

# Genetic Identification of Progeny of Reef-Resident Brown Rockfish (*Sebastes auriculatus*)

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## Abstract

The extent of larval retention and natal homing in demersal fish is a topic central to the design and the efficacy of marine protected areas (MPAs). Unfortunately, little is known about effective larval dispersal in many marine species. The duration of the pelagic phase in many species suggests extensive dispersal, and population genetic studies indicate large-scale exchange of migrants, though there is also recent evidence for surprisingly limited realized dispersal. Here, we use genetic markers (microsatellites) to identify the offspring of resident adult brown rockfish (*Sebastes auriculatus*) among incoming settling juveniles on an isolated artificial reef at Point Heyer in Puget Sound, thus directly estimating rates of self-recruitment on the reef. Due to low marker variability, unambiguous identification of these offspring from empirical data was not possible. Nevertheless, comparison between parent-offspring matches in observed and simulated genetic data suggested that self-recruitment was less than 10%. One of the juveniles genetically matching an adult was confirmed as its offspring by larval otolith marking, which confirms that self-recruitment does occur. Our data suggested

some, but limited, self-recruitment, that corresponded well to expectations at this scale from mean dispersal distances in brown rockfish.

## Introduction

The extent of dispersal in marine species has attracted great interest among ecologists, evolutionary biologists, and resource managers alike, not only because data on dispersal and retention mechanisms provide powerful insights into the distribution, phylogeography (Feral 2002), and evolution of marine species (Palumbi 1996, Lessios et al. 2001), but also because assumptions about self-recruitment of marine stocks underlie many of the commonly used strategies in fish stock assessment (Cowan and Shaw 2002) and conservation (Planes et al. 2000). With the emphasis on marine protected areas (MPAs) as a tool for marine conservation, the question of realized dispersal of pelagic larvae has found renewed significance, because the function of MPAs in a regional context depends critically on the demographic exchange between the MPA and surrounding areas (Botsford et al. 2003, Palumbi 2003). At one extreme, retention of all life history stages within an MPA negates any positive effects on surrounding areas, while at the other extreme, total export of larvae or juveniles from the MPA may limit the conservation value of the protected area (Palumbi 2003). Some information on realized dispersal from MPAs is therefore required; and, although data on adult migration are accumulating (Pittman and McAlpine 2003), little is known about the effect of larval dispersal, which most likely dominates the level of demographic connectivity of protected areas with surrounding regions.

Unfortunately, there is little information on realized dispersal of marine larvae, and most estimates have been derived indirectly by inferences from current speeds, larval duration, or the genetics of adult populations (Bohonak 1999, Palumbi 2003). Such estimates are inherently imprecise; and, although cross-species correlations between genetic population differentiation (estimating migratory exchange) and larval duration could be demonstrated (e.g., Doherty et al. 1995, Bohonak 1999), the predictive value of such indirect inferences for MPA design remains limited. Furthermore, while some more direct evidence, such as the occurrence of larvae of coastal species in the open ocean (Scheltema 1986) and the rapid spread of marine invasive species with pelagic larvae (e.g., green crab, *Carcinus maenas*, Geller 1994) clearly demonstrate the occurrence of long-distance dispersal, its frequency and thus ecological significance in the short term, especially for MPA design, remains questionable (Palumbi 2003).

Indeed, recent evidence from larval biology suggests that long distance dispersal, although important evolutionarily (Strathmann 1978, Duda and Palumbi 1999), may be rare (Palumbi 2001) and that at least

some of the recruitment in demersal fish and benthic invertebrates may stem from local sources. Oceanographic features such as currents or eddies play an important role in dispersal and retention of pelagic larvae and may also strongly affect larval mortality (Bailey et al. 1997, Withler et al. 2001). Furthermore, behavioral mechanisms, such as vertical migrations exploiting currents at different depths, can greatly influence the direction and extent of horizontal advection (Bilton et al. 2002). Evidence for localized self-recruitment comes from unexpected genetic subdivisions in marine species (Awise 1992, Taylor and Hellberg 2003), the persistence of demersal fish with pelagic larvae on isolated oceanic islands (Hourigan and Reese 1987, White 1998), and information on larval distribution (Bailey et al. 1997, Hay and McCarter 1997) and behavior (Bilton et al. 2002). However, although such evidence suggests predominantly localized recruitment, the quantification of larval export vs. self-recruitment, which is so important for MPA design, remains elusive.

Two major approaches to the estimation of larval dispersal are particularly noteworthy; both have striking results, but they also have some complications. First, chemical signatures within the otoliths, either natural or artificial, can be used to track down the origin of juvenile fish. For example, by marking eggs of coral reef damselfish, *Pomacentrus amboinensis*, with tetracycline, a compound producing fluorescent marks in larval otoliths, Jones et al. (1999) demonstrated that 15-60% of larvae originated from the local adult population on Lizard Island, Great Barrier Reef (about 20 km<sup>2</sup> area). Although that paper clearly demonstrated a relatively high degree of self-recruitment, the confidence limits of the quantitative estimate were wide, mainly due to the large size of the adult population, which limited the proportion of marked eggs to 0.5-2%. A similar approach that exploited natural differences in otolith microchemistry was used to estimate self-recruiting rates of 60-81% to natal spawning sites in weakfish (*Cynoscion regalis*), an estuarine spawning fish in the eastern United States (Thorrold et al. 2001). The estimates of self-recruitment were more precise for weakfish, though the technique relied on differences in chemical composition of the water in natal habitats and may have been limited to estuarine species or more large-scale investigations.

The second main approach used for the estimation of effective larval dispersal is based on genetic differentiation among populations. Most genetic studies find only slight, if any, genetic differentiation among populations of marine species, which greatly complicates the interpretation of data in an applied context, because populations that exchange very few migrants (<10 individuals per generation) cannot be distinguished from a single larger randomly interbreeding population. Under such circumstances, classical population genetic analyses based on Wright's  $F_{ST}$  statistic are inadequate to estimate dispersal on

ecological timeframes (Waples 1998). However, new approaches to data analysis that used more realistic models now allow the estimation of mean dispersal distances from low but significant genetic differentiation (Palumbi 2003). Available genetic data suggest mean dispersal distances of 25-150 km in many marine invertebrate and fish species, an estimate that is consistent with observations from invasive species (Shanks et al. 2003). Although this approach revolutionized our perception of large scale or even ocean-wide random interbreeding, it is limited to species with a detectable increase in genetic divergence with geographic distance. In many species, sharp genetic breaks can be detected (Awise 1992, Taylor and Hellberg 2003), but in others there is no clear geographic pattern of genetic divergence (Hauser and Ward 1998). Furthermore, dispersal distance estimates are derived as a mean over wide geographic areas; and, although currents can be incorporated into the models (Palumbi 2003), the prediction of dispersal patterns at specific MPAs is still difficult.

An alternative and potentially very powerful approach to evaluate larval dispersal and retention is the use of molecular markers for parental assignment, which allows identification of recruits originating from local adult fish. Until recently, such parental identification was not feasible because of low variability of markers, lethal sampling, and time-consuming analysis of samples. However, the development of microsatellites as high-variability molecular markers now allows parental identification in wild populations. Microsatellites consist of 1-5 base pair (bp) repeats that form tandem arrays up to 300 bp in length and exhibit high levels of allelic variation in repeat number. Polymorphism exhibited by specific microsatellites is readily detected by amplification of the microsatellite by the polymerase chain reaction (PCR) and estimation of length variation on automated systems. Microsatellites have recently come into widespread use in kinship analyses (reviewed in Wilson and Ferguson 2002) because they offer three critical advantages: opportunities for nonlethal sampling, rapid analysis of samples, and high variability. The approach is currently used extensively in salmonid populations (Bentzen et al. 2001) mainly to estimate reproductive success (Dickerson et al. 2002, Seamons et al. 2004), interactions between wild and hatchery fish (McLean et al. 2003), and the evolution of life history strategies (Garant et al. 2003). A similar approach is also possible for marine fishes, provided that a large proportion of resident adults can be sampled, which increases the chance of detecting at least some offspring with practical sample sizes. That way, offspring could be assigned not only to adults on a specific reef or MPA, but in contrast to otolith tagging studies, also to individual adult fish. By selecting a species with relatively isolated adult populations of small size, which allows the collection of a high proportion of the breeding adults in a specific area, more accurate estimates of self-recruitment could be derived.

Here, we used parental assignment of recruiting juveniles as an alternative approach to estimating the level of self-recruitment and larval dispersal in brown rockfish (*Sebastes auriculatus*). We chose brown rockfish as a target species for several reasons: (1) adult brown rockfish have relatively small home ranges, and rarely move farther than 3 km (Matthews 1990, Stout et al. 2001), which minimizes influx of new and unsampled parents; (2) relatively small and insular populations of brown rockfish inhabit several artificial reefs in Puget Sound, which allows the collection of a large proportion of potential parents; (3) some of these artificial reefs have integrated and adjacent nursery habitat for the collection of recruiting juveniles; and (4) the feasibility of inducing trans-generational chemical marks in otoliths of larvae has been demonstrated recently in brown rockfish (Buckley et al. 2007), which provided an independent verification of genetic assignments.

## Materials and methods

### *Sampling*

The study site was an artificial reef at Point Heyer, on the eastern shoreline of Vashon Island in Puget Sound (47°25.2'N, 122°25.6'W, Buckley et al. 2007). The reef is relatively isolated from other rockfish habitat, and the next artificial reef is more than 7 km away. Adjacent to the reef is smaller boulder habitat suitable for juveniles and allowing the collection of settling recruits. During summer 2004, a total of 137 adult brown rockfish (>20 cm) were caught with hand nets by scuba divers, fin clipped, and released immediately. Thirty-one females were also injected with strontium chloride (SrCl<sub>2</sub>) solution (see Buckley et al. 2007). Between spring 2004 and spring 2005, 209 recruits (<101 mm) were collected from the adjacent nursery habitats by divers who used hand nets. Fin clips of adults and juveniles were stored in 95% ethanol until analysis.

### *Molecular methods*

DNA was extracted from each sample using DNeasy extraction kits (Qiagen), following the manufacturer's protocols. Thirty-five microsatellite loci from eight *Sebastes* species were PCR-amplified following the original protocols for each locus, though annealing temperature and number of cycles were adjusted as needed to optimize PCRs. Of the 35 loci screened, 13 loci from five species were selected for further analysis based on reliability of amplification and scoring (Table 1). All samples were amplified at all 13 loci using forward primers labeled with a fluorescent dye, 0.5 units Taq (GeneChoice), and reagents and conditions detailed in Table 1. Amplified PCR products were purified using ethanol precipitation and genotyped using a MegaBACE 1000 DNA Analysis System (Amersham Biosciences). Raw data were analyzed

**Table 1. Source, amplification conditions, and repeat unit of microsatellite loci. Annealing temperatures and numbers of cycles in the PCR protocol are also presented. In loci where there are two annealing temperatures, the first was used for the first number of cycles, followed by the second number of cycles at the second temperature. Repeat unit size (Rp), focal species (the *Sebastes* species from which microsatellites were isolated), GenBank accession numbers, and reference are also shown.**

Locus	dNTPs ( $\mu$ M)	MgCl <sub>2</sub> (mM)	Primer ( $\mu$ M)	DNA ( $\mu$ L)	Annealing temp. ( $^{\circ}$ C)	Cycles	Rp	Focal species	GenBank accession	Reference
Sal1	80	1	0.3	5	47.5	29	4	<i>alutus</i>	AF153595	Miller et al. 2000
Sal3	80	1.5	0.3	5	53	25	5	<i>alutus</i>	AF153597	Miller et al. 2000
Sma2	200	1.5	0.5	3	53/56	7/18	2	<i>maliger</i>	AY654594	Wimberger et al. 1999
Sma4	200	1.5	0.3	3	55/58	7/18	3	<i>maliger</i>	AY654596	Wimberger et al. 1999
Sma10	200	1.5	0.3	3	57.5/60.5	7/18	2	<i>maliger</i>	AY654602	Wimberger et al. 1999
Sme5	200	1.5	0.15	3	58	34	4	<i>melanops</i>	AF142487	Seeb et al. (unpubl.) <sup>a</sup>
Sme8	200	1.5	0.15	3	54	34	4	<i>melanops</i>	AF142490	Seeb et al. (unpubl.) <sup>a</sup>
Spi4	100	1.5	0.5	5	59.3	30	4	<i>pinniger</i>	AY192599	Gomez-Uchida et al. 2003
Spi6	100	1.5	0.15	1.5	59.3	30	4	<i>pinniger</i>	AY192600	Gomez-Uchida et al. 2003
Stra6-52	200	1.5	0.5	3	54	36	2	<i>rastrelliger</i>	AF269057	Westerman et al. 2005
Stra7-25	200	1.5	0.09	3	66.5	36	2	<i>rastrelliger</i>	AF269056	Westerman et al. 2005
Stra15-8	200	1.5	0.3	3	54	36	4	<i>rastrelliger</i>	AF269059	Westerman et al. 2005
Stra16-5	100	1.5	0.15	3	64	36	4	<i>rastrelliger</i>	AF269061	Westerman et al. 2005

<sup>a</sup>L.W. Seeb et al., Alaska Department of Fish and Game.

with Genetic Profiler software (Amersham Biosciences), and automated allele assignments were manually reviewed for accuracy. DNA from 96 randomly picked samples was re-extracted and used in a blind trial to estimate genotyping error.

### **Data analyses and simulations**

Genotype frequencies were tested for conformance to Hardy-Weinberg and linkage equilibrium by using the software package GENEPOP v3.4 (Raymond and Rousset 1995). Loci were also checked for evidence for mis-scoring due to large allele dropout, stuttering, and null alleles (Micro-Checker, van Oosterhout et al. 2004). Observed and expected heterozygosities, inbreeding coefficient ( $F_{IS}$ ) values, and allelic diversities were estimated using FSTAT (Goudet 1995).

Parentage analysis was carried out based on exclusion, as well as by a method based on breeding likelihood (San Cristobal and Chevalet 1997). As the results of exclusion and likelihood were almost identical, only exclusion results are presented. Parents matching at a minimum of 11 out of 12 loci were considered in the assignment. Average exclusion probabilities (i.e., the average probability of excluding an unrelated individual as a parent) per locus and overall loci were calculated following the methods in Marshall et al. (1998).

To assess the power of the approach, simulations were carried out using the PopTools Add-In for Excel (Add-In for MS Excel, Greg Wood, CSIRO, Australia, available online at [www.cse.csiro.au/poptools](http://www.cse.csiro.au/poptools)). First, allele frequencies estimated from all sampled fish were used to draw 137 random adults and 100 random juveniles and to estimate the number of matches that would be expected to occur by chance alone. This simulation was repeated after including 10% offspring of sampled parents to the sampled juveniles. Means and 95% confidence intervals were calculated from 1,000 permutations. Second, 10,000 random adults were drawn from the population, of which 137 were assumed to have been sampled. These 10,000 parents were used to produce 100 offspring—by chance, about 2.7% of these offspring would have at least one parent in the sample of 137 fish. Because of the large matrices involved in these simulations, means and 95% confidence limits of genotype matches were estimated from only 100 permutations. These simulations were used to estimate the distribution of the number of loci by matching between true parent-offspring pairs and between random matches. We also evaluated the difference in match between the most likely parent and the second most likely parent. In order to evaluate the significance of locus variability, we repeated the above simulations by drawing adult genotypes from allele frequencies of the four most polymorphic loci (Spi6, Sra16-5, Sal1, Sra7-25) three times. In all simulations, genotyping error was included by replacing a proportion of offspring alleles equal to the empirically estimated genotyping error with an allele randomly

drawn from the populations. Although this type of genotyping error may be somewhat unrealistic, distinction between different classes of errors is difficult and currently generally not implemented (Marshall et al. 1998, Duchesne et al. 2002). Furthermore, our mode of genotyping error is probably the most conservative in the present context, because replacing the true allele with a common allele is likely to increase the proportion of false matches.

An alternative approach to parental identification is the calculation of relatedness coefficients (Queller and Goodnight 1989). The program Kinship (Goodnight and Queller 1999) was used to simulate expected distributions of relatedness coefficients between unrelated individuals and parent offspring pairs. These distributions were compared with observed distributions of relatedness coefficients in the real data. Additionally, a log-likelihood test that compared the likelihood of unrelated vs. parent offspring relationships was carried out in Kinship—the number of tests significant at the 0.05 level was then compared within adults and recruits, and between these two groups.

## Results

### ***Locus variability and genotyping error***

After repeating failed PCR amplifications, 99.2% of all genotypes were successfully determined. Variability was moderate; expected heterozygosity ranged between 0.6 and 0.9, and the mean allelic diversity was 13 alleles (Table 2). At most loci, there was no evidence for deviation of genotypic proportions from expected Hardy-Weinberg proportions—only Sra 15-8 and Sma 10 showed a significant deficiency of heterozygotes in both adults and recruits, and there was a significant excess of heterozygote adults at Sme 5. Correspondingly, Micro-Checker provided no significant evidence for mis-scoring due to stuttering or large allele drop out at most loci, with the exception of Sma 10, where there was significant evidence for mis-scoring due to stuttering. The genotyping error varied widely between loci, with no detectable error in 96 samples in seven of the thirteen loci, and an error of more than 7% at Sma 10. Because of the high genotyping error and the evidence for scoring problems, Sma 10 was excluded from subsequent analyses.

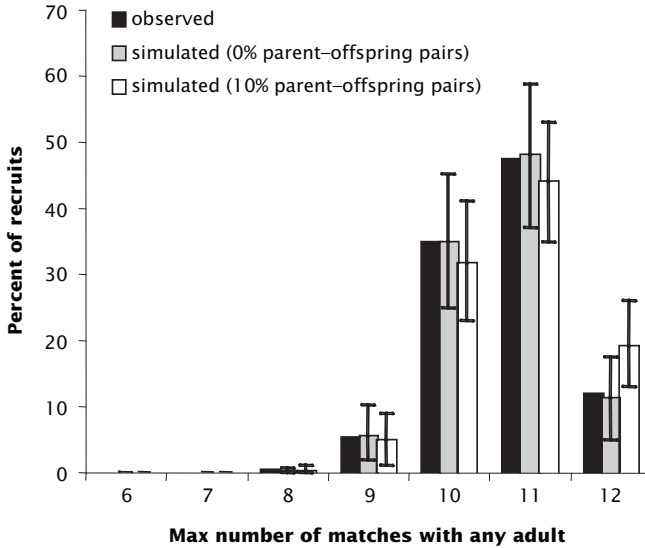
Out of the 66 possible tests for deviations from linkage equilibrium, eleven were significant at the 0.05 level in the adults, and five tests were significant in the recruits. There was no correspondence in apparent linkage between adults and recruits, except for the tests between Spi 4 and Spi 6. It is unlikely that loci other than Spi 4 and Spi 6 were indeed physically linked in a genome, a consideration that is important for the calculation of exclusion probabilities.

As expected from the relatively low variability estimates, exclusion probabilities (that is, average probabilities of excluding a randomly



**Table 2. Vital statistics of loci surveyed in potential parents (A) and recruits (R), including heterozygosities (observed  $H_o$  and expected  $H_e$ ), inbreeding coefficient ( $F_{IS}$ ) and significant deviation from Hardy-Weinberg equilibrium, and allelic diversity (NA adjusted to a sample size of 135). An estimate of genotyping error based on a blind repeat screening of 96 random samples is also shown. Exclusion probabilities are based on allele frequencies from all fish. Bold  $F_{IS}$  values indicate significant deviations of genotype distributions from Hardy-Weinberg equilibrium with a  $P < 0.05$ ; bold underline with a  $P < 0.01$ .**

Locus	A					R				
	$H_o$	$H_e$	$F_{IS}$	NA	$H_o$	$H_e$	$F_{IS}$	NA	Exclusion probabilities	Genotyping error
Sal1	0.774	0.805	0.039	20.9	0.746	0.807	0.076	17.9	0.466	0.0
Sal3	0.737	0.750	0.017	7.0	0.737	0.733	-0.006	6.6	0.333	0.0
Sma2	0.547	0.617	0.112	3.0	0.612	0.615	0.005	3.0	0.193	0.0
Sma4	0.839	0.811	-0.036	11.0	0.823	0.807	-0.019	9.0	0.456	0.0
Sma10	0.725	0.783	<b>0.074</b>	9.9	0.707	0.773	<b>0.086</b>	12.3	0.400	7.4
Sme5	0.774	0.763	<b>-0.014</b>	13.0	0.789	0.765	-0.032	12.6	0.405	0.0
Sme8	0.672	0.699	0.039	5.0	0.703	0.707	0.005	4.6	0.286	0.0
Spi4	0.693	0.663	-0.046	19.9	0.615	0.654	0.060	20.8	0.269	2.1
Spi6	0.861	0.889	0.032	35.9	0.885	0.883	-0.003	37.5	0.662	2.1
Sra6-52	0.650	0.639	-0.017	6.0	0.699	0.655	-0.066	6.5	0.225	2.1
Sra7-25	0.830	0.855	0.029	15.0	0.813	0.820	0.008	16.2	0.504	4.2
Sra15-8	0.715	0.754	<b>0.051</b>	7.0	0.656	0.739	0.113	8.6	0.341	2.1
Sra16-5	0.905	0.923	0.019	23.9	0.919	0.907	<b>-0.013</b>	24.7	0.701	0.0
Average	0.748	0.765	0.023	13.7	0.746	0.759	0.016	13.9	0.403	1.5



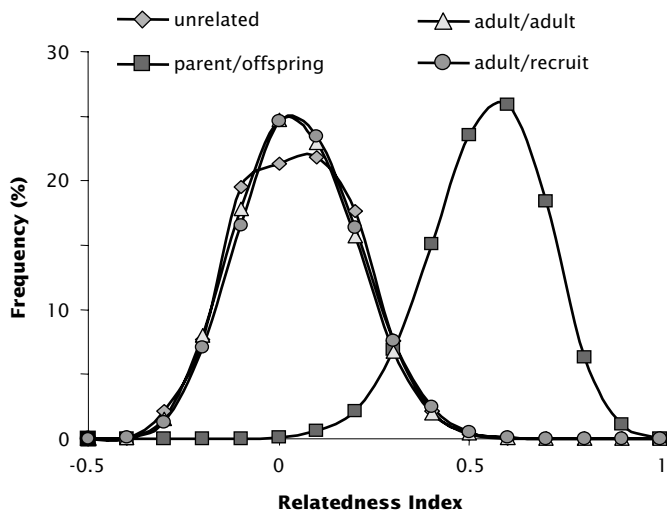
**Figure 1. Frequency distribution of maximum matches between recruits and adults. The observed distribution (black) and simulated distributions with no parent-offspring pairs (gray) and 10% parent-offspring pairs (white) are shown. Error bars represent 95% confidence limit of 1,000 simulations. Note that the observed frequency of 12-locus matches falls below the lower 95% confidence limit of the 10% parent offspring simulation.**

chosen individual from parentage) were also low (Table 2). Nevertheless, over all loci the exclusion probability was 99.88%.

### **Parental assignment**

Of the 209 offspring, 25 matched at least one parent at all 12 loci, and 99 offspring matched at least one parent at 11 loci. Therefore, allowing for genotyping error at one locus, more than half of all offspring had at least one potential parent in the sample, despite the high average exclusion probability. Simulations showed that this distribution of matches is very close to that expected from chance matches among random genotypes, but that it is significantly lower than expected if 10% of juveniles are offspring of sampled adults (Fig. 1). These results indicated that true offspring of resident parents, if present, were relatively rare (<10% of juveniles).

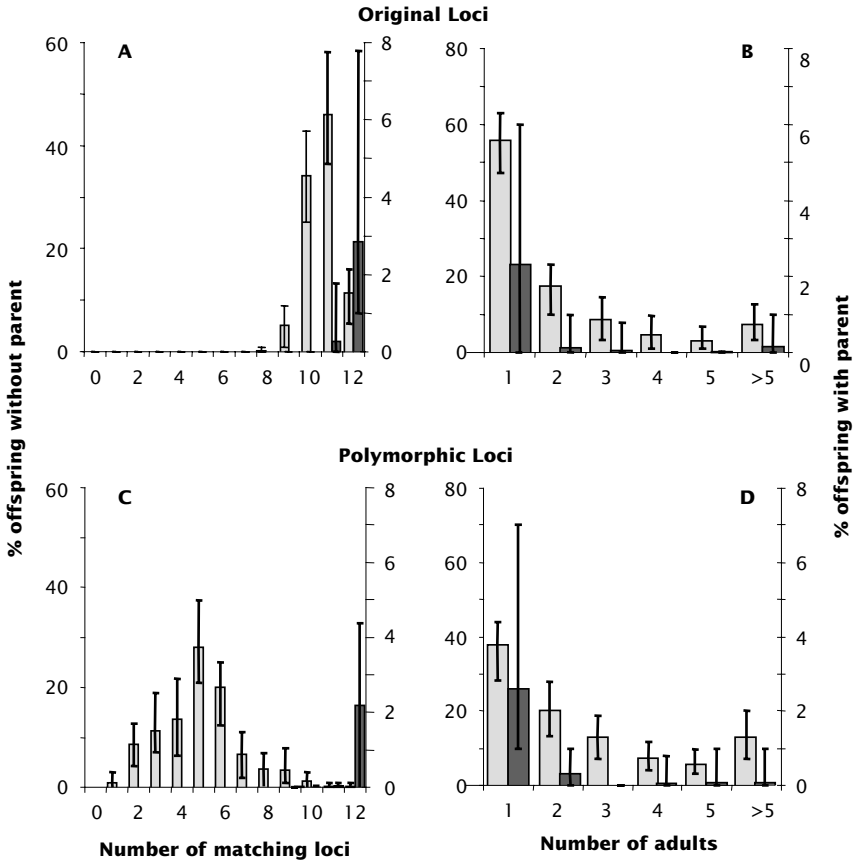
The notion of rarity of true offspring was confirmed by relatedness indices. Both pairwise relatedness between adults and between adults and recruits closely followed the distribution of relatedness



**Figure 2. Frequency distribution of relatedness coefficients. Observed relatedness coefficients between adults and between adults and recruits are shown, as well as simulated relatedness between parent-offspring pairs and unrelated individuals. The distribution of observed relatedness follows closely that expected from unrelated individuals.**

indices expected for unrelated fish (Fig. 2). However, there were some indications for relatedness from the number of log-likelihood tests that showed a significantly higher likelihood ( $P < 0.05$ ) of first order relatedness as compared to the null hypothesis of no relatedness. Of the pairwise tests between adults, 5.2% were significant, while 6.0% were significant between adults and recruits. This slight excess of significant tests above the expected 5.0% may suggest a few, but not many, related individuals.

Simulations with 10,000 candidate parents, of which 137 parents were known, confirmed that about 10% of juveniles without a parent in the sample would have a random match at all 12 loci with one of the sampled adults (Fig. 3A). Although most juveniles with a sampled parent would also show such a match, they would not be obviously distinguishable from random matches. Furthermore, about 10% of the offspring with parents would match their true parent at only 11 loci because of genotyping error. It may be that a better distinguishing feature between true and random matches is the difference in the number of parents matching equally well. However, although the majority of offspring with parents in the sample have only their parent matching at



**Figure 3.** Results from simulations assuming a sample of 137 fish from a population of 10,000 candidate parents. Juveniles with parents in the sample are shown in dark gray and on the right hand scale, whereas juveniles with unsampled parents are shown in light gray and on the left hand scale. Error bars show confidence limits from 100 simulations. (A) Distribution of best matches between adults and juveniles using the original set of loci. (B) Distribution of the number of adults matching offspring at as many loci as the best matching candidate parent. (C) and (D): As in (A) and (B), but using the four most polymorphic loci three times.

all 12 loci, about 40% of random matches also have only one adult that matches best (Fig. 3B). Having a single matching parent is therefore no indication of a true parent-offspring relationship.

Equivalent simulations using the four most polymorphic loci taken three times demonstrated the value of a highly polymorphic data set for parental identification. Most offspring with no parent in the sample match any parent by chance at only five loci, and only 0.2% match at all 12 loci (Fig. 3C). In contrast, 96% of offspring with parent in the sample match that parent at all 12 loci—the other 4% being due to genotyping error. Interestingly, however, the number of potential parents matching at the same number as the maximum match was still not a good indicator for true parentage, as in almost 60% of random matches there was a single sampled parent matching best.

## Discussion

The results of this study suggested that the molecular markers used here were not sufficiently variable to provide an unambiguous identification of offspring of resident rockfish. However, the data did indicate very limited self-recruitment, and in conjunction with SrCl<sub>2</sub> marking, led to the identification of a single offspring of an injected fish (Buckley et al. 2007). Furthermore, simulations showed that a more polymorphic marker set would have the power to identify offspring, despite the large number of potential candidate parents and the very small proportion of parents sampled.

### ***Parental assignments and rates of self-recruitment***

Our study identified 124 potential offspring of the rockfish sampled (matching at 11 or 12 loci to any candidate parent). However, simulations suggested that a very similar number of matching offspring would be expected by chance alone. Simulations that included 10% “true” parent offspring pairs produced significantly more matching pairs than observed, which suggested that the true value of self-recruitment is less than 10%. On the other hand, SrCl<sub>2</sub> marking clearly identified one offspring of an injected female (Buckley et al. 2007). Therefore, genetic and otolith results combined suggested that self-recruitment did occur, but at a rate of less than 10%, though any such conclusions are very preliminary due to the lack of accurate confidence estimates.

Low levels of self-recruitment may be expected, because of the long larval period of brown rockfish (2-2.5 months, Love et al. 2002) and the consequent potential of extensive larval dispersal. However, several lines of evidence also suggest considerable potential for larval retention and limited realized dispersal of brown rockfish. Puget Sound is an enclosed estuary that includes several basins. The artificial reef on Point Heyer is situated in the central basin, which is connected to

northern Puget Sound via Admiralty Inlet (maximum depth 65 m) and to the southern basin via Tacoma Narrows (maximum depth 45 m) (Burns 1985). General circulation patterns are primarily caused by tidal exchange, and consist of outflow through Admiralty Inlet in the upper layer and inflow of marine waters at depth (Stout et al. 2001). In the southern part of the basin, currents generally flow north on the west side of Vashon Island, and south on the east side where Point Heyer is located (Ebbesmeyer et al. 1984). Such oceanographic patterns, bathymetric isolation, and tidal reversal of currents provide ample opportunity for larval retention via mechanisms such as vertical migration, habitat selection, and hydrographic retention (Joyeux 2001, Bilton et al. 2002). Nevertheless, on a very small scale, dispersal would probably be sufficient to explain the apparent low levels of self-recruitment on Point Heyer. Furthermore, the level of interannual variability in circulation patterns is currently understudied, though major circulation patterns, especially the depth of inflowing and outflowing currents, appear to be greatly influenced by decadal climate regimes (Ebbesmeyer et al. 1998). Recruitment estimates from additional years are therefore needed for more long term self-recruitment rates relevant to MPA design.

Expectations of self-recruitment also stem from indirect estimates of dispersal distances in brown rockfish and other *Sebastes* species. Isolation by distance patterns from microsatellite studies on the Pacific east coast indicated mean dispersal distances of less than 10 km in grass rockfish (*Sebastes rastrelliger*, Buonaccorsi et al. 2004), copper rockfish (*S. caurinus*, Buonaccorsi et al. 2002) and brown rockfish (*S. auriculatus*, Buonaccorsi et al. 2005). By assuming that the distribution of individual dispersal distances follows an exponential distribution (Botsford et al. 2001), about 10% of recruits would be expected to settle within 1 km of the point of release, that is, potentially on the same reef. These estimates correspond to our initial results, though more powerful markers are needed to confirm this notion.

An alternative explanation for the low self-recruitment rates estimated here may be a large variance in reproductive success among adults, resulting in a very limited number of adults actually producing recruits. Population genetic studies indicate that in many marine species the effective genetic population size ( $N_e$ ) may be orders of magnitude smaller than the census population size (e.g., Turner et al. 2002, Hauser et al. 2002, Hoarau et al. 2005), which suggests that only one in several thousand fish is successful in reproducing. Maternal effects that lead to much higher larval survival in offspring of older females, in addition to the inherently higher fecundity of older and larger fish (Berkeley et al. 2004), may exacerbate these effects. If such few reproductively successful adults were not included in the adult sample, their offspring might not be identified, and self-recruitment rates would be underestimated. Indeed, most females sampled were smaller than 30

cm and inclusion of the few larger fish on the reef may increase the self-recruitment estimate considerably.

Variation in reproductive success may occur either among individuals within subpopulations (Hauser et al. 2002) or among subpopulations (Turner et al. 2002). Indeed, variation in productivity among subpopulations may decrease  $N_e$  by several orders of magnitude (Turner et al. 2002). Because self-recruitment was estimated as a proportion, it does not only depend on the productivity of the Point Heyer population, but also other populations in the vicinity, and may have been reduced by immigration from very productive populations. Such considerations emphasize the importance of interannual variability in recruitment.

### **Methodological considerations**

Parentage analysis of marine species is not a trivial undertaking. "Biological" assumptions of common parentage methods are that the population is fairly small, that a large proportion of candidate parents have been sampled, that both parents are in the sample, and that the juvenile sample contains a large proportion of offspring of known parents. Violations of these assumptions result in a progressively restrictive range of analysis methods (Jones and Ardren 2003). The population size of brown rockfish in Puget Sound has been estimated as 100,000 individuals (Stout et al. 2001), all of which are potential parents of juveniles recruiting to the Point Heyer reef. However, the adult population of brown rockfish at Point Heyer is 200-250 fish (Buckley et al. 2007) and so about half of the adults were included in this study ( $N = 137$ ). There is therefore a 25% chance that any true offspring of resident adults has both parents in the sample—a probability that is lower than in most other studies of parentage assignment (Jones and Ardren 2001). The proportion of offspring of these adults in the recruiting juveniles is unknown, and may range from 0% given the extensive larval period of 2 to 2.5 months (Love 2002) to 15-60% as was reported for reef fish (*Pomacentrus amboinensis*, Jones et al. 1999) on the Great Barrier reef. All these ecological features of many marine species, such as brown rockfish, complicate parental assignment, but certainly should not prevent it, given the appropriate set of marker loci and some way of verifying assignments.

There are also some assumptions regarding the markers in question. Most importantly, markers should have high variability, and so provide high exclusion probability for random genotypes. Our marker set was less variable than one would like for a parentage assignment study. Two reasons may account for this low variability: first, all loci were isolated from other rockfish species, and it is known that microsatellites are somewhat longer and more variable in the focal species (the species from which it was isolated) than in related species (Neff and Gross 2001). Second, brown rockfish in Puget Sound have lower genetic diversity

than their conspecifics on the Oregon and California coast (Buonaccorsi et al. 2005). The relatively low variability of microsatellites led to low exclusion probabilities (0.2-0.7, Table 2) and further reduced the power of parentage assignment.

Nevertheless, our simulations showed clearly that a more variable microsatellite data set would have high power in detecting true parent-offspring pairs in our samples. Using our suite of loci, about 10% of random pairs of individuals matched at all 12 loci, whereas a more variable set of microsatellites would reduce this percentage to 0.2%, while leaving the distribution of matches between true parent-offspring pairs unchanged. Similar increases in parentage assignment success with increasing diversity of marker loci were observed in other simulation studies (Bernatchez and Duchesne 2000). Interestingly, the distribution of numbers of candidate parents matching the offspring at the same number of loci as the maximum match found did not change noticeably with marker variability. This number of equally well matching parents is closely related to the concept of the difference in match between the most likely parent and the second most likely parent, a criterion often used in parental assignment studies (Marshall et al. 1998). These results suggest that with a small sample from a large number of candidate parents and genotyping error, such a criterion may be unsuitable.

Low genotyping error and low mutation rates are a further assumption of parental assignment. Some approaches allow the consideration of genotyping errors (e.g., Marshall et al. 1998), but in doing so reduce the power of assignment even further. Simulations that used a maximum likelihood approach (Marshall et al. 1998) showed that low marker variability, genotyping error, and a large proportion of unsampled parents together reduce the power sufficiently to prevent confident parental assignment (data not shown). In our study, the locus with the highest genotyping error (Sma 10) was removed from the analysis, and the remaining average genotyping error (1%) was within the average commonly observed in microsatellite studies (Hoffman and Amos 2005). Nevertheless, over the 12 loci, the genotyping error exceeded 12% per multilocus genotype, which resulted in false exclusion of parent offspring pairs. Furthermore, because of the rapidly increasing multilocus genotyping error, the addition of more loci does not always result in higher power of parental assignment, as the limited increase in information content with each additional locus is more than offset by an increase in overall error rate. It would therefore be best to use a limited number of loci with high variability and low genotyping error for parental assignment.



### **Implication for MPA design**

One of the main assertions of MPAs is that protecting a spawning biomass of marine fishes will enhance progeny output, and that the patterns of larval dispersal from the MPAs will contribute to local and/or regional stocks. However, the dispersal of marine fish larvae is poorly documented and this basic assumption remains unverified. Although clearly only a “snapshot” in time, our results suggested that, at least on Point Heyer, recruitment of the 2004 year class occurred primarily from outside the resident population. However, concluding a low conservation priority from these data would be misleading, because data from additional years are needed to establish long-term temporal trends. Furthermore, the destination of Point Heyer offspring is currently unknown, and the population may be an important source of larvae for other reefs. Point Heyer may thus be an important component of the rockfish habitat in Puget Sound. Further surveys including other reefs are needed, optimally using a combination of otolith marking techniques (Buckley et al. 2007) and genetic methods. Our simulations clearly demonstrated the feasibility of the approach of identifying offspring of resident adults, which may be a powerful tool for estimating dispersal into and out of MPAs.

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### **References**

- Avise, J.C. 1992. Molecular population structure and the biogeographic history of a regional fauna: A case history with lessons for conservation biology. *Oikos* 63:62-76.
- Bailey, K.M., P.J. Stabeno, and D.A. Powers. 1997. The role of larval retention and transport features in mortality and potential gene flow of walleye pollock. *J. Fish. Biol.* 51:135-154.
- Bentzen, P., J.B. Olsen, J.E. McLean, T.R. Seamons, and T.P. Quinn. 2001. Kinship analysis of Pacific salmon: Insights into mating, homing, and timing of reproduction. *J. Hered.* 92:127-136.

- Berkeley, S.A., C. Chapman, and S.M. Sogard. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology* 85:1258-1264.
- Bernatchez, L., and P. Duchesne. 2000. Individual-based genotype analysis in studies of parentage and population assignment: How many loci, how many alleles? *Can. J. Fish. Aquat. Sci.* 57:1-12.
- Bilton, D.T., J. Paula, and J.D.D. Bishop. 2002. Dispersal, genetic differentiation and speciation in estuarine organisms. *Estuar. Coast. Shelf Sci.* 55:937-952.
- Bohonak, A.J. 1999. Dispersal, gene flow, and population structure. *Q. Rev. Biol.* 74:21-45.
- 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.
- Botsford, L.W., F. Micheli, and A. Hastings. 2003. Principles for the design of marine reserves. *Ecol. Appl.* 13:S25-S31.
- Buckley, R.M., and L.L. LeClair. 2007. Preliminary results of transgenerational marking of larval marine fish otoliths. 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. (This volume.)
- 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.
- Burns, R. 1985. *The shape and form of Puget Sound*. Washington Sea Grant Program, University of Washington Press, Seattle. 100 pp.
- Cowan, J.H., and R.F. Shaw. 2002. Recruitment. In: L.A. Fuiman and R.G. Werner (eds.), *Fishery science: The unique contribution of early life stages*. Blackwell, London, pp. 88-111.
- Dickerson, B.R., T.P. Quinn, and M.F. Willson. 2002. Body size, arrival date, and reproductive success of pink salmon, *Oncorhynchus gorbuscha*. *Ethol. Ecol. Evol.* 14:29-44.
- Doherty, P.J., S. Planes, and P. Mather. 1995. Gene flow and larval duration in seven species of fish from the Great-Barrier-Reef. *Ecology* 76:2373-2391.
- Duchesne, P., M.H. Godbout, and L. Bernatchez. 2002. PAPA (package for the analysis of parental allocation): A computer program for simulated and real parental allocation. *Mol. Ecol. Notes* 2:191-193.

- Duda, Jr., T.F., and S.R. Palumbi. 1999. Developmental shifts and species selection in gastropods. *Proc. Natl. Acad. Sci. USA* 96:10272-10277.
- Ebbesmeyer, C.C., R.J. Stewart, and S. Albertson. 1998. Circulation in southern Puget Sound's finger inlets: Hammersley, Totten, Budd, Eld, and Case inlets. In: *Proceedings of Puget Sound Research '98 Conference*, March 12-13. Puget Sound Water Quality Action Team, Olympia, pp. 239-258.
- Ebbesmeyer, C.C., C.A. Coomes, J.M. Cox, J.M. Helseth, L.R. Hinchey, G.A. Cannon, and C.A. Barnes. 1984. Synthesis of current measurements in Puget Sound, Washington. Vol. 3. Circulation in Puget Sound: An interpretation based on historical records of currents. NOAA Tech. Memo. NOS-OMS-5. 73 pp.
- Feral, J.P. 2002. How useful are the genetic markers in attempts to understand and manage marine biodiversity? *J. Exp. Mar. Biol. Ecol.* 268:121-145.
- Garant, D., J.J. Dodson, and L. Bernatchez. 2003. Differential reproductive success and heritability of alternative reproductive tactics in wild Atlantic salmon (*Salmo salar* L.). *Evolution* 57:1133-1141.
- Geller, J.B., J.T. Carlton, and D.A. Powers. 1994. PCR based detection of mtDNA haplotypes of native and invading mussels on the Northeastern Pacific coast: Latitudinal pattern of invasion. *Mar. Biol.* 119:243-249.
- Gomez-Uchida, D., E.A. Hoffman, W.R. Ardren, M.A. Banks. 2003. Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species. *Mol. Ecol. Notes* 3:387-389.
- Goodnight, K.F., and D.C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol. Ecol.* 8:1231-1234.
- Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate *F*-statistics. *J. Hered.* 86:485-486.
- Hauser, L., and R.D. Ward. 1998. Population identification in pelagic fish: The limits of molecular markers. In: G.R. Carvalho (ed.), *Advances in molecular ecology*. IOS Press, Amsterdam, pp. 191-224.
- Hauser, L., G.J. Adcock, P.J. Smith, J.H.B. 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. USA* 99:11742-11747.
- Hay, D.E., and P.B. McCarter. 1997. Larval distribution, abundance, and stock structure of British Columbia herring. *J. Fish. Biol.* 51:155-175.
- Hoarau, G., E. Boon, D.N. Jongma, S. Ferber, J. Palsson, H.W. Van der Veer, A.D. Rijnsdorp, W.T. Stam, and J.L. Olsen. 2005. Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proc. R. Soc. Lond. Ser. B* 272:497-503.
- Hoffman, J.I., and W. Amos. 2005. Microsatellite genotyping errors: Detection approaches, common sources and consequences for paternal exclusion. *Mol. Ecol.* 14:599-612.
- Hourigan, T.F., and E.S. Reese. 1987. Midocean isolation and the evolution of Hawaiian reef fishes. *Trends Ecol. Evol.* 2:187-191.

- Jones, A.G., and W.R. Ardren. 2003. Methods of parentage analysis in natural populations. *Mol. Ecol.* 12:2511-2523.
- 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.
- Joyeux, J.C. 2001. The retention of fish larvae in estuaries: Among-tide variability at Beaufort Inlet, North Carolina, USA. *J. Mar. Biol. Assoc. UK* 81:857-868.
- Lessios, H.A., B.D. Kessing, and J.S. Pearse. 2001. Population structure and speciation in tropical seas: Global phylogeography of the sea urchin *Diadema*. *Evolution* 55:955-975.
- Love, M.S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the Northeast Pacific. University of California Press, Berkeley. 405 pp.
- Marshall, T.C., J. Slate, L.E.B. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7:639-655.
- Matthews, K.R. 1990. An experimental study of the habitat preferences and movement patterns of copper, quillback, and brown rockfishes (*Sebastes* spp). *Environ. Biol. Fishes* 29:161-178.
- McLean, J.E., P. Bentzen, and T.P. Quinn. 2003. Differential reproductive success of sympatric, naturally spawning hatchery and wild steelhead trout (*Oncorhynchus mykiss*) through the adult stage. *Can. J. Fish. Aquat. Sci.* 60:433-440.
- 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.
- Neff, B.D., and M.R. Gross. 2001. Microsatellite evolution in vertebrates: Inference from AC dinucleotide repeats. *Evolution* 55:1717-1733.
- Palumbi, S.R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. *J. Exp. Mar. Biol. Ecol.* 203:75-92.
- Palumbi, S.R. 2001. The ecology of marine protected areas. In: M. Bertness, S.D. Gaines, and M.E. Hay (eds.), *Marine ecology: The new synthesis*. Sinauer Associates, Sunderland, Massachusetts, pp. 509-530.
- Palumbi, S.R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. *Ecol. Appl.* 13:S146-S158.
- Pittman, S.J., and C.A. McAlpine. 2003. Movements of marine fish and decapod crustaceans: Process, theory and application. *Adv. Mar. Biol.* 44:205-294.
- Planes, S., R. Galzin, A.G. Rubies, R. Goni, J.G. Harmelin, L. Le Direach, P. Lenfant, and A. Quetglas. 2000. Effects of marine protected areas on recruitment processes with special reference to Mediterranean littoral ecosystems. *Environ. Conserv.* 27:126-143.
- Queller, D.C., and K.F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258-275.
- Raymond, M., and F. Rousset. 1995. Genepop (Version-1.2): Population genetics software for exact tests and ecumenicism. *J. Hered.* 86:248-249.

- Sancristobal, M., and C. Chevalet. 1997. Error tolerant parent identification from a finite set of individuals. *Genet. Res.* 70:53-62.
- Scheltema, R.S. 1986. Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific islands. *Bull. Mar. Sci.* 39:241-256.
- Seamons, T.R., P. Bentzen, and T.P. Quinn. 2004. The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny. *Environ. Biol. Fishes* 69:333-344.
- Shanks, A.L., B.A. Grantham, and M.H. Carr. 2003. Propagule dispersal distance and the size and spacing of marine reserves. *Ecol. Appl.* 13:S159-S169.
- Stout, H.A., B.B. McCain, R.D. Vetter, T.L. Builder, W.H. Lenarz, L.L. Johnson, and R.D. Methot. 2001. Status review of copper rockfish (*Sebastes caurinus*), quillback rockfish (*S. maliger*), and brown rockfish (*S. auriculatus*) in Puget Sound, Washington. NOAA-NWFSC Tech. Memo. 46.
- Strathmann, R.R. 1978. The evolution and loss of feeding larval stages of marine invertebrates. *Evolution* 32:894-906.
- Taylor, M.S., and M.E. Hellberg. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science* 299:107-109.
- Thorrold, S.R., C. Latkoczy, P.K. Swart, and C.M. Jones. 2001. Natal homing in a marine fish metapopulation. *Science* 291:297-299.
- 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* 162:1329-1339.
- Van Oosterhout, C., W.F. Hutchinson, D.P.M. Wills, and P. Shipley. 2004. MicroChecker: Software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4:535-538.
- Waples, R. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *J. Hered.* 89:438-450.
- 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.
- White, M. 1998. Development, dispersal and recruitment: A paradox for survival among Antarctic fish. In: G. di Prisco, E. Pisano, and A. Clarke (eds.), *Fishes of Antarctica: A biological overview*. Springer Verlag, Berlin, pp.53-62.
- Wilson, A.J., and M.M. Ferguson. 2002. Molecular pedigree analysis in natural populations of fishes: Approaches, applications, and practical considerations. *Can. J. Fish. Aquat. Sci.* 59:1696-1707.
- 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.
- 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.

