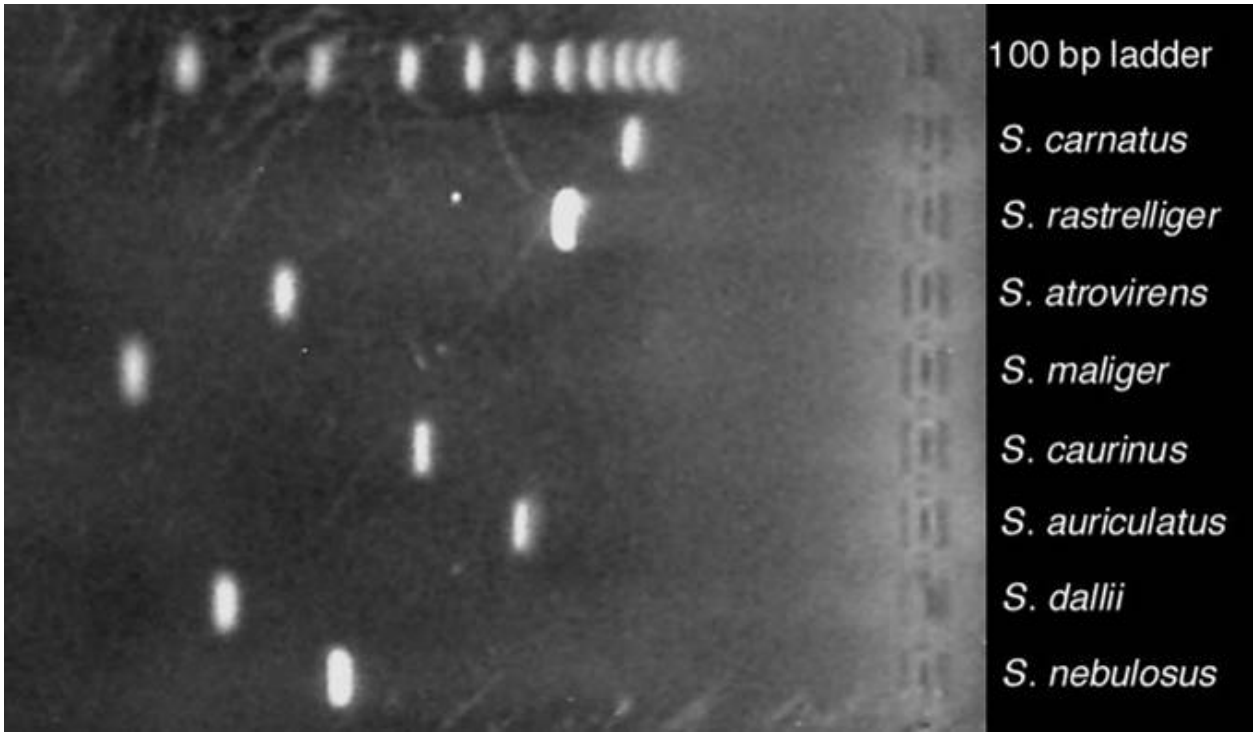

Figure 2. Agarose minigel depicting the results of a species-specific multiplex PCR developed for the *Pteropodus* subgenus. Each lane represents DNA from reference specimens of the indicated species queried using this assay.

Istiophoridae) eggs and larvae off the island of Hawaii from ichthyoplankton samples within 3 hours of acquisition (Hyde et al. 2005). In field application, this method was sensitive and robust and yielded valuable information including the first description of fertilized eggs of the shortbill spearfish (*Tetrapturus angustirostris*).

The second assay utilizes DNA array technology. DNA arrays spotted on epoxy slides allow potentially thousands of taxa to be screened simultaneously. In preliminary work, we have developed an array to screen for 20 species of rockfish, a fraction of the potential available using this platform. The slide is spotted with a series of unique capture probes, each spot containing thousands of capture probes designed to bind to the species-specific probe for a single taxon. Identification of an unknown is accomplished by the incorporation of a fluorescent molecule into the species-specific probe that matches exactly to the unknown DNA sample. The label is incorporated using a technique called allele-specific primer extension, which utilizes *Taq* DNA polymerase to add a labeled deoxynucleotide to the probe bound to the unknown DNA (Chen et al. 2000). Under sufficiently stringent conditions, this incorporation occurs only when the probe and DNA template are an exact match. This reaction is then applied to the DNA array slide where the now labeled probe is captured by a specific capture probe on the surface of the slide. The DNA array is then imaged on a fluorescent scanner. Identity is accomplished by matching of the fluorescent spot against the known x-y coordinates of the capture probes for individual taxa. Figure 3 shows an image of the 20 species DNA array queried with DNA from 3 species of rockfish; spots for each species are replicated 10 times. Though this method is capable of identifying countless taxa, it has some limitations when compared to the previous method. Because more steps are involved, the time required is slightly greater and the potential for sample error is increased. Also, the cost of consumables and equipment is greater using this method, but the more expensive equipment (array printer and reader) is commonly available for use at local universities.

Use of genetic techniques for identification of ichthyoplankton samples is the future for larval and egg surveys. The consistency and high throughput of these assays will ultimately speed sample processing while adding additional taxa and reducing error rates. The ability to cheaply and rapidly analyze survey samples will greatly increase the number of questions that can be addressed and answered using the early life stages of fish. The speed at which some assays can be performed allows near real-time sample identification and can be used to adjust survey parameters to adaptively sample areas where species of interest have been located.

Figure 3. Image of 20 species DNA array probed with DNA from 3 species: stripetail rockfish (*S. saxicola*), cowcod (*S. levis*), and chilipepper (*S. goodei*). Spots containing species-specific capture probes are replicated 10 times.

References

Briggs, J. C. 1974. Marine zoogeography. McGraw-Hill, New York.

Chen, J., M. A. Iannone, M. S. Li, J. D. Taylor, P. Rivers, A. J. Nelson, K. A. Slentz-Kessler, A. Roses, and M. P. Weiner. 2000. A microsphere-based assay for single nucleotide polymorphism analysis using single base chain extension. Genome Res. 10:549–557.

Hyde, J. R., E. Lynn, R. Humphreys, Jr., M. Musyl, A. P. West, and R. Vetter. 2005. Shipboard identification of fish eggs and larvae by multiplex PCR, and description of fertilized eggs of blue marlin, shortbill spearfish, and wahoo. Mar. Ecol. Prog. Ser. 286:269–277.

Kumar, S., K. Tamura, I. Jakobsen, and M. Nei. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Arizona State University, Biodesign Institute, Tempe. Online at http://www.megasoftware.net/mega2.html [accessed 23 October 2006].

Moser, H. G., R. L. Charter, P. E. Smith, D. A. Ambrose, W. Watson, S. R. Charter, and E. M. Sandknop. 2001. Distributional atlas of fish larvae and eggs in the Southern California Bight region: 1951–1998. CalCOFI Atlas No. 34.

Ralston, S., J. R. Bence, M. B. Eldridge, and W. H. Lenarz. 2003. An approach to estimating rockfish biomass based on larval production, with application to *Sebastes jordani*. Fish. Bull. 101(1):129–146.

Raymond, F., and F. Rousset. No date. Genepop on the web. Online at http://wbiomed.curtin.edu.au/genepop/ [accessed 22 November 2006].

Rocha-Olivares, A. 1998. Multiplex haplotype-specific PCR: A new approach for species identification of the early life stages of rockfishes of the species-rich genus *Sebastes Cuvier*. J. Exp. Mar. Biol. Ecol. 231:279–290.

Rousset, F. 1996. Equilibrium values of measure of population subdivision for stepwise mutation processes. Genetics 142:1357–1362.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Integrated Methods to Converge on Estimates of Larval Dispersal

Cynthia A. Taylor
Illumina, Inc.
9885 Towne Centre Drive
San Diego, California 92121
ctaylor@illumina.com

Connectivity at relevant scales for management and conservation of many marine species occurs through dispersal of larvae. By understanding dispersal in the pelagic early life history phase, we can hope to identify barriers to dispersal, recognize conditions that contribute to successful year-classes of marine species, and characterize potential functioning of marine protected areas to better manage harvested stocks. A variety of methods are used to examine pelagic larval dispersal, including chemical tagging (Jones et al. 1999), otolith microchemistry (Swearer et al. 1999), physically following late-stage pelagic fish larvae (Leis and Carson-Ewart 1997), and direct larval observations (Shanks et al. 2003). The cross section of methods have shown that larvae of many species have a lower realized dispersal (and in some cases, natal fidelity) than their time in the pelagic realm might suggest.

Genetic methods, an indirect method of assessing larval dispersal, have been especially useful to identify limitations to gene flow within the range of rockfish (*Sebastes*) species including the Pacific ocean perch (*S. alutus*) (Seeb and Gunderson 1988, Withler et al. 2001), the rosethorn rockfish (*S. helvomaculatus*) (Rocha-Olivares and Vetter 1999), and species within the nearshore *S. Pteropodus* subgenus (Buonaccorsi et al. 2002).

The goal of this study was to assess dispersal and connectivity in the Southern California Bight (SCB) and throughout the range of depth-limited rockfish using a combination of indirect and direct methods. Kelp rockfish (*S. atrovirens*) populations were examined for evidence of genetic structure using 8 coastal and 5 island locations from central California to Baja California, Mexico, using 7 microsatellite loci and 611 adult individuals. Although no significant population structure was detected among all populations treated separately, significant differences were found when populations were pooled based on hydrographic regions identified using average sea surface temperature at the time of spawning and pelagic dispersal (F_{ST} = 0.002). An isolation-by-distance pattern of gene flow among coastal and nearshore island comparisons (Mantel Test, $p = 0.01$) appeared to drive this structure. An estimate of average dispersal was calculated using the relationship between the regression of the isolation by distance and linearized density (see Rousset 1997) at 5–9 km for coastal and nearshore island populations.

Larval distributions were examined directly by collecting early stage (3–16 mm) rockfish samples through the California Cooperative Oceanic Fisheries Investigations (CalCOFI) surveys, completing species-specific identifications on larvae by comparing sequence data to a reference

database of adults for the mitochondrial cytochrome *b* gene, and ageing the larvae using otolith-based methods on the most abundant species in the survey, squarespot rockfish (*S. hopkinsi*). An analysis of covariance showed a significant pattern of higher abundance and younger larvae (mean = 9.2 days) within eddies in the SCB, and lower abundance and older larvae (mean = 15.6 days) outside eddies ($F_1 = 26.13$, $p < 0.001$). For positive stations of the oldest postflexion larvae, 83% (5 of 6 positive stations) were found near (within 20 km of being over) adult habitat. By "tracking" larvae from 1-day-old distributions over natal habitat where they are released by the adults and by examining distributions of aged larvae relative to these natal stations, an overall estimate of dispersal for the 41-day pelagic period was calculated to be 27 km. Using a combination of morphological and molecular methods, 23 additional species were identified from the same cruise, and the youngest larval distributions were examined for concordance with the eddies and adult habitat. The results suggest an overall pattern of retention of these early stage larvae within the SCB and limitation to dispersal beyond that expected for the time in the pelagic stage for both the nearshore kelp rockfish and the more offshore but also depth-limited squarespot rockfish. These data, collected using integrated methods of direct ichthyoplankton observations and indirect genetic methods, support previous work on many species suggesting that pelagic fish larvae have a lower realized dispersal than expected considering the length of their pelagic dispersal period.

References

Buonaccorsi, V. P., C. A. Kimbrell, E. A. Lynn, and R. D. Vetter. 2002. Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. Can. J. Fish. Aquat. Sci. 59:1374–1384.

Jones, G. P., M. J. Milicich, M. J. Emslie, and C. Lunow. 1999. Self-recruitment in a coral reef fish population. Nature 402:802–804.

Leis, J. M., and B. M. Carson-Ewart. 1997. In situ swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. Mar. Ecol. Prog. Ser. 159:165–174.

Rocha-Olivares, A., and R. D. Vetter. 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Can. J. Fish. Aquat. Sci. 56:803–813.

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

Seeb, L. W., and D. R. Gunderson. 1988. Genetic variation and populations structure of the Pacific ocean perch (*Sebastes alutus*). Can. J. Fish. Aquat. Sci. 45:78–88.

Shanks, A., B. A. Grantham, and M. H. Carr. 2003. Propagule dispersal distance and the size and spacing of marine reserves. Ecol. Appl. 13:S159–S169.

Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral-reef fish. Nature 402:799–802.

Withler, R. E., T. D. Beacham, A. D. Schulze, L. J. Richards, and K. M. Miller. 2001. Co-existing populations of Pacific ocean perch, *Sebastes alutus*, in Queen Charlotte Sound, British Columbia. Mar. Biol. 139:1–12.

MHC Evolution in Rockfishes

Andres Aguilar
University of California, Merced
School of Natural Sciences
PO Box 2039
Merced, California 95344
aaguilar2@ucmerced.edu

John Carlos Garza
Southwest Fisheries Science Center
Santa Cruz Laboratory
110 Shaffer Road
Santa Cruz, California 95060
carlos.garza@noaa.gov

The major histocompatibility complex (MHC) is a multigene family found in vertebrates. The MHC contains genes that are involved in the processing and presentation of antigens to cells of the immune system, and are critical to any cell-mediated immune response. MHC class II genes are specifically responsible for the presentation of exogenous or extracellular antigens to the immune system. Genetic variation at MHC class II genes is known to be important in pathogen resistance and may also be important in mate choice. The MHC has only been studied in a relatively small number of fish species, and has not yet been looked at in rockfishes (*Sebastes* spp.). Isolation of MHC genes in rockfishes will provide insight into the genomic organization of MHC genes in higher teleosts, general modes of MHC evolution in fishes, and a valuable tool for the study of selection, mate choice, and population genetics in rockfishes. Our objectives are to 1) isolate MHC class II genes from a variety of rockfish species, 2) perform phylogenetic analysis of rockfish and other teleost MHC sequences, 3) determine whether nonsynonymous substitution rates are elevated compared to synonymous substitutions, and 4) test for locus-specific amplification.

Rockfish tissue samples were obtained from NMFS trawls and longline collections. DNA was extracted using standard protocols. Polymerase chain reaction (PCR) primers were designed to amplify a portion of the MHC class IIB gene, from intron 1 to exon 2 (~500 base pairs [bp] fragment). PCR products were then subcloned and sequenced. An additional set of PCR primers were designed based on intron 1 sequence, and amplified on six greenspotted rockfish (*S. chlorostictus*) and stripetail rockfish (*S. saxicola*) individuals to test for locus-specific amplification. Phylogenetic analysis and estimation of nonsynonymous (amino acid replacement, d_n) and synonymous (silent, d_s) substitution rates was done with MEGA2 (Kumar 2001) for exon 2 fragments only.

MHC class IIB gene fragments were isolated from six rockfish species: aurora rockfish (*S. aurora*), blackgill rockfish (*S. melanostomus*), copper rockfish (*S. caurinus*), greenspotted

rockfish, halfbanded rockfish (*S. semicinctus*), and stripetail rockfish. Multiple loci were that phylogenetic isolated from each of the species. Some sequences contained frameshift mutations, leading to an incorrect open reading frame, indicating that pseudogenes may be present in the rockfish MHC. Figure 1 shows analysis revealed a distinct cluster of rockfish MHC genes when

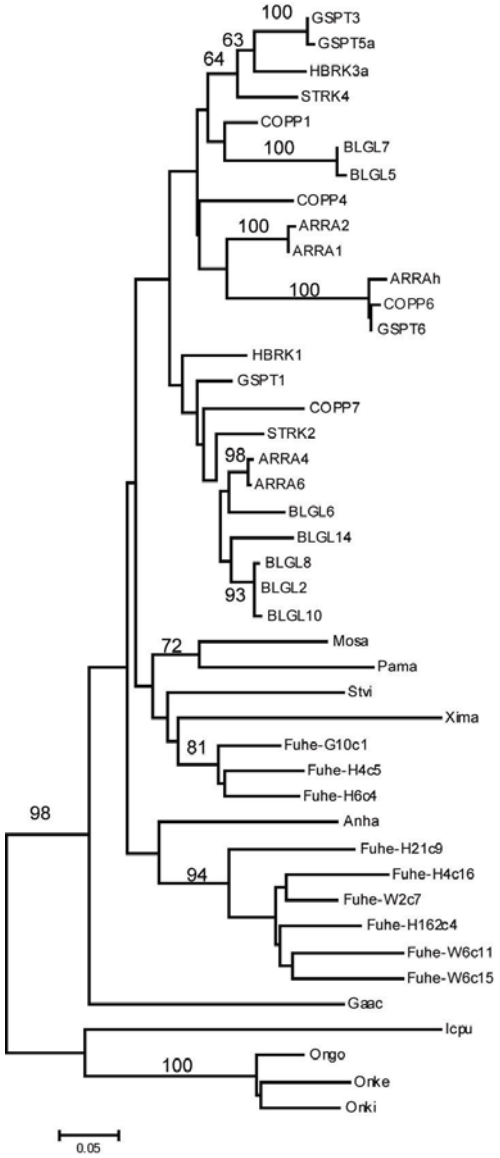

Figure 1. Neighbor-joining tree, estimated using the Tamura-Nei distance measure, for all rockfish MHC class IIB alleles (exon 2) and MHC alleles from other teleost species. Numbers next to nodes indicate percentage of support from 500 bootstrap replicates. Species codes follow. Rockfishes: ARRA: aurora rockfish; BLGL: blackgill rockfish; COPP: copper rockfish; GSPT: greenspotted rockfish; HBRK: halfbanded rockfish; STRK: stripetail rockfish; nonrockfish species: Anha: *Aulonocara hansbaenschi*; Fuhe: mummichog (*Fundulus heteroclitus*); Gaac: threespine stickleback (*Gasterosteus aculeatus*); Icpu: channel catfish (*Ictalurus punctatus*); Mosa: striped bass (*Morone saxitilis*); Ongo: pink salmon (*Oncorhynchus gorbuscha*); Onke: chum salmon (*O. keta*); Onki: coho salmon (*O. kisutch*); Pama: red seabream (*Pagrus major*); Xima: southern platyfish (*Xiphophorus maculatus*); Stvi: walleye (*Stizostedion vitreus*, now *Sander vitreum*).

Table 1. The nonsynonymous (d_n) and synonymous (d_s) substitution rates for the MHC class IIB alleles isolated from greenspotted rockfish and stripetail rockfish and all rockfish species used in this study. Standard errors are given in parentheses.

Species	d_n	d_s	d_n:d_s
Greenspotted rockfish	0.187 (0.036)	0.035 (0.013)	5.34
Stripetail rockfish	0.175 (0.033)	0.056 (0.019)	3.13
All rockfish	0.299 (0.038)	0.086 (0.024)	2.66

compared to other teleost MHC sequences. Transspecific allelism, indicative of a history of balancing selection, is evident in the comparison of *Sebastes* MHC alleles (Figure 1). Redesigning of primers based on intron 1 sequence data gave locus-specific amplification for two of the species: greenspotted rockfish and stripetail rockfish. Elevated d_n:d_s ratios were found for a comparison of all rockfish MHC genes (Table 1) as well as for the alleles isolated from greenspotted rockfish and stripetail rockfish.

The number of MHC loci appears to be variable across rockfish species. This is evident by the number of unique sequences that were isolated from each of the rockfish species. This finding of multiple MHC class II loci within rockfish has also been found in other teleost species (e.g., cichlids [Cichlidae] and sticklebacks [Gasterosteidae]). Elevated d_n:d_s ratios indicate that positive selection has been operating on rockfish MHC sequences, demonstrating historical selection and possible functionality to the genes isolated. Preliminary results suggesting locus-specific amplification in two species (greenspotted rockfish and stripetail rockfish) gives hope for further molecular evolutionary and population genetic studies of MHC class IIB genes in rockfishes.

This is a work in progress, as we are continuing in the isolation of MHC sequences from a larger number of rockfish species. We also plan to verify locus-specific amplification through mother-offspring comparisons. We are also establishing genotyping methods (i.e., single strand conformational polymorphism, or SSCP) for MHC in rockfishes to conduct population-level studies of MHC variation in rockfishes.

References

Kumar, S., K. Tamura, I. Jakobsen, and M. Nei. 2001. MEGA2: Molecular evolutionary genetics analysis software. Arizona State University, Biodesign Institute, Tempe. Online at http://www.megasoftware.net/mega2.html [accessed 23 October 2006].

Microsatellites Reveal Regional Genetic Structure and Effective Population Size in Darkblotched Rockfish

Daniel Gomez-Uchida and Michael A. Banks
Oregon State University
Hatfield Marine Science Center
Coastal Oregon Marine Experiment Station
2030 SE Marine Science Drive
Newport, Oregon 97365
daniel.gomez-uchida@lifetime.oregonstate.edu
michael.banks@oregonstate.edu

The broad diversity among rockfishes (*Sebastes* spp.) complicates the tasks of fisheries management and conservation in the northeastern Pacific. An undesired outcome of historical exploitation of this fish complex (see a review in Parker et al. 2000) has been a growing number of species declared overfished. Darkblotched rockfish (*S. crameri*) is in that delicate status, inhabiting deep areas of the continental shelf and slope. Low biomass estimates of darkblotched rockfish in 1999 (below 25% of the virgin stock) required the implementation of a conservation area from Washington to California, which also restricted catches on other shelf and slope rockfishes, resulting in serious economic impacts to coastal fishing communities. Extensive knowledge of darkblotched rockfish population dynamics is thus necessary to support management strategies such as marine reserves, which may enhance rockfish stock productivity (Lubchenco et al. 2003).

Molecular population genetics provides an understanding of rockfish population dynamics in an evolutionary context. One primary question is whether the long pelagic stage (2 months) of darkblotched rockfish in surface waters (Love et al. 2002) allows effective mixing of individuals by ocean currents on a large scale (e.g., from Washington to northern California). From a conservation standpoint, to determine whether eggs, larvae, juveniles, or adults are exported or retained from or within an area is key for marine reserve design (Hellberg et al. 2002). Microsatellites have demonstrated that highly variable markers may offer more analytical resolution to address this question in rockfish biology (e.g., Roques et al. 2002). Increased resolving power through microsatellites, however, requires larger sample sizes as well as repeated sampling over time in order to give a significant statistical signal (Waples 1998).

Effective population size (N_e) is another parameter of interest in conservation, as it assesses the number of successful breeders. If the effective size is too small, it may jeopardize the long-term viability of a population through inbreeding depression (Frankham 1995). Employing the so-called temporal method in long-lived fishes has shown that N_e can be several orders of magnitude smaller than census sizes (N) (Hauser et al. 2002, Turner et al. 2002), which

might be explained by a large individual variance in reproductive success (Hedgecock 1994), among several causes. Despite the importance of effective population size for conservation genetics, no estimates of this parameter are available for rockfishes in the northeast Pacific.

Here we present results from molecular, population, genetic, and demographic analyses of darkblotched rockfish, collected during 2001 and 2002 NOAA Fisheries annual shelf and slope surveys along the northeastern Pacific coast. Our first objective was to determine whether darkblotched rockfish samples were drawn from a genetically panmictic or subdivided population. To accomplish this goal, 1,268 rockfishes caught between 48°N and 39°N latitude were genotyped across seven microsatellite loci: *Sma*10, *Sma*11 (Wimberger et al. 1999); *Sal*1, *Sal*3 (Miller et al. 2000); and *Spi*4, *Spi*10, and *Spi*12 (Gomez-Uchida et al. 2003). Hardy-Weinberg model fit and Wright's *F*-statistics were calculated to measure genetic variation within and among samples (LeClair and Buckley 2001). Using F_{ST} as an estimate of genetic distance, we tested the hypothesis of isolation by distance using pairwise $F_{ST}/(1 - F_{ST})$ and geographic distance values. We then used an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis to visualize distinct genetic groups using Cavalli-Sforza's chord distance (Felsenstein 1993). Results indicated a weak signal of genetic differentiation in the 2001 and 2002 original collections.

We thus investigated pooling genotypes across locations, creating larger samples comparable in size, based on genetic similarity and geographic proximity, in order to increase resolution. This exercise is summarized in Table 1, which shows that pooling across both years (see Pooled 2001–2002 column) maximized the number of significant loci among five combined samples: Washington (WA), northern Oregon zone 1 (NOR1), northern Oregon zone 2 (NOR2), southern Oregon (SOR), and northern California (NCA). Also, a statistically significant pattern of isolation by distance was revealed; however, F_{ST} values remained relatively constant overall, reflecting low levels of genetic differentiation, regardless of sample sizes (Table 1). Three significant clusters from the five samples can be identified in the UPGMA phenogram of Figure 1.

Our second objective was to compute an estimate of effective population size for the entire darkblotched rockfish population from Washington to California. We preferred a panmictic approach despite our previous findings, because the temporal method for overlapping generations (Jorde and Ryman 1995) relies on allele frequencies calculated on each cohort or

Table 1. Summary statistics, genetic indices of differentiation, and statistical fit to isolation-by-distance model for raw and pooled collections of darkblotched rockfish.

	Raw 2001	**Raw 2002**	**Pooled 2001**	**Pooled 2002**	**Pooled 2001–2002**
Number of samples	17	16	8	6	5
Mean sample size ± SD	25.7 ± 7.2	48.8 ± 34.1	54.3 ± 8.5	128.7 ± 33.6	231.6 ± 52.3
Number of significant loci*	0	0	2	1	5
F_{ST}	0.001	–0.001	0.002	0.000	0.001
Isolation-by-distance model fit (R^2; *P*-value)	0.015; 0.15	0.032; 0.053	0.066; 0.19	0.052; 0.24	0.52; 0.015

*After Bonferroni's adjustment for multiple *k* tests (loci), $k = 7$, $P < 0.0071$.

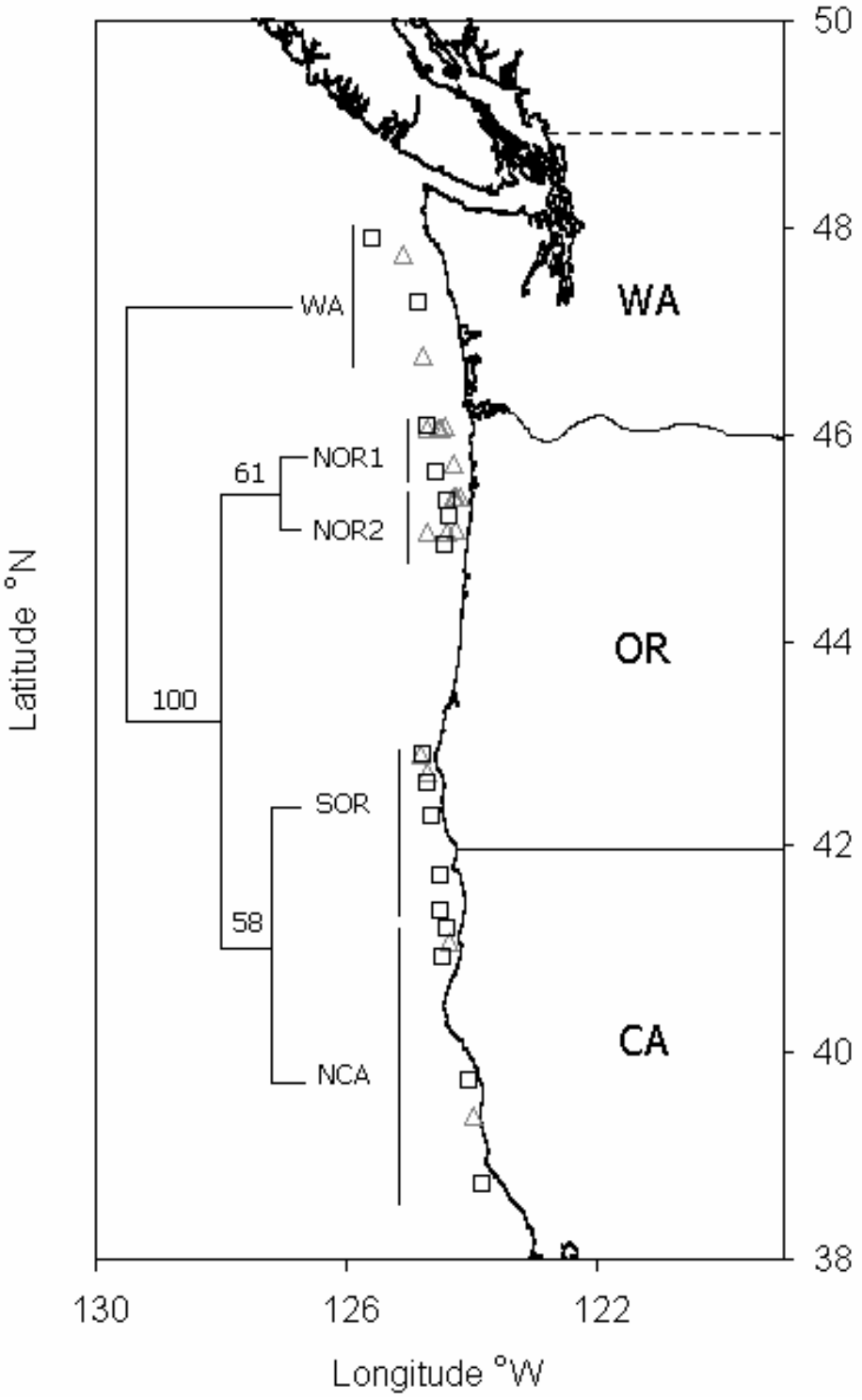

Figure 1. Distribution map of NOAA Fisheries Service 2001 shelf (gray triangles) and 2002 slope (black squares) sample sites in the northeastern Pacific, and associated UPGMA tree drawn from pooled samples across years (see Table 1 and text) using Cavalli-Sforza's chord distance. Numbers on the tree are percentage of bootstrap support for each branch (WA = Washington; NOR1 = northern Oregon zone 1; NOR2 = northern Oregon zone 2; SOR = southern Oregon; NCA = northern California).

year-class. Under subdivision, we reasoned that allele frequencies might have been biased as result of low cohort sample size within each of the three previously described clusters (Figure 1). Demographic parameters such as survival-at-age and reproductive success-at-age are also required for estimating effective size. NOAA Fisheries Service ageing teams determined individual age from otolith readings. Demographic parameters were obtained from available stock assessments (Rogers et al. 2000) and published literature (Nichol and Pikitch 1994). Because low frequency alleles can also bias N_e estimation, we used a binning procedure Turner et al. (2001) developed. The harmonic mean of numbers-at-age across 40 years of fishery data was calculated as darkblotched rockfish census population size (N) (Rogers et al. 2000). From 1911 to 2000 (calendar years), 30 cohorts were found. Nonetheless, 97% of the sample was concentrated among seven cohorts born between 1994 and 2000. Only these year-classes were used during the estimation of N_e. Estimates were approximately three orders of magnitude smaller than census size (N_e = 20,954; N = 22,984,049), with a confidence interval for N_e = [11,977, 32,400].

We derive two main conclusions from this study. First, we were able to reveal a pattern of genetic differentiation in darkblotched rockfish, ranging from Washington to northern California, by simulating larger samples that increased statistical resolution. Despite documenting low levels of genetic differentiation for darkblotched rockfish, we identified three distinct subpopulations as well as a pattern of isolation by distance. These findings should be taken into account in future management measures, since this species has been defined as a single fishery unit. Second, we found that effective population size was at least three orders of magnitude smaller than census sizes, which challenges the intuitive notion that large marine fish populations are "immune" to loss of genetic variation (Hauser et al. 2002). Only several thousand darkblotched breeders rather than millions in the whole population support current conservation initiatives to rebuild this overexploited rockfish stock.

References

Felsenstein, J. 1993. PHYLIP (phylogeny inference package), version 3.57c. Univ. Washington, Department of Genetics, Seattle.

Frankham, R. 1995. Conservation genetics. Ann. Rev. Genet. 29:305–327.

Gomez-Uchida, D., E. A. Hoffman, W. R. Ardren, and M. A. Banks. 2003. Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species. Mol. Ecol. Notes 3:387–389.

Hauser, L., G. J. Adcock, P. J. Smith, J. H. Bernal Ramirez, and G. R. Carvalho. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proc. Natl. Acad. Sci. 99:11742–11747.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? *In* A. R. Beaumont, Genetics and evolution of aquatic organisms, p. 122–134. Chapman and Hall, London.

Hellberg, M. E., R. S. Burton, J. E. Neigel, and S. R. Palumbi. 2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. 70:273–290.

Jorde, P. E. and N. Ryman. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. Genetics 139:1077–1090.

LeClair, L. L., and R. M. Buckley. 2001. Electrophoretic identification of juvenile rockfish (genus *Sebastes*) recruiting to drifting algae and seagrass habitats off the Washington coast. Northwest Sci. 75:53–60.

Love, M. S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the northeast Pacific. University of California Press, Berkeley.

Lubchenco, J., S. R. Palumbi, S. D. Gaines, and S. Andelman. 2003. Plugging a hole in the ocean: The emerging science of marine reserves. Ecol. Appl. 13(1):S3–S7.

Miller, K. M., A. D. Schulze, and R. E. Withler. 2000. Characterization of microsatellite loci in *Sebastes alutus* and their conservation in congeneric rockfish species. Mol. Ecol. 9:240–242.

Nichol, D. G. and E. K. Pikitch. 1994. Reproduction of darkblotched rockfish off the Oregon coast. Trans. Am. Fish. Soc. 123:469–481.

Parker, S. J., S. A. Berkeley, J. T. Golden, D. R. Gunderson, J. Heifetz, M. A. Hixon, R. Larson, B. M. Leaman, M. S. Love, J. A. Musick, V. M. O'Connell, S. Ralston, H. J. Weeks, and M. M. Yoklavich. 2000. Management of Pacific rockfish. Fisheries 25(3):22–29.

Rogers, J. B., R. Methot, T. L. Builder, K. Piner, and M. Wilkins. 2000. Status of the darkblotched rockfish (*Sebastes crameri*) resource in 2000. Appendix: Status of the Pacific coast groundfish fishery through 2000 and recommended acceptable biological catches for 2001. (Available from Pacific Fishery Management Council, 7700 NE Ambassador Place, Suite 200, Portland, OR 97201.)

Roques, S., J. M. Sevigny, and L. Bernatchez. 2002. Genetic structure of deep-water redfish, *Sebastes mentella*, populations across the North Atlantic. Mar. Biol. 140(2):297–307.

Turner, T. F., L. A. Salter, and J. R. Gold. 2001. Temporal-method estimates of N_e from highly polymorphic loci. Conserv. Genet. 2:297–298.

Turner, T. F., J. P. Wares, and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162(3):1329–1339.

Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. J. Hered. 89(5):438–450.

Wimberger, P., J. Burr, A. Lopez, and P. Bentzen. 1999. Isolation and characterization of twelve microsatellite loci for rockfish (*Sebastes*). Mar. Biotech. 1:311–315.

Population Structure of Puget Sound Rockfish Assessed with Mitochondrial DNA and Microsatellites

Jennifer A. Hempelmann
Northwest Fisheries Science Center
Conservation Biology Division
2725 Montlake Boulevard East
Seattle, Washington 98112
jennifer.hempelmann@noaa.gov

Christiane H. Biermann
Portland State University
Department of Biology
1719 SW 10th Avenue
Portland, Oregon 97207
biermann@pdx.edu

Erik E. Sotka
Stanford University
Hopkins Marine Station
120 Oceanview Boulevard
Pacific Grove, California 93950
sotka@stanford.edu

Daniel Halos
University of Washington
Friday Harbor Laboratories
620 University Road
Friday Harbor, Washington 98250
danhalos@gmail.com

Historically, rockfishes (*Sebastes* spp.) and other large vertebrates have played fundamental roles in the ecology of nearshore communities in the northeast Pacific (Dayton et al. 1998). However, over the last three decades, many ecologically and commercially important large rockfish species along the northeastern coastlines of the Pacific Ocean have rapidly declined in abundance to a fraction of their original biomass (Gunderson 1997). This decline can be attributed in part to a popular recreational and commercial fishery of rockfish that arose in the late 1960s and early 1970s and peaked at over 200 million tons in the early 1990s along the west coast of North America (Laidig et al. 1996, Love et al. 2002). In stark contrast to declines in the large rockfishes, a "dwarf" rockfish that has no commercial value or recreational interest—the

Puget Sound rockfish (*S. emphaeus*)—has exploded in population size by several orders of magnitude over the same time period (Fulmer et al. in press). The concern for managers is that huge aggregations of these "dwarf" rockfish may impede the recovery of larger and more commercially valuable rockfish species through competition.

Even though it is informative to study a nonfished species in addition to its exploited congeners, few studies have focused on Puget Sound rockfish. This study uses sequences from the mitochondrial control region and alleles from two microsatellite loci to quantify gene flow among five Puget Sound rockfish populations separated by 10–120 km in Washington waters (Figure 1) and to assess whether there was a genetic signature of recent population expansion.

Our examination of the mitochondrial control region revealed a great deal of genetic variation within populations of Puget Sound rockfish (Table 1). Fifty-six haplotypes—only 16 of which were shared by two or more individuals—were sequenced from the 127 total individuals. On average, individuals differed by approximately 1% (±0.1% SD; Table 1). A phylogenetic analysis delineated two major clades within Puget Sound rockfish, distinguished by two transitions and one transversion (genealogy not shown).

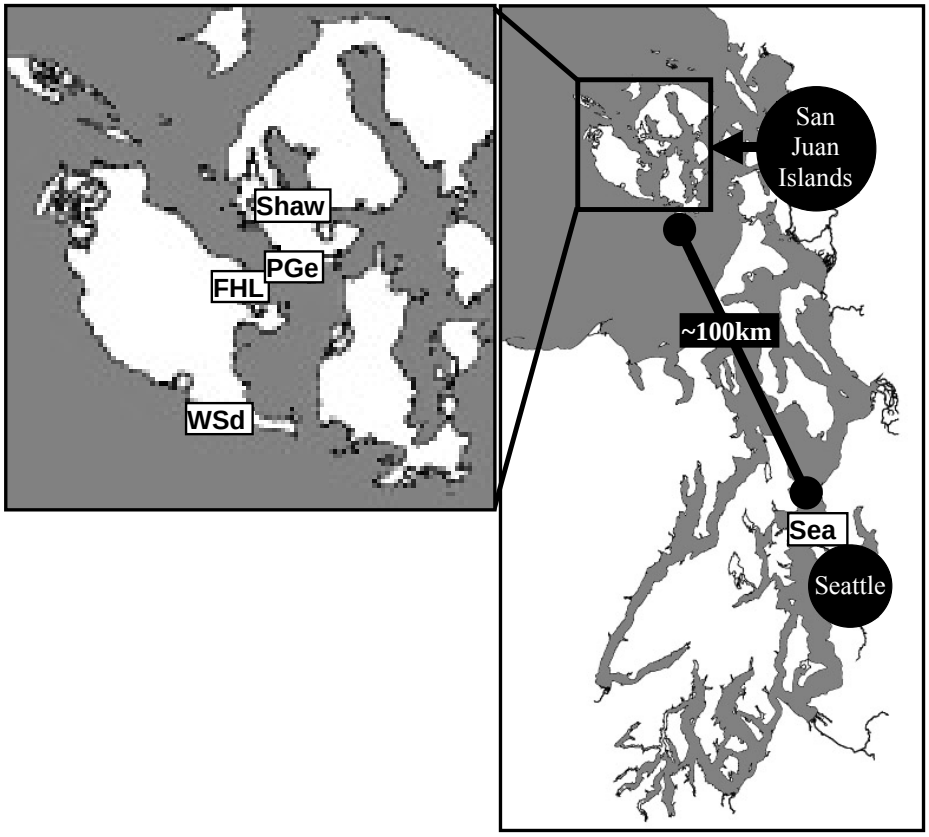

Figure 1. Collection locations (shown in white boxes).
Sea = Off Boeing Creek, Seattle
WSd = West side of San Juan Island, off Eagle Cove
FHL = East side of San Juan Island, near Friday Harbor Laboratories
PGe = Point George, South of Shaw Island
Shaw = North of Shaw Island, Broken Point

Table 1. Indices of genetic diversity, haplotype frequencies, and tests for population expansion (Tajima's D-statistics and Fu's F-statistics) among populations of the Puget Sound rockfish.

Population	*n*	Number of haplotypes	Number of unique haplotypes	Proportions of clades (A:B)	Nucleotide diversity (±SD)	Tajima's D-test		Fu's F-test	
						D	p-value	F	p-value
FHL	28	22	14	0.71:0.29	0.012(±0.002)				
PGe	32	17	5	0.78:0.22	0.008(±0.003)				
Shaw	25	20	7	0.80:0.20	0.010(±0.002)				
Westside	19	15	8	0.79:0.21	0.012(±0.002)				
Seattle	23	17	6	0:70:0.30	0.012(±0.002)				
Total	127	56	40	0.76:0.24	0.010(±0.002)	−2.03	0.021	−47.91	<0.001

Despite the considerable genetic variation within Puget Sound rockfish, there was little evidence of population subdivision across localities, which were separated by up to 120 km (Figure 1). There were no significant differences in the frequencies of the 16 shared haplotypes and no differences in the relative proportions of the two control region clades, which occurred at all locations in approximately a 70:30 mix. An analysis of molecular variance (AMOVA) of the mitochondrial DNA (mtDNA) sequences indicated no significant differentiation among populations. An AMOVA analysis of the two microsatellite loci, which presumably evolve more quickly than mtDNA, also displayed no significant population subdivision.

Because it is unlikely that the noncoding control region locus is under strong selection, the observed genetic homogeneity among these populations of Puget Sound rockfish is probably the consequence of broad gene flow among populations, or a recent cessation of gene flow and incomplete lineage sorting. We currently believe that there is substantial gene flow among rockfish populations from the San Juan Islands and Puget Sound, because even the more quickly evolving microsatellite loci displayed no genetic differentiation with reasonable sample sizes (n = 15 to 27 per locus per population). A more definitive conclusion may be reached with higher sample sizes and more loci.

The mtDNA sequence data contain a signal of population expansion that appears to reflect a relatively old expansion into the Puget Sound region. Analysis of the mitochondrial control region data indicated more new mutations than would be expected from the number of segregating sites (Tajima's D = −2.03, p = 0.021) or from the number of alleles (Fu's F = −47.91, $p < 0.001$; Table 1). Because it is unlikely that the noncoding locus is under selection, these negative D and F values likely indicate an expanding population.

A mismatch distribution analysis (see Lessios et al. 2001) confirmed the results of the Tajima's D-tests and Fu's F-tests and provided an estimate of the time since expansion began. From the mismatch analysis (Figure 2), we estimate that the time since the expansion began in Puget Sound is approximately 12,770 years before present—with a large confidence interval, however. The notion that the expansion began approximately 12 thousand years ago (KYA) matches very closely with estimates of when habitats became available in Puget Sound after the retreat of the last glacial maximum (Pielou 1991).

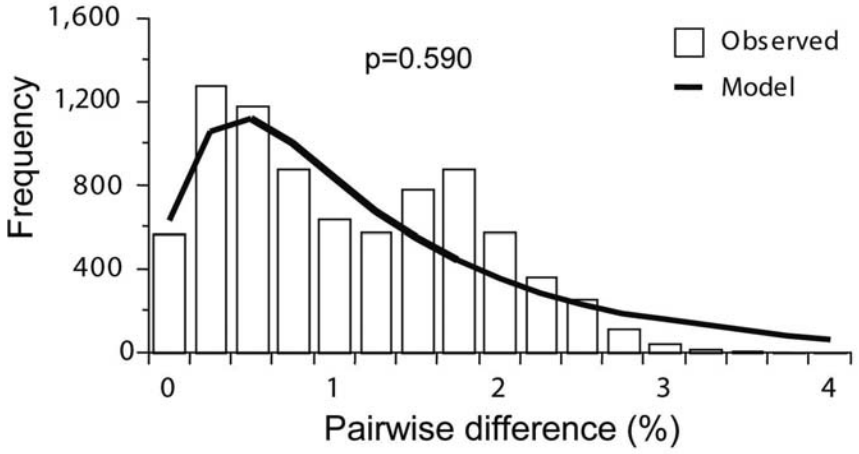

Figure 2. Mismatch distribution of Puget Sound rockfish mtDNA control region sequences.

In summary, we have detected apparently high gene flow among populations of rockfish in Puget Sound and the Northwest Straits and detected a genetic signal of population expansion that was likely initiated with the postglacial colonization of the Georgia Basin about 12 KYA. The most pressing work that remains is to assess whether the high densities of this "dwarf" rockfish may alter the course of recovery of the larger, commercially valuable species. In particular, a focus on the feeding ecologies and interactions among adult Puget Sound rockfish and juvenile individuals of the larger rockfish species should reveal whether these fish guilds compete for food and habitat and thus whether such competition may impede the recovery of the larger rockfish. Alternatively, if Puget Sound rockfish are regularly eaten as prey by larger rockfish (Love et al. 2002), then large abundances of Puget Sound rockfish may actually accelerate recovery of larger rockfish.

Acknowledgments

This project was generated during a spring 2003 research apprenticeship course at Friday Harbor Laboratories at University of Washington. Jennifer Hempelmann received financial support from the Mary Gates Endowment. Erik Sotka was supported in part by the Andrew W. Mellon Foundation. We thank R. Buckley and the Washington Department of Fish and Wildlife for help with fishing.

References

Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1998. Sliding baselines, ghosts, and reduced expectations in kelp forest communities. Ecol. Appl. 8:309–322.

Fulmer, J., D. R. Gunderson, L. LaFrance, B. S. Miller, B. Goetz, and W. A. Palsson. In press. Changes in growth and recruitment of the Puget Sound rockfish (*Sebastes emphaeus*) in northern Puget Sound. *In* J. Heifetz, J. DiCosimo, A. J. Gharrett, M. S. Love, V. M. O'Connell, and R. D. Stanley (eds.), Biology, assessment, and management of North Pacific rockfishes. Alaska Sea Grant, University of Alaska Fairbanks.

Gunderson, D. R. 1997. Spatial patterns in the dynamics of slope rockfish stocks and their implications for management. Fish. Bull. 95:219–230.

Laidig, T. E., K. M. Sakuma, and M. N. Nishimoto. 1996. Description of pelagic larval and juvenile stripetail rockfish, *Sebastes saxicola* (family Scorpaenidae), with an examination of larval growth. Fish. Bull. 94:289–299.

Lessios, H. A., M. J. Garrido, B. D. Kessing. 2001. Demographic history of *Diadema antillarum*, a keystone herbivore on Caribbean reefs. Proc. R. Soc. Lond. B 268:2347–2353.

Love, M. S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the northeast Pacific. University of California Press, Berkeley.

Pielou, E. 1991. After the Ice Age: The return to life to glaciated North America. University of Chicago Press, Chicago.

Genetic Markers Distinguish Populations of Black Rockfish in the Gulf of Alaska

Lisa W. Seeb
Alaska Department of Fish and Game
Division of Commercial Fisheries
Gene Conservation Laboratory
333 Raspberry Road
Anchorage, Alaska 99518
lisa_seeb@fishgame.state.ak.us

The understanding of the genetic structure of discrete stocks and the biological significance of local depletions is a central feature of conservation and restoration of commercially exploited fisheries resources. Marine organisms represent unusual challenges in the study of population genetics. Adult marine organisms may make extensive migrations over huge distances and have highly dispersive larval and early life history stages.

Rockfish (*Sebastes* spp.) stocks are harvested extensively in Alaska by both commercial and sport fisheries, but like rockfishes throughout the North Pacific, they are easily overharvested and slow to rebuild due to longevity and delayed maturation (Love et al. 2002). The harvest for nearshore rockfishes in the northern Gulf of Alaska primarily occurs in state waters, and black rockfish (*S. melanops*) represent an important component of that fishery. Schools of adults often aggregate over shallow rocky areas making them particularly susceptible to fishing. The State of Alaska has sole management and assessment responsibility for black rockfish, since it was believed that the species was not adequately assessed by the trawl surveys conducted by the National Marine Fisheries Service (NMFS).

In this study, we surveyed the population structure of 10 populations of black rockfish ranging from the Alaska Peninsula across the Gulf of Alaska. We also included a representative population from Washington State as an outgroup (Table 1, Figure 1). Samples were collected by hook and line and from commercial and sport fish sampling. Target sample sizes were set at 100, but realized sample sizes varied from 24 to 130 individuals. Fin clips from individual fish were nonlethally sampled, placed in ethanol, and shipped to the laboratory for analysis.

DNA was extracted using a rapid precipitation method; all microsatellite analyses were conducted on an Applied Biosystems (ABI) 377 automated sequencer (Applied Biosystems, Foster City, California). Individuals were analyzed for 10 microsatellite loci including tri- and tetranucleotide loci cloned from black rockfish (*Sme* 2, 3, 4, 5, 8, 9, 11, 14, ADFG unpubl. data) and dinucleotide loci from quillback rockfish (*S. maliger*) (*Sma* 1, 3; Wimberger et al. 1999). A wide range of variation was observed at the 10 loci with the number of alleles per locus varying from 8 to 48 (Table 2). Overall, 235 alleles were observed.

Table 1. Collection locations of black rockfish.

Location	*N*	Collection date
Washington	30	1998
Southeast Alaska—Sitka	100	1999
Yakutat	130	2003
Prince William Sound	48	2000
Resurrection Bay	24	1998
Kodiak Island—East	100	1998
Kodiak Island—West	86	1998
Alaska Peninsula—Chignik	100	2000
Alaska Peninsula—Sand Point	60	1999
Alaska Peninsula—Akutan	100	1999

To evaluate the relationships among sites, F_{ST} was calculated among all collections as well as pairwise between collections using FSTAT version 2.9.3 (Goudet 2001). Significant population structure was detected with an overall value of F_{ST} of 0.008 ($P = 0.01$). This level of structure, although low, is consistent with previous studies of rockfish species over broad geographic ranges based on microsatellite loci. For example, Buonaccorsi et al. (2002), surveying copper rockfish (*S. caurinus*) from California to British Columbia, estimated an F_{ST} of 0.007.

Figure 1. Map of collection sites for black rockfish.

Table 2. Microsatellite loci, number of alleles and locus source for black rockfish.

Locus	Number of alleles	GenBank accession number or source
Sme 2	25	AF142484
Sme 3	38	AF142485
Sme 4	34	AF142486
Sme 5	13	AF142487
Sme 8	15	AF142490
Sme 9	24	AF142491
Sme 11	18	AF142493
Sme 14	8	AF142587
Sma 1	12	Wimberger et al. 2000
Sma 3	48	Wimberger et al. 2000
Overall	235	

A matrix of pairwise F_{ST} values between all pairs of populations was computed. Population structure was visualized using multidimensional scaling (MDS) of the pairwise values as implemented in NtSYS (Exeter Software, Setauket, New York) to reduce the dimensionality of the interpopulation distances to three-dimensional space. Two major clusters are apparent in the multidimensional scaling, a large cluster of populations ranging from the Alaska Peninsula through the central Gulf of Alaska and a second cluster of populations ranging from Yakutat to the Washington population from the Pacific Northwest (Figure 2). These data suggest an isolation by distance model with a distinct discontinuity between Prince William Sound and the Yakutat.

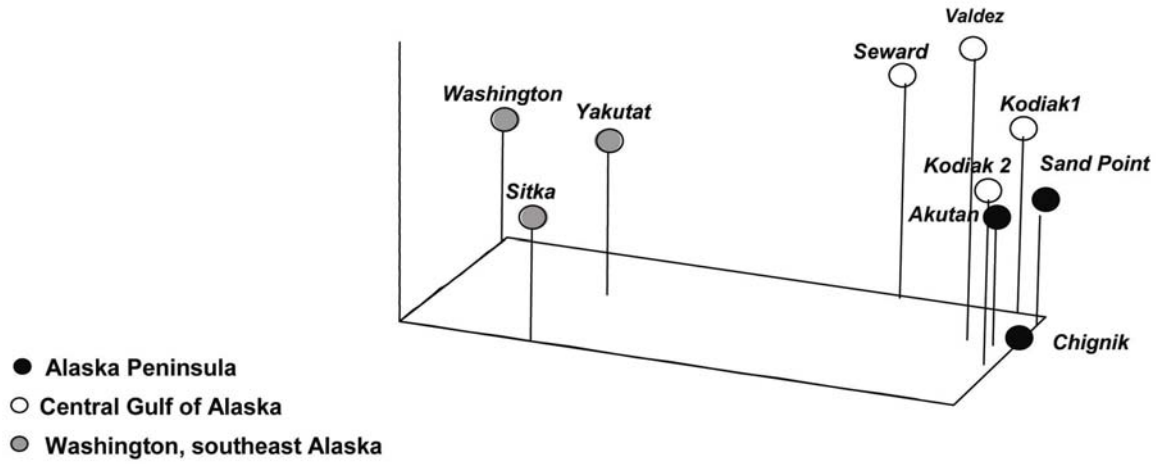

Figure 2. MDS using pairwise F_{ST} values for 10 populations of black rockfish from the Gulf of Alaska and Washington coast.

References

ADFG (Alaska Department of Fish and Game). Unpubl. data. Ten microsatellite loci including tri- and tetranucleotide loci cloned from black rockfish. (Available from Alaska Dept. Fish and Game, 333 Raspberry Rd., Anchorage, AK 99518.)

Buonaccorsi, V. P., C. A. Kimbrell, E. A. Lynn, and R. D. Vetter. 2002. Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. Can. J. Fish. Aquat. Sci. 59:1374–1384.

Goudet, J. 1995. FSTAT (version 1.2): A computer program to calculate F-statistics. J. Hered. 86:485–486.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Updated from Goudet (1995). Online at http://www2.unil.ch/popgen/softwares/fstat.htm.

Love, M. S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the northeast Pacific. University of California Press, Berkeley.

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

Genetic Studies of Rockfish: Identification, Relationships, and Population Structure

Anthony J. Gharrett, Zhuozhuo Li, Andrew P. Matala, and Andrew K. Gray
University of Alaska Fairbanks
School of Fisheries and Ocean Sciences, Juneau Center
11120 Glacier Highway
Juneau, Alaska 99801
a.gharrett@uaf.edu

Jonathan Heifetz
National Marine Fisheries Service
Auke Bay Laboratory
11305 Glacier Highway
Juneau, Alaska 99801
jon.heifetz@noaa.gov

Milton S. Love
University of California, Santa Barbara
Marine Science Institute
Santa Barbara, California 93106
love@lifesci.ucsb.edu

Arthur W. Kendall, Jr.
National Marine Fisheries Service
7600 Sand Point Way NE
Seattle, Washington 98115
art.kendall@noaa.gov

The species-rich genus of rockfish (*Sebastes* spp.) continues to provide many challenges to fisheries scientists. More than 60 species are indigenous to the Pacific coast of North America and over 100 occur worldwide (Kendall 2000). Rockfishes are important to commercial and recreational fisheries, and their numbers and diversity indicate that they are important and complex components of the marine ecosystem. The challenges of rockfish result both from the large number of species and their often close similarities.

Although the life history and ecology of many rockfish species have been studied in detail, little information is available for the majority of species. In addition to the number of species, some of which are cryptic and others probably unrecognized (Seeb 1986), the ontogenetic progression of rockfish obfuscates study efforts. Female rockfish are viviparous. In the marine environment, larvae develop through a series of transformations until, as juveniles, they begin to take on adult characteristics. It is not yet possible to identify visually the species of

many larvae and identification of some juveniles is difficult. Consequently, the early life history of those species is unknown. These problems, related directly to the systematics of the rockfish genus, make it difficult to determine environmental influences on their abundances and roles in the marine ecosystem.

The planktonic nature and dispersal of rockfish larvae also create questions about the sources of rockfish production. Because dispersal of larvae is related to oceanic processes, the nature of the population structure is not immediately obvious for a particular species. The population structure of different species may vary substantially because of the variety of life histories. Knowledge of the population structure translates to practical questions when fisheries scientists attempt to manage fish for harvest, to conserve species, or to protect them from environmental or anthropogenic influences. These questions involve the population genetics of rockfish species.

Approach

We have addressed a broad array of questions concerning systematics and population structure using studies involving genetic analysis. We have examined the genetic diversity among species and characterized the genetic composition within and among selected species of rockfish. The underlying concept is that evolutionarily related individuals share common ancestry but often exhibit some genetic divergence. At the molecular level, the overall DNA base sequence (or gene products) of two related species should be similar, but because of divergence in nucleotide sequences there are some differences. Ordinarily, more differences accumulate as divergence between species (or populations) increases.

Our work had three major facets. The basis of the species identification work involved development of restriction site maps in regions of the mitochondrial DNA that include the nicotinamide adenine dinucleotide, reduced form (NADH), dehydrogenase subunit-3 and subunit-4 genes (ND3/ND4 region), and the 12S and 16S ribosomal RNA (rRNA) genes (12S/16S region). Those data also provided preliminary data for phylogenetic comparisons among rockfish species as well as with two other scorpaenids, shortspine thornyheads (*Sebastolobus alascanus*) and *Helicolenus hilgendorfii*. In the second facet, we applied our database to identify to species larval and juvenile rockfish collected in ichthyoplankton and juvenile surveys. The third facet of the project was to examine the potential of mitochondrial DNA (mtDNA) and microsatellite variation to detect population structure.

Results

Our first studies examined interspecific mtDNA variation in *Sebastes* rockfish. The systematics of rockfish, the assignment of subgenera, and the relationships between Asian and North American species have not been thoroughly evaluated since the advent of molecular methods. We mapped restriction sites in the mtDNA ND3/ND4 and 12S/16S regions and used the presence or absence of sites as characters for comparisons among species. We began by examining the most common species in the eastern Gulf of Alaska (GOA). We were able to distinguish all 15 rockfish species included in the study; although all were distinct, black rockfish (*S. melanops*) was similar to yellowtail rockfish (*S. flavidus*) (both subgenus *Sebastosomus*), harlequin rockfish (*S. variegatus*) was similar to sharpchin rockfish

(*S. zacentrus*) (both subgenus *Allosebastes*), and quillback rockfish (*S. maliger*) was similar to copper rockfish (*S. caurinus*) (both subgenus *Pteropodus*) (Gharrett et al. 2000). Subsequently, we extended our survey of rockfish species and included species from Asian and both the southern and northern North American ranges.

With these samples we addressed questions about 1) the integrity of the subgenus *Pteropodus*, which has representatives both in Asia and along the North American coast, and their relationship to species in other subgenera (e.g., *Mebarus*), and 2) the monophyly of subgenera within rockfish. We discovered that Asian and North American *Pteropodus* and *Mebarus* species are distinct (Li et al. 2006a). In addition, several of the subgenera (e.g., *Acutomentum* and *Allosebastes*) are probably not monophyletic (Li et al. 2006b). Mitochondrial DNA has unique markers because mtDNA is clonal and matrilineally inherited. Nuclear markers provide an independent evolutionary snapshot, and we initiated a study of microsatellite flanking regions. The rationale was that these sequences are unlikely to be heavily influenced by natural selection. The flanking regions in aggregate would provide sequences that reflect generalized divergence among species. That work also indicated that Asian rockfish species represented lineages separate from their North American congeners (Asahida et al. 2004).

From the information that restriction site data provided, the majority of more than 70 species of rockfish could be unequivocally identified. Only a few small groups of species (usually groups of two or three) could not be resolved. One of the challenges rockfish present is that the larvae and many small juveniles are difficult or impossible to identify visually. Consequently, we applied the species-identifying mtDNA methods to collections of juveniles from the Southern California Bight (SCB) and the GOA and to several ichthyoplankton collections from Southeast Alaska. In all of the studies, collaborators (M. Nishimoto in California and A. Kendall and B. Wing in Alaska) examined the morphology of the specimens in an effort to develop or confirm morphological means to identify these small fish. Morphological identification of the SCB specimens was confirmed, but some species groups like gopher rockfish (*S. carnatus*) and black-and-yellow rockfish (*S. chrysomelas*) remain indistinguishable (Li et al. in press). We successfully extracted DNA from 5-mm preflexion larvae and identified them to species or species group, but with exceptions for two species of the subgenus *Pteropodus* and possibly sharpchin rockfish, no morphological markers were found that would facilitate field identification (Gray et al. 2006). The GOA juveniles were also identified genetically to species or species group and many of the fish were identified morphologically. Although some of the specimens could not be identified to species, the number of potential species was reduced to two to four (Kondzela et al. in press). Consequently, it was possible to use morphology to complete the identifications in nearly all cases for juveniles.

One of the controversies that remains is the relationship between gopher rockfish and black-and-yellow rockfish. These species occupy the same range, but seem to be ecologically separated. Recently, microsatellite markers have been identified that are probably species specific (Narum 2000). Previous studies failed to find fixed differences between the species. We examined mtDNA variation within these groups and used an analysis that tested the significance of haplotype distributions in a haplotype tree in a context of geographic distribution. Although both species have many of the same common haplotypes, their distributions differed, especially for presumed ancestral haplotypes, suggesting that one species emerged to the south of Point Conception and the other emerged to the North. In addition, comparison of haplotypes of

copper rockfish from its northern (Canada) and southern (Southern California) ranges reveals overlap in mtDNA haplotype composition, but frequency differences indicate population divergence.

Knowledge of population structure is important to conservation and management missions, and population genetic structure is particularly useful because it can identify maximum bounds of production units. For population genetic studies, microsatellites provide a useful tool because there are literally thousands in the genome, many are probably neutral and noncoding, and they are diploid, which provides information that can be used both to assess their utility and is useful in stock separation computations. Consequently, we used microsatellites in separate studies of the population genetic structure of bocaccio (*S. paucispinis*), rougheye rockfish (*S. aleutianus*), shortraker rockfish (*S. borealis*), and northern rockfish (*S. polyspinis*). Bocaccio along the California coast has been heavily exploited and is in jeopardy. Our work suggests that the bocaccio population structure should be considered in making conservation decisions and that the structure may be influenced by current patterns. The population structure observed in bocaccio is consistent with a neighborhood model in which the distribution of a species is relatively continuous, but the distance moved between birth and reproduction is much less than the species range (Matala et al. 2004a). Shortraker rockfish also has population structure that appears consistent with the neighborhood model (Matala et al. 2004b).

In our population studies of rougheye rockfish, we were at first puzzled by the absence of heterozygotes at one microsatellite locus, but when we observed that two distinct mtDNA haplotypes were correlated with the microsatellite expression, it became clear that there were two distinct species (Gharrett et al. 2005). Both species of rougheye rockfish (Types I and II) (Gharrett et al. in press) also have population structure that appears consistent with the neighborhood model. Weak structure was observed in preliminary studies of northern rockfish.

What Remains to Be Done?

The results summarized above leave many questions. First, several groupings of rockfish cannot yet be distinguished genetically. It is possible that discriminating markers occur in other mtDNA regions. Exploration of other regions also should provide markers that increase the systematic resolution. In addition to better resolving systematic differences, it would be useful to survey nuclear loci. One of the problems with the molecular phylogenies is that they do not provide clear relationships near the interior of the tree. To define those relationships, either a substantial amount of additional data or data from more strongly conserved sequences would be useful.

Additional markers would also be useful for species identification of larvae and juveniles. The RFLP markers could be converted to single nucleotide polymorphism (SNP) markers. SNP markers would make processing samples from ichthyoplankton surveys very fast and could alter the approach to larval assessment surveys in some areas and make such surveys appropriate in other areas.

The differences between Asian and North American species are intriguing. Although we have tissue samples of nearly all of the Asian species, we have not yet analyzed them. The

completion of those analyses will provide interesting information about the evolutionary history of rockfish.

There are more than 70 rockfish species along the North American coast. Many are commercially or recreationally valuable; all are important components in the ecosystem. We know little about their population structure, but because many are long-lived, they are demographically fragile and need to be well understood and carefully managed if we expect them to persist. Knowledge of their population genetic structure is an important facet of learning about their biology and the basis of their productivity.

Acknowledgments

Our work has been supported by the U.S. Geological Survey, the U.S. National Marine Fisheries Service Auke Bay Laboratory, the Saltonstall-Kennedy Fund, and the North Pacific Research Board.

References

Asahida, T., A. K. Gray, and A. J. Gharrett. 2004. Use of microsatellite locus flanking regions for phylogenetic analysis: A preliminary study of *Sebastes* subgenera. Environ. Biol. Fishes 69:461–470.

Gharrett, A. J., A. K. Gray, and J. Heifetz. 2000. Identification of rockfish (*Sebastes* spp.) from restriction site analysis of the mitochondrial ND3/ND4 and 12S/16S rRNA gene regions. Fish. Bull. 99:49–62.

Gharrett, A. J., A. P. Matala, E. L. Peterson, A. K. Gray, Z. Li, and J. Heifetz. 2005. Two genetically distinct forms of rougheye rockfish are different species. Trans. Am. Fish. Soc. 134:242–260.

Gharrett, A. J., A. P. Matala, E. L. Peterson, A. K. Gray, Z. Li, and J. Heifetz. In press. Distribution and population genetic structure of sibling species of rougheye rockfish. *In* J. Heifetz, J. DiCosimo, A. J. Gharrett, M. S. Love, V. M. O'Connell, and R. D. Stanley (eds.), Biology, assessment, and management of North Pacific rockfishes. Alaska Sea Grant, University of Alaska Fairbanks.

Gray, A. K., A. W. Kendall, Jr., B. L. Wing, M. G. Carls, J. Heifetz, Z. Li, and A. J. Gharrett. 2006. Identification and first documentation of larval rockfishes in southeast Alaskan waters was possible using mitochondrial markers but not pigmentation patterns. Trans. Am. Fish. Soc. 135:1–11.

Kendall, A. W., Jr. 2000. An historical review of *Sebastes* taxonomy and systematics. Mar. Fish. Rev. 62:1–23.

Kondzela, C. M., A. W. Kendall, Jr., Z. Li, D. Clausen, and A. J. Gharrett. In press. Species of juvenile rockfish collected in the Gulf of Alaska, 1998–2002. *In* J. Heifetz, J. DiCosimo, A. J. Gharrett, M. S. Love, V. M. O'Connell, and R. D. Stanley (eds.), Biology, assessment, and management of North Pacific rockfishes. Alaska Sea Grant, University of Alaska Fairbanks.

Li, Z., A. K. Gray, M. S. Love, T. Asahida, and A. J. Gharrett. 2006a. Phylogeny of members of the rockfish (*Sebastes*) subgenus *Pteropodus* and their relatives. Can. J. Zool. 84:527–536.

Li, Z., M. M. Nishimoto, M. S. Love, and A. J. Gharrett. 2006b. Comparing the identification of Southern California juvenile rockfishes (genus *Sebastes* spp.) by restriction site analysis of the mitochondrial ND3/ND4 region and by morphological characteristics. Fish. Bull. 104:376–382.

Li, Z., A. K. Gray, M. S. Love, A. Goto, and A. J. Gharrett. In press. Are the subgenera of *Sebastes* monophyletic? *In* J. Heifetz, J. DiCosimo, A. J. Gharrett, M. S. Love, V. M. O'Connell, and R. D. Stanley (eds.), Biology, assessment, and management of North Pacific rockfishes. Alaska Sea Grant, University of Alaska Fairbanks.

Matala, A. P., A. K. Gray, A. J. Gharrett, and M. S. Love. 2004a. Microsatellite variation indicates population genetic structure of bocaccio. N. Am. J. Fish. Man. 24:1189–1202.

Matala, A. P., A. K. Gray, J. Heifetz, and A. J. Gharrett. 2004b. Population structure of Alaskan shortraker rockfish, *Sebastes borealis*, inferred from microsatellite variation. Environ. Biol. Fishes 69:201–210.

Narum, S. 2000. Assortative mating and genetic structure of gopher rockfish (*S. carnatus*) and black-and-yellow-rockfish (*S. chrysomelas*): A case of incipient speciation. Master's thesis. Univ. San Diego, San Diego, CA.

Seeb, L. W. 1986. Biochemical systematics and evolution of the scorpaenid genus *Sebastes*. Doctoral dissertation. Univ. Washington, Seattle.

Conservation Genetics of Rockfishes: The West Coast's Most Species-rich and Imperiled Genus of Fishes

Russell D. Vetter
Southwest Fisheries Science Center
8604 La Jolla Shores Drive
La Jolla, California 92038
russ.vetter@noaa.gov

More than 70 species of rockfish (*Sebastes* spp.) occur off the west coast of North America at depths from the intertidal to over 500 m. All members of the genus share an ovoviviparous system of reproduction with mate selection, copulation, internal fertilization, and the gestation and production of live-born young. Relative to broadcast spawning fishes, the properties of assortative mating and live bearing may promote reproductive isolation and regional retention of larvae. Assortative mating may increase rates of speciation (Rocha-Olivares 1999a, 1999b, Narum et al. 2004) and larval retention may promote a high level of phylogeographic population structure (Rocha-Olivares and Vetter 1999, Buonaccorsi et al. 2004). The high numbers of similar species, the potential for cryptic species, and the likelihood of regional stock structure make rockfish species difficult to assess and manage but also make them good candidates for molecular approaches to their systematics, population structure, and ecology. In the early 1990s the Fish Genetics Group at the Southwest Fisheries Science Center La Jolla Laboratory targeted rockfish and related scorpaenid fishes for molecular studies of their ecology and evolutionary biology. Studies of molecular larval identification (Fish and Chips: Genetic Identification of Rockfish Larvae and Juveniles, p. 35) and larval dispersal patterns (Integrated Methods to Converge on Estimates of Larval Dispersal, p. 42) are covered elsewhere in this volume. First, I summarize studies of the evolution, phylogeny, and subgeneric structure of the genus particularly as it informs studies of morphology, biogeography, niche, and early life history characteristics. Second, I discuss types of population genetic structure that have been observed in different scorpaenid fishes and how this may relate to larval dispersal patterns and metapopulation structure. Finally, I discuss the application of molecular studies to inform management decisions.

Classic systematic investigations of the rockfishes noted morphologic similarities and differences that led to the description of several taxonomic groupings that have at times been elevated to genus-level distinctions although they are presently subsumed within the rockfishes. Phylogenetic studies based on mitochondrial DNA (mtDNA) have generally supported morphologically based subgeneric lineages with the occasional reassignment of a species that is not related by descent but appears similar due to convergent evolution. Cytochrome *b* sequences of 54 rockfish species were originally described by Rocha-Olivares et al. (1999a). Since then almost all remaining species have been sequenced (>98 species or taxa, see Fish and Chips:

Genetic Identification of Rockfish Larvae and Juveniles, p. 35). Subsequent studies have sought to refine subgeneric groupings, for example, the *Sebastosomus* (Rocha-Olivares et al. 1999b) and *Pteropodus* (Taylor 1998). Numerous field studies of adults, juveniles, and larvae have also attempted to group rockfishes by similarities in adult niches or juvenile life history. For example, Lenarz et al. (1995) observed that pelagic juveniles could be grouped by depth of occurrence and relative abundance in El Niño and normal years. They noted that these coherent groups corresponded to adult guilds such as structure schoolers (fish that tend to congregate near large objects like rocky reefs, oil platforms, etc.) and nearshore demersals. It has been particularly gratifying that morphological characters and ecological behaviors tend to overlay phylogenetic groupings determined by molecular methods. The genetic lineage known as the *Pteropodus* rockfishes directly corresponds to the pelagic juvenile and adult assemblages described by Lenarz et al. as the nearshore demersals and the *Sebastomas* lineage to the structure schoolers. The value of considering these subgeneric lineages continues to be a useful organizing principle for interpreting field observations.

Most scorpaenid fishes have a sedentary adult stage with dispersal largely confined to the pelagic larval and juvenile stages. The descriptions of various patterns of adult population genetic structure have allowed us to infer characteristic patterns of larval dispersal, identify geographic or oceanographic barriers to dispersal, and make management recommendations regarding population connectivity and appropriate units of management. In a recent book chapter (Gunderson and Vetter 2006), I proposed four types of metapopulation structure that might be expected in sedentary groundfish species with various pelagic larval dispersal strategies (Figure 1). Possible patterns range from broadly dispersing species with no genetic structure to non-dispersing species with virtually closed populations and a high degree of genetic structure. The shortspine thornyhead (*Sebastolobus alascanus*) and the longspine thornyhead (*S. altivelis*) produce eggs and have mesopelagic juvenile stages that can last more that a year. They are thought to be an example of broad dispersal and recruitment from a common larval pool (Figure 1, far left). In this case there is little to no population genetic structure in mitochondrial control region DNA (Stepien et al. 2000). A study of population genetic structure in the broadly distributed rosethorn rockfish (*Sebastes helvomaculatus*) shows mesoscale population genetic structure with a separation that corresponds with the divergent flows of the Alaska Gyre and the California Current (Rocha-Olivares and Vetter 1999) and Figure 2. In this scenario larvae are thought to be advected away from their natal habitat but are dispersed via oceanographic features (Figure 1, middle left). In other species of rockfishes larvae remain nearshore and may never enter advective currents. Studies of copper rockfish (*S. caurinus*), grass rockfish (*S. rastrelliger*), and brown rockfish (*S. auriculatus*) all seem to show that in nearshore kelp habitats larvae may avoid offshore advective flows and may be largely self-recruiting but with limited diffusive dispersal of larvae to neighboring rocky habitats (Buonaccorsi et al. 2002, 2004, 2005). This dispersal strategy produces a series of small, stepping-stone metapopulations characterized by a strong isolation-by-distance genetic structure (Figure 1, middle right). The pattern of very limited dispersal (Figure 1, far right) has not been observed in scorpaenid fishes but has been observed in the surfperches (Embiotocidae), which produce large precocious juveniles that do not have a pelagic dispersal phase (Bernardi 2000).

Population genetic studies of rockfishes and related scorpaenid fishes have been used to inform management decisions in several important ways: 1) defining appropriate management

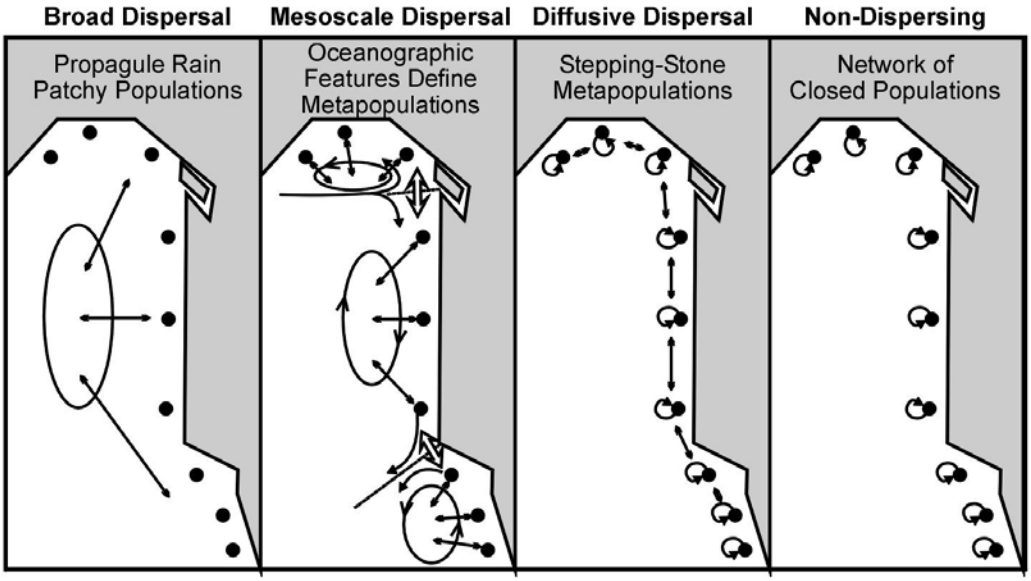

Figure 1. Propagule dispersal models and population structure: (far left) broad advective dispersal typical of species with extended early planktonic stages; (middle left) mesoscale dispersal typical of species whose early life stages develop in the plankton but are retained in an oceanographic feature such as the Southern California eddy; (middle right) diffusive dispersal describes nearshore species whose eggs and larvae remain in nearshore boundary layers subject to diffusive rather than advective flows; and (far right) non-dispersing describes species that produce large precocious young capable of swimming and not subject to passive dispersal in currents (after Gunderson and Vetter 2006).

units for stock assessments, 2) defining distinct population segments (DPSs) for Endangered Species Act (ESA) listings, and 3) estimating realized larval dispersal distances and the design of marine protected area (MPA) networks. The study of copper rockfish (Buonaccorsi et al. 2002) formed the basis for declaring Puget Sound populations of copper rockfish as a DPS under the procedures of the ESA. Avoiding potential pitfalls in the design of MPAs is illustrated by a recent study of grass rockfish (Buonaccorsi et al. 2004). Here we showed that, for nearshore rockfish species targeted for management via MPAs, the genetic signal of isolation by distance could be used to calculate mean dispersal distances (Figure 3). The finding that these species have mean dispersal distances on the order of tens of kilometers is quite remarkable and suggests that many small MPAs spaced over shorter distances rather than a few large reserves might be more effective for the maintenance of population connectivity. It is politically unrealistic for MPAs to be spaced every 10 km. This practical reality suggests that intervening fished areas must be managed such that some individuals live to reproduce and act as multigenerational stepping stones that can provide genetic connectivity between MPAs.

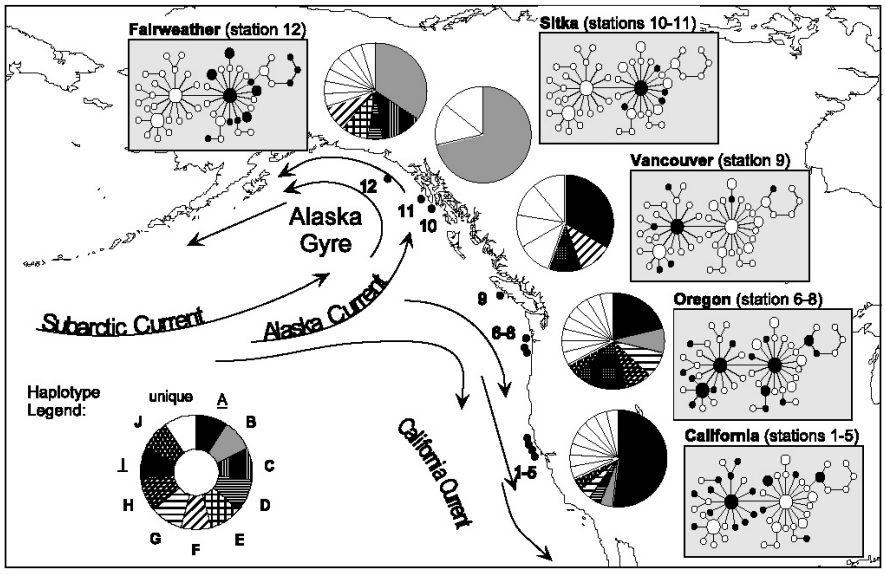

Figure 2. Map of study area showing the sampling stations (1–12) for rosethorn rockfish. In each putative population, pie charts represent the frequency of 10 haplotype classes (A–J) defined by ignoring autapomorphic sites. Classes A and I (underlined) belong to clade A. Classes B–H and J (boldface) belong to group B. Unique haplotypes can be in either one. In each population solid circles in the minimum spanning network indicate the presence of a given haplotype. The major oceanographic features of the northeast Pacific are also presented (after Rocha-Olivares and Vetter 1999).

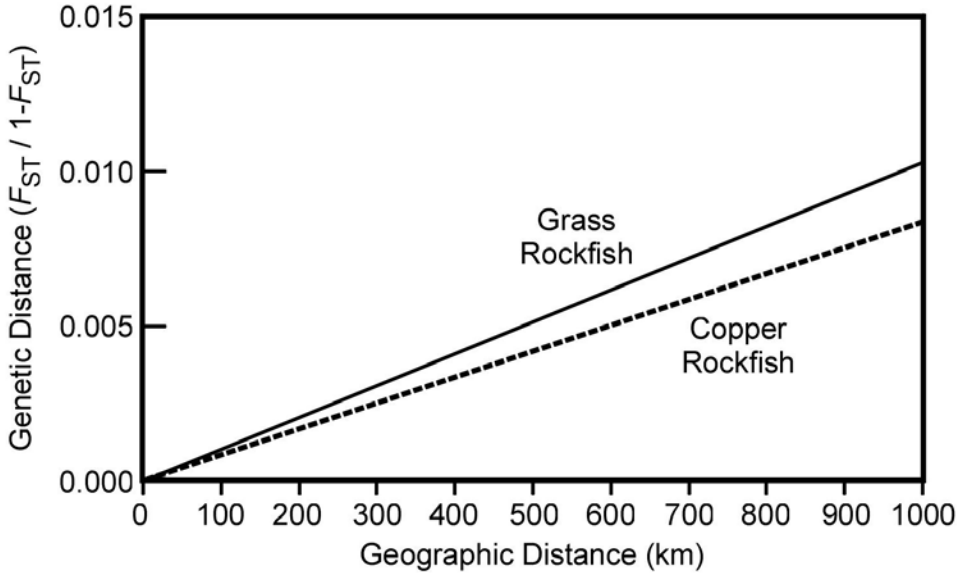

Figure 3. Comparison of isolation-by-distance coastal regression slopes for grass rockfish and copper rockfish. For the purpose of comparing slopes the y-intercept was set to zero (after Buonaccorsi et al. 2004).

References

Bernardi, G. 2000. Barriers to gene flow in *Embiotoca jacksoni*, a marine fish lacking a pelagic larval stage. Evolution 54:226–237.

Buonaccorsi, V. P., C. A. Kimbrell, E. A. Lynn, and R. D. Vetter. 2002. Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. Can. J. Fish. Aquat. Sci. 59:1374–1384.

Buonaccorsi, V. P., M. Westerman, J. Stannard, C. Kimbrell, E. Lynn, and R. D. Vetter. 2004. Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. Mar. Biol. 145:779–788.

Buonaccorsi, V. P., C. A. Kimbrell, E. A. Lynn, and R. D. Vetter. 2005. Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. Conserv. Genet. 6:697–713.

Gunderson, D., and R. D. Vetter. 2006. Temperate rocky reef fishes. *In* J. P. Kritzer and P. F. Sale (eds.), Marine metapopulations. Academic Press, Boston.

Lenarz, W. H., D. A. Ventresca, W. M. Graham, F. B. Schwing, and F. Chavez. 1995. Explorations of El Niño events and associated biological population dynamics off Central California. CalCOFI Rep. 36:106–119.

Narum, S. R., V. P. Buonaccorsi, C. A. Kimbrell, and R. D. Vetter. 2004. Genetic divergence between gopher rockfish (*Sebastes carnatus*) and black-and-yellow rockfish (*Sebastes chrysomelas*). Copeia 2004(4):926–931.

Rocha-Olivares, A., C. A. Kimbrell, B. J. Eitner, and R. D. Vetter. 1999a. 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*. Mol. Phylogenet. Evol. 11:426–440.

Rocha-Olivares, A., R. H. Rosenblatt, and R. D. Vetter. 1999b. Molecular evolution, systematics and zoogeography of the rockfish subgenus *Sebastosomus* (*Sebastes*, Scorpaenidae) based on mitochondrial cytochrome *b* and control region sequences. Mol. Phylogenet. Evol. 11:441–458.

Rocha-Olivares, A., and R. D. Vetter. 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus)*. Can. J. Fish. Aquat. Sci. 56:803–813.

Stepien, C. A., A. K. Dillon, and A. K. Patterson. 2000. Population genetics, phylogeography, and systematics of the thornyhead rockfishes (*Sebastolobus*) along the deep continental slopes of the North Pacific Ocean. Can. J. Fish. Aquat. Sci. 57:1701–1717.

Taylor, C. A. 1998. Phylogenetics and larval identification of rockfishes (genus *Sebastes*) using mitochondrial DNA techniques. Master's thesis. San Diego State Univ., San Diego, CA.

Appendix A: Rockfish Microsatellite Loci in GenBank

GenBank is a collection of publicly available DNA sequences, which is maintained by the National Center for Biotechnology Information, a part of the National Institute of Health. The following species in the genus *Sebastes* are identified by scientific name in Table A-1:

acadian redfish (*S. fasciatus*)
black rockfish (*S. melanops)*
blue rockfish (*S. mystinus*)
bocaccio (*S. paucispinus*)
brown rockfish (*S. auriculatus*)
canary rockfish (*S. pinniger*)
China rockfish (*S. nebulosus*)
copper rockfish (*S. caurinus*)
darkblotched rockfish (*S. crameri*)
deepwater redfish (*S. mentella*)
dusky rockfish (*S. ciliatus*)
grass rockfish (*S. rastrelliger*)
kitsunemebaru (*S. vulpes*)
Schlegel's black rockfish (*S. schlegeli*)
darkbanded rockfish (*S. inermis*)
Norway or small redfish (*S. viviparus*)
ocean perch (*S. marinus*)
Pacific ocean perch (*S. alutus*)
quillback rockfish (*S. maliger*)
redbanded rockfish (*S. babcocki*)
rougheye rockfish (*S. aleutianus*)
shortraker rockfish (*S. borealis*)
silvergray rockfish (*S. brevispinis*)
splitnose rockfish (*S. diploproa*)
tiger rockfish (*S. nigrocinctus*)
togotto-mebaru (*S. joyneri*)
vermilion rockfish (*S. miniatus*)
widow rockfish (*S. entomelas*)
yellowtail rockfish (*S. flavidus*)
yelloweye rockfish (*S. ruberrimus*)
goldeye rockfish (*S. thompsoni*)

Table A-1. Rockfish (*Sebastes* spp.) microsatellite loci found in GenBank. H_O is the observed heterozygosity: 0 indicates a value of zero and a dash (—) indicates no data. If the variability of the locus was tested in other species, the results are noted here.

Locus	Primer sequences (5'–3')	Repeat motif	Species	H_O	Source[a]	GenBank number[b]	Comments
*Sal*1	CCAAGTGTTTGTTTGAT ATGCTCAGCACAGTAATTA	tet	*S. alutus*	0.87	Miller et al. 2000	AF153595	Linked to *Sal*6; variable in 12 of 13 species tested
*Sal*2	CTCTGTTTGGTATTTAACCTG GTAAAAGGAATCCACCTAAC	tet		0.78	Miller et al. 2000	AF153596	Variable in 11 of 13 species tested
*Sal*3	TCCCCATAAAGACAAACTGTAGC ACAGCAGACAGCAGTTCT	pent		0.83	Miller et al. 2000	AF153597	Variable in all 13 species tested: *S. auriculatus*, *S. brevispinis*, *S. caurinus*, *S. entomelas*, *S. flavidus, S. maliger, S. melanops*, *S. nigrocinctus*, *S. pinniger*, *S. babcocki*, *S. aleutianus*, *S. ruberrimus, S. mentella*
*Sal*4	GGTGATATGATAGATTGCAG AATGATGGACGGATGTATAG	tet		0.79	Miller et al. 2000	AF153598	Variable in 9 of 13 species tested
*Sal*5	GGTGCGAGTGGAAATAATCT AATAAAGTTTACTATCTATC	tet		0.71	Miller et al. 2000	AF153599	Redesigned F primer to ATTCAAGGACTGTGGGTGC and was polymorphic in 10 of 13 species but had 1–2 nonoverlapping loci
*Sal*6	GGGCGTCCAGGGTTTCCTC AATCACCACATGCATCA	di		0.50	Miller et al. 2000	AF153595	Linked to *Sal*1; polymorphic in 9 of 13 species
*Seb*9	AAGGCTGACTCTGAGTGGGA CTCTGAGTCTATGTATCTGGCT	di	See comments	0.48–0.96	Roques et al. 1999	AF103018	Designed from DNA pooled from *S. fasciatus, S. mentella, S. marinus,* and *S. viviparus*
*Seb*25	CAGCTTGACGTGAGGGGA GTGCCTGTTTAGGGTGTTCTT	di	See comments	0.71–0.96	Roques et al. 1999	AF103023	Designed from DNA pooled from *S. fasciatus, S. mentella, S. marinus,* and *S. viviparus*
*Seb*30	CTGTTGGACAGATAAAGACGC GGTGATATTGCTGCTGGTAGAT	di	See comments	0.60–0.90	Roques et al. 1999	AF103025	Designed from DNA pooled from *S. fasciatus, S. mentella, S. marinus,* and *S. viviparus*
*Seb*31	GTGAGACCAGTAATAAGGGCA TACTTCTCGACTGTGGTG	di	See comments	0.67–0.93	Roques et al. 1999	AF103019	Designed from DNA pooled from *S. fasciatus, S. mentella, S. marinus,* and *S. viviparus*

Table A-1 continued. Rockfish (*Sebastes* spp.) microsatellite loci found in GenBank. H_O is the observed heterozygosity: 0 indicates a value of zero and a dash (—) indicates no data. If the variability of the locus was tested in other species, the results are noted here.

Locus	Primer sequences (5'–3')	Repeat motif	Species	H_O	Source[a]	GenBank number[b]	Comments
*Seb*33	CAGATGTTGGTAGACGCAAGCA AGTCCAGTGTCCATCCTCCTT	di	See comments	0.89–1.00	Roques et al. 1999	AF103020	Designed from DNA pooled from *S. fasciatus*, *S. mentella*, *S. marinus*, and *S. viviparus*
*Seb*37	GTACAGTCCATTCAGCTTTGA AGGGTGTGTGGAAGAAATAGT	di	See comments	0.61–0.89	Roques et al. 1999	AF103022	Designed from DNA pooled from *S. fasciatus*, *S. mentella*, *S. marinus*, and *S. viviparus*
*Seb*45	GAGGAGGAAAAGACTGGACAGA GAAAGATGGTGAGCAGCGATGA	di	See comments	0.54–0.73	Roques et al. 1999	AF103021	Designed from DNA pooled from *S. fasciatus*, *S. mentella*, *S. marinus*, and *S. viviparus*
*Seb*46	GCTGATGTTGCTCCTAAAGAA CTCTTCATGTCAATCCTGCCT	di	See comments	0.67–0.87	Roques et al. 1999	AF103024	Designed from DNA pooled from *S. fasciatus*, *S. mentella*, *S. marinus*, and *S. viviparus*
*Sma*1	AAGTGAGTGGGTTTCATTGAGATAC TACCTGTGTCAGCAAGTAAACTCTG	di	*S. maliger*	—	Wimberger et al. 1999	AY654593	Appears to amplify two loci; variable in *S. caurinus*, *S. auriculatus*, *S. melanops*, *S. ciliatus*, *S. borealis*, *S. aleutianus*, *S. paucispinis*, and *S. alutus*
*Sma*2	TTACTTGTTTTCTTTGTCTCATGTGG CACAGGACTATCAGCAGGGAAG	di	*S. maliger*	0.47	Wimberger et al. 1999	AY654594	Variable in *S. caurinus*, *S. auriculatus*, *S. ciliatus*, *S. borealis*, *S. aleutianus*, *S. paucispinus*, and *S. alutus*
*Sma*3	GCAGACTTACAGCGGTTTCAC ACCATCCAGTCATACGAGCAC	di	*S. maliger*	0.68	Wimberger et al. 1999	AY654595	Variable in *S. maliger*, *S. caurinus*, *S. ruberrimus*, *S. melanops*, *S. ciliatus*, *S. borealis*, *S. aleutianus*, *S. paucispinis*, and *S. alutus*
*Sma*4	CATAACATATGATGGAAAATAAAACCC CAAATTGCCCCCACTGAAG	tri	*S. maliger*	0.66	Wimberger et al. 1999	AY654596	Variable in *S. maliger*, *S. caurinus*, *S. auriculatus*, *S. borealis*, *S. aleutianus*, *S. paucispinis*, and *S. alutus*
*Sma*5	ATTCCCACCCACTCACACTT GAGATTTCTGGAGTCCACGC	di	*S. maliger*	0.38	Wimberger et al. 1999	AY654597	Variable in *S. maliger*, *S. caurinus*, *S. borealis*, *S. aleutianus*, *S. paucispinis*, and *S. alutus*
*Sma*6	ATGATGAAGTGTCGGTTGCTC AAGGGAGGGCACCCCAAAC	di	*S. maliger*	0	Wimberger et al. 1999	AY654598	Not enough information to determine variability in other species
*Sma*7	CATAGGTCATTTCTCAAAGGTGTG GAGAAACAAACAGGAACTGAGAGAG	di	*S. maliger*	0	Wimberger et al. 1999	AY654599	Variable in *S. melanops*, *S. ciliatus*, *S. borealis*, *S. aleutianus*, *S. paucispinis*, *S. alutus*, and *Helicolenus* sp.

Table A-1 continued. Rockfish (*Sebastes* spp.) microsatellite loci found in GenBank. H_O is the observed heterozygosity: 0 indicates a value of zero and a dash (—) indicates no data. If the variability of the locus was tested in other species, the results are noted here.

Locus	Primer sequences (5'–3')	Repeat motif	Species	H_O	Source[a]	GenBank number[b]	Comments
*Sma*8	TCACACATCGTCTGGCTATTTC AATTCTAAAGTCTATGGGGACACG	di	*S. maliger*	0	Wimberger et al. 1999	AY654600	Not enough information to determine variability in other species
*Sma*9	GATCACCGTTATTGCTTGAGTG CTTTTAACGGTTTTTACCGATAGC	di	*S. maliger*	0	Wimberger et al. 1999	AY654601	Not enough information to determine variability in other species
*Sma*10	CAGCAGCATTTGAAGCACATC CACCAGTGGAACACACGCAC	di	*S. maliger*	0.79	Wimberger et al. 1999	AY654602	Variable in *S. maliger*, *S. caurinus*, *S. auriculatus*, *S. ciliatus*, *S. borealis*, *S. aleutianus*, *S. paucispinis*, *S. alutus*, and *Helicolenus* sp.
*Sma*11	AATATGAGGACGGGCAACG TAGTAGGAGTGAGCATCGTACCAG	di	*S. maliger*	0	Wimberger et al. 1999	AY654603	Variable in *S. caurinus*, *S. ciliatus*, *S. borealis*, *S. aleutianus*, *S. paucispinis*, and *S. alutus*
*Sma*12	AACTGAGAAGAAAAGGGAGCG GACAAAGTGGAGCAGAGAGTCC	irreg.	*S. maliger*	0	Wimberger et al. 1999	AY654604	Not enough information to determine variability in other species
Sme1	GCAACAACTCTGGCACAC GTGGAAAAAATGTCCTCCTC	tet	*S. melanops*	—	ADFG unpubl. data	AF141663	Primer sequences estimated from GenBank
Sme2	TGCCAGCTCTTTACTGTTATT CATCCTTCACTCCTTCACTC	tet	*S. melanops*	—	ADFG unpubl. data	AF142484	Primer sequences estimated from GenBank
Sme3	CAGCAAGGTAAGAGTAAACTGC GCTTTCTGGAGAAGTCGAGT	tet	*S. melanops*	—	ADFG unpubl. data	AF142485	Primer sequences estimated from GenBank
*Sme*4	GCAGCCAACTACTAACTAACAGC TTGCACGCTTTGCTAATGTA	tet	*S. melanops*	—	ADFG unpubl. data	AF142486	Primer sequences estimated from GenBank
Sme5	TGTTTTGCACTATGGAACTGT CCAAATTCATCTCAGAACTCTC	tet	*S. melanops*	—	ADFG unpubl. data	AF142487	Primer sequences estimated from GenBank
Sme6	ATTCAAACCTTAGCCACAGT CACGGGGATAGATAAAGC	tet	*S. melanops*	—	ADFG unpubl. data	AF142488	Primer sequences estimated from GenBank
Sme7	GGAACTGTGATGCTGGAAAC CAGGGAAAACCCCAAACTAT	tet	*S. melanops*	—	ADFG unpubl. data	AF142489	Primer sequences estimated from GenBank; amplifies 2 loci in *S. flavidus* (Young et al. unpubl. data)
Sme8	GGAACTGTGATGCTGGAAAC GGGTCAACTATGATGAACGG	tet	*S. melanops*	—	ADFG unpubl. data	AF142490	Primer sequences estimated from GenBank
*Sme*9	TGATGCACAACACTAAAACAAG TCCCTTGGGATCAATAAAGT	tet	*S. melanops*	—	ADFG unpubl. data	AF142491	Primer sequences estimated from GenBank

Table A-1 continued. Rockfish (*Sebastes* spp.) microsatellite loci found in GenBank. H_O is the observed heterozygosity: 0 indicates a value of zero and a dash (—) indicates no data. If the variability of the locus was tested in other species, the results are noted here.

Locus	Primer sequences (5'–3')	Repeat motif	Species	H_O	Source[a]	GenBank number[b]	Comments
Sme10	TGTTCAAAGGTCAAGGTCACT GTGTCGCTGTTTCCACTGT	tet	*S. melanops*	—	ADFG unpubl. data	AF142492	Primer sequences estimated from GenBank
*Sme*11	GACAATAACCTCCCATGTCTG CCCTCGGGATCAATAAAGT	tet	*S. melanops*	—	ADFG unpubl. data	AF142493	Primer sequences estimated from GenBank
*Sme*12	TGAATTTCCCTCAGGATCAA ATGGGAGAACCGTGATCATA	tet	*S. melanops*	—	ADFG unpubl. data	AF142494	Primer sequences estimated from GenBank
Sme13	AACGCTCCGTTTTAGACAGAC ATGGAACTGTGTCGCTGTAAA	tet	*S. melanops*	—	ADFG unpubl. data	AF142495	Primer sequences estimated from GenBank
Sme14	TTATACAAAGGACCTCCCCC TTCAGATGTCAGGTCACCCT	tet	*S. melanops*	—	ADFG unpubl. data	AF142587	Primer sequences estimated from GenBank
Sme15	TTGAATTTCCCTCTGGATCA TGTCGCTTCACACAGGTAGA	tet	*S. melanops*	—	ADFG unpubl. data	AF142496	Primer sequences estimated from GenBank
*Spi*4	GTCAGAGTTACATAGCGTGCCCT GCACTATGGAACTGTGATTCTGGA	tet	*S. pinniger*	0.86	Gomez-Uchida et al. 2003	AY192599	Variable in *S. ruberrimus, S. caurinus, S. crameri, S. diploproa, S. mystinus, S. maliger, S. nigrocintus, S. miniatus,* and *S. melanops*
*Spi*6	AGTGGAAGTGAACACGTAGGTTAG CACTATGGAACTGTGATGCTGG	tet	*S. pinniger*	0.94	Gomez-Uchida et al. 2003	AY192600	Variable in *S. nebulosus, S. ruberrimus, S. caurinus, S. crameri, S. diploproa, S. mystinus, S. maliger, S. nigrocintus, S. miniatus, S. melanops,* and *S. auriculatus*
*Spi*7	CTGTCTTTGTCACTGTAATCATAGTCA GATCTGGAGTCAGATGGATAGATG	tet	*S. pinniger*	0.07	Gomez-Uchida et al. 2003	AY192601	Departure from Hardy-Weinberg—possible null alleles; variable in *S. mystinus, S. miniatus,* and *S. melanops*
*Spi*9	CATTCTTACGCACCGATCTG GAGTTTTCTTCATCTCCTTGTATTTT	tet	*S. pinniger*	0.11	Gomez-Uchida et al. 2003	AY192602	Departure from Hardy-Weinberg—possible null alleles; variable in *S. mystinus, S. miniatus, S. melanops,* and *S. flavidus*
*Spi*10	TTTGATGGCCTGAAACTGAG GTTCAAACACACAGTAGCTAAACTATC	tet	*S. pinniger*	0.77	Gomez-Uchida et al. 2003	AY192603	Variable in *S. nebulosus, S. ruberrimus, S. caurinus, S. crameri, S. diploproa, S. mystinus, S. maliger, S. nigrocintus, S. miniatus, S. melanops, S. flavidus,* and *S. auriculatus*

Table A-1 continued. Rockfish (*Sebastes* spp.) microsatellite loci found in GenBank. H_O is the observed heterozygosity: 0 indicates a value of zero and a dash (—) indicates no data. If the variability of the locus was tested in other species, the results are noted here.

Locus	Primer sequences (5'–3')	Repeat motif	Species	H_O	Source[a]	GenBank number[b]	Comments
*Spi*12	GGGAGTATGAGAGAGGATCATGC CAATACGCCTCCAAGCTAGATC	di	*S. pinniger*	0.40	Gomez-Uchida et al. 2003	AY192604	Variable in *S. nebulosus*, *S. ruberrimus*, *S. caurinus*, *S. crameri*, *S. diploproa*, *S. maliger*, and *S. nigrocintus*
*Spi*14	CCAGCAGCTTGGATAGATAGTTAG GCTGGAAATACATTACACTGTTTAGTC	tet	*S. pinniger*	0.91	Gomez-Uchida et al. 2003	AY192605	Variable in *S. nigrocintus*
*Spi*17	TGTTGGTTAATTACATGCTGGA TATTCCCAGCAGCTTGGATA	tet	*S. pinniger*	0.91	Gomez-Uchida et al. 2003	AY192606	Didn't amplify in any other species
*Spi*18	GTACAAGAAGTTAAAAAGCAAGTTGCAG GCGTGTTTGCACTAACCTTTGT	tet	*S. pinniger*	0.76	Gomez-Uchida et al. 2003	AY192607	Variable in *S. caurinus* and *S. miniatus*
Sra.5-9	CTTGCTACTGCAGAGTGACTAC CCTCATAATAGAGCTTGTAATAACG	di	*S. rastrelliger*	0.35	Westerman et al. 2005	AF269052	
Sra.5-32	GTGAGGAGGTTAAGATGACCG AGCACACACGTCTAAAACACT	di	*S. rastrelliger*	—	Westerman et al. unpubl. data	AF269053	Primer sequences estimated from GenBank
Sra.6-52	ATCGGGTGTCCTTCAGTCAG CGCTTTAATTTCCCGTTGAA	di	*S. rastrelliger*	—	Westerman et al. unpubl. data	AF269057	Primer sequences estimated from GenBank
Sra.7-2	GAACATCCCTCCTTCCGACGC GTCAAACAACTGCAGAATGTTCG	di	*S. rastrelliger*	0.43	Westerman et al. 2005	AF269054	
Sra.7-7	GCATGAAAGTGTATGAAAGGC CATGTGATTCTGTGTCTAACTGAG	di	*S. rastrelliger*	0.72	Westerman et al. 2005	AF269055	
Sra.7-25	GACCTTTCCCTGAACACACTCG CAAGAGGCGGTGGTGCTGATGG	di	*S. rastrelliger*	0.85	Westerman et al. 2005	AF269056	
Sra.11-103	CTTGCAGGTAACGGGAAGG GGCTGATGACATTGCAACCTTG	tri	*S. rastrelliger*	—	Westerman et al. 2005	AF269058	
Sra.15-8	GGAGATGTGCGTGGCTCGTCTGG GGGTTTACTCATTGTAGAC	tet	*S. rastrelliger*	0.87	Westerman et al. 2005	AF269059	
Sra.15-23	CCCCAAATACTGTCTTGCCAG CCGTCTTGATCCAGATGGTACATGTC	di	*S. rastrelliger*	—	Westerman et al. unpubl. data	AF269060	Primer sequences estimated from GenBank; monomorphicin design organism
Sra.16-5	CCATCTGTGCTGAGCTGTCACTG GAGAAGAGGCCTACAAGTACC	tet	*S. rastrelliger*	0.93	Westerman et al. 2005	AF269061	
*Ssc*1	AAGTCAACCCATCAGAAGCTGT GGTTGTTCCACTGTGAAGTCTG	di	*S. schlegeli*	0.07	Yoshida et al. 2005	AB058404	

Table A-1 continued. Rockfish (*Sebastes* spp.) microsatellite loci found in GenBank. H_O is the observed heterozygosity: 0 indicates a value of zero and a dash (—) indicates no data. If the variability of the locus was tested in other species, the results are noted here.

Locus	Primer sequences (5'–3')	Repeat motif	Species	H_O	Source[a]	GenBank number[b]	Comments
*Ssc*12	AACACGCTGAACAGAGAACAAA GCTCCGACTATAGCTGGTCCTA	di	*S. schlegeli*	0.84	Yoshida et al. 2005	AB058405	
*Ssc*71	GATCCCTCCTTTCCTTTCAGGAT GCCTCATCGCATTGCTACAACA	di	*S. schlegeli*	0.43	Yoshida et al. 2005	AB058409	
*Ssc*23	AGTGTCATGCCCTCTTCCAG CACTCGGCATTCTCACCTCA	di	*S. schlegeli*	0.90	Yoshida et al. 2005	AB058406	
*Ssc*51	GTGCTGATGGAAAACACTACCA GTGACCTTTCCCTGAACACACT	di	*S. schlegeli*	0.89	Yoshida et al. 2005	AB058407	
*Ssc*69	GGCACCGAGCTCAACCTTACTG TGCTGTGACTATTTCCCTCTGGC	di	*S. schlegeli*	0.82	Yoshida et al. 2005	AB058408	
Sth 3A	ATGGTGACAAGCTAGCAGTGCATTC GACAATGTCCATCTAGGCATGACTG	di	*S. thompsoni*	0.60	Sekino et al. 2000	AB033424	Variable in *S. inermis, S. joyneri*, and *S. vulpes*
Sth 3B	GTCATGCCTAGATGGACATTGTCTAC GAGATAAGAGGAGTTTGAAGGCAGAG	di	*S. thompsoni*	0.92	Sekino et al. 2000	AB033425	Variable in *S. inermis* and *S. vulpes*
Sth 24	AGGACAGGATGTGCCCTTTTACCA GCCTCAGAGGCCGATTTCCTTATT	di	*S. thompsoni*	0.46	Sekino et al. 2000	AB033426	Variable in *S. joyneri*
Sth 37	TACAGGAAACAAGACCACGGGTACAG GCAACATCCCTTTAAGTCACCTGCAG	di	*S. thompsoni*	0.88	Sekino et al. 2000	AB033427	Variable in *S. inermis, S. joyneri*, and *S. vulpes*
Sth 45	CTGGGACCTAGCCTGATTACAGCA AAACTCAGCGACAGCAGACCACA	di	*S. thompsoni*	0.71	Sekino et al. 2000	AB033428	Variable in *S. inermis* and *S. vulpes*
Sth 56	CAGCAGCTCCAGTTCAGTGTATGT GGATTGATCCTCATGTGGTGCTCT	di	*S. thompsoni*	0.74	Sekino et al. 2000	AB033429	Variable in *S. inermis, S. joyneri*, and *S. vulpes*
Sth 86	ACCATCACCCACTGTAAACTGCA TACCAGGAAACGTCGTGTCTCAA	di	*S. thompsoni*	0.82	Sekino et al. 2000	AB033430	Variable in *S. inermis* and *S. vulpes*
Sth 91	TTTCGATATGCTTCGCTAGGGTGTT CCATCAAACTGCACCAACAAAGACA	di	*S. thompsoni*	0.30	Sekino et al. 2000	AB033431	Variable in *S. inermis* and *S. vulpes*

[a] ADFG (Alaska Department of Fish and Game). Unpubl. data. Fifteen microsatellite loci including tri- and tetranucleotide loci cloned from black rockfish. (Available from Alaska Dept. of Fish and Game, 333 Raspberry Rd., Anchorage, AK 99518.)

Gomez-Uchida, D., E. A. Hoffman, W. R. Ardren, and M. A. Banks. 2003. Microsatellite markers for the heavily exploited canary (Sebastes pinniger) and other rockfish species. Mol. Ecol. Notes 3:387–389.

Miller, K. M., A. D. Schulze, and R. E. Withler. 2000. Characterization of microsatellite loci in *Sebastes alutus* and their conservation in congeneric rockfish species. Mol. Ecol. 9:240–242.

Roques, S., D. Pallotta, J.-M. Sevigny, and L. Bernatchez. 1999. Isolation and characterization of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei: Scorpaenidae, genus *Sebastes*). Mol. Ecol. 8:685–702.

Sekino, M., N. Takagi, M. Hara, and H. Takahashi. 2000. Microsatellites in rockfish *Sebastes thompsoni* (Scorpaenidae). Mol. Ecol. 9:634.

Westerman, M. E., V. P. Buonaccorsi, J. A. Stannard, L. Galver, C. Taylor, E. A. Lynn, C. A. Kimbrell, and R. D. Vetter. 2005. Cloning and characterization of novel microsatellite DNA markers for the grass rockfish, *Sebastes rastrelliger*, and cross-species amplification in 10 related *Sebastes* spp. Mol. Ecol. Notes 5:74–76.

Westerman, M. E., V. P. Buonaccorsi, and R. D. Vetter. Unpubl. data. Isolation and characterization of novel polymorphic microsatellite markers from the grass rockfish, *Sebastes rastrelliger*. Cited in http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=10179897 [accessed 24 April 2007].

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

Yoshida, K., M. Nakagawa, and S. Wada. 2005. Multiplex PCR system applied for analyzing microsatellite loci of Schlegel's black rockfish, *Sebastes schlegeli*. Mol. Ecol. Notes 5:416–418.

Young, S. F., A. W. Pichahchy, and J. F. Von Bargen. Unpubl. data. A microsatellite DNA analysis of population subdivision in yellowtail rockfish (*Sebastes flavidus*) along the Pacific coast of North America. (Available from S. F. Young, Washington Dept. of Fish and Wildlife, youngsfy@dfw.wa.gov.)

[b] The GenBank database is online at http://www.ncbi.nlm.nih.gov/GenBank [accessed 27 October 2006].

Recent NOAA Technical Memorandums

published by the
Northwest Fisheries Science Center

NOAA Technical Memorandum NMFS-NWFSC-

79 **Lawson, P.W., E.P. Bjorkstedt, M.W. Chilcote, C.W. Huntington, J.S. Mills, K.M.S. Moore, T.E. Nickelson, G.H. Reeves, H.A. Stout, T.C. Wainwright, and L.A. Weitkamp. 2007.** Identification of historical populations of Coho salmon *(Oncorhynchus kisutch)* in the Oregon coast evolutionarily significant unit. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-79, 129 p. NTIS number pending.

78 **Ruckelshaus, M.H., K.P. Currens, W.H. Graeber, R.R. Fuerstenberg, K. Rawson, N.J. Sands, and J.B. Scott. 2006.** Independent populations of Chinook salmon in Puget Sound. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-78, 125 p. NTIS number PB2007-104920.

77 **Sloan, C.A., D.W. Brown, G.M. Ylitalo, J. Buzitis, D.P. Herman, D.G. Burrows, G. Yanagida, R.W. Pearce, J.L. Bolton, R.H. Boyer, and M.M. Krahn. 2006.** Quality assurance plan for analyses of environmental samples for polycyclic aromatic compounds, persistent organic pollutants, fatty acids, stable isotope ratios, lipid classes, and metabolites of polycyclic aromatic compounds. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-77, 30 p. NTIS number PB2007-104919.

76 **Gustafson R.G., J. Drake, M.J. Ford, J.M. Myers, E.E. Holmes, and R.S. Waples. 2006.** Status review of Cherry Point Pacific herring *(Clupea pallasii)* and updated status review of the Georgia Basin Pacific herring distinct population segment under the Endangered Species Act. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-76, 182 p. NTIS number PB2007-104918.

75 **Keller, A.A., B.H. Horness, V.J. Tuttle, J.R. Wallace, V.H. Simon, E.L. Fruh, K.L. Bosley, and D.J. Kamikawa. 2006.** The 2002 U.S. West Coast upper continental slope trawl survey of groundfish resources off Washington, Oregon, and California: Estimates of distribution, abundance, and length composition. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-75, 189 p. NTIS PB2006-111432.

74 **Pool, S.S., and R.D. Brodeur. 2006.** Neustonic mesozooplankton abundance and distribution in the northern California Current, 2000 and 2002. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-74, 76 p. NTIS PB2006-109275.

73 **Myers, J., C. Busack, D. Rawding, A. Marshall, D. Teel, D.M. Van Doornik, and M.T. Maher. 2006.** Historical population structure of Pacific salmonids in the Willamette River and lower Columbia River basins. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-73, 311 p. NTIS PB2006-109278.

72 **Keller, A.A., E.L. Fruh, K.L. Bosley, D.J. Kamikawa, J.R. Wallace, B.H. Horness, V.H. Simon, and V.J. Tuttle. 2006.** The 2001 U.S. West Coast upper continental slope trawl survey of groundfish resources off Washington, Oregon, and California: Estimates of distribution, abundance, and length composition. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-72, 175 p. NTIS PB2006-109277

71 **Nash, C.E., P.R. Burbridge, and J.K. Volkman (editors). 2005.** Guidelines for ecological risk assessment of marine fish aquaculture. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-71, 90 p. NTIS PB2006-109287.

Most NOAA Technical Memorandums NMFS-NWFSC are available online at the Northwest Fisheries Science Center web site (http://www.nwfsc.noaa.gov).