

Multiple paternity and maintenance of genetic diversity in the live-bearing rockfishes *Sebastes* spp.

John R. Hyde^{1,2,*}, Carol Kimbrell², Larry Robertson², Kevin Clifford³,
Eric Lynn², Russell Vetter²

¹Scripps Institution of Oceanography, 9500 Gilman Drive, La Jolla, California 92093-0203, USA

²Southwest Fisheries Science Center, NOAA/NMFS, 8604 La Jolla Shores Dr., La Jolla, California 92037, USA

³Oregon Coast Aquarium, 2820 SE Ferry Slip Rd, Newport, Oregon 97365, USA

ABSTRACT: The understanding of mating systems is key to the proper management of exploited species, particularly highly fecund, *r*-selected fishes, which often show strong discrepancies between census and effective population sizes. The development of polymorphic genetic markers, such as codominant nuclear microsatellites, has made it possible to study the paternity of individuals within a brood, helping to elucidate the species' mating system. In the present study, paternity analysis was performed on 35 broods, representing 17 species of the live-bearing scorpaenid genus *Sebastes*. We report on the finding of multiple paternity from several species of *Sebastes* and show that at least 3 sires can contribute paternity to a single brood. A phylogenetically and ecologically diverse sample of *Sebastes* species was examined, with multiple paternity found in 14 of the 35 broods and 10 of the 17 examined species, we suggest that this behavior is not a rare event within a single species and is likely common throughout the genus. Despite high variance in reproductive success, *Sebastes* spp., in general, show moderate to high levels of genetic diversity. We suggest that multiple paternity may be a form of bet hedging that serves to maximize genetic diversity within broods and that, regardless of the selective value at the level of individual fitness, the net effect at the population level may be a genetic buffer to the consequences of severe overexploitation.

KEY WORDS: Multiple paternity · *Sebastes* · Rockfish · Mating system · Polyandry · Polygynandry

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The accumulation and maintenance of genetic diversity is a key topic in conservation genetics. A species' life history, particularly its reproductive behaviors (i.e. mating system [Nunney 1993], age/size-dependent effects on fecundity and offspring quality [Berkeley et al. 2004], degree of parental care), can have a significant effect upon genetic diversity. Ecological effects (e.g. Cushing's [1990] match–mismatch hypothesis) can also significantly influence levels of genetic diversity, perhaps leading to large discrepancies between census and effective population sizes (sensu Hedgecock 1994). As such, all of these parameters should be included in management plans to conserve genetic diversity in populations that are under intensive

exploitation or declining due to habitat degradation and/or climate change.

Of the 8 subfamilies of the scorpionfish family Scorpaenidae, viviparity is confined to the subfamily Sebastinae, with the genus *Sebastes* the most advanced. At parturition, *Sebastes* larvae are primitively developed (e.g. pre-flexion yolk-sac stage with limited swimming ability) in comparison to other live-bearers (e.g. embiotocids, poeciliids, and most sharks). However, this compromise between maternal investment (Boehlert & Yoklavich 1984) and high fecundity results in broods of comparable size to oviparous, broadcast-spawning fishes (i.e. 1000s to at least 2.7 million larvae; Moser 1967, Love et al. 2002), while avoiding the period of greatest mortality (i.e. egg and early larval stages). Most species produce a single brood annually,

*Email: jrhyde@ucsd.edu

though a few species, mainly within the Southern California Bight, show evidence of multiple annual broods (e.g. *S. paucispinis*) (Moser 1967). Despite both high fecundity and enhanced larval survivorship, *Sebastes* spp. still suffer high levels of larval mortality, resulting in highly variable recruitment success (i.e. dominant year-classes occurring only once per decade; Tolimieri & Levin 2005). The genetic diversity within these dominant year-classes may represent a disproportionate contribution from a very small subset of the spawning females (sensu Hedgecock 1994). Though at the population level this bias is likely ameliorated by the long reproductive lifetimes of these fishes (e.g. de Bruin et al. 2004), this buffer may be diminished by the dramatic truncation of age distributions caused by current levels of exploitation (Love et al. 1998).

Of the 100+ *Sebastes* species, courtship behavior has been formally described for only two, *S. inermis* and *S. mystinus*, a demersal and a semi-pelagic species, respectively (see Love et al. 2002). Anecdotal observations of *S. melanops* and *S. miniatus* have been reported in Love et al. (2002). In all cases, an elaborate courtship dance is performed by the male in an attempt to entice the female; however, the ultimate decision to mate resides with the female. Despite the practice of mate choice, both females and males of *S. inermis* have been observed mating with multiple partners, suggesting a polygynandrous mating system in these fishes.

Unlike the closely related *Helicolenus dactylopterus* (Muñoz et al. 2000), no sperm storage structure within the ovaries has been found in the examined species of *Sebastes*. However, the asynchrony between mating, hormonal changes (Mori et al. 2003), maturation of oocytes, and the development of embryos, as well as the finding of both free-swimming and embedded spermatozoa in the ovaries, suggests that sperm are stored in some manner and that insemination may precede fertilization by up to 6 mo (Moser 1967, Takahashi et al. 1991).

Observation of females engaged in multiple mating events with different males leads to the question: Can multiple males sire offspring within the same brood? To address this question, we examined broods from both captive and wild populations of several species.

MATERIALS AND METHODS

Sample collection. Aquarium studies: Broods from captive populations of grass *Sebastes rastrelliger* and yelloweye rockfish *S. ruberrimus*, both species that are subject to heavy exploitation by commercial and recreational fisheries, were examined in detail to determine patterns of paternity. These captive populations were

composed of 12 grass rockfish, maintained at the Southwest Fisheries Science Center experimental aquarium (La Jolla, CA), and 80 yelloweye rockfish, maintained in an exhibit at the Oregon Coast Aquarium (Newport, OR). All fish in both populations were captured, individually tagged (i.e. PIT or Floy T-Bar tags), measured, and had tissue samples taken from the caudal end of the anal fin. Tissue samples were stored in 95% undenatured ethanol. Both populations contained an equal mixture of both sexes, and all fish were assumed to be reproductively mature based upon published size–maturity relationships (Love et al. 2002).

Gravid females were identified visually by their distended abdomens and were placed in isolation tanks until parturition to ensure that all collected larvae could be correctly associated with the mother. After parturition, larvae were randomly collected from the tank and stored whole in 95% undenatured ethanol. All samples from *Sebastes rastrelliger* were collected over a 2 yr period (2000 to 2001), from 4 broods of similar size (~400 000 larvae), produced by the same female. The broods from *S. ruberrimus* were collected during a single season from different females.

Wild-caught fish: Samples from wild populations were collected opportunistically using various methods (i.e. otter trawl, pole spear, hook and line) and selected to represent a phylogenetically diverse sample of species (sensu Hyde & Vetter 2007). Specimens were identified to species level using Love et al. (2002). In total, 28 gravid females and associated larvae, representing 16 species, were selected for analysis. A portion of the adult fish's pectoral fin was preserved in 95% undenatured ethanol. In most cases, barotrauma caused by overexpansion of the swim bladder caused forced expulsion of larvae upon capture. These larvae were mixed to provide a representative sample of the brood and preserved in mass in 95% undenatured ethanol. In some cases, whole ovaries were excised and larvae were sampled from the anterior, middle, and posterior parts of each ovary.

DNA extraction. For all adults and the larvae of the wild-caught fish, DNA was extracted by a Proteinase K digestion followed by nucleic acid separation and purification using a LiCl:chloroform protocol and subsequent ethanol precipitation (see Hyde & Vetter 2007). DNA pellets were resuspended with 0.1× Tris-EDTA (TE) buffer (pH 8.0); quantity and quality were assessed via UV spectrophotometry, and 100 ng μl^{-1} working stocks were prepared by dilution in 0.1× TE. In order to expedite the analyses of multiple paternity from wild-caught fish, 100 randomly sampled larvae were co-extracted in a single extraction. For larvae from the aquarium husbandry studies, a Chelex (Bio-Rad Laboratories)-based boiling protocol (see Hyde &

Vetter 2007) was employed using a single, whole larva for each extraction. All DNA extractions were stored frozen at -20°C pending analysis.

PCR amplification of microsatellites. Microsatellite loci were chosen from the libraries developed by Westerman et al. (2005) (Sra5-9.1, Sra7-2.2, Sra7-7.3, Sra7-25.4, Sra11-103.6, Sra16-5-8, Sra15-8.9) and Gomez-Uchida et al. (2003) (Spi4, Spi6, Spi10). All fish within the captive populations were genotyped at 6 (*Sebastes rastrelliger*) or 8 (*S. ruberrimus*) microsatellite loci (see Table 1). For wild-caught specimens, the goal of the study was to identify instances of multiple paternity rather than the assignment of paternity to an individual, so only 5 loci were surveyed. As Neff & Pitcher (2002) showed that multiple paternity can be detected with $>90\%$ probability using as few as 2 mildly polymorphic (e.g. 5 alleles) loci on samples of >20 larvae, it was expected that our use of 100 larvae and 5 loci was sufficient to address this issue. All loci were amplified by polymerase chain reaction (PCR) following the conditions described in Gomez-Uchida et al. (2003) and Westerman et al. (2005). Fluorescently labeled PCR products were separated by size via electrophoresis through either a 6% polyacrylamide gel (using an ABI 377XL automated sequencer) or capillaries filled with POP-7 polyacrylamide polymer (using an ABI 3130XL Genetic Analyzer). All samples were run with an internal size standard (ROX 500) and scored using Genemapper v3.7 software (Applied Biosystems). To control for size-calling variance between runs and machines, reference samples were included within each PCR and genotyping batch.

Determination of paternity. Paternal alleles were deduced by subtraction of the maternal alleles from each larval genotype. Paternity of larvae from the aquarium husbandry studies was assigned by comparison of deduced paternal alleles to the genotypes of potential sires using Cervus v2.0 (Marshall et al. 1998). Cervus assigns paternity by exclusion of individuals with mismatching genotypes. In cases where there remain 2 or more possible sires for an individual, a likelihood-based probability is used to assign the most probable sire. In all instances in this study, larvae were unambiguously assigned to a single sire by genotype exclusion alone.

For wild-caught specimens, paternal alleles were deduced by exclusion of maternal alleles as before, with the presence of 3 or more paternal alleles, at any locus, coded as a positive finding of multiple paternity. As the genotypes of the potential sires in the wild populations were unknown, the measure of paternity by this method is conservative, providing only a minimum estimation of the number of sires. For example, if sires share alleles with each other or with the mother it is possible to have instances of multiple paternity

Table 1. *Sebastes* spp. Number of paternal alleles detected at each locus after subtraction of the maternal alleles, the deduced sires, and their percentage of paternity of the examined larvae. N/E: microsatellite loci not examined; ID: identification sequence

Mother's ID	No. of larvae	Sra5-9.1	Sra7-2.2	Sra7-7.3	Sra7-25.4	Sra11-103.6	Sra16-5.8	Sra15-8.9	Spi4	Spi6	Spi10	ID of sires (percent of brood)
<i>S. rastrelliger</i> (Brood 1)	24	2	2	2	2	2	2	N/E	N/E	N/E	N/E	MA9 (100)
<i>S. rastrelliger</i> (Brood 2)	20	2	2	2	2	2	2	N/E	N/E	N/E	N/E	MA9 (100)
<i>S. rastrelliger</i> (Brood 3)	11	2	2	3	4	2	4	N/E	N/E	N/E	N/E	MA6 (27), MA9 (9), MA10 (64)
<i>S. rastrelliger</i> (Brood 4)	24	2	2	3	2	2	4	N/E	N/E	N/E	N/E	MA3 (21), MA6 (79)
<i>S. ruberrimus</i>	96	N/E	2	2	4	N/E	3	4	1	0	1	MQ8 (98), MQ30 (1), MQ43 (1)
<i>S. ruberrimus</i>	48	N/E	1	2	3	N/E	1	2	1	3	2	MQ24 (94), MQ54 (2), MQ65 (4)
<i>S. ruberrimus</i>	48	N/E	2	1	1	N/E	1	2	0	2	2	MQ6 (4), MQ31 (96)

within a brood while only detecting 2 or fewer paternal alleles at any locus.

Modeling the effect of multiple paternity on the inbreeding coefficient and genetic diversity. To examine the potential effect of multiple paternity on the genetic diversity of a brood, we resampled genotypic data from several fish in our aquarium husbandry studies. Genotypic data, from the pairing of a female with each of the implicated breeding males, were sampled with replacement for 1000 iterations and used to construct composite offspring genotypes. The genotypes of these 'pseudo-larvae' were then tested in every possible combination, considering the number of sires of a brood and assuming equal levels of paternity for all males. The inbreeding coefficient, F_{IS} (Weir & Cockerham 1984), and the number of alleles present at each locus were determined using GenePop v3.4 (Raymond & Rousset 1995). Means and the standard deviations of F_{IS} (see Fig. 2) and total allele number across all loci (see Fig. 3) are presented for up to 4 (*Sebastes rastrelliger*) or 5 (*S. ruberrimus*) sires.

RESULTS

Aquarium husbandry studies

As seen in Table 1, >1 father was identified in Broods 3 and 4 of *Sebastes rastrelliger*. The finding of 3 fathers in Brood 3 supports the assertion of Neff & Pitcher (2002) that multiple fathers can be detected with low sample size ($n = 11$ of approximately 400 000 larvae). Similarly, for the *S. ruberrimus* broods, it was found that multiple males contributed to the paternity of a single brood (see Table 1). In all cases, broods that were positive for multiple paternity showed unequal paternal contribution, with a skew towards a single male.

Wild-caught specimens

All wild-caught females and larvae were successfully genotyped at 4 or 5 loci. As 100 larvae were pooled and co-extracted, the resulting allelogram represented all alleles at each locus within a brood, with no quantification as to the number of larvae with a particular genotype. After subtracting the maternal alleles from this composite genotype, the number of paternal alleles was counted.

If >2 paternal alleles were found at any of the 5 analyzed loci, the brood was scored as positive for multiple paternity. As stated previously, this is a conservative assay for multiple paternity as shared alleles between dam and sire, as well as between different sires, can easily mask the true number of sires (see Table 1 to compare the number of deduced paternal alleles at each locus with the number of sires found in the aquarium studies). Of the 28 wild-caught individuals, representing 16 species, broods from 9 species showed evidence of multiple paternity (see Table 2).

DISCUSSION

Our results show occurrences of multiple paternity in 10 species (*Sebastes atrovirens*, *S. brevispinis*, *S. diploproa*, *S. elongatus*, *S. goodei*, *S. jordani*, *S. proriger*, *S. rastrelliger*, *S. ruberrimus*, and *S. rufus*) from both captive and wild populations. The remaining 7

Table 2. *Sebastes* spp. Number of deduced paternal alleles from wild-caught specimens after subtraction of maternal alleles from collective genotypes of 100 co-extracted larvae from each brood. NA: failed amplification of microsatellite locus; ID: identification sequence. Values in bold indicate the presence of more than 2 paternal alleles

Species	Collection ID	Sra4	Sra8	Spi4	Spi6	Spi10
<i>S. atrovirens</i> ^a	SWFSC_15-95	2	3	2	2	2
<i>S. auriculatus</i>	SWFSC_36-82	1	2	2	2	1
<i>S. aurora</i>	SWFSC_42-2	1	1	0	1	2
<i>S. aurora</i>	SWFSC_55-31	0	1	1	NA	0
<i>S. brevispinis</i> ^a	SWFSC_92-4	0	3	1	1	1
<i>S. brevispinis</i>	SWFSC_91-57	1	NA	2	2	2
<i>S. chlorostictus</i>	SWFSC_155-81	1	2	1	2	2
<i>S. chlorostictus</i>	SWFSC_185-20	1	2	2	2	1
<i>S. diploproa</i> ^a	SWFSC_74-8	2	1	2	2	3
<i>S. diploproa</i>	SWFSC_45-37	2	0	1	2	2
<i>S. diploproa</i>	SWFSC_45-41	2	0	2	2	1
<i>S. elongatus</i>	SWFSC_89-67	0	1	1	2	1
<i>S. elongatus</i>	SWFSC_50-56	1	2	0	2	1
<i>S. elongatus</i> ^a	SWFSC_50-58	2	1	1	4	2
<i>S. eos</i>	SWFSC_176-11	1	1	1	2	0
<i>S. goodei</i> ^a	SWFSC_236-25	2	2	2	3	2
<i>S. goodei</i>	SWFSC_236-26	2	2	2	NA	1
<i>S. helvomaculatus</i>	SWFSC_73-45	2	2	1	2	1
<i>S. helvomaculatus</i>	SWFSC_80-68	2	1	1	1	2
<i>S. jordani</i> ^a	SWFSC_56-4	1	3	3	0	2
<i>S. proriger</i> ^a	SWFSC_89-65	3	1	3	NA	2
<i>S. proriger</i>	SWFSC_66-25	1	0	1	NA	2
<i>S. proriger</i>	SWFSC_69-52	1	1	2	NA	0
<i>S. ruberrimus</i> ^a	SWFSC_70-81	4	0	2	2	2
<i>S. rufus</i> ^a	SWFSC_176-14	3	0	0	NA	0
<i>S. wilsoni</i>	SWFSC_69-81	2	2	1	2	2
<i>S. wilsoni</i>	SWFSC_69-83	2	2	1	2	1
<i>S. zacentrus</i>	SWFSC_88-6	1	NA	2	2	1

^aMultiple paternity

species showed no evidence of multiple paternity, but such events may have been missed due to the limited and conservative nature of this study. Finding positive evidence of this in 10 of the 17 (59%) species and 14 of the 35 broods (40%) examined, using a fairly conservative approach, suggests that multiple paternity within the genus is somewhat common. Further, examples of multiple paternity were found in species representing the majority of clades and ecotypes (i.e. deep soft sediment [*S. diploproa* and *S. elongatus*], deep high-relief reef [*S. ruberrimus* and *S. rufus*], schooling semi-pelagic [*S. jordani*], midwater near-shore [*S. atrovirens*], and shallow high-relief reef [*S. rastrelliger*]), in the northeast Pacific (see Fig. 1). However, due to the limited number of individuals examined within individual species, we were unable to estimate the frequency of this behavior in natural populations.

Ng et al. (2003) found evidence for multiple paternity in *Sebastes marmoratus*, an appropriate outgroup for *Sebastes* (Hyde & Vetter 2007), suggesting that this may be a pleisiomorphic trait and that examination of additional species within the genus and subfamily will likely yield additional occurrences of this behavior. However, species within the genera *Helicolenus* and *Hozukius* should be examined to determine the pleisiomorphic state with certainty.

This finding begs the question as to the possible advantages gained through mating behaviors that result in multiple paternity. This can be considered from the point of view of both individual reproductive fitness and the subsequent consequences on population level genetic diversity. Rockfish females seem to gain no direct material (e.g. food and shelter) or protective (e.g. egg guarding by males) benefits from the mating event, gaining little more than gametes. This contrasts with other animals such as birds, where females often gain material benefits (e.g. access to male's territory or shelter, food, protection) through mating.

Numerous studies, in mostly *K*-selected organisms, have suggested that the choice of a high-quality mate may confer enhanced reproductive fitness on the female. However, in *r*-selected marine species that produce large quantities of progeny, most of which die at early life stages, chance and environmental matching (sensu Cushing 1990) is thought to play the greatest role in subsequent reproductive success. Under these environmental constraints, bet-hedging to match unpredictable environmental conditions may be an important component of reproductive success. In the present study, we consider a few ideas that may have direct bearing on *Sebastes* spp. and give a few examples where benefits of these strategies have been realized in other taxa.

Genetic diversity

Enhancement of genetic diversity is a particularly compelling theory to explain this trait in *r*-selected species. Though direct comparison of allelic diversity at selectively neutral loci does not represent the true extent of phenotypic variation possible within a population, estimates of genetic diversity using these markers may act as a proxy for assessing the potential for adaptive variability within a population. Using genotypic data from our captive populations, we show that the average number of alleles in a single brood, across all microsatellite loci examined, increases significantly when the brood is the result of multiple sires (see Fig. 2). Though our simulation is based upon a possibly unrealistic assumption of equal paternity among sires, even broods with a strong skew toward 1 sire will show increased diversity, though the magnitude may be dampened. Such increases in diversity may ameliorate the negative effects on genetic diversity that are possible with sweepstakes recruitment. Beyond the direct benefits of increased diversity, several indirect benefits relevant to *Sebastes* spp. have been realized by other taxa (i.e. increased schooling competence and predator avoidance [Evans & Magurran 2000] and resistance to parasites [Baer & Schmid-Hempel 1999]).

Inbreeding depression

Inbreeding depression, the loss of genetic heterozygosity in a population as a result of related individuals mating, can have profound effects upon reproductive success and subsequent conservation and management strategies of exploited species. By resampling the genotypic data from our captive populations we modeled the change in the inbreeding coefficient of a brood (F_{IS}) when multiple sires were present (see Fig. 3), showing that F_{IS} tends toward zero with increasing number of sires. Given the remarkable site fidelity (Mitamura et al. 2002), lack of movement of adult rockfish, and low geographic dispersal of larvae in some demersal species (Buonaccorsi et al. 2005), there is a reasonable concern for inbreeding.

Population ramifications

Under ideal conditions, the effective (N_E) and census population sizes (N_C) should be equal. However, several factors may act to decrease N_E (e.g. unequal sex ratio, mating system, age/size-biased reproductive success, overlapping generations, and populations that experience a high variance in reproductive success). Hedgecock (1994) showed that sweepstakes recruit-

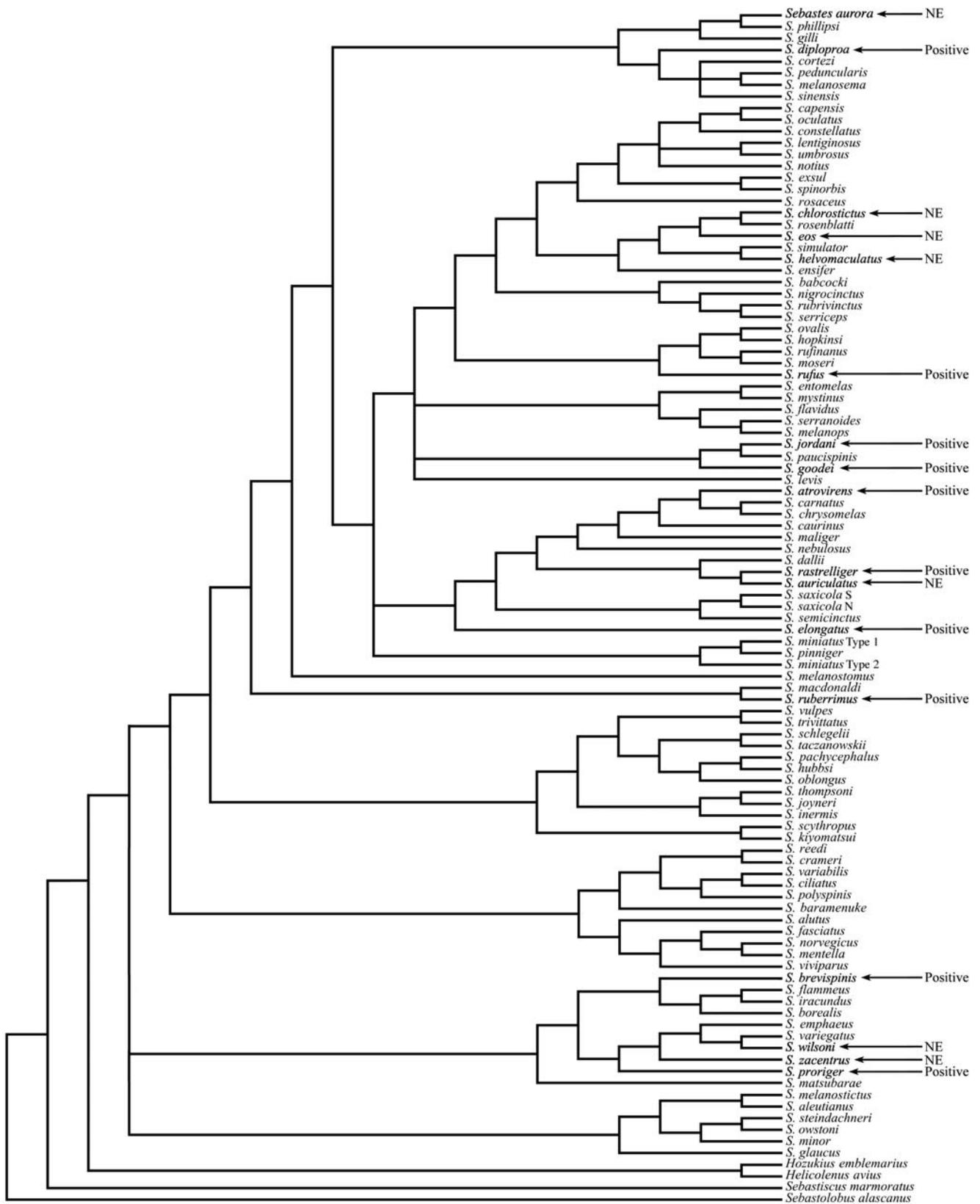


Fig. 1. Phylogeny of *Sebastes* spp. (adapted from Hyde & Vetter 2007). Species positive for multiple paternity are indicated. NE: species examined, but with no evidence for multiple paternity. Species names in bold indicate species examined in this study

ment in oyster populations created a difference between N_E and N_C of 5 orders of magnitude. Recent studies of N_E in several r -selected marine fishes including squirefish *Chrysophrys auratus* (Hauser et al. 2002), red drum *Sciaenops ocellatus* (Turner et al. 2002), canary *Sebastes pinniger* (Gomez-Uchida 2006) and darkblotched *S. crameri* rockfish (Gomez-Uchida & Banks 2006), and Atlantic cod *Gadus morhua* (Hutchinson et al. 2003) have shown similar discrepancies of 3 to 5 orders of magnitude.

The studies of Hauser et al. (2002) and Hutchinson et al. (2003) found that despite populations in the millions, heavy fishery exploitation over the past 50 yr had caused a significant decline in both N_E and genetic diversity. This disparity was due to the large difference between N_E and N_C in these species, requiring large census population sizes (i.e. millions to 10s of millions) to maintain N_E at a level that would preserve long-term genetic diversity. This realization suggests that many of the world's ex-

ploited species may have already undergone similar reductions in genetic diversity and perhaps adaptability due to the intense levels of exploitation over the past 50 to 100 yr.

Interestingly, in a study of the heavily over-fished canary rockfish *Sebastes pinniger*, Gomez-Uchida (2006) did not find a reduction in genetic diversity from samples spanning 31 yr. This finding may be due in part to the lack of samples from the pre- or early exploitation period, as well as the greater lifespan of canary rockfish (~75% greater than Hauser et al.'s [2002] fish), a life-history trait that could temporarily dampen the effect of overfishing on genetic diversity. Though canary rockfish were not assayed in the present study, the occurrence of multiple paternity in this species could serve as a buffer against loss of genetic diversity.

Multiple paternity creates broods with more possible genetic combinations than possible with single father parentage, aiding in the maintenance of genetic diversity and preventing the loss of rare alleles (Waples 1987) within populations that undergo sweepstakes-like recruitment. Interestingly, Gilbert-Horvath et al. (2006) found no evidence for genetic heterogeneity between individual recruitment pulses of young-of-the-year and adult populations of kelp rockfish *Sebastes atrovirens*, a species shown in the present study and that of Sogard et al. (2008) to practice multiple paternity. The authors suggest this as evidence that sweepstakes recruitment does not occur in this species; however, this lack of heterogeneity could also be due to the increased genetic diversity of multiply sired broods.

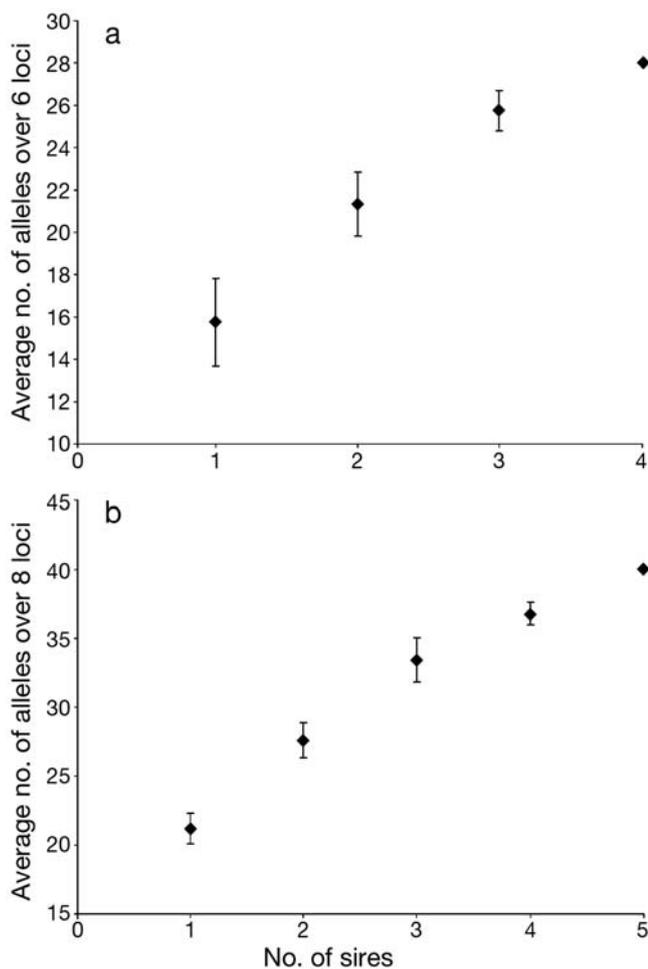


Fig. 2. (a) *Sebastes rastrelliger* and (b) *S. ruberrimus*. Means and standard deviations of modeled genetic diversity of a brood, in relation to increasing number of sires, are plotted

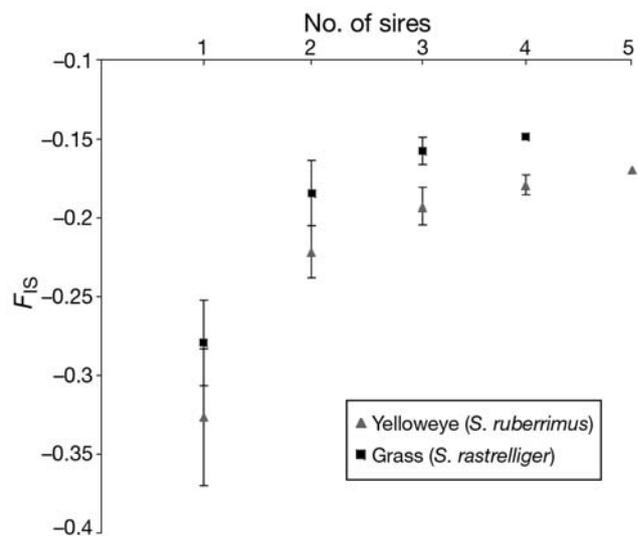


Fig. 3. *Sebastes rastrelliger* and *S. ruberrimus*. Means and standard deviations of modeled inbreeding coefficient (F_{IS}) (Weir & Cockerham 1984) of a brood, in relation to increasing number of sires, are plotted. F_{IS} of adult populations were -0.1011 (*S. rastrelliger*) and 0.0505 (*S. ruberrimus*)

Concomitant with increased genetic diversity, multiple paternity carries with it an increase in N_E (Waples 1987, Sugg & Chesser 1994). Waples (1987) calculated that multiple paternity can almost double N_E . Though a 2-fold difference may seem minor, when coupled with the large discrepancies between N_C and N_E (i.e. 3 to 4 orders of magnitude in *Sebastes crameri* and *S. pinniger*) this equates to a potential buffer against loss of genetic diversity of millions to 10s of millions of fish. For example, consider a species similar to *S. pinniger* with $N_E = 4694$ and $N_C = 5\,453\,000$ (Gomez-Uchida 2006), doubling N_E would result in the genetic diversity of this species being equal to that of a similar though monogamously mating species with $N_C = 10\,906\,000$. Such a buffer should help recover and maintain genetic diversity during bottleneck (e.g. overfishing, severe environmental fluctuations, disease) and/or founding events.

Acknowledgements. We thank the captain and crew of the FV 'Dominator' for assistance in collection of samples during the National Marine Fisheries Service—Alaska Fisheries Science Center 1998 RACE survey. Additionally, we thank S. Grossberg, J. Stannard, and C. Taylor for collection of samples from the Southern California Bight. P. Hastings, R. Rosenblatt, N. Warner, and 3 anonymous reviewers provided comments on earlier versions of this manuscript. Disclaimer: The authors and their agencies do not necessarily approve, recommend, or endorse any proprietary product or material mentioned in this publication. The views expressed herein are those of the authors and do not necessarily reflect the views of their agencies or institutions.

LITERATURE CITED

- Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumblebee. *Nature* 397:151–154
- Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology* 85:1258–1264
- Boehlert GW, Yoklavich M (1984) Reproduction, embryonic energetics, and the maternal-fetal relationship in the viviparous genus *Sebastes*. *Biol Bull* 167:354–370
- Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD (2005) Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. *Conserv Biol* 6: 697–713
- Cushing DH (1990) Plankton production and year class strength in fish populations: an update of the match/mismatch hypothesis. *Adv Mar Biol* 26:249–293
- de Bruin JP, Gosden RG, Finch CE, Leaman BM (2004) Ovarian aging in two species of long-lived rockfish, *Sebastes aleutianus* and *S. alutus*. *Biol Reprod* 71:1036–1042
- Evans JP, Magurran AE (2000) Multiple benefits of multiple mating in guppies. *Proc Natl Acad Sci USA* 97: 10074–10076
- Gilbert-Horvath EA, Larson RJ, Garza JC (2006) Temporal recruitment patterns and gene flow in kelp rockfish (*Sebastes atrovirens*). *Mol Ecol* 15:3801–3815
- Gomez-Uchida D (2006) Spatial and temporal scales of genetic change in two overfished rockfishes. PhD thesis, Oregon State University, Corvallis, OR
- Gomez-Uchida D, Banks MA (2006) Estimation of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*. *J Hered* 97:603–606
- Gomez-Uchida D, Hoffman EA, Arden WR, Banks MA (2003) Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species. *Mol Ecol Notes* 3:387–389
- Hauser L, Adcock GJ, Smith PJ, Ramirez JHB, Carvalho GR (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
- Hedgecock D (1994) Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont AR (ed) *Genetics and evolution of aquatic organisms*. Chapman & Hall, London, p 122–134
- Hutchinson WF, van Oosterhout C, Rogers SI, Carvalho GR (2003) Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proc R Soc Lond B Biol Sci* 270: 2125–2132
- Hyde JR, Vetter RD (2007) The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). *Mol Phylogenet Evol* 44:790–811
- Love MS, Caselle JE, Buskirk WV (1998) A severe decline in the commercial passenger fishing vessel rockfish (*Sebastes* spp.) catch in the Southern California Bight, 1980–1996. *CCOFI Rep* 39:180–195
- Love MS, Yaklovich M, Thorsteinson L (2002) The rockfishes of the Northeast Pacific. UC Press, Berkeley, CA
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* 7:639–655
- Mitamura H, Arai N, Sakamoto W, Mitsunaga Y and others (2002) Evidence of homing of black rockfish *Sebastes inermis* using biotelemetry. *Fish Sci* 68:1189–1196
- Mori H, Nakagawa M, Soyano K, Koya Y (2003) Annual reproductive cycle of black rockfish *Sebastes schlegeli* in captivity. *Fish Sci* 69:910–923
- Moser HG (1967) Reproduction and development of *Sebastes paucispinis* and comparison with other rockfishes off California. *Copeia* 1967:773–779
- Muñoz M, Casadevall M, Bonet S, Quaggio-Grassiotto I (2000) Sperm storage in the ovary of *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaenidae): an ultrastructural study. *Environ Biol Fishes* 58:53–59
- Neff BD, Pitcher TE (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fishes. *J Fish Biol* 61:739–750
- Ng W, Sadovy Y, Leung FCC (2003) Mating system of the rockfish, *Sebastes marmoratus*, as revealed by DNA fingerprinting. *Ichthyol Res* 50:339–348
- Nunney L (1993) The influence of mating system and overlapping generations on effective population size. *Evolution* 47:1329–1341
- Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Sogard SM, Gilbert-Horvath E, Anderson EC, Fisher R, Berkeley, SA, Garza JC (2008) Multiple paternity in viviparous kelp rockfish, *Sebastes atrovirens*. *Environ Biol Fishes* 81:7–13, doi:10.1007/s10641-006-9170-9
- Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. *Genetics* 137:1147–1155

- Takahashi H, Takano K, Takemura A (1991) Reproductive cycles of *Sebastes taczanowskii*, compared with those of other rockfishes of the genus *Sebastes*. *Environ Biol Fishes* 30:23–29
- Tolimieri N, Levin PS (2005) The roles of fishing and climate in the population dynamics of bocaccio rockfish. *Ecol Appl* 15:458–468
- Turner TF, Wares JP, Gold JR (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
- Waples RS (1987) Sperm storage, multiple insemination, and genetic variability in mosquitofish: a reassessment. *Copeia* 1987(4):1068–1071
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370
- Westerman ME, Buonaccorsi VP, Stannard JA, Galver L and others (2005) Cloning and characterization of novel micro-satellite DNA markers for the grass rockfish, *Sebastes rastrelliger*, and cross-species amplification in 10 related *Sebastes* spp. *Mol Ecol Notes* 5:74–76

*Editorial responsibility: Otto Kinne,
Oldendorf/Luhe, Germany*

*Submitted: February 13, 2007; Accepted: October 23, 2007
Proofs received from author(s): March 14, 2008*