UNIVERSITY OF CALIFORNIA, SAN DIEGO

The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier): Insights into speciation and biogeography of temperate reef fishes.

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Marine Biology

by

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ABSTRACT OF THE DISSERTATION

The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier): Insights into speciation and biogeography of temperate reef fishes.

by

John R. Hyde

Doctor of Philosophy in Marine Biology

University of California, San Diego, 2007

Philip A. Hastings, Chair

Dispersal and speciation in the marine environment have long been key topics in evolutionary biology and more recently in conservation biology. The genus *Sebastes*, with approximately 110 species, is found throughout most of the world's cold-temperate seas. Their breadth of phenotypic and species diversity, ecological dominance, and importance to world fisheries has made them a focus of intensive study by countless researchers. In this dissertation, DNA sequence and microsatellite data were used to investigate patterns of speciation, distribution, and mating habits.

In Chapter I, DNA sequence data from seven mitochondrial and two nuclear genes, were used to evaluate possible geographic origin, patterns and timing of dispersal, speciation patterns and drivers, and inter-species relationships. The data support a middle

Miocene origin for *Sebastes* spp. in deep habitats of the Northwest Pacific followed by dispersal into the Northeast Pacific and eventually into the southern hemisphere, driven by progressive cooling and strengthening of major ocean currents. Previous hypothesized relationships, shown to be mostly poly- or para- phyletic, were revised in light of the findings.

Chapter I provided evidence for two new "cryptic" species. In Chapter II, sequence of the mitochondrial cytochrome *b* gene, in combination with nine microsatellite loci, was used to evaluate the geographic and bathymetric range of the vermilion rockfish species complex, as well as test for reproductive isolation between the putative species. Genetic analyses supported the presence of two species, separated primarily by depth of adult occurrence. This finding, in association with distribution patterns of 12 recent species pairs, suggests a novel speciation mode based upon the loss/truncation of an ontogenetic migration phase.

In Chapter III, DNA sequence data from Chapter II was used to evaluate patterns of population connectivity and gene flow within the vermilion rockfish. A range-wide analysis showed high levels of genetic heterogeneity, likely driven by limited larval dispersal and barriers to gene flow across major oceanographic features.

Patterns of paternity and mating system were investigated in Chapter IV using microsatellite loci. Paternity analyses were performed on larvae from both captive and wild populations, representing most major lineages found in Chapter I. Ten of the 17 examined species, showed evidence for multiple sires within a single brood. The implications for genetic diversity produced by this polygynandrous mating system are discussed.

I.

The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier)

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The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier)

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Abstract

The evolutionary relationships of the livebearing rockfishes of the genus *Sebastes* have been a point of interest since their original description. With over 65 species found in the northeast Pacific (NEP), 27 in the northwest Pacific (NWP), seven in the Gulf of California (GC), four in the north Atlantic (NA) and at least two in the southern hemisphere (SH), they represent a fascinating lineage for studies of spatial and temporal patterns of dispersal, vicariance and speciation in the marine environment. Previous studies of *Sebastes* species have attempted to reconstruct their phylogeny using allozyme patterns or portions of a single mitochondrial gene while incompletely sampling the genus, resulting in a partial picture with low statistical support. In this study, genetic analyses using sequence data (5581 characters) from seven mitochondrial genes (cytochrome *b*, cytochrome *c* oxidase subunit 1, 12S rRNA, 16S rRNA, tRNA proline, tRNA threonine and the control region) and two nuclear genes (recombination activating gene 2 and internal transcribed spacer 1), along with a near complete sampling of species, have produced a well supported phylogenetic hypothesis of the relationships between *Sebastes* species as well as clarifying their position within the scorpaenid subfamily, Sebastinae. Though studies of similar magnitude have been conducted at the family and subfamily level, this represents the most detailed and extensive examination of biogeography and marine speciation within a single, widely distributed marine fish genus. Both Bayesian posterior and maximum parsimony analyses produced highly similar phylogenies suggesting an origin for *Sebastes* at high-latitudes in the NWP. The majority of previously proposed sub-generic groupings based upon morphology are found to be either para- or polyphletic. Using Bayesian-derived genetic distance measures together with rate smoothing techniques, a molecular clock was applied to the phylogeny. The clock-calibrated data suggest that *Sebastes* originated in the middle Miocene, concordant with fossil data, and began substantial diversification and dispersal in synchrony with high-latitude cooling and establishment of productive upwelling systems across the north Pacific (NP) in the late Miocene. Contrary to contemporary taxonomic criteria that often group Asian and North American species based on common morphology, the molecular phylogenies tend to indicate geographically circumscribed lineages with no evidence for repeated long distance dispersal between disjunct biogeographic provinces (e.g., Asian species nested within a North American lineage). No examples of large-scale glacial vicariance as would be suggested by Asian and North American sibling species were observed. To the contrary, sibling species tended to be in geographic proximity. While occasional long distance dispersal may occur, such as the single colonization of the SH, and thermal barriers presently exist between the NP, NA, GC, and SH taxa, the observable patterns in *Sebastes* suggest colonization occurs by stepwise invasion of newly available habitat when temperature conditions permit. Colonization events are spread throughout the sub-generic lineages. Vicariant isolation processes may occur on smaller geographic scales perhaps due to local isolating mechanisms such as glacial advance and retreat, sea level change, and ocean currents. Allopatric differences may be enhanced by a tendency for female mate choice and assortative mating in these livebearing species. The ongoing process of thermal advance and retreat is reflected in contemporary patterns of phylogeographic population genetic structure within species and may be enhanced under climate warming.
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Keywords: *Sebastes*; Rockfish; Sebastinae; Scorpaenidae; Evolution; Speciation; Phylogeny

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1. Introduction

The processes of genetic divergence, reproductive isolation, and species formation in the marine environment are poorly understood. Typically, marine species are characterized by large population sizes, extensive planktonic dispersal, and large geographic ranges; all processes that tend to limit genetic differentiation and species formation (Palumbi, 1994). For marine fishes, the study of speciation is compounded by the great age and deep genetic separation typical of most fish taxa (Grant and Bowen, 1998). Phylogenetic lineage reconstruction is often obscured or poorly supported due to saturation and back mutation at informative genetic loci while paleo-geographic landmarks are often so distant they do not provide good mileposts for estimating the timing of speciation events. Differences in the rates of nucleotide substitution based on body size, metabolic rate and generation time can confound molecular clock comparisons between distant fish taxa (Martin et al., 1992; Martin and Palumbi, 1993). The genus *Sebastes* has attracted the attention of marine evolutionary biologists because of: (1) The comparatively recent (Miocene) origin of the genus (Wourms, 1991), (2) The incredible richness and ecological diversity of species (Love et al., 2002), (3) A variety of well defined paleo-geographic colonization events that can serve as calibration points for molecular clocks, (4) Phylogenetically informative levels of inter-specific genetic variance (Seeb, 1986; Rocha-Olivares et al., 1999a,b), (5) A wide array of recently evolved sibling species pairs (Orr and Blackburn, 2004; Narum et al., 2004; Gharrett et al., 2005), and (6) High levels of intra-specific genetic structure that can be correlated with ocean currents (Rocha-Olivares and Vetter, 1999) and alterations in sea level and ice cover (Buonaccorsi et al., 2002). Studies using innovative analytical approaches to the study of marine speciation, such as the "ancient species flocks" concept, have already been applied to *Sebastes* (Johns and Avise, 1998; Ruber and Zardoya, 2005) but limited species numbers, sample size, genetic loci, and paleo-oceanographic information have severely limited the full understanding of the radiation of the genus and its utility in understanding general mechanisms of marine speciation.

Sebastes is the most speciose scorpaenid genus with approximately 110 species known worldwide (Eitner et al., 1999; Love et al., 2002; Kai et al., 2003; Orr and Blackburn, 2004; Nelson, 2006). Together with *Helicolenus*, *Hozukius*, and *Sebastiscus* they are grouped into the scorpaenid subfamily Sebastinae. This group is notable for possession of a developed swimbladder with associated musculature (Hallacher, 1974) as well as the evolution of viviparity (Wourms, 1991). Species of *Sebastes* occur in cold-temperate waters throughout the northeast Pacific (NEP) ($n \sim 70$ species) (Love et al., 2002), northwest Pacific (NWP) ($n \sim 33$ species) (Kai et al., 2002a,b, 2003), north Atlantic (NA) ($n = 4$ species) (Nedreaas et al., 1994; Johansen et al., 2000, 2002; Roques et al., 2002), and across the southern hemisphere (SH) ($n \sim 2$ species) (Rocha-Olivares et al., 1999c). Along both coasts of North America, many species are heavily exploited in both commercial and recreational fisheries (Love et al., 2002).

Morphologically and ecologically, *Sebastes* species are notable for their diversity in form and function. They can be found from tide pools (i.e., *S. rastrelliger*) to depths in excess of 1000 m (i.e., *S. cortezi*) (Chen, 1975; Love et al., 2002). Body shape, size, and head spination seem strongly correlated with the life history parameters of individual species or clades. Mobile, semi-pelagic species (e.g., *S. brevispinis, S. goodei, S. jordani, S. owstoni* and *S. paucispinis*) tend to have an elongated body, greatly reduced head spines, and a general drab to dusky coloration. Sedentary, benthic species (e.g., *S. chrysomelas, S. hubbsi, S. levis, S. oblongus, S. serriceps*, and *S. simulator*) have more typical scorpaenid morphologies with deep bodies, strong head spination, and distinctive, often striking color patterns. With over 100 species, intermediates between these morphologic extremes are common.

Ecologically, *Sebastes* species show a wide range of roles. Near-shore species are partitioned by depth, benthic habitat relief, and relationship to macro-algal environments (e.g., stands of *Macrocystis pyrifera*). Further offshore, this trend continues with species partitioned again by depth, benthic relief, and substrate type (e.g., soft sediment, cobble, boulder fields, etc.) (Allen, 1982; Gunderson and Vetter, 2005). To further add complexity to the habitat partitioning by adults, many species show strong ontogenetic shifts in affinity. In the extreme example, *S. diploproa* juveniles may spend up to a year associated with drifting surface algal rafts, followed by a period of mid-water residence, eventually settling as adults onto soft bottom habitat in depths to almost 800 m (Boehlert, 1977; Love et al., 2002). A common pattern in the NEP is recruitment of pelagic juveniles to near-shore environments, often kelp (*Macrocystis* and *Nereocystis*) and seagrass (*Phyllospadix* and *Zostera*) beds, followed by a transition to adult habitat ranging from kelp forest (e.g., *S. atrovirens*), deep soft sediment (e.g., *S. saxicola*), to deep offshore high relief reefs (e.g., *S. paucispinis*) (Love et al., 1991). Once settled, adult *Sebastes* spp. tend to show strong site fidelity (Mitamura et al., 2002; Starr et al., 2002).

Despite their broad geographic distribution and diversity in form and function, *Sebastes* species are limited to cool-temperate, upwelling driven systems. Warm, oligotrophic waters represent a significant barrier to their spread. Short-term disruptions of upwelling events (e.g., ENSO events) can have profound effects upon reproduction and survival (Eldridge et al., 1991; Boehlert and Yoklavich, 1984; Ventresca et al., 1995; Woodbury, 1999). On local scales, larval and pelagic juvenile distributions can be strongly influenced by the unique physical parameters of these upwelling systems (Moser and Boehlert, 1991; Moser and Smith, 1993; Ross and Larson, 2003).

The phylogeny and evolution of the group has been the subject of much debate and has resulted in a great deal of confusion (see Kendall, 2000; for a comprehensive and entertaining review). Early studies on the relationships

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between species used both morphologic (e.g., head spination, body shape, gas bladder musculature, and color patterns) as well as meristic characters to identify up to 23 subgeneric lineages (several sub-genera have at times been raised to genera) within the currently recognized genus *Sebastes*. These groupings have been variously lumped and split by different authors, mostly based upon their personal belief on the scoring of character states and the inclusion and weighting of phylogenetic characters. Clearly, the presence of such great morphological variation coupled with the propensity of *Sebastes* species for ecological partitioning of different habitats suggests that there could be a fair amount of convergent evolution of body shape and morphology. Additionally, though certain morphologic characters, such as the presence of particular head spines, may seem like novel features, larvae of all examined *Sebastes* species typically possess a full or nearly full, scorpaenid compliment of head spines, some of which are subsequently lost during development (Moser, 1996). Character states that are lost during ontogeny may confuse relational studies unless scored at appropriate life stages.

More recently, protein and mitochondrial DNA (mtDNA) data have been used to test the validity of several of these sub-genera and to provide an overview of the biogeography of the genus (Seeb, 1986; Rocha-Olivares et al., 1999a,b; Kai et al., 2003). The majority of the species ($n = 75$) have had a portion of their mitochondrial cytochrome *b* gene (751 bases) sequenced and subjected to phylogenetic analyses (Rocha-Olivares et al., 1999a,b; Kai et al., 2003). The relationships hypothesized by these studies have both supported (Rocha-Olivares et al., 1999b) and refuted (Kai et al., 2003; Li et al., 2006) previous classifications.

These mtDNA-based studies have helped provide valuable information on the possible area of origin and timing of speciation events for *Sebastes* as well as evidence of their relation to other members of the subfamily Sebastinae (Rocha-Olivares et al., 1999b; Kai et al., 2003). However, to better understand the pattern and timing of dispersal and speciation it is necessary to more completely sample the genus and collect data from additional mitochondrial and nuclear loci. This approach is necessary to corroborate phylogenetic hypotheses and to improve statistical support of groupings by increasing the number of informative characters available for analysis (Zwickl and Hillis, 2002). Unfortunately, many remaining species have proven difficult to obtain for genetic analysis because of their rarity. In some cases the only known specimens have been fixed in formalin and thus are not ideal for genetic analysis (i.e., Lea and Fitch, 1972, 1979; Chen, 1975). Obtaining genetic data from these rare, often type, specimens in museum collections, while difficult, is a necessity to ultimately answer questions on the relationships between members of the genus and to understand the radiation of the genus as well as the role of environmental factors such as the advent of ocean cooling and development of upwelling systems.

One of the primary motivations for this study was to test Barsukov's (1981) hypothesis of origin for *Sebastes* in the NWP as there are no other extant genera of Sebastinae in the NEP. Traditional use of the fossil record to infer origin is difficult due to the relatively recent origin of the genus and the paucity of fossil data. The best fossil deposits of *Sebastes* spp. are found in the late Miocene diatomite deposits in Lompoc, California (Jordan and Gilbert, 1920; Barsukov, 1989) and Tertiary deposits in Japan (Niino, 1951). Species diversity "hot spots", likely due to the interaction between cold and warm temperate waters in these areas, occur on both sides of the Pacific, one centered off Japan and the other in the southern California bight. The Goldilocks dilemma of temperate reef fishes is that expansion to the south is blocked by waters that are too warm while expansion to the north is blocked by cold Arctic waters. Clearly this was not always the case as disjunct lineages exist in the NA, Gulf of California (GC), and the SH.

In this paper we will provide the most comprehensive and robust phylogenetic analysis of *Sebastes* to date. Clades with high statistical support will be used to reconcile past disagreements over the correct placement of species within sub-generic classifications. Additionally, genetic distance metrics will be applied to these groups in an attempt to understand the timing and possible mechanisms of speciation. Finally sibling species will be examined to evaluate the role of vicariance and dispersal in speciation processes and how this may relate to previously hypothesized evolutionary patterns (e.g., Barsukov, 1981).

This study examined 101 species, 103 individuals and nine loci and as such represents, to our knowledge, the most detailed and extensive examination of biogeography and marine speciation within a single, widely distributed marine fish genus. As such, *Sebastes* presents a unique opportunity to examine the paleo-biogeography of fish colonization of the NP during the past 10 million years.

2. Materials and methods

2.1. Sample collection

Fish were collected using various techniques including hook and line, bottom trawl, pole spear, trap, and surface dip netting. In most cases, specimens were captured fresh and identified to species using Phillips (1957), Chen (1971, 1975), Miller and Lea (1972), Eschmeyer et al. (1983), Kramer and O'Connell (1988), Masuda et al. (1992), Love et al. (2002), and Nakabo (2002). Tissues from the majority of the NWP species were kindly donated by Y. Kai and T. Nakabo, Kyoto University, Japan. Tissues, either white muscle or pectoral fin, were preserved in 95% un-denatured ethanol pending genetic analysis. In a few cases, DNA was obtained from formalin-fixed museum specimens. Due to the difficulty of obtaining sufficient genetic data from formalin-fixed specimens, all species in this analysis that had DNA extracted from fixed specimens were ultimately replaced with ethanol-preserved larvae

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(i.e., *S. rufinanus*) or pelagic juveniles (i.e., *S. cortezi, S. peduncularis,* and *S. sinensis*) that were positively identified to species by comparison of morphology and DNA sequence to reference museum specimens (J. Hyde, unpublished data). Collection location and sample data are provided in Table 1.

The dataset consists of 97 currently recognized species of *Sebastes*, two new cryptic species (*S. miniatus* Type 1 and *S. saxicola* N, J. Hyde unpublished data), a single species from each of the three other Sebastinae genera, and one *Sebastolobus* species as an outgroup. Previous phylogenetic studies of the Scorpaeniformes (Smith and Wheeler, 2004) have shown that the Sebastinae is a valid subfamily and that *Sebastolobus* is an appropriate outgroup for this subfamily. Several nominal NWP *Sebastes* species were not included in this analysis as they are not reciprocally monophyletic at the examined mitochondrial genes, forming species complexes that are still undergoing lineage sorting (Y. Kai, 2005, pers. comm.). In these cases, a single species from each of the complexes was used for the analyses: (nominal species used in this study in brackets): [*S. hubbsi*]—*S. longispinis*; *S. cheni*—[*S. inermis*]—*S. ventricosus*; *S. chalcogrammus*—*S. nigricans*—*S. nudus*—[*S. pachycephalus*]; *S. ijimae*—[*S. vulpes*]—*S. zonatus*. Though *S. carnatus* and *S. chrysomelas* in the NEP are still undergoing mitochondrial lineage sorting (Alesandrini and Bernardi, 1999), the work of Narum et al. (2004) shows that these are valid species and we therefore feel they warrant inclusion in this analysis. Additionally, the inclusion of this species pair allows us to roughly approximate the minimum time necessary for sympatric species to reach reciprocal monophyly of mitochondrial lineages at the examined genes. Other than members of the four NWP complexes, only five of the currently recognized 110 *Sebastes* species are missing from this analysis; *S. itinus, S. koreanus, S. nivosus, S. varispinis*, and *S. wakiyai*.

2.2. DNA extraction

2.2.1. Ethanol preserved specimens

DNA was extracted from ethanol preserved tissue using various protocols, most often using a standard proteinase K digestion followed by a lithium chloride:chloroform nucleic acid purification and subsequent ethanol precipitation (Gemmel and Akiyama, 1996). DNA from the remaining samples was extracted using either the DNeasy kit (Qiagen) following the manufacturer's protocol or by use of a Chelex™ (Bio-Rad Laboratories) boiling technique (Hyde et al., 2005).

2.2.2. Formalin preserved specimens

Amplifiable DNA was extracted from formalin-fixed tissues using a modified antigen retrieval method based upon the protocol of Shi et al. (2002). This technique was successful for specimens that had been preserved for several months to nearly 100 years (J. Hyde unpublished data). Briefly, tissue was soaked overnight in 95% ethanol to help remove residual formalin. Approximately 100 mg of tissue was allowed to air dry and then placed in a boil-proof 1.5 ml tube containing 180 μL of the extraction buffer (28.6 mM citric acid, 28.6 mM KH_2PO_4, 28.6 mM H_3BO_3, pH 11). The sample was then placed in an autoclave and subjected to high temperature (~120 °C) and pressure for 20 min after which the pressure was allowed to slowly vent in order to minimize boiling of the sample. The alkaline buffer in conjunction with the high temperature and pressure is believed to reverse the fixative's cross-linking effect on the proteins, allowing them to be better digested by the proteinase. Once the sample had cooled to room temperature, 1.5 μL of 3 M sodium acetate (pH 5.2) was added to lower the pH to an acceptable level for proteinase K digestion. At this point the protocol followed that of the manufacturer for the QiaAmp DNA extraction kit (Qiagen) with a few exceptions. After digestion of the tissue with the proteinase K, 0.5 μL of 3 M sodium acetate (pH 5.2) and 1 μL of carrier RNA (1 μg/μL) (Qiagen) was added to help facilitate adsorption of DNA to the silica matrix of the spin column. DNA was eluted in 50 μL of the provided elution buffer.

2.3. PCR amplification

DNA was amplified from seven mitochondrial genes (cytochrome *b* (cytb), cytochrome *c* oxidase subunit 1 (cox1), 12 S rRNA, 16S rRNA, tRNA proline, tRNA threonine and the non-coding mitochondrial control region). Additionally, two nuclear genes (recombination activating gene 2 (RAG2) and internal transcribed spacer 1 (ITS1)) were sequenced. In the case of DNA extracted from formalin preserved tissue, small fragments (200–300 bp) were amplified and subsequently assembled from cytb only. Primer information is presented in Table 2.

For all primer pairs, 10 μL reaction volumes containing (67 mM Tris–HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercapto-ethanol, 2 mM $MgCl_2$, 800 μM dNTPs, 0.4 μM each primer, 0.5 units *Taq* DNA polymerase (New England Biolabs), and 1 μL of DNA template) were amplified using the following temperature profile in a PTC200 DNA Engine (MJ Research); 94 °C (2:00), 35 cycles of [94 °C (0:30), 59 °C (1:00), 72 °C (1:00)], followed by 3 min at 72 °C. All PCR batches contained at least one no template negative control to monitor for possible DNA contamination. Products were electrophoresed through a 2% (w/v) agarose gel in 1× Tris–Borate–EDTA buffer, stained with ethidium bromide and visualized via an UV-transilluminator. Reactions were digested using ExoSAP-IT (USB Corp.) to remove unincorporated primers and deoxynucleotides prior to cycle sequencing. Products had both strands individually cycle sequenced with BigDye v.1.1 Dye Terminators (Applied Biosystems) and analyzed on an ABI 3100 automated capillary sequencer (Applied Biosystems). DNA sequences from both strands were aligned and edited using Sequencher v4.5 (GeneCodes, Inc.).

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Table 1
Collection information and GenBank accession numbers for specimens used in this study

Genus	Species	Collection location				GenBank Accession Nos.								
		Collection number	Latitude (N)	Longitude (W)	Date	cytb	D-loop	cox1	12S rRNA	16S rRNA	ITS1	RAG2	tRNA Thr and Pro	
Helicolenus	*avius*	SWFSC 112-37	Tokyo, Japan		990116	DQ678505	DQ678608	DQ678402	DQ678196	DQ678299	DQ678711	DQ678807	DQ678910	
Hozukius	*emblemarius*	FAKU 81635	34.05	135.35	NA	DQ678499	DQ678602	DQ678396	DQ678190	DQ678293	DQ678705	DQ678801	DQ678904	
Sebastes	*aleutianus*	SWFSC 4-71	41.23	124.42	950709	DQ678418	DQ678521	DQ678315	DQ678109	DQ678212	DQ678624	DQ678720	DQ678823	
	alutus	SWFSC 121-65	55.69	157.47	990604	DQ678416	DQ678519	DQ678313	DQ678107	DQ678210	DQ678622	DQ678718	DQ678821	
	atrovirens	SWFSC 166-27	32.83	117.25	020717	DQ678423	DQ678526	DQ678320	DQ678114	DQ678217	DQ678629	DQ678725	DQ678828	
	auriculatus	SWFSC 36-82	33.00	117.28	020304	DQ678513	DQ678616	DQ678410	DQ678204	DQ678307	NA	DQ678815	DQ678918	
	aurora	SWFSC 42-2	34.91	121.05	980609	DQ678417	DQ678520	DQ678314	DQ678108	DQ678211	DQ678623	DQ678719	DQ678822	
	babcocki	SWFSC 179-35	58.63	−151.19	990623	DQ678422	DQ678525	DQ678319	DQ678113	DQ678216	DQ678628	DQ678724	DQ678827	
	baramenuke	FAKU 81605	39.48	141.32	NA	DQ678491	DQ678594	DQ678388	DQ678182	DQ678285	DQ678697	DQ678793	DQ678896	
	borealis	SIO 01-186	36.44	121.91	010904	DQ678506	DQ678609	DQ678403	DQ678197	DQ678300	DQ678712	DQ678808	DQ678911	
	brevispinis	SWFSC 92-4	48.41	126.05	980731	DQ678419	DQ678522	DQ678316	DQ678110	DQ678213	DQ678625	DQ678721	DQ678824	
	capensis	SWFSC 10-91	South Africa		9601	DQ678420	DQ678523	DQ678317	DQ678111	DQ678214	DQ678626	DQ678722	DQ678825	
	carnatus	SWFSC 147-86	31.42	116.67	001029	DQ678424	DQ678527	DQ678321	DQ678115	DQ678218	DQ678630	DQ678726	DQ678829	
	caurinus	SWFSC 124-4	43.13	124.45	990830	DQ678425	DQ678528	DQ678322	DQ678116	DQ678219	DQ678631	DQ678727	DQ678830	
	chlorostictus	SWFSC 156-89	32.85	117.30	0205	DQ678435	DQ678538	DQ678332	DQ678126	DQ678229	DQ678641	DQ678737	DQ678840	
	chrysomelas	SWFSC 163-11	33.22	119.50	010328	DQ678426	DQ678529	DQ678323	DQ678117	DQ678220	DQ678632	DQ678728	DQ678831	
	ciliatus	UW 043242-#27	53.73	165.54	960525	DQ678515	DQ678618	DQ678412	DQ678206	DQ678309	NA	DQ678817	DQ678920	
	constellatus	SWFSC 153-81	30.33	116.08	001027	DQ678436	DQ678539	DQ678333	DQ678127	DQ678230	DQ678642	DQ678738	DQ678841	
	cortezi	SWFSC 225-76	28.98	113.43	050408	DQ678497	DQ678600	DQ678394	DQ678188	DQ678291	DQ678703	DQ678799	DQ678902	
	crameri	SWFSC 134-40	36.78	122.12	920111	DQ678437	DQ678540	DQ678334	DQ678128	DQ678231	DQ678643	DQ678739	DQ678842	
	dalli	SWFSC 114-21	34.05	119.00	990227	DQ678427	DQ678530	DQ678324	DQ678118	DQ678221	DQ678633	DQ678729	DQ678832	
	diploproa	SWFSC 143-51	36.79	122.13	920112	DQ678438	DQ678541	DQ678335	DQ678129	DQ678232	DQ678644	DQ678740	DQ678843	
	elongatus	SWFSC 89-67	48.24	125.17	980730	DQ678434	DQ678537	DQ678331	DQ678125	DQ678228	DQ678640	DQ678736	DQ678839	
	emphaeus	SWFSC 7-84	47.92	122.58	950413	DQ678439	DQ678542	DQ678336	DQ678130	DQ678233	DQ678645	DQ678741	DQ678844	
	ensifer	UW 2003-016	32.61	117.31	50817	DQ678440	DQ678543	DQ678337	DQ678131	DQ678234	DQ678646	DQ678742	DQ678845	
	entomelas	SWFSC 3-50	37.24	122.72	950626	DQ678441	DQ678544	DQ678338	DQ678132	DQ678235	DQ678647	DQ678743	DQ678846	
	eos	SWFSC 176-11	32.80	117.80	020303	DQ678442	DQ678545	DQ678339	DQ678133	DQ678236	DQ678648	DQ678744	DQ678847	
	exsul	SWFSC 144-75	28.98	113.43	950327	DQ678443	DQ678546	DQ678340	DQ678134	DQ678237	DQ678649	DQ678745	DQ678848	
	fasciatus	SWFSC 14-29	North Atlantic		980213	DQ678444	DQ678547	DQ678341	DQ678135	DQ678238	DQ678650	DQ678746	DQ678849	
	flammeus	FAKU 81606	39.48	141.32	NA	DQ678486	DQ678589	DQ678383	DQ678177	DQ678280	DQ678692	DQ678788	DQ678891	
	flavidus	SWFSC 20-72	36.91	122.18	980615	DQ678445	DQ678548	DQ678342	DQ678136	DQ678239	DQ678651	DQ678747	DQ678850	
	gilli	SWFSC 129-60	32.75	117.75	000405	DQ678446	DQ678549	DQ678343	DQ678137	DQ678240	DQ678652	DQ678748	DQ678851	
	glaucus	FAKU 82532	43	144.38	NA	DQ678488	DQ678591	DQ678385	DQ678179	DQ678282	DQ678694	DQ678790	DQ678893	
	goodei	SWFSC 155-75	32.60	117.42	020331	DQ678461	DQ678564	DQ678358	DQ678152	DQ678255	DQ678667	DQ678763	DQ678866	
	helvomaculatus	SWFSC 128-68	32.87	117.87	000318	DQ678496	DQ678599	DQ678393	DQ678187	DQ678290	DQ678702	DQ678798	DQ678901	
	hopkinsi	SWFSC 156-91	32.85	117.30	0205	DQ678447	DQ678550	DQ678344	DQ678138	DQ678241	DQ678653	DQ678749	DQ678852	
	hubbsi	FAKU 130191	NA	NA	NA	DQ678501	DQ678604	DQ678398	DQ678192	DQ678295	DQ678707	DQ678803	DQ678906	
	inermis	FAKU 86581	NA	NA	NA	DQ678483	DQ678586	DQ678380	DQ678174	DQ678277	DQ678689	DQ678785	DQ678888	
	iracundus	FAKU 81604	39.48	141.32	NA	DQ678495	DQ678598	DQ678392	DQ678186	DQ678289	DQ678701	DQ678797	DQ678900	
	jordani	SWFSC 110-84	58.90	122.58	930223	DQ678448	DQ678551	DQ678345	DQ678139	DQ678242	DQ678654	DQ678750	DQ678853	
	joyneri	FAKU 130109	NA	NA	NA	DQ678489	DQ678592	DQ678386	DQ678180	DQ678283	DQ678695	DQ678791	DQ678894	
	kiyomatsui	FAKU 81567	34.05	135.35	NA	DQ678487	DQ678590	DQ678384	DQ678178	DQ678281	DQ678693	DQ678789	DQ678892	
	lentiginosus	SWFSC 8-89	29.16	118.27	960808	DQ678449	DQ678552	DQ678346	DQ678140	DQ678243	DQ678655	DQ678751	DQ678854	
	levis	SWFSC 164-66	32.66	117.97	030115	DQ678450	DQ678553	DQ678347	DQ678141	DQ678244	DQ678656	DQ678752	DQ678855	
	macdonaldi	SWFSC 36-91	32.60	117.42	020312	DQ678451	DQ678554	DQ678348	DQ678142	DQ678245	DQ678657	DQ678753	DQ678856	
	melanger	SWFSC 26-74	49.08	126.20	980806	DQ678428	DQ678531	DQ678225	DQ678119	DQ678222	DQ678634	DQ678730	DQ678833	

(continued on next page)

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Table 1 (*continued*)

Genus	Species	Collection location				GenBank Accession Nos.							
		Collection number	Latitude (N)	Longitude (W)	Date	cytb	D-loop	cox1	12S rRNA	16S rRNA	ITS1	RAG2	tRNA Thr and Pro
	matsubarae	FAKU 81639	34.05	135.35	NA	DQ678498	DQ678601	DQ678395	DQ678189	DQ678292	DQ678704	DQ678800	DQ678903
	melanops	SWFSC 37-50	35.65	121.52	981114	DQ678453	DQ678556	DQ678350	DQ678144	DQ678247	DQ678659	DQ678755	DQ678858
	melanosema	UW 112698	32.92	117.53	040221	DQ678514	DQ678617	DQ678411	DQ678205	DQ678308	NA	DQ678816	DQ678919
	melanosticius	SWFSC 7-92	36.28	121.97	950802	DQ678414	DQ678517	DQ678311	DQ678105	DQ678208	DQ678620	DQ678716	DQ678819
	melanostomus	SWFSC 129-66	32.75	117.75	000405	DQ678454	DQ678557	DQ678351	DQ678145	DQ678248	DQ678660	DQ678756	DQ678859
	mentella	SWFSC 12-36	69.67	18.93	950626	DQ678455	DQ678558	DQ678352	DQ678146	DQ678249	DQ678661	DQ678757	DQ678860
	miniatus type 1	SWFSC 156-86	32.83	117.25	020527	DQ678456	DQ678559	DQ678353	DQ678147	DQ678250	DQ678662	DQ678758	DQ678861
	miniatus type 2	SWFSC 159-72	32.83	117.25	020527	DQ678457	DQ678560	DQ678354	DQ678148	DQ678251	DQ678663	DQ678759	DQ678862
	minor	FAKU 83713	44	145	NA	DQ678502	DQ678605	DQ678399	DQ678193	DQ678296	DQ678708	DQ678804	DQ678907
	moseri	SIO 95-33	32.63	117.96	950901	DQ678509	DQ678612	DQ678406	DQ678200	DQ678303	DQ678715	DQ678811	DQ678914
	mystinus	SWFSC 118-37	34.01	119.39	990703	DQ678458	DQ678561	DQ678355	DQ678149	DQ678252	DQ678664	DQ678760	DQ678863
	nebulosus	SWFSC 151-60	45.85	124.00	000616	DQ678429	DQ678532	DQ678326	DQ678120	DQ678223	DQ678635	DQ678731	DQ678834
	nigrocinctus	SWFSC 7-71	56.33	136.00	960105	DQ678459	DQ678562	DQ678356	DQ678150	DQ678253	DQ678665	DQ678761	DQ678864
	norvegicus	SWFSC 12-24	69.67	18.93	940712	DQ678452	DQ678555	DQ678349	DQ678143	DQ678246	DQ678658	DQ678754	DQ678857
	notius	SWFSC 8-91	25.59	113.37	960814	DQ678460	DQ678563	DQ678357	DQ678151	DQ678254	DQ678666	DQ678762	DQ678865
	oblongus	FAKU 130128	NA	NA	NA	DQ678503	DQ678606	DQ678400	DQ678194	DQ678297	DQ678709	DQ678805	DQ678908
	ocultatus	SWFSC 10-78	N35.5	32.59		DQ678421	DQ678524	DQ678318	DQ678112	DQ678215	DQ678627	DQ678723	DQ678826
	ovalis	SWFSC 161-60	33.03	118.56	010326	DQ678431	DQ678534	DQ678328	DQ678122	DQ678225	DQ678637	DQ678733	DQ678836
	owstoni	FAKU 83264	SW Sea of Japan		NA	DQ678493	DQ678596	DQ678390	DQ678184	DQ678287	DQ678699	DQ678795	DQ678898
	pachycephalus	FAKU 130088	NA	NA	NA	DQ678490	DQ678593	DQ678387	DQ678181	DQ678284	DQ678696	DQ678792	DQ678895
	paucispinis	SWFSC 153-79	30.33	116.08	001027	DQ678462	DQ678565	DQ678359	DQ678153	DQ678256	DQ678668	DQ678764	DQ678867
	pedunculoris	SWFSC 225-68	28.98	113.43	050408	DQ678504	DQ678607	DQ678401	DQ678195	DQ678298	DQ678710	DQ678806	DQ678909
	phillipsi	SWFSC 128-62	32.87	117.87	000318	DQ678463	DQ678566	DQ678360	DQ678154	DQ678257	DQ678669	DQ678765	DQ678868
	pinniger	SWFSC 76-19	45.74	124.20	980716	DQ678464	DQ678567	DQ678361	DQ678155	DQ678258	DQ678670	DQ678766	DQ678869
	polyspinis	SWFSC 121-69	58.40	−153.65	990620	DQ678512	DQ678615	DQ678409	DQ678203	DQ678306	NA	DQ678814	DQ678917
	proriger	SWFSC 89-65	48.24	125.17	980730	DQ678465	DQ678568	DQ678362	DQ678156	DQ678259	DQ678671	DQ678767	DQ678870
	rastrelliger	NA	32.85	117.32	0101	DQ678430	DQ678533	DQ678327	DQ678121	DQ678224	DQ678636	DQ678732	DQ678835
	reedi	SWFSC 74-11	45.08	124.71	980712	DQ678415	DQ678518	DQ678312	DQ678106	DQ678209	DQ678621	DQ678717	DQ678820
	rosaceus	SWFSC 163-20	33.22	119.50	010328	DQ678466	DQ678569	DQ678363	DQ678157	DQ678260	DQ678672	DQ678768	DQ678871
	rosenblatti	SWFSC 146-85	32.45	119.15	940526	DQ678467	DQ678570	DQ678364	DQ678158	DQ678261	DQ678673	DQ678769	DQ678872
	ruberrimus	SWFSC 132-12	45.74	124.61	980715	DQ678468	DQ678571	DQ678365	DQ678159	DQ678262	DQ678674	DQ678770	DQ678873
	rubrivinctus	SWFSC 126-77	32.85	117.32	990416	DQ678469	DQ678572	DQ678366	DQ678160	DQ678263	DQ678675	DQ678771	DQ678874
	rufinanus	SWFSC LT22	32.42	119.96	9904	DQ678508	DQ678611	DQ678405	DQ678199	DQ678302	DQ678714	DQ678810	DQ678913
	rufus	SWFSC 176-33	32.80	117.80	020303	DQ678470	DQ678573	DQ678367	DQ678161	DQ678264	DQ678676	DQ678772	DQ678875
	saxicola type N	SWFSC 128-83	36.83	122.16	921120	DQ678433	DQ678536	DQ678330	DQ678124	DQ678227	DQ678639	DQ678735	DQ678838
	saxicola type S	SWFSC 181-43	32.67	117.33	0210	DQ678432	DQ678535	DQ678329	DQ678123	DQ678226	DQ678638	DQ678734	DQ678837
	schlegeli	FAKU 130219	NA	NA	NA	DQ678481	DQ678584	DQ678378	DQ678172	DQ678275	DQ678687	DQ678783	DQ678886
	scythropus	FAKU 81566	34.05	135.35	NA	DQ678485	DQ678588	DQ678382	DQ678176	DQ678279	DQ678691	DQ678787	DQ678890
	semicinctus	SWFSC 155-99	32.67	117.31	020525	DQ678471	DQ678574	DQ678368	DQ678162	DQ678265	DQ678677	DQ678773	DQ678876
	serranoides	SWFSC 147-84	32.67	117.25	001015	DQ678472	DQ678575	DQ678369	DQ678163	DQ678266	DQ678678	DQ678774	DQ678877
	serriceps	SWFSC 153-60	30.37	116.08	001027	DQ678473	DQ678576	DQ678370	DQ678164	DQ678267	DQ678679	DQ678775	DQ678878
	simulator	SWFSC 187-15	32.61	117.31	050123	DQ678507	DQ678610	DQ678404	DQ678198	DQ678301	DQ678713	DQ678809	DQ678912
	sinensis	SWFSC 225-80	28.98	113.43	050408	DQ678511	DQ678614	DQ678408	DQ678202	DQ678305	NA	DQ678813	DQ678916
	spinorbis	SWFSC 144-68	28.98	113.43	950328	DQ678474	DQ678577	DQ678371	DQ678165	DQ678268	DQ678680	DQ678776	DQ678879
	steindachneri	FAKU 83715	44	145	NA	DQ678484	DQ678587	DQ678381	DQ678175	DQ678278	DQ678690	DQ678786	DQ678889
	taczanowskii	SWFSC 14-57	41.77	140.72	960721	DQ678494	DQ678597	DQ678391	DQ678185	DQ678288	DQ678700	DQ678796	DQ678899
	thompsoni	FAKU 130111	NA	NA	NA	DQ678482	DQ678585	DQ678379	DQ678173	DQ678276	DQ678688	DQ678784	DQ678887
	trivittatus	SWFSC 126-75	39.64	141.95	9904	DQ678492	DQ678595	DQ678389	DQ678183	DQ678286	DQ678698	DQ678794	DQ678897

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umbrosus	SWFSC 153-62	30.37		116.08	001027	DQ678475	DQ678578	DQ678372	DQ678166	DQ678269	DQ678681	DQ678777	DQ678880
variabilis	UW 043203-#40	54.11		161.73	960601	DQ678510	DQ678613	DQ678407	DQ678201	DQ678304	NA	DQ678812	DQ678915
variegatus	SWFSC 178-3	43.38		124.55	950315	DQ678476	DQ678579	DQ678373	DQ678167	DQ678270	DQ678682	DQ678778	DQ678881
vivparus	SWFSC 35-88	Iceland			991020	DQ678477	DQ678580	DQ678374	DQ678168	DQ678271	DQ678683	DQ678779	DQ678882
vulpes	FAKU 130099	NA	NA		NA	DQ678478	DQ678581	DQ678375	DQ678169	DQ678272	DQ678684	DQ678780	DQ678883
wilsoni	SWFSC 174-76	55.4	134.44		9901	DQ678479	DQ678582	DQ678376	DQ678170	DQ678273	DQ678685	DQ678781	DQ678884
zacentrus	SWFSC 20-99	37.24	122.83		980616	DQ678480	DQ678583	DQ678377	DQ678171	DQ678274	DQ678686	DQ678782	DQ678885
Sebastiscus	*marmoratus*	SWFSC 112-51	Tokyo, Japan		990116	DQ678516	DQ678619	DQ678413	DQ678207	DQ678310	NA	DQ678818	DQ678921
Sebastolobus	*alascanus*	SWFSC IIF10	33.03	117.37	9003	DQ678500	DQ678603	DQ678397	DQ678191	DQ678294	DQ678706	DQ678802	DQ678905

Institutional abbreviations follow Leviton et al. (1985) except for SWFSC which corresponds to the Southwest Fisheries Science Center, La Jolla, California. NA, not available. Institutional abbreviations follow Leviton et al. (1985) except for SWFSC which corresponds to the Southwest Fisheries Science Center, La Jolla, California. NA, not available.

2.4. Sequence alignment and phylogenetic analysis

Sequence alignments between species were accomplished using ClustalW (Higgins et al., 1994) as implemented in MEGA v3.1 (Kumar et al., 2004) with the default settings. Additional manipulation and alignment optimizations were done manually. The propensity of the control region and ITS1 for insertions and deletions made automated sequence alignment nearly impossible. A small portion of ITS1 that was difficult to unambiguously align was removed from analysis. Sequence alignment, including gap characters, produced a data matrix containing 5581 nucleotides. Of these, 3682 sites are constant, 1134 are parsimony informative, and 765 are variable and parsimony uninformative (see Table 3).

Pairwise comparisons of uncorrected *p*-distance for each gene were conducted between all species pairs using PAUP*(v4.b10) (Swofford, 2001). These distances were used to evaluate the relative evolutionary rate of the different genes examined. Using cytb distance as a reference, ratios to individual gene distance were examined. The means and standard deviations of these measures are presented in Table 4.

As a heuristic examination for saturation of substitution sites, plots were generated to compare substitution number at 1st, 2nd, and 3rd codon positions versus total uncorrected genetic distance. An unsaturated dataset should show a linear relationship between substitution number at a particular codon position and increasing genetic distance. In the case of saturation of substitution sites one would expect to see a plateau in this relationship at larger genetic distances. The three protein coding genes (cytb, cox1, and RAG2) were examined in this fashion and did not show evidence for saturation (see Fig. 1).

2.4.1. Maximum parsimony

Gene partitions were subjected to a partition homogeneity test as implemented in PAUP* using 1000 replicates, each including 10 random sequence addition replicates. Test results indicated that there was no significant conflict in phylogenetic signal between gene partitions, so all sequence data was concatenated and analyzed as a single unit in parsimony analyses. Maximum parsimony analysis was conducted using PAUP*. Character state optimization was set to ACCTRAN, multi-state characters treated as polymorphic, gaps treated as a "5th base", TBR branch swapping was employed, and multiple saved trees and "max trees" were set to automatically increase if needed. The "5th base" rather than "missing" setting for gaps was chosen as gaps often prove to be informative phylogenetic characters. This was observed to be the case with preliminary analyses showing that several otherwise highly supported clades contain one or more synapomorphic gap characters (J. Hyde unpublished data). All other settings were left at the default values. Heuristic tree searches were done using 1000 replicates of random sequence addition.

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Table 2
PCR primers used in this study for DNA amplification

Amplified region	Primer	Sequence 5′–3′	Reference
Control region	D-RF	CCT GAA AAT AGG AAC CAA ATG CCA G	This study
	Thr-RF	GAG GAY AAA GCA CTT GAA TGA GC	This study
cytb	Glu-RF2	AAC CAT CGT TGT TAT TCA ACT ACA AGA ACC	This study
	RevThr-RF2	TTT ACA AGA CCA GGG CTC TG	This study
cox1	Cox1-RF-R	CCT GAG AAT AGT GGG AAT CAG TG	This study
	Cox1-Tyr-RF-F	TAC AAT CCA TCG CTT AAA AAC TCA GC	This study
16S	16SA	CGC CTG TTT ATC AAA AAC AT	Palumbi et al. (1991)
	16SB	CCG GTC TGA ACT CAG ATC ACG T	Palumbi et al. (1991)
12S	12SA	AAA CTG GGA TTA GAT ACC CCA CTA T	Palumbi et al. (1991)
	12SB	GAG GGT GAC GGG CGG TGT GT	Palumbi et al. (1991)
RAG2	RAG2-RF-F	GTA GAG CTC CTC GGA GTC TTC GAG	This study
	RAG2-RF-R	ACC ATG GAT AGC CGT GGC TGC	This study
ITS1	18d	CAC ACC GCC CGT CGC TAC TAC CGA TT	Hillis and Dixon (1991)
	5.8c	GTG CGT TCG AAG TGT CGA TGA TCA A	Hillis and Dixon (1991)

Table 3
Nucleotide base composition and character number for genes used in this study

Gene	A	C	G	T	No. of characters	No. of parsimony informative characters
cytb	0.25046	0.29186	0.15671	0.30097	1141	349
tThr-tPro	0.27044	0.2648	0.19134	0.27342	143	14
dloop	0.39439	0.16457	0.12436	0.31667	512	224
12s	0.27726	0.28893	0.23748	0.19633	387	34
16s	0.29114	0.25299	0.23125	0.22462	590	32
cox1	0.24112	0.26372	0.189	0.30616	1161	265
rag2	0.22359	0.27522	0.2816	0.2196	713	42
its1	0.18092	0.32951	0.31269	0.17688	934	174
				Total	5581	1134

Table 4
Pairwise comparisons of uncorrected *p*-distance between species at selected genes expressed as a ratio to cytochrome *b*

Gene	Mean ratio	SD
dloop	1.85885458	0.94247422
cox1	0.69475085	0.15226424
12S	0.31133226	0.14986064
16S	0.13326596	0.08049313
RAG2	0.16529698	0.23730285
ITS1	0.25389092	0.18723527

This is used as a metric to compare relative evolutionary rates between different gene regions.

In addition to the standard heuristic search for the most parsimonious trees, a parsimony ratchet (Nixon, 1999) was implemented to further search treespace. The parsimony ratchet has been shown to quickly and thoroughly search treespace often finding shorter trees in much less time than simple heuristic searches. This was accomplished through 200 iterations of the random re-weighting of 15% of the characters and subsequent short heuristic searches. When compared to the unperturbed dataset this method acts to "warp" tree space, facilitating the rapid exploration of many tree islands at the expense of thorough searching of any one island. The key element of this method is that the ratchet proceeds only to progressively better islands. In analysis of the *Sebastes* dataset, 20 independent runs of the parsimony ratchet were done using PAUP* and batch files generated using PAUPrat (Sikes and Lewis, 2001). The batch files were edited to treat multi-state characters as polymorphic and gaps as "5th bases". Saved trees from all runs were combined and filtered for the shortest trees. These trees were then combined into a single 50% majority-rule consensus tree.

To assess statistical support values for the nodes, a non-parametric bootstrap resampling scheme was applied in PAUP*. Trees were assembled by stepwise addition using 10 random sequence addition replicates for each of 1000 bootstrap iterations. To reduce computational time, max trees were limited to 1000 trees saved per iteration. Though there was some minor tree buffer overflow, the trees saved from the majority of the replicates were well below this ceiling suggesting that any effect on the results is likely negligible.

2.4.2. Bayesian posterior analysis

In addition to the maximum parsimony analysis, the genetic data was subjected to a Bayesian posterior analysis. Genetic data were evaluated for evolutionary model testing using MrModeltest v2.2 (Nylander, 2004) as implemented using the PAUP* framework. This program uses both the hierarchical likelihood ratio test (Huelsenbeck and Crandall, 1997) and Akaike (Akaike, 1974) information criterion to test the fit of the data to 24 different evolutionary models. This analysis was done both on the concatenated sequence data and on the individual genes. In all but one case the model chosen was a general time reversible (GTR)

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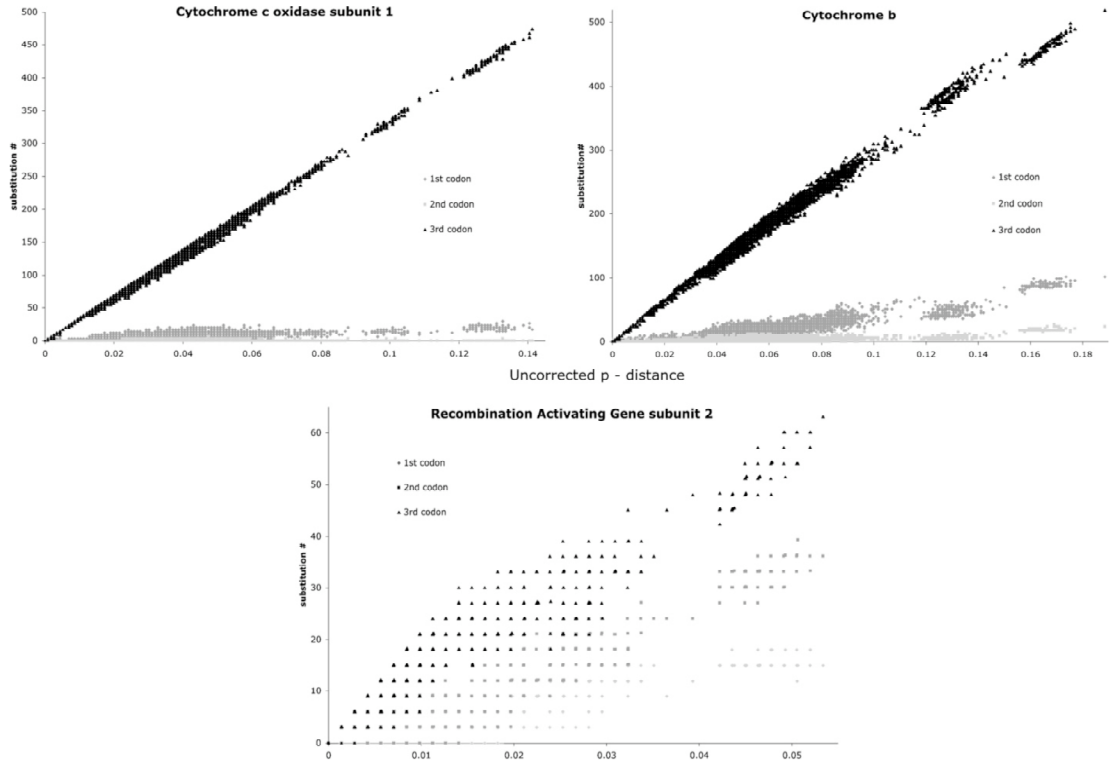

Fig. 1. Graphical presentation of the number of nucleotide substitutions at 1st (black), 2nd (dark gray), and 3rd (light gray) codon sites as a function of uncorrected *p*-distance. Used as an evaluation tool to check for saturation of substitution sites in protein coding genes. Saturation would be indicated as a plateau in substitution number at increasing genetic distance.

model (Rodriguez et al., 1990) considering an empirically derived proportion of invariant sites (I) and gamma shape distribution (Γ). The one exception, RAG2, fit the Hasegawa et al., 1985) (HKY) + I + Γ model best. MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was used to generate a Bayesian inferred phylogenetic hypothesis using Metropolis coupled Markov chain Monte Carlo analysis. The results of the MrModeltest analysis showed that although the same evolutionary model fit most of the genes, both I and Γ varied between them. For this reason, the data was partitioned into eight unlinked sets each with its own estimation of I and Γ (tRNA threonine and tRNA proline were analyzed together). All partitions except RAG2 were set to nst = 6, Dirichlet (1,1,1,1), and the program empirically estimated both I and Γ. The RAG2 partition settings were the same as above with the exception of nst = 4, corresponding to the HKY + I + Γ model.

Two independent runs of four Markov chains each were allowed to proceed for 4,000,000 cycles. Each run consisted of a single "cold" chain and three "heated" chains with a temp setting of 0.3. The heated chains act to flatten the likelihood surface being explored by the chains so as to avoid becoming trapped in local minima and to facilitate state changing between the chains. The utilization of two independent runs serves as a diagnostic tool for checking the status of the analysis. When the "average standard deviation of split frequencies" between the two runs decreases towards zero it is indicated that the runs have both converged upon the same region of tree space and the analysis has reached stationarity, giving an accurate representation of the posterior probability distribution. In addition to this diagnostic, the first 25% of the analysis was discarded to eliminate the inclusion of data from the "burn-in" phase of the analysis where the posterior probabilities may have not yet reached stationarity.

3. Results

3.1. Nucleotide composition

Nucleotide base composition analyses showed similar results to other studies of mitochondrial genes. As noted by Rocha-Olivares et al. (1999a,b) and Kai et al. (2003) for *Sebastes* there is a strong anti-g bias in the cytochrome *b* gene. This same bias is seen in the cytochrome *c* oxidase I, tRNA proline, and tRNA threonine genes. The

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mitochondrial control region shows both a strong anti-c and anti-g bias. In contrast, internal transcribed spacer 1 shows a strong anti-a and anti-t bias. Base composition values and number of characters for each gene are presented in Table 3.

3.2. Maximum parsimony

Both standard heuristic and ratchet-based parsimony analyses produced shortest trees of the same length (7621 steps). Standard heuristic searches were fairly short and seemed to perform comparably to the ratchet method. This is likely due to the large number of parsimony informative characters in the dataset ($n = 1134$) facilitating resolution of the phylogeny despite the relatively large number of taxa. Fig. 2 presents the 50% majority rule consensus tree of 204 equally parsimonious trees obtained by combining trees from both the standard heuristic and ratchet searches with a consistency index (Kluge and Farris, 1969) of 0.35 and retention index (Farris, 1989) of 0.58, when uninformative characters are retained. Prior to combining the trees from these two analyses, a Kishino-Hasegawa test was applied in PAUP* to test for any significant difference between trees from both methods. As no significant difference was found, the trees were combined. In fact, both methods of tree searching produced the same set of 204 equally parsimonious trees. This strong concordance between methods suggests that tree space had been thoroughly searched and that the saved trees represent the true most parsimonious set of trees. Bootstrap support values >50 are noted above the branch at all nodes (see Fig. 2).

3.3. Bayesian inference

The MCMC analysis was stopped after 4,000,000 cycles as the "average standard deviation of split frequencies diagnostic" indicated that the two runs had converged on the same region of treespace. The Bayesian analysis produced a credible set of 20,708 trees. The consensus tree with the maximum posterior probability (see Fig. 3) is very similar in topology to that produced using maximum parsimony (see Fig. 2). Minor differences in topologies are possibly due to the inability for current distance-based metrics such as maximum likelihood and Bayesian inference to incorporate alignment gaps in analyses, instead coding these characters as missing (Felsenstein, 1981). Despite the exclusion of these potentially informative gap characters, most posterior support values (Fig. 3) are similar to or greater than the bootstrap values (Fig. 2) from the maximum parsimony analysis.

3.4. Construction of a clock calibrated ultra-metric tree

In order to better understand the timing of speciation events, an ultra-metric tree was constructed using branch length measures obtained from the consensus tree generated during the Bayesian posterior analysis. Bayesian-derived branch lengths were chosen as they were derived from a parameter-rich model that independently analyzed each data partition. This should act to minimize rate inconsistencies between genes, reducing biases that are present in currently available likelihood and distance based programs (Huelsenbeck et al., 2002). The included branch length measures represent the average of the branch lengths from the posterior distribution of credible trees. The outgroup, *Sebastolobus alascanus*, was removed from this analysis in order to minimize the effect of unequal evolutionary rate between it and the ingroup taxa. Raw branch length data were run through the program r8s v1.7 (Sanderson, 2003) in order to determine node age and correct for inconsistency of evolutionary rate between lineages. Branch length correction was accomplished using the penalized likelihood function (Sanderson, 2002) with a truncated Newton method with bound constraints. The penalty function was set to additive. A cross validation analysis was conducted to choose the proper rate smoothing parameter (parameters between 0.1 and 1000 were assessed). The best smoothing parameter was determined to be around 1.05.

The tree was age calibrated by setting the most recent common ancestor to *S. alutus* and *S. norvegicus* at 3 million years ago (MYA). This calibration point was previously used by Rocha-Olivares et al. (1999a,b) for cytb data as high-latitude cooling and reduced sea level caused a cessation of favorable conditions for genetic interchange between the NA and NP at this time. Both Bayesian inference and maximum parsimony analyses supported *S. alutus*, from the NP, as the sister taxon to the four closely related Atlantic species. The resulting chronogram was drawn using Mesquite v1.1 (Maddison and Maddison, 2002) and is presented in Fig. 5. In addition to the presented chronogram, data on diatom mass accumulation rates (MAR) at three NP sites and historic eustatic sea level are presented on the same time scale. Data were adapted from Barron's (1998) study of diatom deposition rates throughout the NP and Miller et al.'s (2005) studies of paleo-sea level fluctuation.

4. Discussion

This study represents the most robust and comprehensive phylogenetic analysis of the rockfishes of the genus *Sebastes*. Both maximum parsimony and Bayesian inference analyses of the genetic data produce similar and well-supported phylogenies. These studies provide supported phylogenetic hypotheses for relationships between individual species, relationships between clades (sub-genera), as well as the relationship between *Sebastes* species and the other members of Sebastinae. The extraordinary amount of new genetic data, some of it extracted from rare formalin fixed material, is a database so rich it cannot be discussed fully in this paper and invites analyses by other scientists interested in the general properties of marine speciation. The application of a molecular clock, based on multiple genes, constrained within a single taxonomic lineage, and

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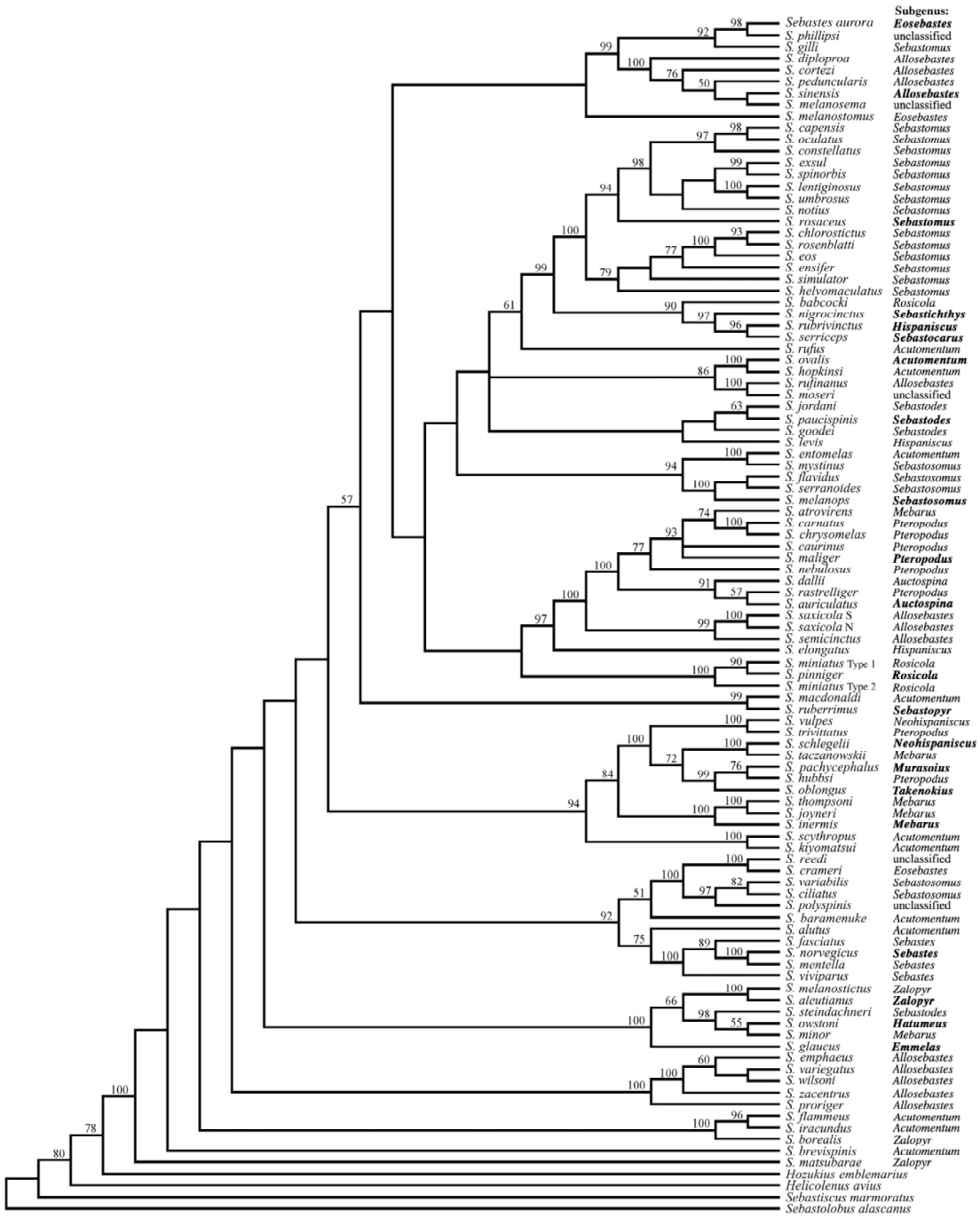

Fig. 2. Maximum parsimony 50% majority rule consensus tree of 204 equally parsimonious trees. Tree length is 5581 steps. Numbers above nodes indicate bootstrap support values >50%. Current morphology based sub-generic classification (Kendall, 2000) is indicated to the right of the species name with "Type" species in bold.

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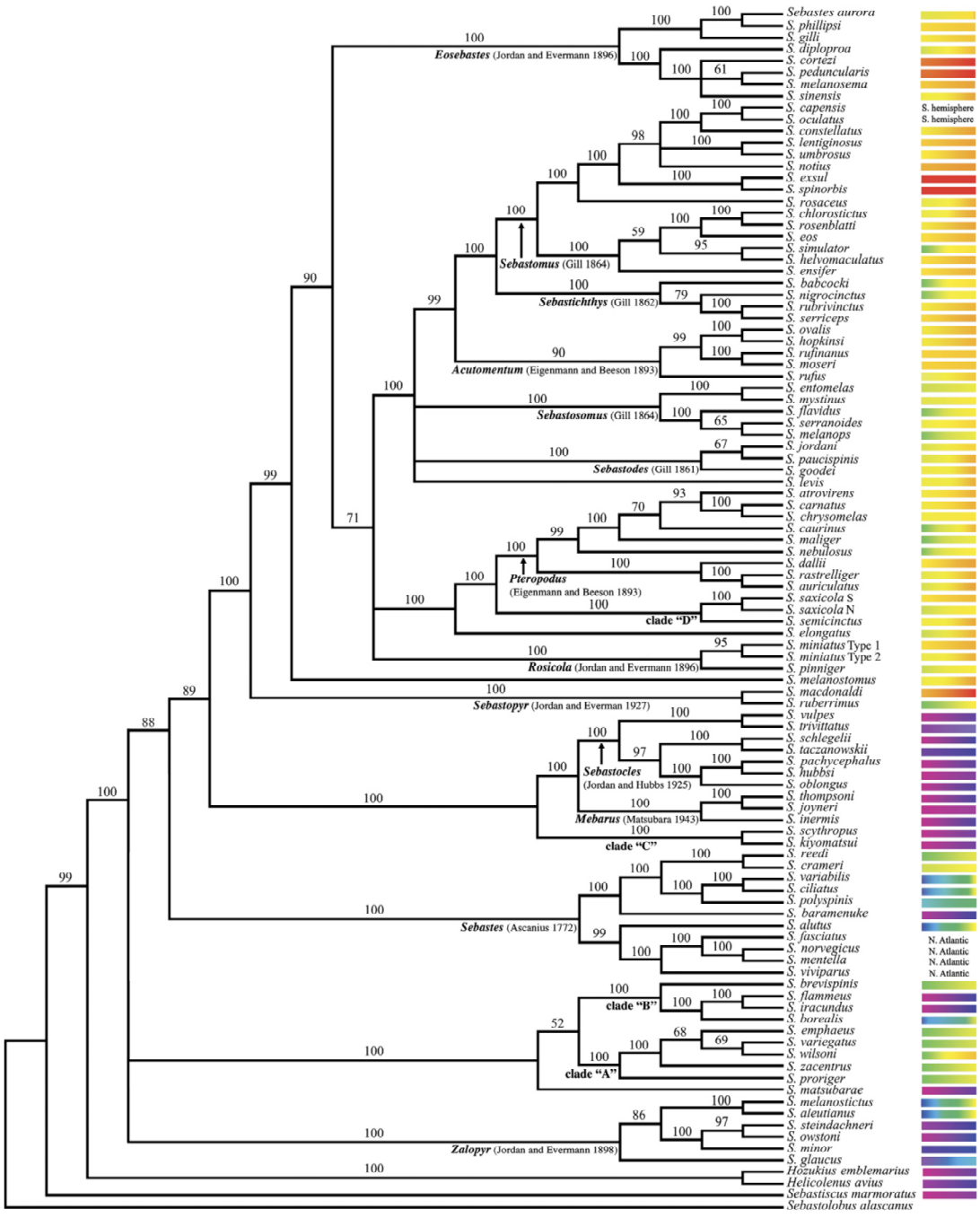

Fig. 3. Consensus tree with maximum posterior probability generated from Bayesian posterior analysis using MrBayes v3.1. Numbers above nodes indicate Bayesian posterior probabilities >50%. Color spectrums next to species names indicate common range in reference to Fig. 4. Revised sub-generic names are listed below nodes.

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anchored by known paleo-oceanographic events is a particularly important result.

Maximum likelihood methods were not employed in this paper due to computational constraints and the similarity in method to Bayesian inference. Though maximum likelihood has proved amenable to analyses of single genes, we believe that the analysis of multiple genes under this methodology, each with different evolutionary rates, introduces unacceptable biases. When multiple genes are analyzed together, Bayesian inference should prove superior given its ability to set optimal model parameters for each data partition (Castoe et al., 2004). Current maximum likelihood models are unable to accommodate such model optimization across multiple genetic loci, instead creating a single model for the data as a whole (Huelsenbeck et al., 2002).

When considering statistical support for clades, it is important to consider known biases associated with these measures. Bootstrap resampling of phylogenies has been shown to be an overly conservative measure of support and tends to decrease with increasing number of analyzed taxa (Cummings et al., 2003). Conversely, Bayesian posterior probability may tend to overestimate support, (Cummings et al., 2003; Simmons et al., 2003) especially when incorrect model parameters are employed. As it is clearly worse to overly support the wrong phylogeny than to inadequately support the correct phylogeny we advocate consideration of these biases when assessing the support values, especially when they show large discrepancies.

Though five of the 110 species are missing from these analyses, their inclusion is not expected to substantially alter tree topology as these species are likely contained within currently represented clades. According to Y. Kai (2005) pers. comm., cytochrome *b* data suggest that *S. koreanus* and *S. nivosus* are both closely related to *S. pachycephalus*, *S. oblongus*, and *S. hubbsi.* Barsukov (1981) suggested that *S. wakiyai* is closely related to *S. steindachneri*; both of these are very similar in morphology to *S. itinus* (Y. Kai, pers. comm. 2005). Three of the Gulf of California species (*S. cortezi, S. peduncularis*, and *S. sinensis*) are all very similar to *S. varispinis* (Chen, 1975) and this is likely a species complex that is still undergoing genetic lineage sorting (J. Hyde unpublished data).

4.1. Species flocks

Greenwood (1984) defined species flocks as geographically circumscribed, monophyletic groups of species that have undergone an explosive burst of speciation, in comparison to sister taxa, usually due to the evolution of novel adaptive traits or colonization of new habitat. Johns and Avise (1998), using an algorithm devised by Wollenberg et al. (1996), had posited that *Sebastes* might be an ancient marine species flock. This conclusion was based on partial sequence of a single mitochondrial gene from 28 species representing multiple phylogenetic lineages. As noted by the authors, support was low for the majority of the internal nodes. This was likely due to the inadequate resolution provided by relying upon a single gene for phylogenetic information. The combination of small sample size and a limited number of characters in phylogenetic analyses often creates long branches and polytomies, which supports the hypothesis of rapid flock-like radiations. Also, the results of their tests were highly contingent on outgroup choice and its effect on cumulative distribution functions. No close sebastine outgroup was used, but rather the more distantly related *Sebastolobus* or *Scorpaena* species. While a formal reanalysis awaits, the data presented here clearly show a wide variety of speciation patterns between different lineages, some ancient and some modern. It is interesting that some lineages such as *Sebastodes*, containing *S. paucispinis* and *S. jordani*, that are epibenthic and maintain large population sizes, have single lineages stretching back almost 6 million years despite presumed displacements due to glacial advance and retreat. At the other extreme, lineages such as *Eosebastes* and *Sebastomus*, that have endemic species within the Gulf of California, have rapidly formed multiple new species within the past half to one million years. Thus this analysis indicates there are variable speciation rates within *Sebastes* (see Fig. 6), an idea which is inconsistent with the expectations of a species flock. Future analyses of species flocks involving *Sebastes* should focus on the monophyletic and geographically constrained clades within the genus (e.g., *Acutomentum*, *Pteropodus*, *Sebastocles*, *Sebastomus*) rather than analyzing the genus as a whole.

4.2. Comparison to previous studies

Phylogenetic hypotheses generated in this study are similar to those put forth by Rocha-Olivares et al. (1999a,b) and Kai et al. (2003). The genus *Sebastes* remains monophyletic. Interestingly the genus *Sebastiscus*, once considered a subgenus of *Sebastes*, proves to be the sister group to the remainder of the Sebastinae. The subgenus *Sebastomus* (sensu Chen, 1971) has withstood a more thorough analysis, *Pteropodus* (sensu Matsubara 1943) remains polyphyletic, and "clade NWP" and "clade NEP" of Kai et al. (2003) are recovered. Increased character and taxon sampling in this study have, however, dramatically improved tree stability resulting in higher statistical support, especially at the deeper nodes. This increase in clade support allows us to make revisions to previous sub-generic classifications (see Fig. 3). For this, we chose to use the phylogenetic hypothesis produced by Bayesian inference both because of its typically higher support values, enhancing resolution for clades near the base of the tree, and its further use in construction of the molecular clock based analyses (Fig. 5). When possible, we used previously proposed named sub-genera (see Table 5). When multiple generic type species were nested within a clade, the subgenus with historical precedence was used (e.g., *Sebastichthys*). If no type species resided within a clade a simple name was applied (i.e., clade "A", clade "B", clade "C", clade "D").

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Table 5
List of valid subgenera (Kendall, 2000), type species, and descriptive authority

Subgenus	Type species	Authority
Acutomentum	*Sebastes ovalis*	Eigenmann and Beeson (1893)
Allosebastes	*S. sinensis*	Hubbs (1951)
Auctospina	*S. auriculatus*	Eigenmann and Beeson (1893)
Emmelas	*S. glaucus*	Jordan and Evermann (1898)
Eosebastes	*S. aurora*	Jordan and Evermann (1896)
Hatumeus	*S. owstoni*	Matsubara (1943)
Hispaniscus	*S. rubrivinctus*	Jordan and Evermann (1896)
Mebarus	*S. inermis*	Matsubara (1943)
Murasoius	*S. pachycephalus*	Matsubara (1943)
Neohispaniscus	*S. schlegelii*	Matsubara (1943)
Primospina	*S. mystinus*	Eigenmann and Beeson (1893)
Pteropodus	*S. maliger*	Eigenmann and Beeson (1893)
Rosicola	*S. pinniger*	Jordan and Evermann (1896)
Sebastes	*S. norvegicus*	Ascanius (1772)
Sebastichthys	*S. nigrocinctus*	Gill (1862)
Sebastocarus	*S. serriceps*	Jordan and Evermann (1927)
Sebastocles	*S. hubbsi*	Jordan et al. (1925)
Sebastodes	*S. paucispinis*	Gill (1861)
Sebastomus	*S. rosaceus*	Gill (1864)
Sebastopyr	*S. rubberimus*	Jordan and Evermann (1927)
Sebastosomus	*S. melanops*	Gill (1864)
Takenokius	*S. oblongus*	Matsubara (1943)
Zalopyr	*S. aleutianus*	Jordan and Evermann (1898)

Despite improvements to the genetic phylogeny, the majority of previously proposed morphologic sub-genera were found to be either para- or poly-phyletic (see Fig. 2). This disagreement between phenotype and genotype is likely due to episodes of convergent evolution driven by the habitat and life history of individual species or clades. As the sister genera to *Sebastes*, *Helicolenus* and *Hozukius*, are deep water, demersal fishes with strong head spines, it would seem likely that the ancestral form of *Sebastes* shared similar traits (Barsukov, 1981). This seems to be supported by the observation that several of the early branching lineages of *Sebastes* (i.e., *S. borealis, S. flammeus, S. iracundus*, and *S. matsubarae*) fit this description (see Figs. 2 and 3). The reduction and loss of head spines in association with a semi-pelagic lifestyle seems, in general, to be a derived condition. In comparison to a demersal lifestyle, a semi-pelagic lifestyle would seem more conducive to adult dispersal, perhaps facilitating the spread of *Sebastes* species throughout the NP. Starr et al. (2002) showed that *S. paucispinis*, a semi-pelagic species, exhibited larger individual home range size than *S. chlorostictus*, a demersal species.

The genetic analyses corroborate Barsukov's (1981) hypothesis that the plesiomorphic trait of strong head spination in association with a more demersal lifestyle has reappeared in several clades (e.g., *Sebastomus*, *Pteropodus*, and *Sebastocles*). However, Barsukov's (1981) hypothesis that speciation occurs in threes, due primarily to depth segregation and competitive exclusion of the intermediate depth form, seems to be incongruent with our finding that the majority of species within a clade co-occur and often share very similar morphologies. Additionally, the molecular clock based analysis (Fig. 5) yields few examples of "triple synchronous" speciation events, instead suggesting that speciation events occur in a progressive stepwise fashion. Our findings are more suggestive of an evolutionary model in which species disperse into newly available habitat and subsequently segregate based upon fine-scale ecological (e.g., Allen, 1982) and assortative mating preferences (e.g., Narum et al., 2004). This may ultimately be enhanced by fluctuations in temperature and sea level leading to the isolation of populations, potentially resulting in allopatric speciation.

4.3. Convergent evolution of morphology

Pteropodus (sensu Matsubara, 1943) and *Mebarus* (sensu Chen, 1985) are polyphyletic in both this analysis and that of Kai et al. (2003) and Li et al. (2006). Both sub-genera contain species found primarily at mid-latitudes on both sides of the Pacific, suggesting a vicariant origin for such a distribution, perhaps by high-latitude glaciation (as suggested by Barsukov, 1981). However, this does not seem to be the case. These two sub-genera are perhaps the best examples in the Sebastinae for taxonomic confusion due to convergent evolution. Kai et al. (2003) termed the two clades, containing species previously classified mostly as *Pteropodus* or *Mebarus*, as "clade NWP" (*S. hubbsi*, *S. inermis*, *S. joyneri*, *S. oblongus*, *S. pachycephalus*, *S. schlegelii*, *S. taczanowskii*, *S. thompsoni*, *S. trivittatus*, and *S. vulpes*) and "clade NEP" (*S. atrovirens*, *S. auriculatus*, *S. carnatus*, *S. caurinus*, *S. chrysomelas*, *S. dallii*, *S. maliger*, *S. nebulosus*, and *S. rastrelliger*). The sister species to both of these clades (*S. scythropus* and *S. kiyomatsui*, "clade NWP"; *S. elongatus*, *S. saxicola* N, *S. saxicola* S, and *S. semicinctus*, "clade NEP") are deep water, low relief reef/soft bottom dwellers with weak to moderate head spines. In both clades there seems to be a progression toward more demersal, shallow, high relief reef dwelling species with strong head spination.

Within Kai et al.'s (2003) "clade NWP" there are two clades with different morphologies. Members of the first clade, *Mebarus*, containing *S. inermis, S. joyneri*, and *S. thompsoni*, dwell on coastal rocky reefs at shallow to moderate depths (<200 m) and have weak to moderate head spines. Both *S. inermis* and *S. thompsoni* are known to associate with drifting algal rafts as juveniles, exhibiting a semi-pelagic lifestyle, at least during this life stage. The second clade, *Sebastocles*, contains some of the shallowest occurring species in the NWP (i.e., *S. hubbsi, S. nivosus, S. trivittatus*) and tend to be demersal, high relief rocky reef dwellers with strong head spination.

In agreement with Li et al. (2006) we choose to maintain the subgenus *Pteropodus* for species within "clade NEP". Most species within this clade are shallow dwelling (<100 m), associated with high relief reef, and have strong head spination. The exception is *S. atrovirens*, previously classified as *Mebarus*, which shows reduced head spination, likely associated with its preferential midwater affinity for

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stands of macro-algae (e.g., *Macrocystis pyrifera*) rather than rocky reef. The sister clade to *Pteropodus*, clade "D", is closely related to and shares several traits with *Pteropodus* species (e.g., larval pigment pattern and juvenile habitat) yet differ in general adult morphology (e.g., reduced head spination), depth (occur deeper than *Pteropodus* species), and adult habitat preference (i.e., soft bottom or low-relief reef).

It seems that the similar trends in morphology found in clades on opposite sides of the NP are driven more by the similarity between their habitats than their evolutionary lineage. Within lineages it appears that morphology evolves rapidly depending on the habitat or life history preferences of individual species (e.g., reduction of head spination in *S. atrovirens*). Such examples of ecology-linked morphology seem relatively common throughout *Sebastes* leading to numerous examples of species that appear superficially similar but are not closely related in genetic analyses (e.g., *S. alutus*—*S. goodei*; *S. brevispinis*—*S. macdonaldi* - *S. paucispinis*; *S. aleutianus*—*S. borealis*—*S. melanostomus*; *S. ciliatus*—*S. mystinus*).

4.4. Paleo-oceanography of the north Pacific and evolution of Sebastes

Barsukov (1981) proposed an origin for *Sebastes* in the NWP based upon his reasoning that the more primitive members of Sebastinae are not found in the NEP. The phylogenies produced by both maximum parsimony and Bayesian inference support the NWP as the center of origin. The oldest *Sebastes* fossils are found in late Miocene (~6–10 million years ago) diatomite deposits in Lompoc, California (Barsukov, 1989) and are similar to extant NEP species (i.e., S. *brevispinis, S. flavidus, S. gilli, S. goodei*, and S. *semicinctus*). Interestingly, these are mostly semi-pelagic species with weak head spination, supporting the hypothesis that dispersal into the NEP was facilitated by this derived lifestyle. Unfortunately, the NWP is depauperate in accessible Miocene deposits so there is little comparative fossil record from this area. The ages of these fossils are in agreement with the molecular clock estimates in Fig. 5.

The development of paleo-oceanographic features conducive to the evolution and spread of *Sebastes* species seems to have begun with the middle Miocene (~15–17 MYA) shoaling of the Indonesian Seaway (Tsuchi, 1997). This disruption of the circum-global Tethyan Sea and its associated westward currents led to a dramatic change of currents in the Pacific. Of particular importance were the strengthening of the warm northward Kuroshio current in the NWP, increased ocean gyre circulation, and the subsequent strengthening of the cool southward California current in the NEP. Coincident with these changes, significant diatom deposits began to appear and gradually increase in the NWP (Barron, 1998). These patterns were further intensified with the progressive shoaling of the Central American Seaway (Marincovich, 2000). In addition to surface current flows, these changes marked the beginning of cold deepwater formation in the NA and establishment of its global circulation (Haug and Tiedemann, 1998). This deepwater circulation would play a key role in the provisioning of cool, nutrient laden water to the north Pacific (NP), altering climate and promoting dramatic increases in primary production.

Intense geologic shifts in the middle Miocene (~15 MYA) led to the rotation of the back-arc shelf of Southwest Japan creating the Japan Sea (Itoh et al., 1997). This caused a dramatic change in the faunal assemblage of the region. The northeast islands were subjected to a dramatic cooling as they began to be influenced by the cool, southward Oyashio current (Tsuchi, 1997). Fossils of cold-temperate species began to appear in NE Japan at this time. In contrast, the newly opened Japan Sea was fed by the warm Kuroshio-derived Tsushima current through the Tsushima Strait and was dominated by species of tropical affinity. The transition from the middle to late Miocene (~10–11 MYA) epoch in this region was marked by further geologic activity resulting in the uplifting of the Tsushima Strait, ceasing the warm water input to the Japan Sea from the south. This dramatic cooling of the Japan Sea likely opened the door for invasion of *Sebastes* species from the northeast. Cool conditions dominated the Japan Sea until warm water input was restored in the middle Pliocene (~3.5 MYA) (Tsuchi, 1997).

The increase and subsequent fluctuations of upwelling driven primary and secondary production during the Miocene epoch was suggested as the driving force behind population expansion/contraction and species radiations of cetaceans and pinnipeds (Lipp and Mtichell, 1976). This hypothesis suggests that increased upwelling brings with it the growth of a species range, due to the expansion of suitable habitat and prey resources, while subsequent contractions of these favorable conditions may lead to the extinction or isolation of populations. Additionally, the development of productive ecosystems likely favored the evolution of specialist species through niche partitioning. By these mechanisms, similar trends in abundance and species radiation should be expected in other upwelling dependent taxa (e.g., *Sebastes* spp.).

Primary production dramatically increased in the NWP, primarily off the Kamchatka peninsula (site 884, Detroit Seamount (51°27′ N, 168°20′ E)), beginning ~9 MYA (Barron, 1998). This first period of high productivity was likely due to the input of nutrients into the NWP through increased deepwater circulation but was not associated with dramatic cooling. A second peak in primary production began ~8.3 MYA across the NP, coincident with the initial diversification of *Sebastes* (node A, Fig. 5), and peaked ~6.5–5.5 MYA. This protracted period of enhanced primary production was possibly driven by increased upwelling caused by high-latitude cooling and increasing deepwater and ocean gyre circulation due to the continued shoaling of the Central American Seaway (Barron, 1998). Interestingly, this bloom of primary production seems to have peaked off California ~6.5 MYA (Isaacs, 1983, 1985),

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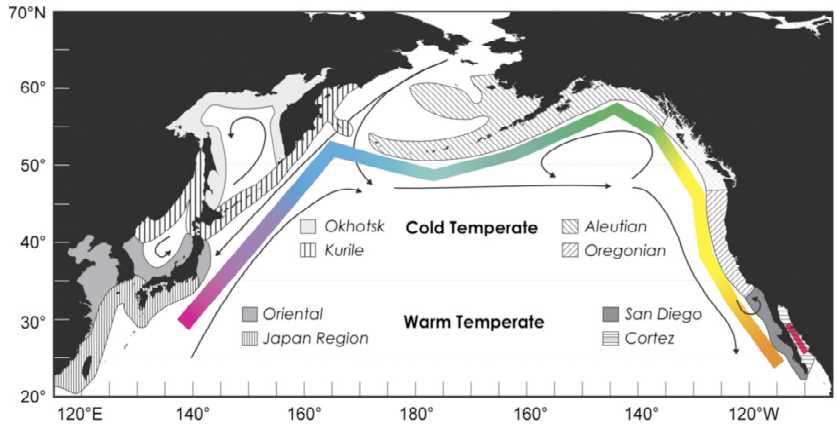

Fig. 4. Biogeographic map of the north Pacific. Provinces correspond to those proposed by Briggs (1974) and Brusca and Wallerstein (1979) with areas of disagreement represented by overlap of provinces. Arrows indicate general direction of major ocean and basin currents.

coincident with a period of initial diversification of *Sebastes* in this region (node E, Fig. 5), and spread westward to central Japan ~5.5 MYA (site 438 (40°38′N, 143°13′E)). This time of major upwelling, resulting in increased primary production and cooling of mid and high-latitudes, coincides with the initial diversification (see Figs. 3–5) and rapid spread of *Sebastes* species throughput the NP, both to the east and south to mid-latitudes on both sides of the Pacific.

Approximately 4–3.5 MYA, during a period of high latitude warming, the Bering Straits opened and allowed biotic exchange from the NP into the Bering Sea and eventually the NA (Vermeij, 1991; Briggs, 1995). Dramatic cooling and glaciation at high latitudes began ~3.0–2.7 MYA and ceased favorable conditions for biotic exchange between the two oceans. Many taxa are documented to have made this journey into the NA, among them was the ancestor of the *S. alutus* clade (node F, Fig. 5). This lineage successfully colonized much of the NA eventually forming four closely related species (*S. fasciatus, S. mentella, S. norvegicus*, and *S. viviparus*). This dispersal event allows us the unique opportunity for calibrating a molecular clock model of genetic evolution. Such a calibration allows placement of approximate times on the nodes of the phylogeny (see Fig. 5).

4.5. Sea level change as a promoter of speciation

With the cessation of flow through the Central American Seaway (~3 MYA), modern ocean circulation patterns were largely established. This event also marked the beginning of a period of frequent glacial-interglacial cycles. Periods of intense glaciation are notable not only for the great ice sheets that formed at high latitudes but also for a significant decrease in global sea level of 50–100 m (Haq et al., 1987; Miller et al., 2005), cooler sea surface temperatures, and alteration of major ocean currents (Herbert et al., 2001). Periods of low sea level occurred primarily at 6.3–5.6 MYA, 5.1–4.6 MYA, 4.2–4.0 MYA, 3.5–3.2 MYA, 2.9–2.5 MYA, 2.3–2.0 MYA, 1.9–1.6 MYA, 1.3–1.9 MYA and repeatedly throughout the past 800,000 years (Haq et al., 1987; Graham et al., 2003; Miller et al., 2005) (see Figs. 5 and 6). Such large changes in sea level can have profound implications on regional ocean circulation patterns as previous submarine ridges become barriers to circulation. This is especially important in the SCB and Japan where low sea levels create semi-isolated basins, possibly restricting gene flow, promoting divergence and subsequent speciation. Lower sea level not only altered current flows but dramatically increased near-shore macro-algal and rocky habitat, at least in the SCB (Graham et al., 2003). Perhaps this spurred the evolution of specialist species to these habitats (i.e., *S. atrovirens, S. auriculatus, S. carnatus, S. caurinus, S. chrysomelas, S. dallii, S. maliger, S. melanops, S. nebulosus, S. rastrelliger, S. serranoides*), many of which have evolved during a protracted period of low sea level during the past 3 million years.

Recent studies of population structure in several *Sebastes* species have shown that despite a lengthy pelagic dispersal phase there can be fairly low geographic dispersal (Buonaccorsi et al., 2002, 2004, 2005; Taylor, 2004; Taylor et al., 2004). Regional signals of genetic isolation can be present due to interactions of major ocean currents (Rocha-Olivares and Vetter, 1999), persistent regional excursions and eddies of current systems (Buonaccorsi et al., 2002, 2004, 2005; Cope, 2004; Matala et al., 2004), and even local bathymetric and current features (Withler et al., 2001; Buonaccorsi et al., 2002). Clearly this indicates a tendency for *Sebastes* species to become genetically isolated during periods of expansion/contraction of cool water systems, changes in sea level, or major alterations of current flow.

To test whether changing sea level acted to promote evolution of new species in *Sebastes*, sea level data (Miller et al., 2005) was averaged over 100 kyr periods and com-

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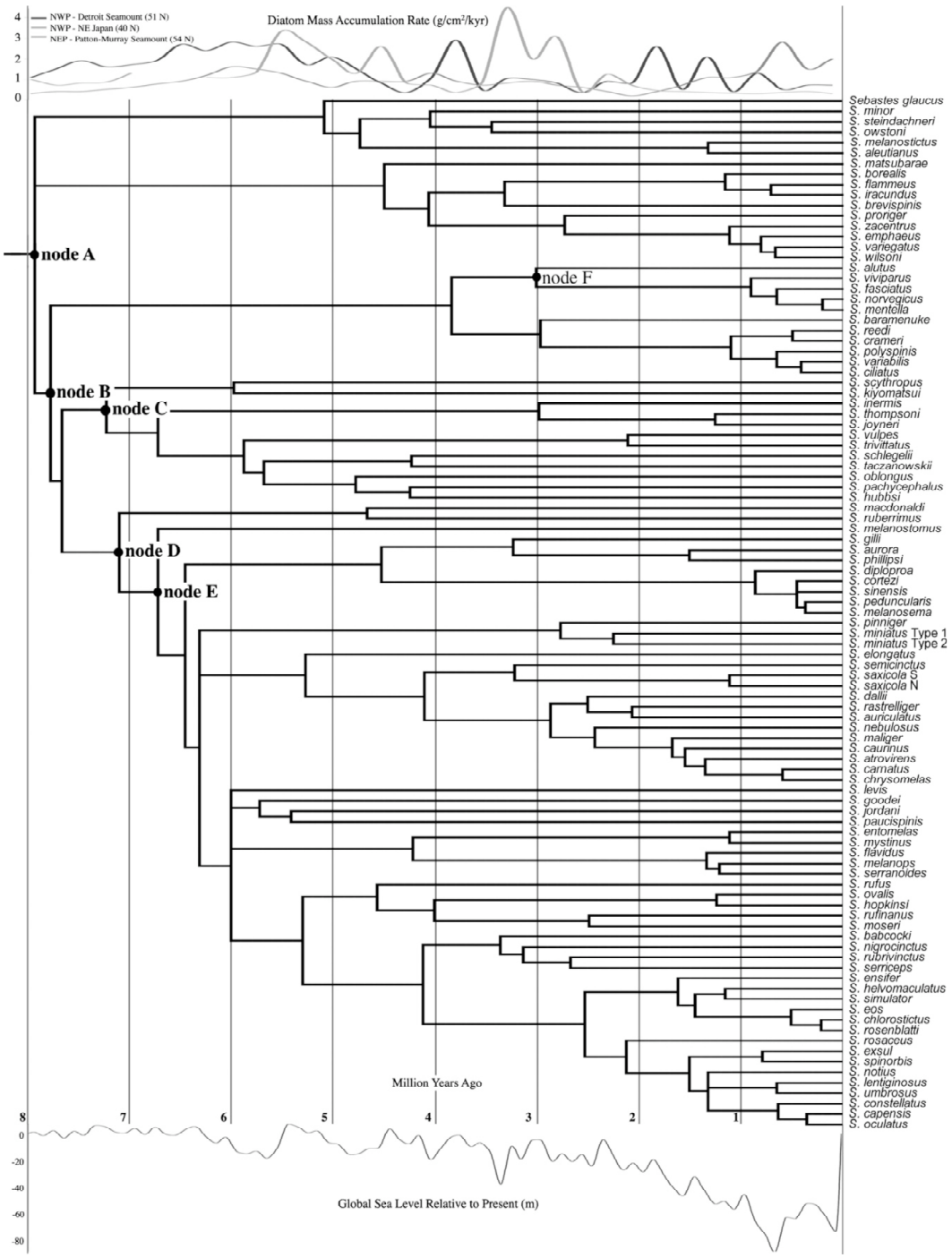

Fig. 5. Hypothesized chronogram for the evolution of *Sebastes* generated using Bayesian derived branch lengths adjusted with a penalized likelihood method as implemented in r8s (Sanderson, 2002). Paleo sea levels are 100 kyr averaged data adapted from Miller et al. (2005). Diatom mass accumulation rate graphs are adapted from Barron (1998) and are included as proxies for primary production. Labeled nodes are referenced in the text.

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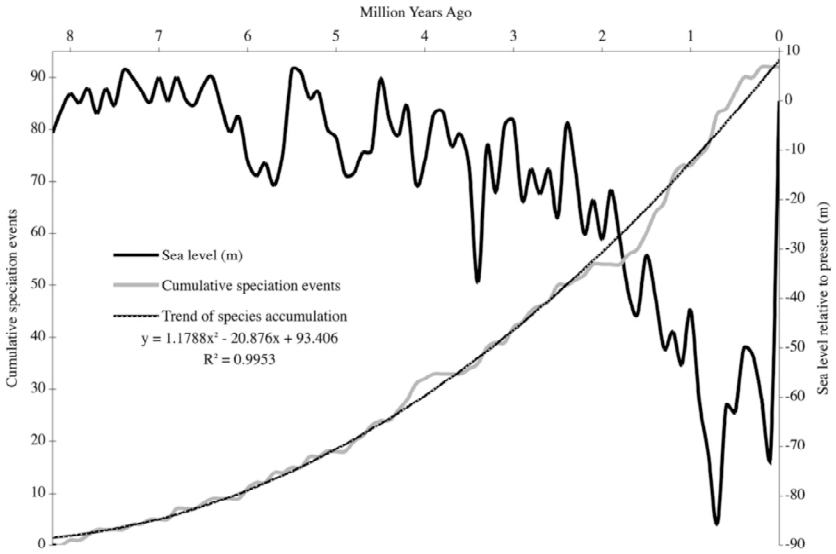

Fig. 6. Paleo sea levels (black) relative to the present, (Miller et al. 2005) averaged over 100,000 year intervals, are plotted on the right axis. Cumulative speciation events (gray) from the clock-calibrated tree analysis (Fig. 5) were binned over 100 kyr intervals and plotted on the left axis. The overall species accumulation trend is consistent through time, strong deviations from this trend, both positive and negative, may be related to changes in sea level.

pared to deviations of the instantaneous speciation rate from the average rate. To do this, node ages were binned into 100 kyr periods and assembled into a graph of cumulative speciation events. A polynomial curve of best fit was applied and used to calculate the deviation between the observed and expected number of speciation events at each time period, effectively creating a measure of the instantaneous change in speciation rate. Correlation analysis between change in sea level and change in speciation rate was not significant. However, the graph of sea level and cumulative speciation events (Fig. 6) appears to show a general increase in speciation events in association with large drops in sea level and conversely a decrease in speciation events during periods of increasing or stable sea level. The lack of significance may be due to small node-age errors introduced during the rate smoothing and calibration processes resulting in slight phase shifts in this relationship.

As *Sebastes* species have complex mating behavior (Helvey, 1982; Shinomiya and Ezaki, 1991; J. Hyde unpublished data) including internal fertilization and possible pheromone (Helvey, 1982) and sound production (Hallacher, 1974), isolation events would be expected to be particularly effective at promoting speciation. It is easy to envision drift in mating preference between isolated regions leading to the maintenance of reproductive isolation even after subsequent reconnection of populations. Development of divergent and heritable mating preferences are hallmarks of incipient stages of speciation (Haesler and Seehausen, 2005).

4.6. What does the future hold for the genus Sebastes?

Our ability to recognize new species is dependent upon the evolution of unique physical and/or genetic characters, a process that can take a great deal of time. There are examples in several *Sebastes* species where color morphs, which are not reciprocally monophyletic at the examined mitochondrial genes, show evidence for assortative mating when examined using faster evolving nuclear markers (Kai et al., 2002a and Kai et al., 2002b; Narum et al., 2004). These species complexes are likely in the early stages of speciation and will continue to diverge in the future. As genetic techniques continue to advance and additional species are examined in detail, we will likely find more incidences of cryptic species, indicating that the process of speciation is ongoing in this genus.

These magnificent fishes have succeeded in spreading around the globe due in large part to the cooling of the world oceans since the middle Miocene. However, anthropogenic drivers over the past century seem to be forcing the oceans to gradually warm (Field et al., 2006). This likely marks the end of southward expansion in the northern hemisphere but may reopen the door to the north as arctic waters warm and again become hospitable to these fishes. Perhaps additional lineages will colonize the NA from the NP, leading to eventual speciation. Species at mid-latitudes will likely face additional temperature challenges as seasonal periods of cool, favorable conditions for larval survival are reduced. Endemic species within the GC will likely be maintained as the unique geology and oceanography of

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this region promotes strong upwelling of cool, nutrient-rich water (López et al., 2006). However, thermal barriers that currently exist will likely be strengthened. Additionally, expansion of persistent low oxygen "dead zones" (Dybas, 2005) across the NP may act to further restrict population connectivity, perhaps fostering the speciation process.

Though climate change may alter the long-term evolutionary trajectory of *Sebastes* spp., the short-term threats of pollution and overfishing are likely more disruptive. Commensurate with the growth of human populations, there is an increasing demand for fishery resources worldwide. Recent studies project dire consequences for world fisheries within the next 40–50 years unless current trends of exploitation are changed (e.g., Worm et al., 2006). *Sebastes* spp. have been heavily exploited by both commercial and recreational fishermen for over 50 years throughout the NP. Several NEP *Sebastes* spp. are currently classified by the Pacific Fisheries Management Council as overfished (i.e., *S. crameri, S. entomelas, S. levis, S. paucispinis, S. pinniger, S. ruberrimus*), while many others are well below their historic levels of abundance.

5. Summary

The phylogenetic hypotheses put forth in this study support the origin of *Sebastes* during the middle Miocene epoch at high-latitudes in the NWP. The dramatic cooling and increase in primary production that began ~9–8 MYA in the NWP seems to coincide with the initial diversification of the genus as seen in the ultra-metric tree analysis (node A, Fig. 5). Pronounced upwelling with increased primary production and ocean cooling began ~8–6 MYA across most of the NP and seems to coincide with a second burst of diversification both to the east and south. It would seem that the radiation initially was to the east into the Gulf of Alaska (node B, Fig. 5) but was soon followed by expansion south to the mid-latitude Japanese Islands (node C, Fig. 5). This was soon followed by further expansion east and south into the California Current system and to mid-latitudes in the NEP (node D, Fig. 5). More recent tectonic events and additional cooling allowed further expansion southward and subsequent speciation in the Gulf of California (*S. cortezi, S. exsul, S. macdonaldi, S. peduncularis, S. sinensis, S. spinorbis*, and *S. varispinis*) and eventually the SH (*S. capensis* and *S. oculatus*). In all cases, both ancient and recent, colonization of newly created temperate habitat appears to have been progressive and stepwise. Examination of the biogeographic map and color-coded tree (Figs. 3 and 4) did not reveal a single case of trans-Pacific dispersal such that an Asian species occurs in a strictly North American lineage or vise versa. Colonization of both the Gulf of California and southern hemisphere came from the lineages with the most southern affinity. It is also interesting to note that all of the basic ecological morphotypes commonly observed in NP habitats, (e.g., soft bottom vs. hard bottom, shallow reef vs. deep reef, schooling vs. solitary), (Gunderson and Vetter, 2005) had evolved prior to 3 million years ago. Subsequent Pleistocene glacial cycles have mostly developed variations on these basic ecological themes.

From these analyses it would seem that the evolution of a more fusiform morphology and semi-pelagic lifestyle, in conjunction with increased upwelling and cooler ocean temperatures, facilitated the spread of *Sebastes* species throughout the NP. This hypothesis is similar to those proposed in previous studies (e.g., Barsukov, 1981). Such innovations away from the typical demersal existence of a deep bodied, spiny scorpaenid fish is not surprising considering recent genetic (Smith and Wheeler, 2004) and morphologic (Imamura and Yabe, 2002) analyses of the Scorpaeniformes. Their results clearly show the order to be polyphyletic with numerous percoids nested within the Scorpaeniformes. Such results suggest that the morphologic characters typical of scorpaenid fishes are more labile than previously believed. Nowhere is this more evident than within *Sebastes* where species range from small, relatively head-spine free, semi-pelagic species (e.g., *S. jordani* and *S. owstoni*) to large, strong head spination, demersal species (e.g., *S. aleutianus*, *S. levis*, and *S. melanostomus*).

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II.

Cryptic speciation in the vermilion rockfish (*Sebastes miniatus*) and the role of bathymetry in the speciation process

Abstract of Chapter II:

A recent phylogenetic review of the genus *Sebastes* suggested the existence of a cryptic species of vermilion rockfish (*S. miniatus*). To evaluate the geographic and bathymetric range of the Type 1 and Type 2 forms reported in Hyde & Vetter (2007), cytochrome *b* sequences were examined from 548 fish. Type 1 fish were found primarily south of Point Conception on reefs deeper than 100m. Type 2 fish were common range-wide at sites shallower than 100m. Reproductive isolation between the two types was tested using nine microsatellite loci. Estimates of genetic divergence were made using the fixation index (F_{ST}) and correspondence between haplotype and genotype was tested by Bayesian population assignment and multivariate plotting of individual genotypes. Microsatellite analyses gave strong support for the presence of two distinct groups of genotypes. All fish with Type 1 haplotypes and fish with Type 2 haplotypes from <100m depth had genotypes unique to their haplotype group. However, most (68%) fish with Type 2 haplotypes from >100m depth assigned strongly to the Type 1 genotype group. Morphometric comparisons between the two genotypic groups revealed significant differences at three of the six examined measurements. Differences in both genetics, depth of occurrence, and morphology suggests these are separate species. This finding along with evidence of depth segregation in many recent species pairs led us to hypothesize a speciation model for *Sebastes* spp. by which the loss or truncation of a depth related ontogenetic migration can lead to the creation of reproductively isolated populations.

Introduction:

Failure to recognize the existence of two reproductively isolated entities (cryptic species) within an exploited stock is one of the most critical errors in management. This error can be compounded if management strategies include spatial or depth related closures that favor one unrecognized entity over the other. Such a potential problem came to light in a recent phylogenetic examination of the heavily exploited rockfishes, genus *Sebastes* (Hyde & Vetter 2007). The subgenus *Rosicola* historically contained two species, the canary rockfish, *S. pinniger*, and the vermilion rockfish, *S. miniatus* that together represent an important component of the west coast commercial and recreational fisheries. The investigations of Hyde & Vetter (2007), based on seven mitochondrial and two nuclear loci, suggested the presence of a third taxon that was reasonably old (~2.3MYA) but previously unrecognized. This situation was not unlike the recent discovery of a cryptic species pair within *S. aleutianus* (Gharrett et al. 2005; Gharrett et al. 2006). This study examines the presence of a cryptic species within *Rosicola,* the common occurrence of bathymetric parapatry in recent sister taxa of *Sebastes*, and the proposal of a previously unrecognized general mechanism for reproductive isolation based upon the truncation of a bathymetric ontogenetic migration (sensu Gunderson & Vetter 2006).

Canary rockfish, *S. pinniger*, are abundant from British Columbia to Point Conception. They are generally found on the inner continental shelf from 80-200m depth and are susceptible to trawl and hook & line fisheries (Love et al. 2002; Williams & Ralston 2002). They release live young that spend three to four months in the pelagic

environment before settling as juveniles in shallow kelp forest habitats. This benthic settlement phase is followed by an ontogenetic migration to greater depths as they mature (Vetter & Lynn 1997). Presently canary rockfish are considered overfished, placing stringent management on the directed fishery and limiting other fisheries, both commercial and recreational, due to their presence as bycatch.

Vermilion rockfish (*S. miniatus*) are very similar in morphology and appearance to canary, tending to be more red than orange (see color plates in Love et al. 2002). They generally inhabit shallower water north of Point Conception where they typically occur inshore of canary habitat in depths <100m. The timing of reproduction, typically in the fall, differs from the winter spawning canary but spawning and settlement peaks are not well defined (Love et al. 2002), perhaps due to the presence of more than one unrecognized taxon. South of Point Conception, where canary are uncommon, vermilion rockfish expand their known depth range to include habitat down to 200m on coastal reefs and offshore banks, again hinting at the possibility of an unrecognized taxon (Love et al. 2002). Vermilion populations are heavily exploited (currently ranked #1 in the southern California recreational fishery and #3 statewide, MacCall 2005) and are partly protected by extensive area and depth closures designed to protect cowcod (*S. levis*).

The discovery of a deep phylogenetic division (Hyde & Vetter 2007) followed by a fresh look at long known anomolies in life history characters (e.g., multiple settlement peaks and a wide depth expansion south of Pt. Conception, Love et al. 2002) prompted a detailed examination of the habitat and genetic relationships between canary and the Type 1 and Type 2 vermilion of Hyde & Vetter (2007). This required an exhaustive sampling effort that paid strict attention to location, depth of capture, and morphology.

In this paper we present mitochondrial cytochrome *b* sequence data, coupled with an examination of allelic variation at nine nuclear microsatellite markers, to examine 548 vermilion rockfish from throughout the known latitudinal and depth range of the species. We also examined the depth preferences for 12 pairs of recent sister species within the genus and offer a general evolutionary scenario of how paedomorphosis (i.e., truncation of a life history trait such as ontogenetic bathymetric migration) can lead to reproductive isolation and perhaps speciation within the genus.

Methods:

Sample collection:

Fish were collected throughout the entirety of the species common range using various techniques (i.e., hook and line, bottom trawl, pole spear) and identified to species using Love et al. (2002). In addition to standard sample information, care was taken to obtain accurate collection location and depth of capture (see Table 2-1). Tissues, either white muscle or pectoral fin, were preserved in 95% un-denatured ethanol pending DNA extraction and genetic analysis.

DNA extraction:

DNA was extracted using various protocols. Most samples had DNA extracted using a standard proteinase K digestion followed by a lithium chloride:chloroform nucleic acid purification and subsequent ethanol precipitation (Gemmel & Akiyama 1996). DNA from the remaining samples was extracted using either the DNeasy kit

(Qiagen) following the manufacturer's protocol or by use of a Chelex (BioRad Laboratories) boiling technique (Hyde et al. 2005).

PCR amplification:

Mitochondrial DNA:

DNA was amplified for sequencing from the mitochondrial cytochrome *b* (cytb) gene using primers GluRF2 5' AAC CAT CGT TGT TAT TCA ACT ACA AGA ACC and CB3RF2 5' CGA ACA GGA ART ATC AYT CTG G in a 10µL reaction volume containing (67mM Tris-HCl pH 8.8, 16.6mM $(NH_4)_2SO_4$, 10mM ß-mercapto-ethanol, 2mM $MgCl_2$, 800µM dNTPs, 0.4µM each primer, 0.5 units *Taq* DNA polymerase (New England Biolabs), and 50-100ng of DNA template) and amplified using the following temperature profile in a PTC200 DNA Engine (MJ Research); 94ºC (2:00), 35 cycles of [94ºC(0:30), 59ºC(1:00), 72ºC(1:00)], followed by three minutes at 72ºC. All PCR batches contained at least one no template negative control to monitor for possible DNA contamination. Products were electrophoresed through a 2% (w/v) agarose gel in 1 X Tris-Borate-EDTA buffer, stained with ethidium bromide and visualized via an UV-transilluminator. Reactions were digested using ExoSAP-IT (USB Corp.) to remove unincorporated primers and deoxynucleotides prior to cycle sequencing. Products had both strands individually cycle sequenced using BigDye v.3.1 Dye Terminators and analyzed on an ABI 3130XL automated capillary sequencer (Applied Biosystems). DNA sequences from both strands were aligned and edited using Sequencher v4.5 (GeneCodes, Inc).

Microsatellites:

Nine microsatellite loci (see Table 2-2) were chosen from the libraries developed by Gomez-Uchida et al. (2003) and Westerman et al. (2005). These loci were selected based upon previous experience showing them to be robust, easily scored, and moderately polymorphic. All microsatellite 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 sized using an ABI 3130XL Genetic Analyzer with the ROX 500 size standard (Applied Biosystems) and scored using Genemapper v3.7 software. The computer program MicroChecker (van Oosterhaut et al. 2004) was used to examine the data for common genotyping errors.

Phylogenetic analysis:

Partial sequence (782bp) of cytb from all currently described species of *Sebastes* in the northeast Pacific (Hyde & Vetter 2007) were combined with 548 sequences of vermilion and eight of canary rockfish generated in this study. These sequences represented 44 unique vermilion rockfish haplotypes and four unique canary rockfish haplotypes. Sequence data of unique haplotypes have been deposited in GenBank, accession numbers EF587183-EF587231. Genetic data were evaluated for evolutionary model testing using MrModeltest v2.2 (Nylander 2004) as implemented within the PAUP*(v4.b10) (Swofford 2001) framework. This program uses both the hierarchical likelihood ratio test (Huelsenbeck & Crandall 1997) and Akaike information criterion (Akaike 1974) to test the fit of the data to 24 different evolutionary models. The model chosen by both methods was the GTR model of Rodriguez et al. (1990), considering an

empirically derived proportion of invariant sites (I) and gamma shape distribution (Γ). MrBayes v3.1.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was used to generate a Bayesian inferred phylogenetic hypothesis using Metropolis coupled Markov chain Monte Carlo analysis. The evolutionary model was set to nst=6, Dirichlet (1,1,1,1), and the program empirically estimated both I and Γ. Two independent runs of four Markov chains each were allowed to proceed for 2,000,000 cycles using the default heating values. The first 25 percent of the analysis was discarded to eliminate the inclusion of data from the "burn-in" phase of the analysis where the posterior probabilities may have not yet reached stationarity.

Microsatellite analyses:

To test for reproductive isolation between the two mtDNA clades of vermilion rockfish we chose to examine allelic variation at nine codominant nuclear microsatellite loci. As the canary rockfish clade is of similar divergence as the two vermilion rockfish clades (Hyde & Vetter 2007), we chose to use it as a comparative benchmark for measuring inter-specific divergence. A range-wide population genetic study using microsatellite markers is the subject of a separate paper (J. Budrick in prep), so we chose to limit the geographic scope of our analysis in this study. As canary rockfish are exceedingly rare south of Point Conception, we used samples from the central California coast, for our comparisons.

Samples were grouped and analyzed first by collection site (i.e., central California, southern California <100m, southern California >100m) and then by mitochondrial clade (i.e., Type 1, Type 2, canary rockfish). Standard measures of

molecular diversity, expected and observed heterozygosity, and measures of the fixation index F_{ST} (Weir & Cockerham 1984) were performed for all loci individually and collectively using Genepop v3.4 (Raymond & Roussett 1995). The local inbreeding coefficient (F_{IS}) was calculated for each locus in order to measure conformance to the expectation of Hardy-Weinberg equilibrium (F_{IS}=0). Probabilities for Hardy-Weinberg and linkage equilibrium were calculated using an exact test based upon the Markov chain algorithm as described by Guo & Thompson (1992) and implemented in Genepop with 1,000,000 steps. Probabilities were combined across all loci using Fisher's method (Sokal & Rolf 1995). Significance probabilities at individual loci were corrected for type 1 error using the sequential Bonferroni correction for multiple comparisons (Rice 1989).

The program Structure v2.1 (Pritchard et al. 2000) was used to generate a Bayesian inference of population structure. By this method, a model of *k* populations is assumed, where *k* is often unknown, and samples are grouped in order to minimize linkage disequilibrium and maximize conformity to Hardy-Weinberg equilibrium across all analyzed loci. At diploid nuclear loci, the erroneous grouping of genetically divergent populations can result in departures, at multiple loci, from both the expected values of Hardy-Weinberg and linkage equilibrium for a randomly mating population. By assessing the change in Markov chain Monte Carlo (MCMC) derived likelihood scores for increasing values of *k*, it is possible to deduce the number of populations with the maximum posterior probability. Starting with *k*=1, the likelihood scores decrease dramatically with increasing values of *k* until reaching a relative plateau when the most likely value of *k* has been reached. All samples were originally grouped as "populations" either by their site of origin or clade of origin (i.e., Type 1, Type 2, and canary), values of

k=1 to *k*=5 were tested, admixture between populations was allowed and 1,000,000 iterations of MCMC were performed after a burn-in period of 200,000 iterations. Though putative population assignment is provided in the input file, program output results in a reassignment of individuals to the most likely of the *k* populations. This assignment metric also allows us to assess the possibility of hybrid ancestry of individuals within the sample.

Morphometric comparisons:

To evaluate the two vermilion types for differences in phenotype we measured several morphometric parameters following Phillips (1957). In particular we measured; head length, orbit length, inter-orbit width, caudal peduncle depth, lower jaw length, and snout to dorsal-fin length. Only a subset of our genotypically analyzed fish (92 of 548) were available in their entirety for this analysis. Measurements were made with digital calipers to the nearest 0.01mm and expressed as a fraction of the standard length (SL) of the fish. The ratios of measure/SL were compared within and between the two vermilion rockfish groups and tested using a two-tailed t-test in order to test for significant differences between the groups.

Results:

Phylogenetic and Phylogeographic analyses:

Analysis of the sequence data from vermilion rockfish, sampled throughout their common range, produced two distinct clades with high Bayesian posterior support values

(Figure 2-1). The level of intra-specific haplotypic diversity within both of these clades is similar to that found within most other species of *Sebastes* (J Hyde unpublished data), including the sister species, canary rockfish. Additionally, the inter-specific divergence between these clades is of similar or greater magnitude than that observed for most sister species of *Sebastes* (see Hyde & Vetter 2007) including the canary rockfish (*S. pinniger*).

Phylogeographic analysis revealed a high degree of geographic and bathymetric segregation of the two clades. Type 1 haplotypes were found primarily south of Point Conception and were most abundant in samples collected from offshore banks and submarine canyons in depths greater than 100m, but were also found in much lower abundance at shallow (<100m), nearshore sites. All fish with Type 1 haplotypes collected at these shallow sites were young of the year or sub-adult fish (i.e., <250mm TL). Type 2 haplotypes were found throughout the common range of vermilion rockfish and were found primarily in collections from shallow (<100m), nearshore sites, but were also found in much lower abundance on deep (>100m), offshore banks (Figure 2-2).

Microsatellite analyses:

Hardy-Weinberg and linkage equilibrium:

When grouped by sampling site, all vermilion rockfish collections from southern California showed a strong departure from both Hardy-Weinberg (Table 2-3) and linkage equilibrium. Alternatively, the samples were grouped by mitochondrial clade (i.e., Type 1, Type 2) and reanalyzed. In this analysis the Type 1 clade showed no departure from Hardy-Weinberg equilibrium at all but the Spi6 locus (Table 2-3). Linkage disequilibrium between loci was also greatly reduced. However, the Type 2 clade

remained out of equilibrium at most loci. Such strong departures, across multiple loci, are usually indicative of mixed populations being analyzed together. Analysis of genetic data with MicroChecker (van Oosterhaut et al. 2004) found no evidence for genotyping errors at most loci, however, excess homozygosity at the Spi6 locus suggested the presence of null alleles.

Structure analysis:

Structure v2.1 (Pritchard et al. 2000) acts to group samples into *k*-populations by combining samples in order to minimize departures from both Hardy-Weinberg and linkage equilibrium. When all vermilion and canary rockfish samples were analyzed together, the value of *k* with the highest likelihood was k=3 ($\log_e$ (P) = -19540, Table 2-4). The resultant groupings (canary, Genotype A, Genotype B) largely conformed to mitochondrial clade assignment (i.e., canary, Type 1, Type 2, see Figure 2-3A). All canary rockfish and all but one Type 1 vermilion rockfish assigned with high probability to their respective group. However, though the majority of Type 2 vermilion rockfish assigned strongly together (Genotype A), there were a number of fish collected from depths >100m that assigned strongly with Type 1 fish (Genotype B, n=57 of 347 fish, see Figure 2-3B). Despite these examples of disagreement between mtDNA haplotype and nuclear genotype there were few potential hybrid genotypes, with only one a potential F1 hybrid. To better visualize the positions of individual samples in multivariate space we used the program PCAGEN v.1.21 (Goudet 1999) to perform a principal component analysis (PCA) of the genotype data. As suggested by the Structure results, the primary component axis of PCA produced three distinct distributions of genotypes (Figure 2-3A).

As before, these distributions largely conformed to mtDNA haplotype grouping with the exception of a subset of the vermilion Type 2 haplotypes nested within the Genotype B distribution.

Genetic fixation measurements:

The use of F-statistics to measure genetic distance is common in studies examining intra-specific divergence of populations, but this metric may be inappropriate when measuring inter-specific divergence. However, due to the close relationship between the canary and vermilion rockfish (Hyde & Vetter 2007) we feel that this metric is useful for measurements of genetic distance between the two vermilion clades. Despite the strong violation of both Hardy-Weinberg and linkage equilibrium found in the first two groupings, we chose to retain them in our analyses and compare the samples by sampling site, mtDNA clade, and the genotypic groups determined by Structure. Individual fish that showed strong disagreement between mtDNA haplotype and genotype assignment were removed from the genotypic groups prior to pairwise measurement of F_{ST}. In all comparisons, highly significant F_{ST} values were obtained across the nine loci (Table 2-5). However, the highest and most significant values of F_{ST} were found between the groups determined by Structure. The divergence between the two vermilion groups ($F_{ST} = 0.1013$) was similar to that between canary rockfish and either of the vermilion groups ($F_{ST} = 0.0915 - 0.1161$).

Morphometric analysis:

In total, 92 of the 548 vermilion rockfish used for genetic analysis were available for morphometric examination. As mitochondrial haplotype and nuclear genotype were in disagreement for some fish, we chose to label fish with the genotypic group assignment produced by the Structure analysis. Of the six morphometric characters, three were significantly different between the two groups (orbit length, inter-orbit width, and caudal peduncle depth) (Table 2-6). In general, Type 1 fish had smaller eyes, greater inter-orbit width, and a more slender caudal peduncle than Type 2 fish.

Discussion:

Speciation in the sea is generally thought to be reduced due to the great dispersal capacity and consequent high gene flow of pelagic larval phases of most organisms. Despite this generality, many cases of restricted gene flow, population genetic structure, and ultimately speciation do occur and the mechanisms that promote limited gene flow are becoming better characterized (e.g., Palumbi 1994; Hellberg 2006). Environmental factors such as persistent oceanographic features and discontinuous habitats (e.g., temperate rocky reefs) coupled with life history characteristics such as assortative mating, larval retention, homing behavior, migration and local adaptation can create barriers to gene flow. The temperate rocky reef systems of the Pacific Northwest include many of the environmental conditions that can lead to restricted gene flow and metapopulation structure. *Sebastes* spp. dominate these temperate rocky reefs and exhibit many life history characteristics that can further restrict gene flow including mate selection, internal

fertilization, release of free swimming live young, short distance homing, and long distance patterns of ontogenetic migration (Gunderson & Vetter 2006). Thus it is no surprise that *Sebastes* is the most speciose scorpaenid genus with approximately 110 species found throughout the world's cold temperate seas (Nelson 2006; Hyde & Vetter 2007). The evolutionary relationships between species have been a point of contention and confusion for decades (Kendall 2000), but most of the subgeneric and specific relationships are now well characterized on the basis of molecular phylogenetic approaches (Rocha-Olivares et al. 1999; Hyde & Vetter 2007). Additionally, a recent flurry of population genetic studies has shown a strong tendency for several rockfish species to exhibit a greater degree of intra-specific genetic structure and restricted gene flow than is typical of most *r*-selected marine species (e.g., Withler et al. 2001; Buonaccorsi et al. 2002, 2004, 2005; Cope 2004; Matala et al. 2004; Taylor 2004; J Hess, NMFS, pers comm).

In addition to present day barriers to dispersal, the north Pacific has had a very dynamic history during the evolution of *Sebastes* (see Hyde & Vetter 2007). Numerous periods of great fluctuations of major ocean currents (e.g., Herbert et al. 2001), sea level (Haq 1987; Miller et al. 2005), ocean temperature (Miller et al. 2005) and upwelling (Barron 1998) have occurred since the middle Miocene. These fluctuations bring with them the opportunity for habitat expansion, contraction, and the subsequent isolation of populations. This may be particularly important in the Southern California Bight (SCB), a transition zone between the San Diegan and Oregonian biogeographic provinces (Briggs 1974). Periods of past sea level drop have dramatically altered the ecology and oceanography of the SCB, greatly enhancing the amount of nearshore macro-algal and

rocky habitat (e.g., Graham et al. 2003). This drop in sea level also resulted in the effective separation of the SCB from the cool California Current flow due to the shoaling of the Channel Islands and offshore banks during periods of glacial maxima (see Figure 2-2, 100m depth contour is similar to the paleo-coastline at glacial maxima). It is easy to imagine how such scenarios of separation and subsequent behavioral and genetic drift may enhance the speciation process, especially in fishes such as *Sebastes* which show little dispersal as adults.

Phylogeography:

Phylogenetic analysis of the vermilion rockfish samples produced two distinct clades with high posterior support values, partitioned by both depth and location. The Type 2 clade was found from Neah Bay, Washington to Punta Baja, Baja California, Mexico. The Type 1 clade was found from Monterey, California to Colnett Bank, Baja California, Mexico and offshore to Guadalupe Island. In addition to the partial geographic separation, there was a strong bathymetric segregation with Type 2 fish dominating collections shallower than 100m (93.7%) and Type 1 fish dominating collections deeper than 100m (64.2%) (see Table 2-1 and Figure 2-2). Additionally, all of the collections of Type 1 fish from shallow sites were of young of the year or sub-adult fish (i.e., <250mm TL). This as well as the lack of juveniles and sub-adults observed on offshore banks (J Butler, NMFS, pers comm.) suggests that though juveniles of both clades settle in relatively shallow, nearshore habitats (J Hyde unpublished data), Type 1 fish must subsequently undertake an ontogenetic migration to greater depths and offshore banks whereas Type 2 fish remain resident to these shallow habitats.

Cryptic species:

Our results clearly show two distinct genetic units within the currently recognized vermilion rockfish, defined primarily by mitochondrial haplotype (i.e., Type 1, Type 2). These evolutionary units showed segregation both geographically and bathymetrically with a small degree of overlap. The question at hand is what to make of this finding? Are these differences merely the result of a high degree of population subdivision caused by restricted dispersal between nearshore and offshore reefs? Fortunately, the canary rockfish can serve as an appropriate metric for comparison, as it and the two clades of vermilion rockfish are of similar levels of divergence (Hyde & Vetter 2007). Analysis of microsatellite data from our canary and vermilion rockfish samples, using Structure, strongly supported the existence of three distinct genetic groups (Table 2-4). Further analyses of the microsatellite data showed similar levels of divergence between all members of this triumvirate at all loci when measured using the fixation index, F_{ST} (see Table 2-5). Using principal component analysis, these results were further corroborated by the finding of three distinct groupings in multivariate space (see Figure 2-3A). As suggested by the F_{ST} values, there were marked differences in allele frequencies at most loci, including a large number of private alleles (see Figure 2-4). These results are incongruent with the expectations of intra-specific population comparisons at the regional, or even sub-specific level.

Our finding of significant differences in both genetics and morphology suggest that the two types of vermilion rockfish should be afforded equal evolutionary status as that enjoyed by the canary rockfish. We therefore recommend that the Type 1 and Type

2 vermilion rockfish of Hyde & Vetter (2007) be recognized as separate species. The vermilion rockfish, *S. miniatus*, was originally described by Jordan and Gilbert (1880) from specimens collected off Monterey and Santa Barbara, California. A junior synonym, *S. eigenmanni*, was described by Cramer (in Jordan 1896) from a specimen collected off Monterey. A survey of our sample collections showed three (of 110 specimens examined) Type 1 fish north of Point Conception and only one (of 38 specimens examined) was found in the Santa Barbara collection. It is therefore assumed that the type specimens represent only the Type 2 species. The two forms can be distinguished by color with the Type 1 fish having an orange-red coloration in contrast to the more uniform red coloration typical of the Type 2 fish (see Figure 2-5).

Mitochondrial introgression:

Despite the existence of two distinct genetic groups, the reproductive barrier does not seem to have been 100% effective. Though all fish with Type 1 mtDNA haplotypes had genotypes that assigned with high likelihood to a single group (Genotype B), many (67.6%) of the fish with Type 2 haplotypes found in depths >100m also assigned to this group (see Figure 2-3B). Interestingly, this disagreement between haplotype and genotype was not seen in fish with Type 2 haplotypes collected from shallow (<100m) sites, however such occurrences should be expected with increased sampling. These misassigning fish were either derived from either hybrid ancestry or incomplete lineage sorting. Assignment tests using Structure showed little evidence for F1 or F2 hybrids among these fish, with the exception of one fish, of 548 sampled. Additionally, one of the Type 2 haplotypes (Hap18) was unique to these misassigned fish. Given the low

level of haplotypic divergence of Hap18 (1bp), the presence of a shared common haplotype (Hap 1), as well as the long divergence time between clades (~2.3 MYA), it seems unlikely that these misassigned fish represent incomplete lineage sorting. Rather, this suggests that a one-way mitochondrial introgression occurred from Type 2 into Type 1 fish. This finding is not unexpected as studies of other rockfish species have uncovered evidence that such hybridization events have occurred and continue to occur (e.g., Seeb 1998; Buonaccorsi et al. 2005; Gharrett et al. 2005).

The role of upwelling, sea level recession and bathymetry in the speciation process:

It is tempting to speculate as to the possible drivers behind the divergence of these three species. Using sequence data from seven mitochondrial and two nuclear genes, as well as a near complete sampling of species, Hyde & Vetter (2007) provided an estimate of the timing of divergence at ~2.7 million years ago (MYA) for the canary rockfish and ~2.3 MYA for the vermilion rockfish clades. With the final shoaling of the Isthmus of Panama ~3 MYA, modern ocean current patterns were largely established. Concurrent with this event was the beginning of a period of increased global cooling and more frequent glacial-interglacial cycles. The strengthening of the California Current as well as decreasing global temperature likely opened the door for colonization of the Southern California Bight (SCB) and may have created more favorable conditions in shallow water habitats. Barron (1998) showed a rapid onset of diatom accumulation, indicative of greatly enhanced upwelling, at ~2.7 MYA in the SCB. This coincides with the hypothesized time of divergence between vermilion and canary rockfish (Hyde & Vetter 2007). Perhaps a colonization event of the SCB by the ancestor of vermilion and canary

rockfish, followed by subsequent restriction of geneflow, led to a speciation event through peri- or parapatric mechanisms. This is not unreasonable as population genetic studies of several rockfish species have shown that Point Conception can act as a strong barrier to geneflow on an evolutionary time-scale (Buonaccorsi et al. 2002, 2004, 2005; Matala et al. 2004; Taylor 2004; J Hyde in prep). Similarly, analysis of catch data from three years of coast-wide pelagic juvenile rockfish surveys showed strong declines in abundance at Point Conception, Cape Blanco, and Cape Mendocino (Sakuma et al. 2006), suggesting that these features continue to act as barriers to gene flow on a contemporary time-scale.

Large-scale glaciation at high latitudes had profound effects upon global sea level (Haq 1987; Miller 2005), major ocean currents (e.g., Herbert et al. 2001), and the amount of nearshore macro-algal and rocky habitat (e.g., Graham et al. 2003; Kinlan et al. 2005). During periods of peak glaciation, sea level dropped 50 to 125m relative to current levels (Haq 1987; Graham et al. 2003; Miller 2005). As a result, the offshore banks in the SCB offered new areas of shallow rocky habitat and likely fostered extensive macro-algal communities (Graham et al. 2003; Kinlan et al. 2005). In addition to the new shallow habitat created by the shoaling of these offshore banks, Graham et al. (2003) and Kinlan et al. (2005) hypothesized that both the island and mainland coasts fostered greater rocky and macro-algal habitat than at present. The rapid proliferation of these highly productive habitats would likely have attracted numerous species of shallow dwelling rockfishes, including the ancestor of *S. miniatus*. The hypothesized time of divergence between Type 1 and Type 2 fish of ~2.3 MYA coincides with the beginning of this period

of increasing shallow reef productivity, inferred from the progressive decline in sea level observed between ~2.3 to ~1 MYA (Haq 1987; Miller et al. 2005).

Phylogenetic review of the genus (Hyde & Vetter 2007) showed that the biogeographic expansion of *Sebastes* spp. into the northeast Pacific and the evolution of the major ecological groups were accomplished by the early Pliocene (e.g., shallow structure schooling (*Sebastosomus*), deep structure schooling (*Sebastodes*), shallow solitary demersal (*Pteropodus*), deep solitary demersal (*Sebastomus*), deep soft sediment (*Eosebastes*), solitary lurker predator (*Sebastichthys*)). Hyde & Vetter (2007) suggested that the ancestor of *Sebastes* was likely a demersal species, strongly associated with deep high-relief reefs and therefore the evolution of both semi-pelagic and shallow dwelling species was novel. However, the recruitment of pelagic juveniles to shallower habitats, followed by an ontogenetic migration to depth is likely plesiomorphic, as this trait is common in many scorpaenid species. With this in mind, examination of recently evolved sister species suggests a remarkable pattern. In contrast to the typical patterns of vicariant formation of sister species (e.g., across the Isthmus of Panama (Knowlton 1993), the Florida peninsula (Avise 1992), the Baja peninsula (Bernardi et al. 2003)), recently evolved sister species of *Sebastes* tend to be sympatric in a geographic sense but segregated by preferred adult habitat depth. Though there obviously exist some closely related taxa separated primarily by latitude (e.g., *S. babcocki*/*S. rubrivinctus*, *S. miniatus* Type 1/*S. pinniger*, *S. simulator*/*S. helvomaculatus*), it is common for recent sister species to co-occur across a wide latitudinal range while being separated from each other by depths of only 10's of meters. Numerous examples of this exist throughout the genus (see Table 2-7). We suggest that for temperate rocky reef fishes, depth and the patchy

distribution of rocky habitats (Gunderson & Vetter 2006) can play a significant role in the speciation process, often greater than latitudinal thermal gradients or topographically constrained oceanographic features.

Genetic studies such as the present study and that of Hyde & Vetter (2007) have presented several hypothesized mechanisms by which speciation may proceed in these fishes (e.g., allopatry, sexual selection, niche partitioning). A common theme among *Sebastes* spp. is the recruitment of juvenile fish to shallow nearshore habitats followed by an ontogenetic migration to adult habitats, usually at greater depths (Love et al. 1991). Such migrations necessitate not only large-scale movement but also great physiological adaptation (Vetter et al. 1994, Vetter & Lynn 1997). Truncation of this cycle, particularly where habitat is discontinuous, such as offshore banks and islands, may result in the creation of reproductively isolated populations with different habitat affinities. For example, the ancestral depth habitat for adult vermilion and canary rockfish was likely deep (100-200m) rocky reef while the juvenile habitat was shallow (<30m) macro-algal dominated reef (Scenario A, Figure 2-6). A loss or truncation of the ontogenetic migration phase in some fish would lead to their retention in shallow juvenile habitats (Scenario B, Figure 2-6). Assuming subsequent survival to maturity, sufficient prey resources, and heritability of this character loss, the offspring of these fish would likely recruit to similar habitats and reproduce with others that share this habitat affinity, fostering the speciation process. Such a mechanism is simple in that it requires only a loss of a behavior rather than the complex evolution of novel characters (i.e., physiological adaptations to greater depth). This concept of speciation by life history truncation (i.e., paedomorphosis) is a well-documented phenomenon but we believe that

the loss of an ontogenetic migration as a speciation driver is a novel concept for benthic coastal fishes. It is becoming increasingly clear that in conjunction with a solid understanding of their phylogenetic relationships, *Sebastes* spp. offer us a unique opportunity to study speciation patterns in the marine realm, both modern and historic.

Management implications:

Vermilion rockfish have historically supported important commercial and recreational fisheries in California, Oregon, and Washington. During the 14-year period 1990-2004, the California commercial and recreational fisheries took 2,386 and 3,341 metric tons, respectively (MacCall 2005). For the period 2000-2006, among recreationally caught rockfishes, vermilion rockfish ranked #1 in the southern California fishery and #3 statewide (http://www.psmfc.org/recfin).

The presence of a cryptic species within this important fishery has considerable implications for management. In 2001, the 4300 mile^2 Cowcod Conservation Area was created in southern California to protect the severely overfished cowcod, closing all fishing in depths greater than 36m. Prompted by declining stocks of other rockfish species (i.e., pacific ocean perch (*S. alutus*), bocaccio (*S. paucispinis*), canary, darkblotched (*S. crameri*), widow (*S. entomelas*), and yelloweye (*S. ruberrimus*) rockfish), additional depth closures soon followed throughout California waters and have since varied temporally and regionally between 55-110m as maximum allowable fishing depths. These total closures have afforded an ancillary benefit to other rockfish species that co-occur with these overfished species, including the sunset and vermilion rockfish.

Our data show a strong segregation in abundance of both species in association with depth. Throughout their ranges, *S. miniatus* Type 2 were most abundant in depths shallower than 100m while *S. miniatus* Type 1 occurred primarily south of Point Conception and were most abundant in depths between 100-200m. Clearly the difference in depth preference between these two species complicates current management strategies. For example, with the closure of the rockfish fishery in depths greater than 55-110m, additional effort was placed upon shallow, nearshore populations of rockfishes. When considering vermilion rockfish as a single species this seems acceptable as fish are abundant within both the fished and unfished areas. However, when considered as two species, separated by depth, it becomes clear that Type 1 fish are afforded a disproportionate amount of protection at the expense of Type 2 fish, which now receive a disproportionately large amount of the fishing effort. This increased effort results in a severe decline in the means of both age and size frequency (e.g., Love et al. 1998) with the remaining populations of large vermilion rockfish restricted to small marine reserves (e.g., Parnell et al. 2005). This loss of older and larger fish can be especially detrimental to the future reproductive quality and capacity of the population (e.g., Berkeley et al. 2004).

Our finding of a cryptic species of vermilion rockfish within southern California suggests a significant need for revised management strategies for these species. As the #1 recreationally caught rockfish in southern California and #3 statewide, there exists a great potential for fishery decline if improperly managed. This is especially relevant during the current move to establish marine reserves as a solution to fishery management. Without proper scientific study and knowledge of the component species managed by

these reserves, it is unlikely that their promise of fishery management and enhancement will be realized, a fact that is becoming increasingly clear as genetic studies continue to uncover instances of cryptic speciation (i.e., Rocha-Olivares et al. 1999; Kai et al. 2002a, 2002b; Gharrett et al. 2005; M Burford pers comm.) and strong population subdivision within these magnificent fishes (e.g., Rocha-Olivares & Vetter 1999; Withler et al. 2001; Buonaccorsi et al. 2002, 2004, 2005).

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Table 2-1
Collection data for samples used in this study. Samples of vermilion rockfish are binned in depths < and > 100m. Percentage of Type 1 and Type 2 haplotypes (see Figure 2-1) present in each sample are noted. Detailed collection data are available from the authors.

vermilion rockfish						Latitude		Longitude	
	Location	n	collection period	% Type 1	% Type 2	deg	min	deg	min
	Halfmoon Bay	69	2001-2005	0	100	37	28	122	26
S. miniatus	Piedras Blancas	41	1994-2003	0	100	35	39	121	16
	San Miguel Island	31	1993-2001	0	100	34	5	120	32
Collections <100m	Santa Barbara	38	2001-2005	2.6	97.4	34	24	119	51
	San Diego	89	1999-2004	16.9	98.1	32	42	117	16
	San Quintin, Baja	59	2000-2006	6.8	93.2	30	30	116	8
	Total =	327							
vermilion rockfish						Latitude		Longitude	
	Location	n	collection period	% Type 1	% Type 2	deg	min	deg	min
	Monterey	10	1998	30	70	36	54.5	122	11
S. miniatus	Tanner Bank	82	1994-2004	65.9	34.1	32	42	119	4
	9-mile Bank	2	2002	50	50	32	37	117	18
Collections >100m	60-mile Bank	10	1994	80	20	32	6	118	14
	Colnett Bank, Baja	116	2000-2006	64.7	35.3	30	53	116	30
	Guadalupe Island	1	1996	100	0	29	10	118	16
	Total =	221							
canary rockfish						Latitude		Longitude	
	Location	n	collection period	% Type 1	% Type 2	deg	min	deg	min
S. pinniger	Fort Bragg -					39	28	123	50
	San Miguel Island	133	1994-2003	NA	NA	34	5	120	32
	Total =	133							

Table 2-2
Molecular diversity values for individual microsatellite loci. Sra Loci are from Westerman et al. (2005) and Spi loci are from Gomez-Uchida et al. (2003). Groups correspond to those produced through Structure (Pritchard et al. 2000) analysis. Microsatellite repeat motif, number of alleles (A), expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), and associated p-value (*p*) are presented.

	Locus	repeat motif	A	H_E	H_O	F_{IS}	p
	Sra. 7-2.2	di	20	116.59	114	0.0223	0.1183
	Sra. 7-7.3	di	26	137.46	139	-0.0113	0.2824
	Sra. 7-25.4	di	24	143.47	139	0.0313	0.6097
S. miniatus Type 1	Sra. 15-23.7	tetra	5	19.93	21	-0.0541	1
	Sra. 16-5.8	tetra	11	124.67	120	0.0375	0.8451
n=155 fish	Sra. 15-8.9	tetra	17	133.64	132	0.0123	0.5584
	Spi. 4	tetra	13	128.94	128	0.0073	0.0604
	Spi. 6	tetra	22	138.92	118	0.151	0
	Spi 10	tetra	8	102.6	109	-0.0626	0.92

	Locus	repeat motif	A	H_E	H_O	F_{IS}	p
	Sra. 7-2.2	di	28	292.97	272	0.0717	0.2516
	Sra. 7-7.3	di	25	287.45	271	0.0573	0.6959
	Sra. 7-25.4	di	25	213.59	214	-0.0019	0.9073
S. miniatus Type 2	Sra. 15-23.7	tetra	9	247.96	233	0.0604	0.4401
	Sra. 16-5.8	tetra	23	273.57	261	0.046	0.0373
n=321 fish	Sra. 15-8.9	tetra	14	275.3	256	0.0702	0.2288
	Spi. 4	tetra	20	151.08	141	0.0669	0.0021
	Spi. 6	tetra	15	144.004	89	0.3829	0
	Spi 10	tetra	10	224.13	209	0.0676	0.9059

	Locus	repeat motif	A	H_E	H_O	F_{IS}	p
	Sra. 7-2.2	di	79	131.06	125	0.0464	0.0014
	Sra. 7-7.3	di	16	110.75	111	-0.0023	0.4395
	Sra. 7-25.4	di	18	103.22	96	0.0702	0.4111
S. pinniger	Sra. 15-23.7	tetra	2	9.66	10	-0.0356	1
	Sra. 16-5.8	tetra	30	118.35	120	-0.014	0.2874
n=133 fish	Sra. 15-8.9	tetra	13	111.7	102	0.0872	0.0249
	Spi. 4	tetra	20	108.71	108	0.0066	0.5072
	Spi. 6	tetra	45	127.41	127	0.0032	0.4318
	Spi 10	tetra	13	111.84	109	0.0255	0.8826

Table 2-3
Probability of Hardy-Weinberg equilibrium by sampling site (A.), species (B.), mitochondrial clade (C.), and Structure determined groups (D.).

A.

Locus	Half Moon Bay	Piedras Blancas	San Miguel I	Santa Barbara	San Diego	San Quintin	Tanner Bank	Colnett Bank
Sra7-2	**	*	**	ns	ns	ns	*	**
Sra7-7	ns	ns	ns	ns	ns	ns	ns	*
Sra7-25	ns	ns	ns	ns	**	ns	*	**
Sra15-23	ns	ns	ns	ns	**	ns	***	***
Sra16-5	ns	ns	ns	ns	**	ns	ns	ns
Sra15-8	ns	ns	*	ns	ns	ns	ns	ns
Spi4	NA	*	NA	NA	***	*	ns	***
Spi6	NA	**	NA	NA	***	***	***	***
Spi10	ns	ns	ns	**	ns	ns	ns	ns

B.

Locus	vermilion	canary
Sra7-2	***	**
Sra7-7	***	ns
Sra7-25	***	ns
Sra15-23	***	ns
Sra16-5	**	ns
Sra15-8	***	*
Spi4	***	ns
Spi6	***	ns
Spi10	**	ns

C.

Locus	mtDNA Type 1	mtDNA Type 2
Sra7-2	ns	***
Sra7-7	ns	*
Sra7-25	ns	***
Sra15-23	ns	***
Sra16-5	ns	ns
Sra15-8	ns	*
Spi4	ns	***
Spi6	***	***
Spi10	ns	ns

D.

Locus	Genotype B	Genotype A
Sra7-2	ns	ns
Sra7-7	ns	ns
Sra7-25	ns	ns
Sra15-23	ns	ns
Sra16-5	ns	*
Sra15-8	ns	ns
Spi4	ns	**
Spi6	***	***
Spi10	ns	ns

NA = no data available for this comparison
ns > 0.05
* <0.05
** < 0.01
*** < 0.001

Table 2-4
Likelihood output from Structure v2.1. Values of k represent the putative number of source populations contained within the sample of genotypes. The most likely value of k is the value after which the likelihood surface plateaus.

k	-ln likelihood
1	-23934.1
2	-21232.4
3	-19540
4	-19472.1
5	-19453.9

Table 2-5
Pairwise comparisons of F_{ST} and associated *p*-values between sampling depths (A.), mitochondrial clade groups (B.), and groups determined by Structure analysis (C.). Shallow refers to samples collected in depths <100m while deep refers to samples collected in depths >100m.

A.

Locus	shallow vs. deep		shallow vs. canary		deep vs. canary	
Sra7-2	0.0906	***	0.0435	***	0.1156	***
Sra7-7	0.0523	***	0.0836	***	0.1033	***
Sra7-25	0.1415	***	0.2517	***	0.1234	***
Sra15-23	0.3594	***	0.4856	***	0.8324	***
Sra16-5	0.0567	***	0.0138	***	0.0669	***
Sra15-8	0.0318	***	0.0724	***	0.075	***
Spi4	0.0647	***	0.0533	***	0.062	***
Spi6	0.0319	***	0.0314	***	0.0335	***
Spi10	0.0096	***	0.0551	***	0.0982	***
Overall	0.0942	***	0.1302	***	0.1734	***

B.

Locus	Type 1 vs. Type 2		Type 1 vs. canary		Type 2 vs. canary	
Sra7-2	0.0905	***	0.1245	***	0.0282	***
Sra7-7	0.0521	***	0.0943	***	0.0544	***
Sra7-25	0.1255	***	0.0878	***	0.1707	***
Sra15-23	0.2008	***	0.4934	***	0.2908	***
Sra16-5	0.0478	***	0.0457	***	0.0023	***
Sra15-8	0.0479	***	0.0468	***	0.0617	***
Spi4	0.0503	***	0.0484	***	0.0303	***
Spi6	0.0201	***	0.0373	***	0.0200	***
Spi10	0.0118	***	0.0483	***	0.0455	***
Overall	0.0730	***	0.1162	***	0.0818	***

C.

Locus	Genotype B vs. Genotype A		Genotype B vs. canary		Genotype A vs. canary	
Sra7-2	0.1174	***	0.1209	***	0.0302	***
Sra7-7	0.0699	***	0.0932	***	0.0576	***
Sra7-25	0.1742	***	0.0877	***	0.2125	***
Sra15-23	0.2588	***	0.4933	***	0.3022	***
Sra16-5	0.0618	***	0.0458	***	0.0044	***
Sra15-8	0.0665	***	0.0477	***	0.0736	***
Spi4	0.0887	***	0.0483	***	0.0492	***
Spi6	0.0396	***	0.0365	***	0.0254	***
Spi10	0.0140	***	0.0495	***	0.0430	***
Overall	0.1013	***	0.1157	***	0.0920	***

p>0.05 ns
p<0.05 *
p<0.01 **
p<0.001 ***

Table 2-6
Morphometric comparisons between types/species of vermilion rockfish, means and standard deviations (SD) presented. Significance was tested with a two-tailed t-test. Individual measures are expressed in relation to standard length (SL) of individual fish.

species		Orbit length/SL		Interorbital width/SL		Caudal peduncle depth/SL		Head length/SL		Lower jaw length/SL		Snout to Dorsal fin/SL	
	n	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
S. miniatus Type 2	30	0.0822	0.0052	0.0626	0.0027	0.0906	0.0054	0.2936	0.0065	0.1593	0.0055	0.2739	0.0095
S. miniatus Type 1	62	0.0794	0.0037	0.0708	0.0050	0.0831	0.0068	0.2936	0.0098	0.1572	0.0050	0.2638	0.0358
		p=0.0044		**p<0.0001**		**p<0.0001**		p=0.9662		p=0.0824		p=0.1295	

Table 2-7
Depth and common occurrence of recent species pairs. Hypothesized divergence times from Hyde and Vetter (2007). "*" indicates species pairs that exhibit incomplete mtDNA lineage sorting

species	divergence time	common depth	common latitude	reference
S. chlorostictus		60-240	39-28	Love et al. 2002
S. rosenblatti	0.21 MYA	100-490	37-28	J. Butler pers. comm.
S. ciliatus		10-153	59-55	Orr and Blackburn 2004
S. variabilis	* 0.40 MYA	100-300	59-55	
S. reedi		180-275	58-45	Love et al. 2002
S. crameri	0.51 MYA	140-210	51-36	
S. carnatus		12-35	38-30	Love et al. 2002
S. chrysomelas	* 0.58 MYA	1-18	38-34	
S. lentiginosus		75	33-29	Love et al. 2002
S. umbrosus	0.63 MYA	45-60	34-27	
S. variegatus		100-300	59-48	Love et al. 2002
S. wilsoni	* 0.66 MYA	60-150	59-32	
S. flammeus		200-500	42-34	Nakabo 2002
S. iracundus	0.70 MYA	450-1000	44-33	
S. exsul		110-150	29	Love et al. 2002
S. spinorbis	0.77MYA	150-200	29	J. Hyde pers. obs.
S. aleutianus		<250m	59-54	Hawkins et al. 2005
S. melanostictus	1.32 MYA	>250m	59-54	
S. melanops		1-55	59-37	Love et al. 2002
S. flavidus	1.34 MYA	90-180	59-35	
S. miniatus Type 1		100-200	34-30	Love et al. 2002
S. miniatus Type 2	2.3 MYA	30-100	42-30	this study
S. rubrivinctus		60-200	36-30	Love et al. 2002
S. serriceps	2.66 MYA	1-60	35-30	

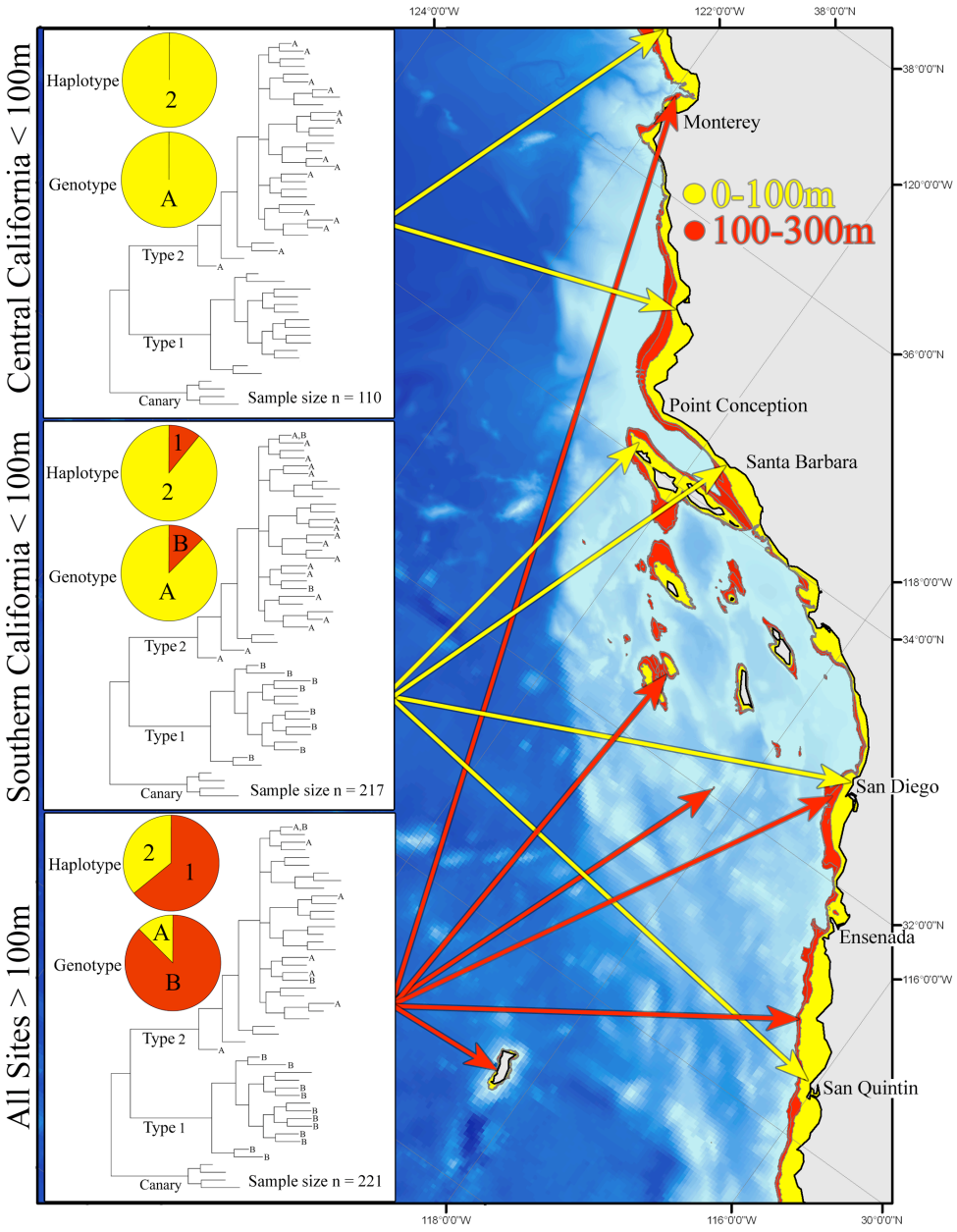

Figure 2-1
Map showing sampling locations for vermilion rockfish used in this study. Bathymetry is color coded with yellow (0-100m) and red (100-300m). Arrows indicate sampling sites and are color coded by collection depth to match the colored depth contours. Haplotypic (i.e., Type 1, Type 2) and genotypic assignment (i.e., Group A, Group B) for each sample group are presented as pie charts. The consensus Bayesian posterior tree of all haplotypes is presented for each sample group, haplotypes present in each group are indicated with an A or B, corresponding to genotypic assignment of individuals at that haplotype.

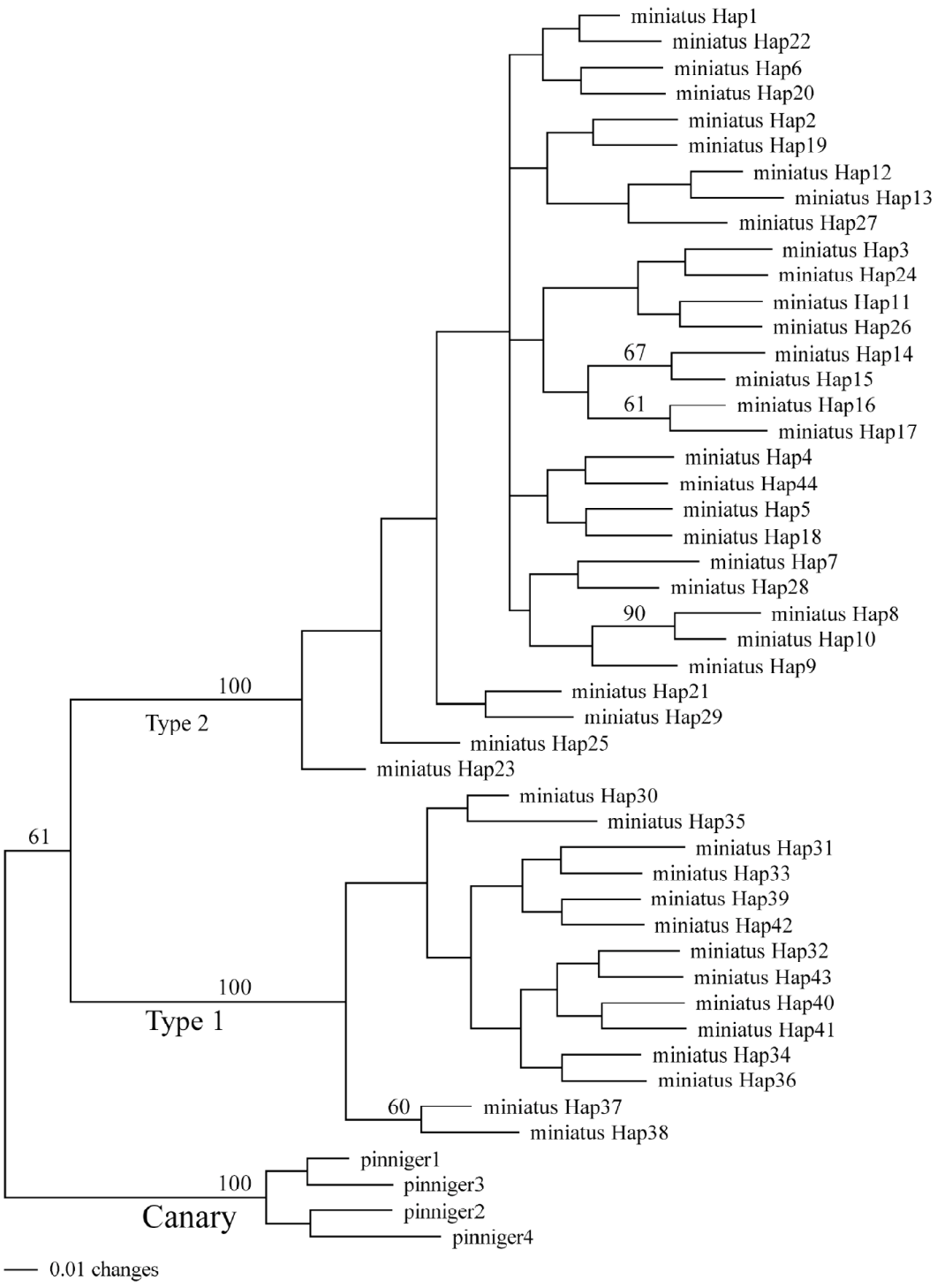

Figure 2-2
Sub-tree of Bayesian derived phylogenetic tree based upon cytochrome *b* sequence data from all northeast Pacific *Sebastes* spp. Branch lengths represent genetic distance and measures of posterior support >50 are presented.

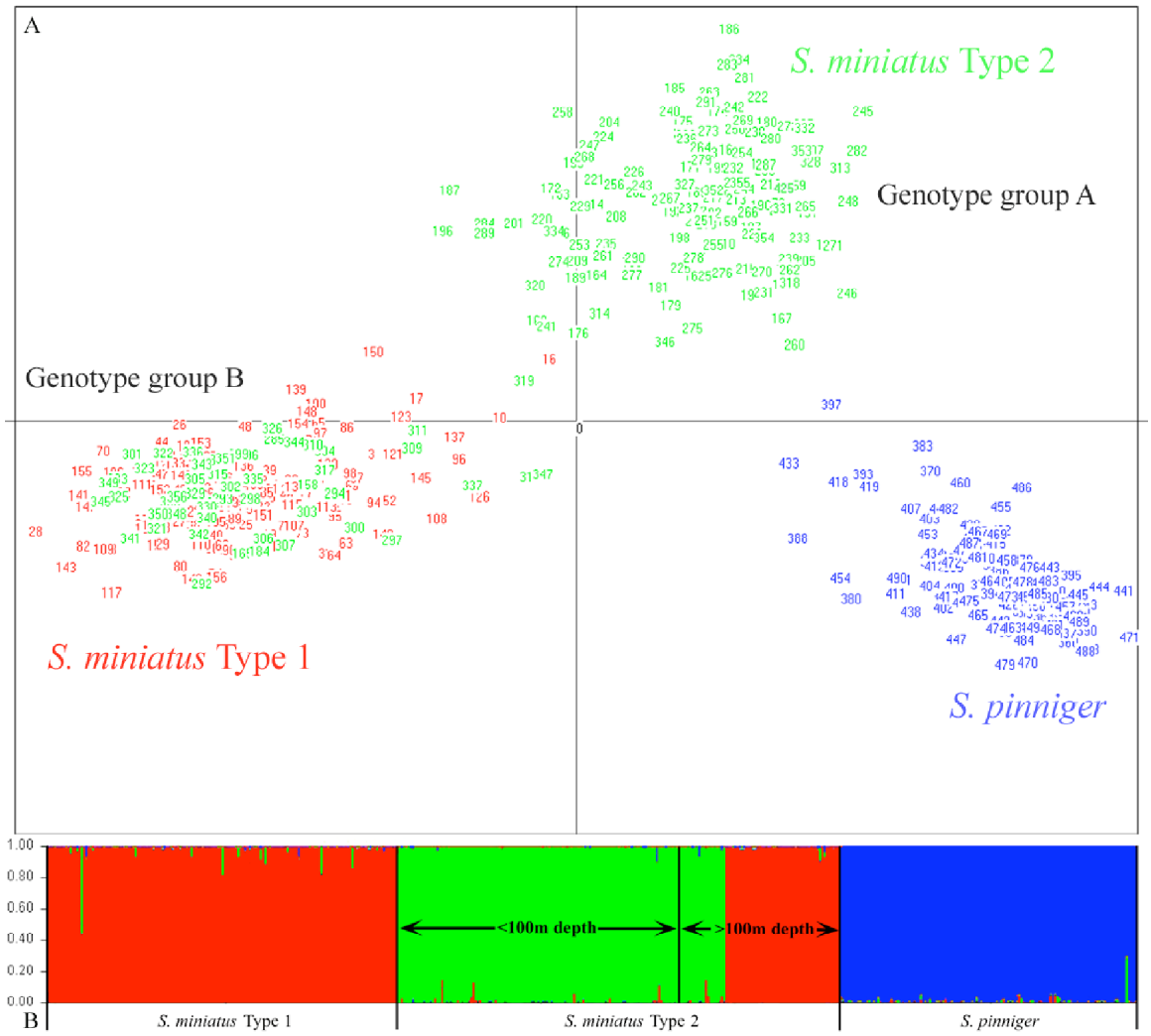

Figure 2-3

A. Primary component axis of principal component analysis of individual genotypes from this study. Samples are color-coded based upon mitochondrial clade membership (Green = *S. miniatus* Type 2, Red = *S. miniatus* Type 1, Blue = *S. pinniger*).

B. Output of population assignment from Structure analysis considering the most likely number of populations (k=3). Assignment probability of individual fish are represented by individual vertical bars on a scale of 0-1. Samples are grouped by mitochondrial clade and Type 2 samples are separated into two depth bins.

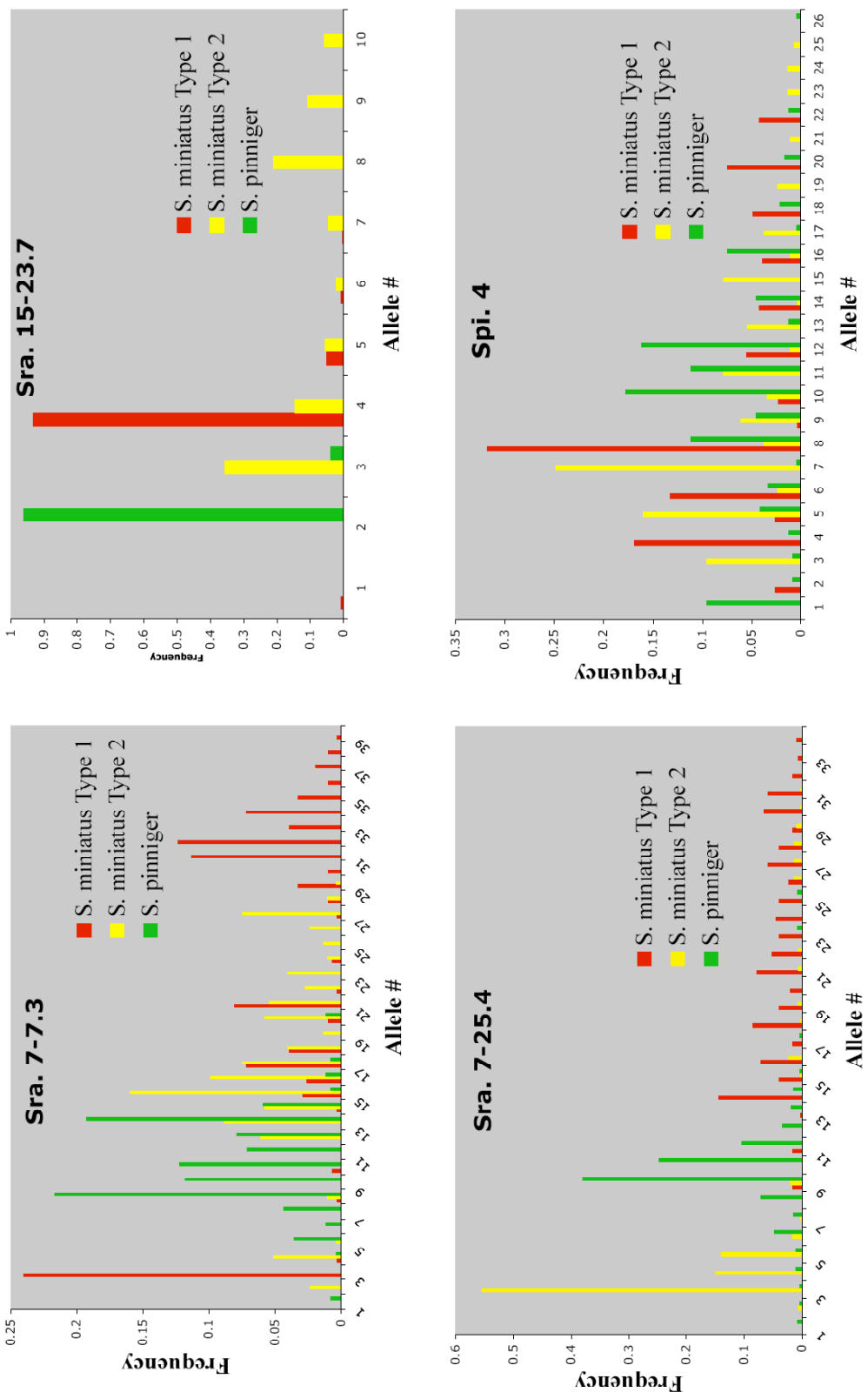

Figure 2-4
Frequency histograms for selected microsatellite loci.

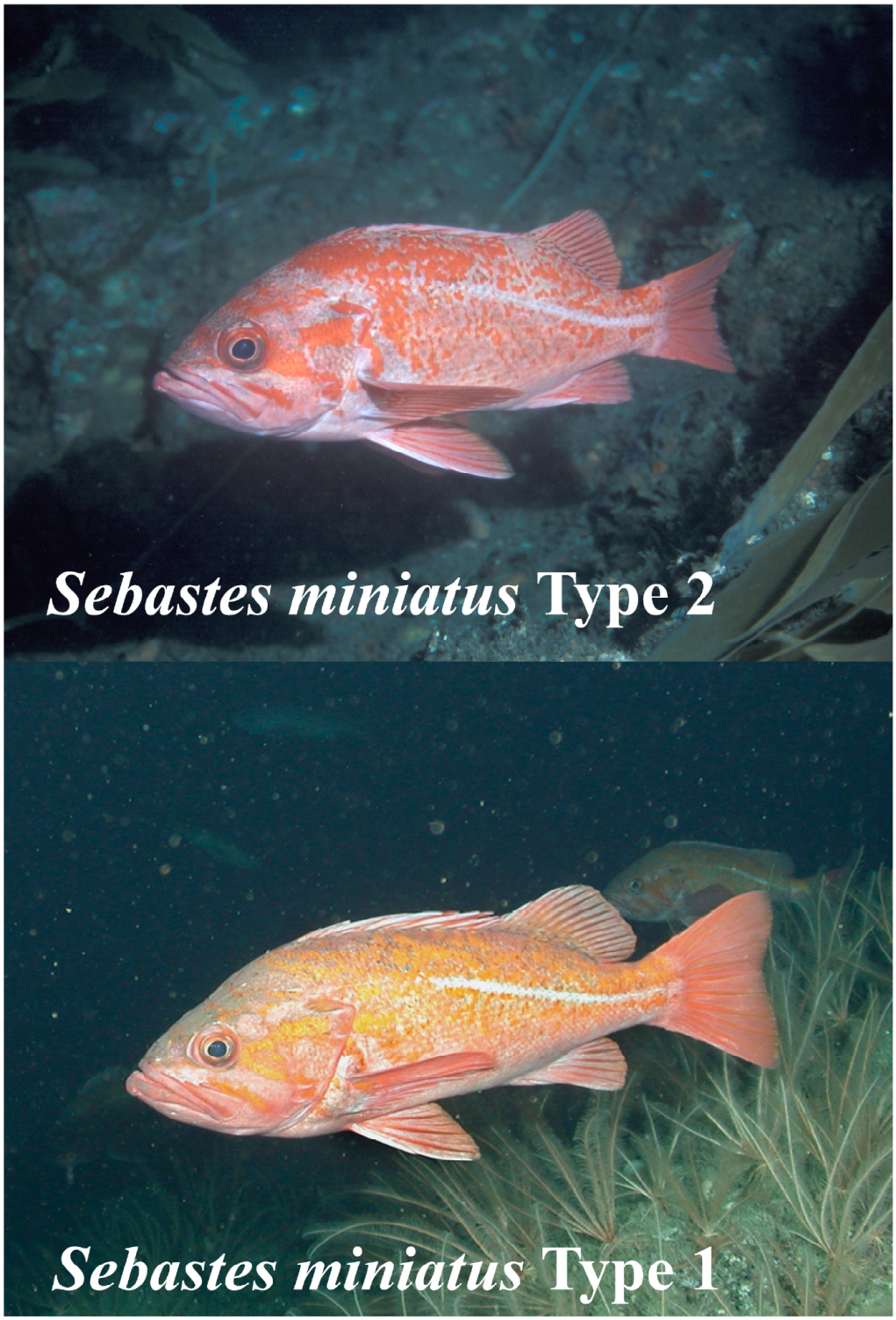

Figure 2-5
Underwater photographs of *Sebastes miniatus* Type 1 (depth ~30m) and *S. miniatus* Type 2 (depth ~150m).

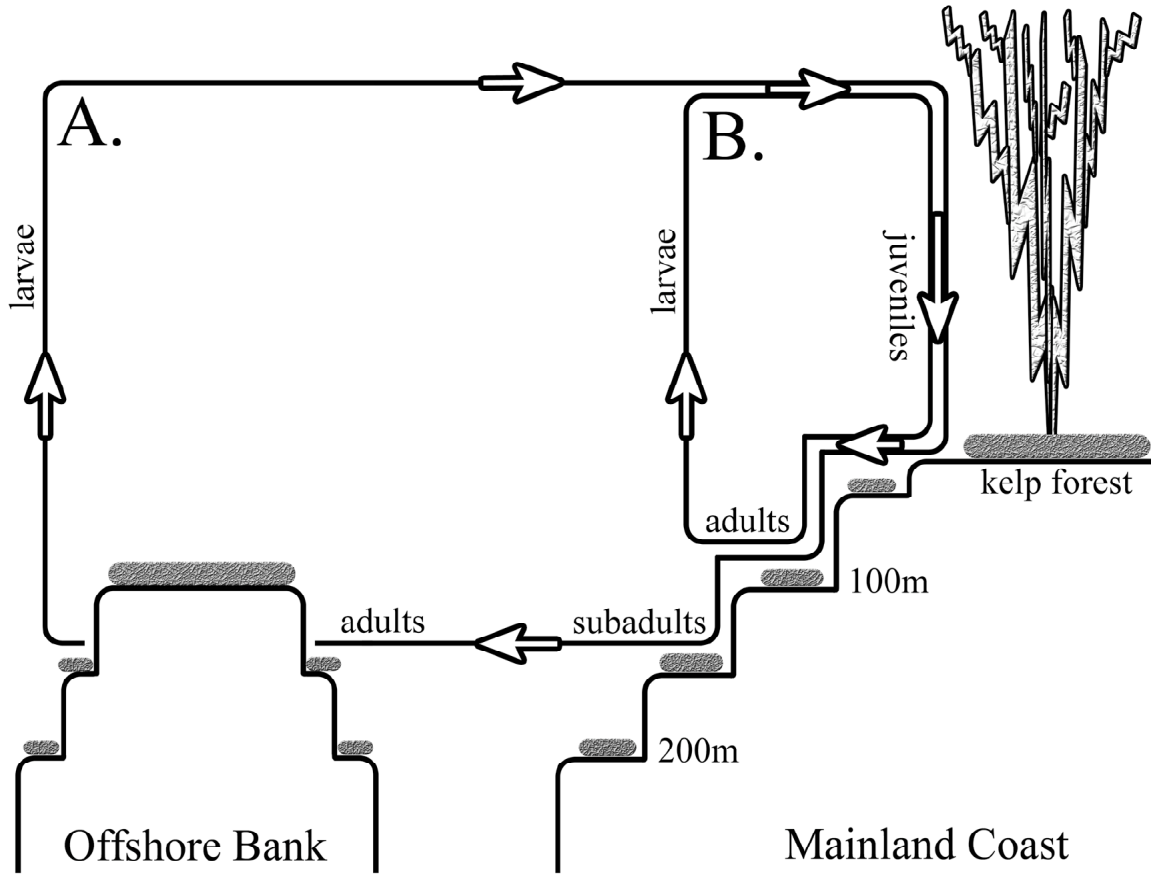

Figure 2-6
Hypothesized pattern of larval release, juvenile settlement, and ontogenetic migration to adult habitat for *S. miniatus* Type 1 (A.) and *S. miniatus* Type 2 (B.). Truncation or loss of the ontogenetic migration present in scenario A. can result in the retention and establishment of reproductive populations near juvenile habitat (scenario B).

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III.

Population structure and limited larval dispersal in the vermilion rockfish (*Sebastes miniatus*) as revealed by mtDNA analysis

Abstract of Chapter III:

Recent studies strongly support the splitting of vermilion rockfish into two species separated primarily by depth of adult occurrence. Evaluation of population connectivity is necessary as current depth-based management policies have placed increased fishing effort upon the already heavily exploited vermilion rockfish. Analysis of gene flow between populations and calculations of larval dispersal values, were accomplished using 782bp of DNA sequence data from the mitochondrial cytochrome *b* gene of 365 vermilion rockfish sampled from Cape Blanco, Oregon to San Quintin, Mexico. A hierarchical Analysis of Molecular Variance (AMOVA) showed significant partitioning of genetic variance across Point Conception (Φ_{CT}=0.09690, p=0.02861), Cape Blanco (Φ_{CT}=0.09724, p=0.00347), and Cape Mendocino (Φ_{CT}=0.09231, p=0.00463). Isolation by distance analysis produced a strong and significant correlation (Φ_{ST}=0.11/1000km, Mantel r=0.77621, p=0.00074). Calculated values of average larval dispersal distance were < 25km. The finding of both strong population structure and limited larval dispersal in the number one caught rockfish in the Southern California fishery and third statewide, has profound implications for current management strategies and the future design of marine reserve networks.

Introduction:

Recent phylogenetic investigations (Hyde & Vetter 2007; Chapter II) have provided evidence for a second species, within the previously described vermilion rockfish, *S. miniatus* (Hyde & Vetter 2007). The two species are separated primarily by depth, with *S. miniatus* Type 2 found shallower (typically <100m) than *S. miniatus* Type 1 (typically >100m). This segregation by depth, coupled with the current practice of management by depth closure, has produced a dichotomy in their exploitation. Due to severe overexploitation of several rockfish species (i.e., Pacific Ocean perch (*S. alutus*), bocaccio (*S. paucispinis*), canary (*S. pinniger*), cowcod (*S. levis*), darkblotched (*S. crameri*), widow (*S. entomelas*), and yelloweye (*S. ruberrimus*) rockfish) a series of depth restricted management areas were created. These closures began in 2001 with the establishment of the 4300 mile^2 Cowcod Conservation Area, soon followed in 2002 with the creation of the California Rockfish Conservation Area, together restricting much of the fishery, both temporally and regionally, with restrictions varying between 36-110m as the maximum allowable fishing depth. These closures have resulted in the near-total protection of *S. miniatus* Type 1 populations while placing increased fishing effort upon the already heavily exploited nearshore *S. miniatus* Type 2 populations.

Together, the two species of vermilion rockfish represent a significant portion of the current and historical commercial and recreational fishery along much of the west coast of North America. During the period 2002-2006, vermilion rockfish ranked number one in the Southern California recreational fishery and third statewide

(http://www.psmfc.org/recfin). The first stock assessment for vermilion rockfish was done in 2005 and it was found that despite the species being overfished in the 1990's, abundance estimates are currently above the precautionary management threshold (MacCall 2005). However, catch values and biomass estimates used in the 2005 stock assessment were based upon the assumption of a single species, potentially a critical management error. Though not currently overfished, at least when assessed as a species complex, vermilion rockfish populations have shown severe declines in both size and age frequencies since the early 1980's with a near complete loss of larger size classes (Love et al. 1998). Such a loss of older and larger fish can be particularly detrimental to the future reproductive quality and capacity of populations (e.g., Berkeley et al. 2004).

Tagging (Miller & Geibel 1973; Lea et al. 1999) and acoustic tracking (Mitamura et al. 2002, 2005; Starr et al. 2002) studies of several *Sebastes* species, including vermilion rockfish, have shown remarkable site fidelity and homing behavior in these fishes, suggesting that the majority of geographic dispersal is accomplished during larval and pelagic juvenile stages. *Sebastes* spp. exhibit a simplified form of viviparity: fertilization and gestation are internal and poorly developed larvae are released alive. Though live-bearing is found in other fishes (e.g., Embiotocidae, Poeciliidae and many elasmobranchs), *Sebastes* spp. are unique in their high fecundity, which ranges from thousands to 2.7 million larvae per brood (Wourms 1991; Love et al. 2002). Larvae and subsequent pelagic juveniles may spend from a month to a year in the pelagic realm before recruiting to benthic habitat (Boehlert 1977; Love et al. 2002). Such extended pelagic dispersal phases offer the opportunity for large-scale geographic transport, especially in quasi-permanent oceanographic features such as those found within the

California Current system (e.g., Parrish et al. 1981). Organisms with both high fecundity and lengthy periods of larval dispersal are expected to show a high-degree of gene flow with little or no genetic differentiation between populations. Despite such traits in *Sebastes* spp., there seems to be overwhelming evidence for genetic heterogeneity among populations of most species studied to date (e.g., Withler et al. 2001; Buonaccorsi et al. 2002, 2004, 2005; Matala et al. 2004; Taylor 2004; Gomez-Uchida & Banks 2005).

The understanding of population connectivity is key to ensuring the persistence of species throughout their historic ranges. This topic is especially relevant, as the establishment of no-take marine reserves is being promoted as a solution to fishery management (e.g., Polachek 1990; McArdle 1998; Murray et al. 1999). However, great care must be taken that reserve networks and management regions are designed to properly preserve population connectivity while also providing an exploitable fishery resource. In this paper we use DNA sequence data from the mitochondrial cytochrome *b* gene to examine population connectivity between vermilion rockfish populations throughout their common range, test for barriers to gene flow, and provide estimates of average larval dispersal.

Materials and Methods:

Sample collection:

Fish were collected throughout common range of the species using various techniques (i.e., hook and line, bottom trawl, pole spear) and identified to species using Love et al. (2002). Sampling locations were chosen to represent regions of the west coast

that correspond to either current management zones (i.e., Northern, Central, Southern, http://www.dfg.ca.gov/mrd) or areas between putative phylogeographic breaks recognized for other species in this region (e.g., Buonaccorsi et al. 2002, 2004, 2005; Cope 2004; Matala et al. 2004; J. Hess, NMFS pers. comm.) (see Figure 3-1). Tissues, either white muscle or pectoral fin clips, were preserved in 95% un-denatured ethanol pending DNA extraction and genetic analyses.

DNA extraction:

DNA was extracted from preserved tissue using various protocols. The majority of samples had DNA extracted using a standard proteinase K digestion followed by a lithium chloride:chloroform nucleic acid purification and subsequent ethanol precipitation (Gemmel & Akiyama 1996). DNA from the remaining samples was extracted using either the DNeasy kit (Qiagen) following the manufacturer's protocol or by use of a Chelex (BioRad Laboratories) boiling technique (see Hyde et al. 2005).

PCR amplification:

DNA was amplified for sequencing from the mitochondrial cytochrome *b* gene using primers GluRF2 5' AAC CAT CGT TGT TAT TCA ACT ACA AGA ACC and CB3RF2 5' CGA ACA GGA ART ATC AYT CTG G in a 10µL reaction volume containing (67mM Tris-HCl pH 8.8, 16.6mM $(NH_4)_2SO_4$, 10mM ß-mercapto-ethanol, 2mM $MgCl_2$, 800µM dNTPs, 0.4µM each primer, 0.5 units *Taq* DNA polymerase (New England Biolabs), and 50-100ng of DNA template) and amplified using the following temperature profile in a PTC200 DNA Engine (MJ Research); 94ºC (2:00), 35 cycles of

[94ºC(0:30), 59ºC(1:00), 72ºC(1:00)], followed by three minutes at 72ºC. All PCR batches contained at least one no template negative control to monitor for possible DNA contamination. Products were electrophoresed through a 2% (w/v) agarose gel in 1 X Tris-Borate-EDTA buffer, stained with ethidium bromide and visualized via an UV-transilluminator. Reactions were digested using ExoSAP-IT (USB Corp.) to remove unincorporated primers and deoxynucleotides prior to cycle sequencing. Products had both strands individually cycle sequenced using BigDye v.3.1 Dye Terminators (Applied Biosystems) and analyzed on an ABI 3130XL automated capillary sequencer (Applied Biosystems). DNA sequences from both strands were aligned and edited using Sequencher v4.5 (GeneCodes, Inc).

Genetic Analyses:

Measures of molecular diversity:

Standard indices of molecular diversity, including haplotype (h) and nucleotide diversity (π) were calculated for individual sample sites and the dataset as a whole using Arlequin v3.1 (Excoffier et al. 2005). To test for neutrality (equilibrium) among individual samples, Tajima's D was calculated using Arlequin and significance tested using 10,000 bootstrap replicates. Demographic history can be estimated using the D statistic, with negative values signaling an excess of low frequency haplotypes, possibly due to recent demographic expansion.

Population structure and gene flow:

In order to assess the geographic partitioning of genetic variance, a hierarchical analysis of molecular variance (AMOVA) was performed using Arlequin. To investigate whether previously proposed phylogeographic breaks may act to restrict gene flow, samples were evaluated using an adjacent sample pooling analysis (sensu Buonaccorsi et al. 2004, 2005). If such a barrier to gene flow exists, it would be expected that populations on either side would be relatively homogeneous while populations across the barrier would be distinct. By this method, adjacent samples were pooled to create two to five groups, in all possible combinations. The best grouping of populations would show the greatest and most significant levels of among-group heterogeneity (Φ_{CT}) while at the same time showing the smallest and least significant levels of within-group heterogeneity (Φ_{SC}).

Pairwise comparisons of Φ_{ST}, between all samples (Table 3-2), were compiled using Arlequin to assess the level of gene flow between populations. These values were linearly transformed (Φ_{ST} /(1- Φ_{ST})) (Slatkin 1993) and plotted against geographic distance data (Figure 3-2) obtained using the path measurement tool in Google Earth (http://earth.google.com). A Mantel test (100 000 permutations) was performed to test the correlation between genetic and geographic distance and the slope of the resultant regression was used to calculate values for average larval dispersal distance. Buonaccorsi et al. (2004, 2005) suggested that the slope of this relationship, combined with estimates of adult density, assuming linear habitat, is amenable to the calculation of average larval dispersal distance for nearshore species of *Sebastes*. Roussett (1997) suggested that habitat can be treated as linear when the habitat width is much less than

the distance over which it is compared. Though vermilion rockfish are found in low abundance on offshore banks (Chapter II) and around islands in the Southern California Bight, their distribution along much of the coast is primarily within a narrow band of moderately shallow (10-100m), nearshore rocky habitat (Love et al. 2002; Chapter II), conforming well to Rousset's requirement. By comparing the inverse of the slope of the genetic and geographic regression to the product $D(\sigma^2)$, while inserting reasonable values for adult density (D), the standard deviation or average dispersal distance (σ) can be calculated. Using this relationship we calculated values of average larval dispersal, for a wide range of reasonable adult densities, assuming symmetrical, exponential dispersal along a linear habitat (Table 3-3).

Statistical Parsimony and Nested Clade Phylogeographic Analysis:

The computer program TCS v1.21 (Clement et al. 2000) was used to generate a statistical parsimony network among all vermilion haplotypes, rooted with a single canary rockfish haplotype, and using the default 95% parsimony connection limit (Figure 3-3). The haplotype network was then partitioned into sequentially nested groupings of 1-step clades following Templeton et al. (1995). Nested clade analysis was performed using the computer program GeoDis v2.5 (Templeton et al. 1995) in conjunction with the geographic distance matrix used in the isolation by distance analysis. The results of this analysis were interpreted using the dichotomous key provided on the GeoDis website (http://darwin.uvigo.es/).

Results:

Haplotype and nucleotide diversity:

In total, 782bp of DNA sequence were analyzed from 365 individuals from nine geographic locations throughout the species common range (Table 3-1). We found 27 unique haplotypes among the nine sites. Haplotype sequences have been deposited in GenBank, accession numbers EF587183-EF587231. The most common haplotype was found in high abundance in all populations and dominated the samples south of Point Conception (see Figure 3-1). A general trend of decreasing haplotype diversity with decreasing latitude was observed. Values for Tajima's D were significant only at the Southern California sample sites, suggesting recent demographic expansion in this region.

Population subdivision as revealed by mtDNA sequence:

AMOVA revealed significant partitioning of genetic variance across multiple geographic features within the study area (see Table 3-4). The strongest population subdivision occurred between groups of populations across Point Conception (2 groups, Φ_{CT}=0.09690, p=0.02861). When additional subdivisions were considered, significant restrictions to gene flow were found across Cape Blanco (3 groups, Φ_{CT}=0.09724, p=0.00347), Cape Mendocino (4 groups, Φ_{CT}=0.09231, p=0.00463), and Monterey Bay (5 groups, Φ_{CT}=0.08710, p=0.01468).

Pairwise Φ_{ST} values were highly significant between most population pairs across Point Conception, even after Bonferroni correction for multiple comparisons (see Table

3-2). The one non-significant comparison was between the Piedras Blancas and San Miguel Island sites. Comparisons between adjacent population pairs north of Point Conception were not significant at the α=0.05 level, however significance was obtained at greater geographic distance. South of Point Conception, all mainland samples were significantly different at the α=0.05 level.

Isolation by distance analyses revealed a significant positive correlation between increasing genetic and geographic distance when all sample locations were compared (Figure 3-2A, Mantel r=0.77621, p=0.00074, r^2=0.6025) and also when only sites within the Oregonian Province (sites 1-5) were compared (Figure 3-2C, Mantel r=0.81065, p=0.01638, r^2=0.6571). The plotted relationships between Φ_{ST} and geographic distance produced a regression with a slope of Φ_{ST}=0.11 per 1000 km when all samples were compared and a Φ_{ST}=0.08 per 1000 km when only sites within the Oregonian Province were compared. Interestingly, removal of the Santa Barbara site greatly improved the fit of the overall regression (Figure 3-2B, Mantel r=0.91280, p=0.00067, r^2= 0.8332), perhaps a consequence of the complicated current patterns within the SCB resulting in non-linear dispersal. Using a range of adult densities, we calculated possible values for average larval dispersal distance (σ) (see Table 3-3). These values ranged from 21 km (D=10 adults/km) to 2 km (D=1000 adults/km) (Φ_{ST}=0.11 per 1000 km) and 25 km (D=10 adults/km) to 2.5 km (D=1000 adults/km) (Φ_{ST}=0.08 per 1000 km). Such values are similar to those found for other nearshore *Sebastes* species (e.g., Buonaccorsi et al. 2002, 2004, 2005; Taylor 2004).

Nested Clade Phylogeographic Analysis:

Nested clade analysis revealed a significant phylogeographic pattern of restricted gene flow. This pattern was driven by the near-complete restriction of 12 haplotypes to populations north of Point Conception and a progressive change, with latitude, in the relative frequencies of several dominant haplotypes (see Figure 3-3). This finding is in agreement with the results of the isolation by distance analysis. The predominance of several derived haplotypes in northern samples, as well as the southern affinity of the ancestral haplotypes, suggests that the historical trend of gene flow has been primarily northward.

Discussion:

Phylogeography and population structure:

Analysis of population structure revealed significant genetic heterogeneity throughout the range of vermilion rockfish. AMOVA partitioned the genetic variance mostly within the separate coastal upwelling regions as proposed by Parrish et al. (1981) with the exception of an additional partition across Cape Mendocino. The strongest barrier to gene flow was across Point Conception, a well-documented biogeographic break between the Oregonian and San Diegan provinces (Briggs 1974). Recent studies have suggested that Point Conception may not function as a genetic barrier for all species (e.g., Burton 1998) and that stronger barriers may be present elsewhere within the Southern California Bight. However, studies of other *Sebastes* species have shown that populations north and south of Point Conception are often significantly different (i.e.,

Buonaccorsi et al. 2002, 2004, 2005; Matala et al. 2002). After Point Conception, AMOVA supported further barriers to gene flow across Cape Blanco, Oregon and Cape Mendocino, California. These oceanographic features have been shown to function as strong genetic barriers for other species, including the blue (*S. mystinus*) (Cope 2004) and yellowtail (*S. flavidus*) rockfish (J. Hess, NMFS, pers comm.). Similarly, analysis of catch data from three years of coast-wide pelagic juvenile rockfish surveys showed strong declines in abundance at Point Conception, Cape Blanco, and Cape Mendocino (Sakuma et al. 2006), further supporting the hypothesis that these features act as barriers to gene flow for many *Sebastes* species.

A significant and positive correlation was found between increasing genetic and geographic distance. This was true when all samples were compared, as well as when only samples within the Oregonian Province were compared, however, there was no significant relationship found between samples south of Point Conception. This lack of a signal south of Point Conception is likely due to the complicated dispersal patterns caused by the unique oceanography and bathymetry of this region, including the presence of ten islands and numerous offshore banks. The impingement of the California Current on the SCB produces a topographically constrained, quasi-permanent cyclonic eddy, a factor complicating the understanding of population connectivity. The regressions fit to the entire dataset and the Oregonian Province subset differed slightly in their slopes, Φ_{ST}=0.11 and Φ_{ST}=0.08 per 1000 km, respectively. This slight difference may be an artifact of comparing populations across the genetic barrier found at Point Conception and we therefore suggest a consideration of both regressions when interpreting our calculated values of larval dispersal distance.

Using the equations of Rousset (1997), the slopes of the isolation by distance regressions, and reasonable values for adult density, we calculated a range of values for average larval dispersal distance. The obtained values (Table 3-3) were much less than would be expected for a species with a larval and pelagic juvenile phase of two to three months. However, studies of other nearshore rockfish species (i.e., Buonaccorsi et al. 2002, 2004, 2005) have produced even lower values for geographic dispersal. Buonaccorsi et al. (2004, 2005) suggested that this limited dispersal may be due in part to the existence of a coastal boundary layer (sensu Largier 2003) acting to retain larvae nearshore, reducing entrainment in the bulk alongshore flow, thereby limiting dispersal.

Vermilion rockfish, in a pattern atypical of most *Sebastes* spp., show peak larval release during fall and early winter months (Love et al. 2002). In contrast to the typical south-ward flow driven by the California Current throughout much of the year, fall and early winter (Sept-Feb) are characterized by weak north-ward flow, at least in the nearshore, driven by sporadic pulses of the northerly flowing Davidson Current. This pattern of weak northward transport is in agreement with the results of the nested clade and isolation by distance analyses. Larval release during a period of low-level, sporadic pole-ward flow may help account for the high degree of population structure and low-levels of larval dispersal observed. Within the Santa Barbara channel (SBC), northward of Santa Rosa Island, the interaction between the Davidson and California Currents creates a strong and persistent cyclonic eddy, especially in the fall and early winter. This eddy may act as a barrier to dispersal within the SBC, perhaps explaining the unexpected behavior of the Santa Barbara site in the isolation by distance analysis (see Figure 3-2).

Settlement of juveniles to shallow, nearshore habitat followed by an ontogenetic migration to deeper adult habitat is a common theme among *Sebastes* spp. (Love et al. 1991). In Chapter II, we suggested that a truncation or loss of this ontogenetic migration in *S. miniatus* may have resulted in a speciation event and the subsequent divergence of Type 1 and Type 2 vermilion rockfish. It is easy to envision how such a mechanism, when combined with limited adult dispersal (e.g., Miller and Geibel 1973; Lea et al. 1999; Mitamura et al. 2002, 2005; Starr et al. 2002), may effectively shorten the dispersal capacity of this species.

Management implications:

Vermilion rockfish have historically represented an important commercial and recreational fishery in California, Oregon, and Washington. During the 14-year period 1990-2004, the California commercial and recreational fisheries took 2,386 and 3,341 metric tons, respectively (MacCall 2005). Among recreationally targeted rockfishes, vermilion rockfish currently rank #1 in the Southern California fishery and #3 statewide.

At current levels of exploitation, there exists a great potential for fishery decline. Our analyses showed significant barriers to gene flow across several geographic features that impinge into the California Current system (i.e., Point Conception, Cape Blanco, Cape Mendocino), suggesting that vermilion rockfish should be managed on a regional scale between these features rather than on a coast-wide basis. Current rockfish management regions within California (i.e., Northern, Central, and Southern) fit our results well, however, separate regional harvest guidelines should be considered. Isolation by distance analysis suggested that larval dispersal is relatively small (i.e. <

25km), suggesting a stepping-stone model of dispersal between adjacent reefs. This finding has obvious implications for the planning of marine reserve networks and is especially relevant during the current move to establish marine reserves as a solution to fishery management. In order to maintain sustainable populations within the reserves, while also providing access and benefit for the fishery, these reserves must be spaced close enough to effectively communicate larvae and maintain gene flow. Without proper scientific study and knowledge of the component species managed by these reserves, it is unlikely that their promise of fishery management and enhancement will be realized. This realization is becoming increasingly clear as the use of genetic tools continues to uncover instances of strong population subdivision within these fishes (e.g., Rocha-Olivares and Vetter 1999; Withler et al. 2001; Buonaccorsi et al. 2002, 2004, 2005; Cope 2004).

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Table 3-1
Measures of molecular diversity for *Sebastes miniatus*. Site # refers to map points in Figure 3-1. "*" indicates values signicant at the α=0.05 level

Sampling Location	site #	N	No. of haplotypes	haplotype diversity	nucleotide diversity	Tajima's D
Cape Blanco, OR	1	50	9	0.856 +/- 0.019	0.002 +/- 0.002	-0.18
Brookings, OR	2	45	8	0.853 +/- 0.027	0.002 +/- 0.001	-0.39
Half Moon Bay, CA	3	53	11	0.809 +/- 0.033	0.002 +/- 0.001	-1.23
Piedras Blancas, CA	4	36	10	0.832 +/- 0.042	0.002 +/- 0.001	-1.33
San Miguel Island, CA	5	30	9	0.635 +/- 0.097	0.001 +/- 0.001	-1.96*
Santa Barbara, CA	6	47	8	0.528 +/- 0.074	0.001 +/- 0.001	-1.66*
San Diego, CA	7	54	12	0.452 +/- 0.085	0.001 +/- 0.001	-2.27*
San Quintin, Mexico	8	50	9	0.598 +/- 0.075	0.001 +/- 0.001	-1.39
Overall		365	27	0.751 +/- 0.022	0.002 +/- 0.001	-1.89*

Table 3-2

Paiwise Φ_{ST} comparisons between sampling sites below diagonal and associated p-values above diagonal. Values significant at the α=0.05 level are highlighted in bold.

Sampling Site	Cape Blanco	Brookings	Half Moon Bay	Piedras Blancas	San Miguel I	Santa Barbara	San Diego	San Quintin
Cape Blanco	-	0.15955	**0.04008**	**0.00308**	**0.00116**	**0**	**0**	**0**
Brookings	0.01223	-	0.29719	0.06697	**0.00725**	**0**	**0**	**0**
Half Moon Bay	**0.02845**	0.00316	-	0.27774	**0.01122**	**0**	**0**	**0**
Piedras Blancas	**0.06627**	0.02397	0.00424	-	0.45763	**0.00026**	**0.00294**	**0.00004**
San Miguel Island	**0.09509**	**0.05989**	**0.05034**	-0.00147	-	0.12596	0.33405	**0.04866**
Santa Barbara	**0.18061**	**0.14364**	**0.14712**	**0.08036**	0.01638	-	**0.0187**	**0.02976**
San Diego	**0.1565**	**0.13309**	**0.09988**	**0.04252**	0.00281	**0.05335**	-	**0.00221**
San Quintin	**0.16685**	**0.13726**	**0.12946**	**0.07444**	**0.0295**	**0.03394**	**0.04254**	-

Table 3-3
Calculations of average larval dispersal distance based upon isolation by distance regression from all sample sites (A.) and sites within the Oregonian Province (B.). Various values for adult density per km of linear coastline (D) and associated dispersal values are presented.

A.

σ^2	D	average dispersal (km)
909.09	10	21.3
90.91	100	6.7
9.09	1000	2.1
0.91	10000	0.7

B.

σ^2	D	average dispersal (km)
1250	10	25.0
125	100	7.9
12.5	1000	2.5
1.25	10000	0.8

Table 3-4
Results of adjacent pooling AMOVA. For each hypothesized number of groups, we show the group with the largest and most significant between group heterogeneity (FCT) and smallest and least significant within group heterogeneity (FSC).

# of partitions	Groups	Φ_{CT}	*p*	Φ_{ST}	*p*	Φ_{SC}	*p*
2 groups	sites **1-4** & sites **5-8**	0.0969	0.0286	0.1207	<0.0001	0.0263	0.0011
3 groups	site **1**, sites **2-4**, & sites **5-8**	0.0972	0.0035	0.1105	<0.0001	0.0147	<0.0001
4 groups	site **1**, site **2**, sites **3-4**, & sites **5-8**	0.0923	0.0046	0.1033	<0.0001	0.0121	0.0022
5 groups	site **1**, site **2**, site **3**, site **4**, & sites **6-8**	0.0871	0.0147	0.0995	<0.0001	0.0136	0.2367

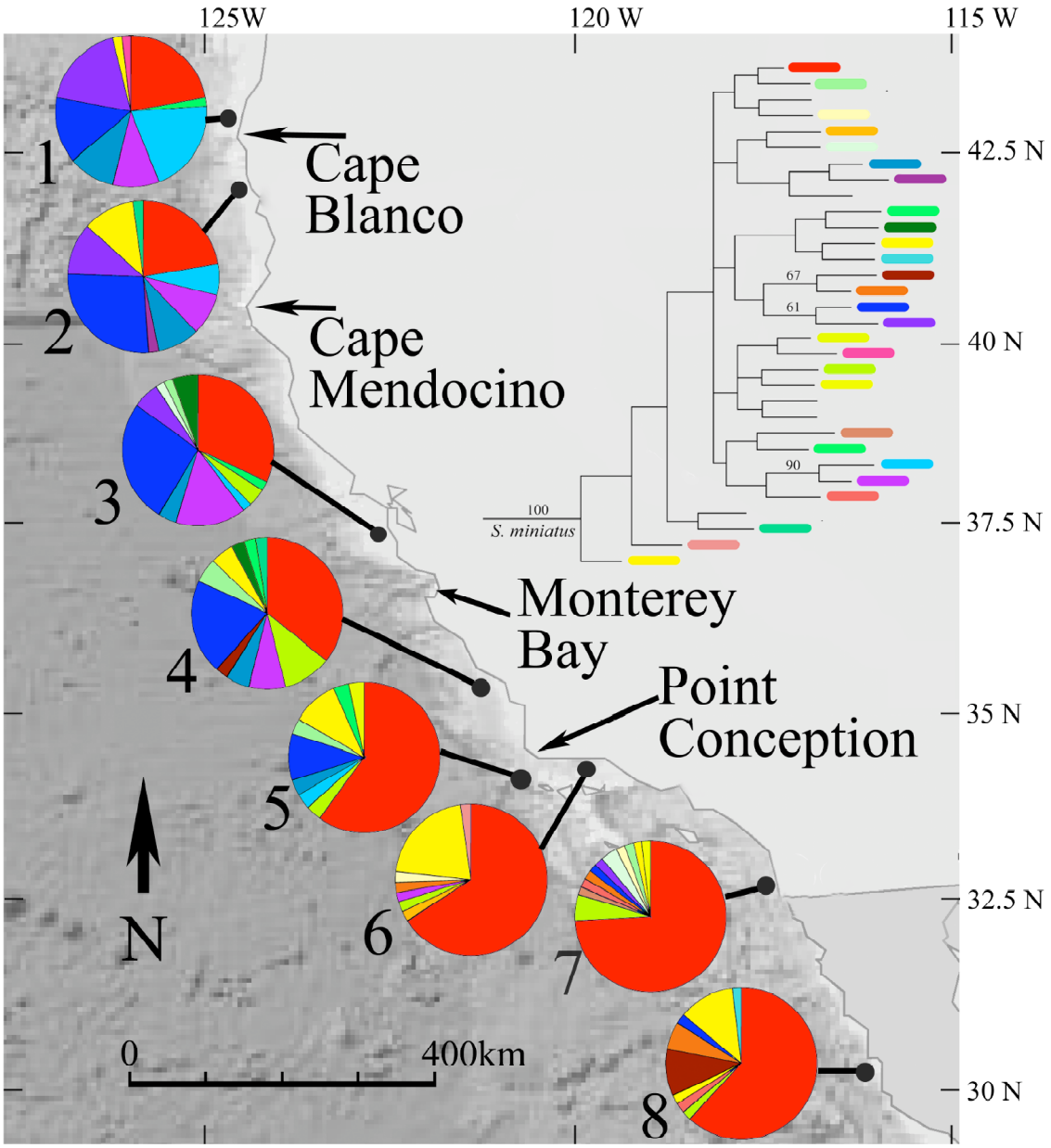
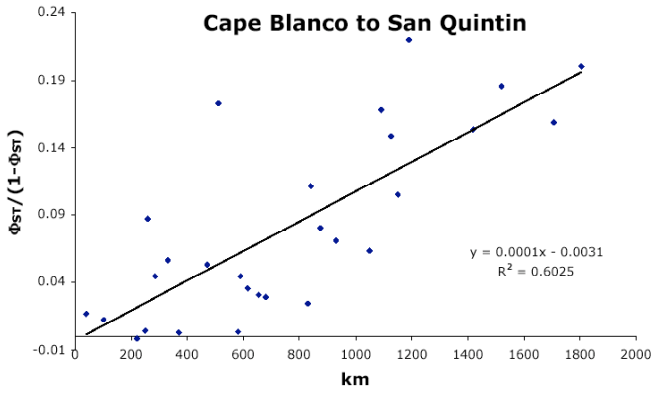
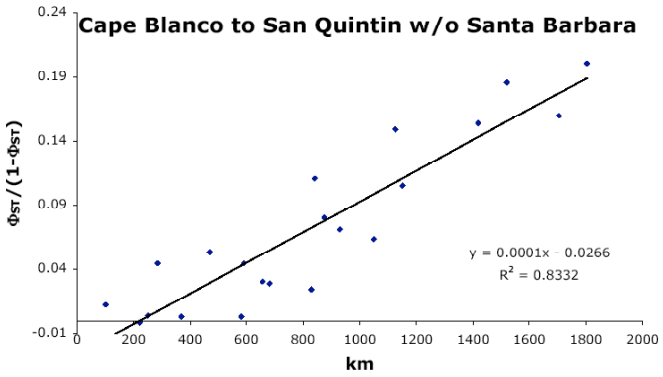


Figure 3-1
Sampling locations for vermilion rockfish used in this study with pie charts depicting relative mitochondrial haplotype content at each site. Hypothesized phylogeographic breaks are indicated with arrows.

A.



B.



C.

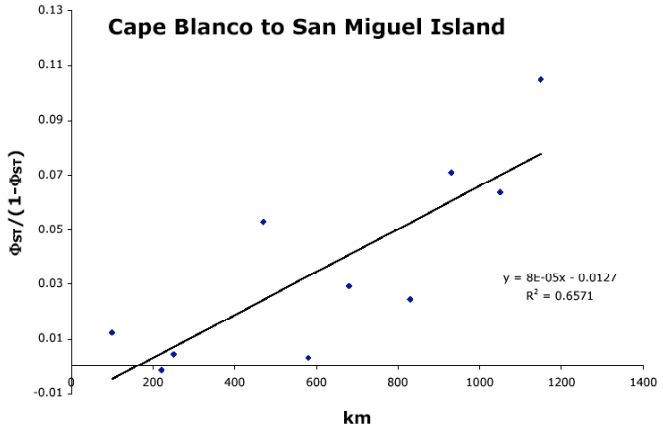


Figure 3-2
Plots of linearized (Slatkin 1995) genetic distance (F_{ST}), between pair-wise comparisons of sampling sites, in relation to geographic distance. Relationships are presented for all sampling sites (A.), all sites except Santa Barbara (B.), and sites within the Oregonian biogeographic province (C.).

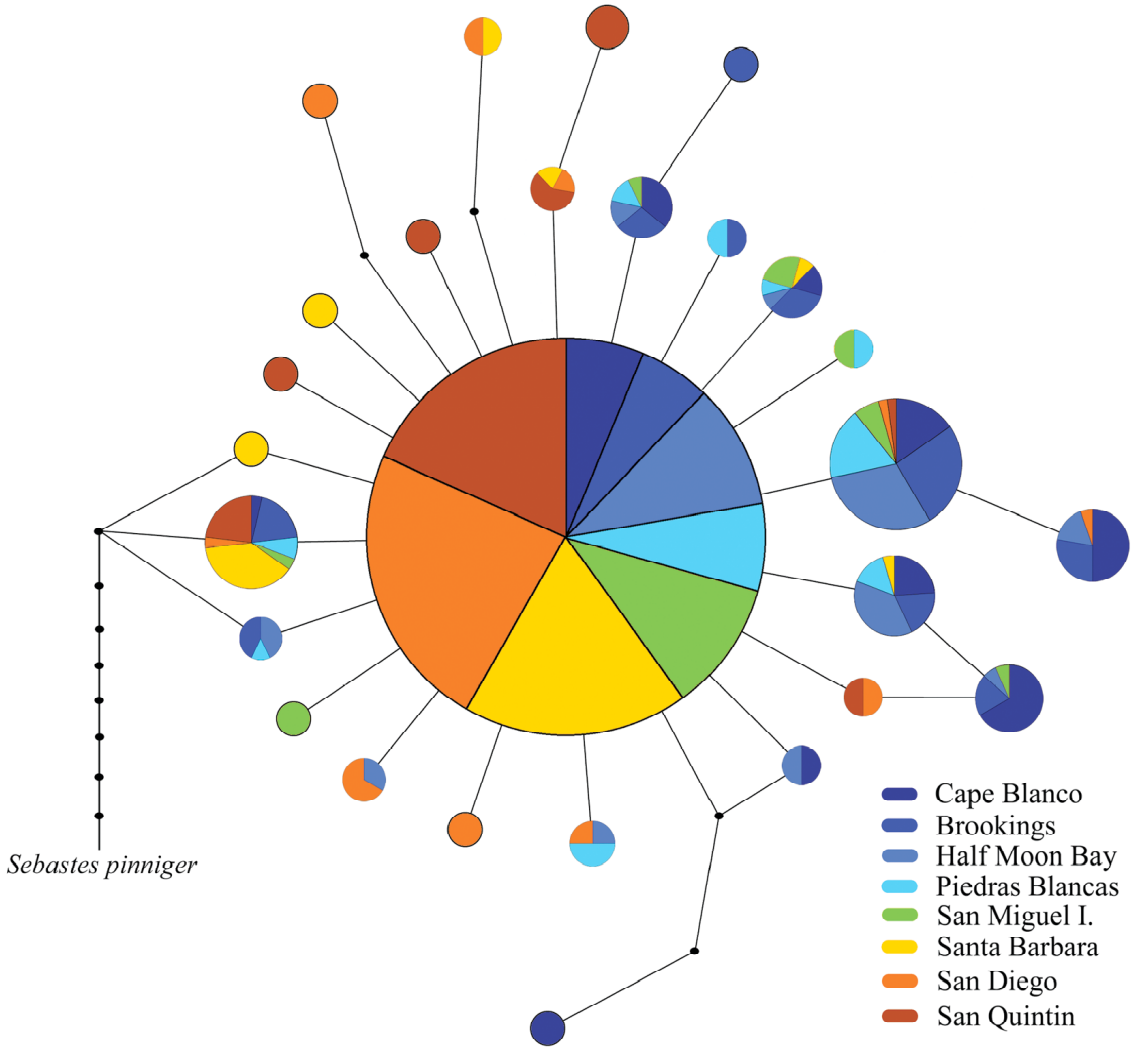


Figure 3-3
Statistical parsimony network among 27 *S. miniatus* haplotypes, rooted with a single *S. pinniger* haplotype. Node size is proportional to haplotype frequency and the colored pie charts indicate the relative contribution of individual sampling sites.

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IV.

Multiple paternity and the maintenance of genetic diversity in the live-bearing rockfishes, genus *Sebastes*

Abstract of Chapter IV:

The understanding of mating systems and their effect on genetic diversity is of strong interest to conservation geneticists and those tasked with managing harvested populations. This is particularly true for marine species that produce large numbers of pelagic eggs or larvae and that often exhibit large discrepancies between effective and census population sizes. The development of polymorphic genetic markers, such as codominant nuclear microsatellite loci, has made it possible to study the paternity of individual offspring within a brood. This has precipitated a host of studies on breeding strategies in birds, mammals, and fishes. Concerns about the loss of genetic diversity in long-lived, viviparous fishes has led to interest in their mating systems. Multiple paternity is common in broadcast spawning fishes, but was thought to be less common in fishes that have internal fertilization. By examining broods from pregnant females in field and aquarium studies we have been able to examine paternity patterns within the live-bearing scorpaenid genus *Sebastes*. We report the first finding of multiple paternity within individual broods of 10 species. Through examination of larvae from aquarium studies we show that at least three sires can contribute paternity to a single brood. Using field caught specimens of gravid fish we surveyed a phylogenetically and ecologically diverse sample of *Sebastes* species. In total, we found instances of multiple paternity in 10 of the 17 examined species, showing that this is not a rare event within a single species and is likely common throughout the genus. *Sebastes* spp. are notable for their highly variable reproductive success, with strong recruitment events occurring approximately once a decade. Such sweepstakes-like reproductive events are likely due to

the chance matching of larval parturition of a subset of the population with favorable environmental conditions. In broadcast-spawning fishes, such high variance in reproductive success is often correlated with low genetic diversity. Despite this high variance in reproductive success, *Sebastes* species, 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 discrete recruitment pulses and subsequently maintain genetic diversity of the population as a whole. Regardless of the selective value of multiple paternity at the level of individual fitness, the net effect at the population level may be a genetic buffer to the consequences of severe overexploitation. This is especially relevant as population sizes of many species of *Sebastes* are currently below 10 percent of historical, unfished levels, with five species considered severely overfished.

Introduction:

The accumulation and maintenance of genetic diversity is a key topic in conservation genetics. Ultimately the amount of genetic diversity in a population at equilibrium is a function of both mutation and drift. The interpretation of present day patterns of spatial and temporal genetic diversity (i.e., effective population size (N_E), allelic richness and levels of heterozygosity) and population connectivity requires not only an understanding of historical patterns of migration, population expansion and contraction but also of basic life history parameters that may affect genetic diversity (i.e., through drift and selection). These parameters include; mating system (e.g., Nunney 1993), age and/or size dependent effects on fecundity and offspring quality (e.g., Berkeley et al. 2004), and ecologically linked effects on larval survival (e.g., Cushing 1975, 1990). All of these parameters can have important ramifications upon genetic effective population size and management plans that aim to conserve genetic diversity in fishes that are under intensive fishery exploitation or declining due to habitat degradation and/or climate change.

Of the eight subfamilies of the scorpionfish family Scorpaenidae, viviparity is confined to the subfamily Sebastinae. Among the four sebastine genera, species within the genus *Sebastes* exhibit the most advanced form of viviparity (Barsukov 1981; Wourms 1991). Though the form of viviparity exhibited by *Sebastes* spp. is primitive when compared to other live-bearers (e.g., Embiotocids, Poeciliids, and most sharks), their compromise between maternal investment (Boehlert & Yaklovich 1984; Boehlert et al. 1986) and high fecundity results in broods comparable in size to those of oviparous,

broadcast spawning fishes, ranging from a few thousand to over 2.7 million larvae (Moser 1967; Love et al. 2002). Most species produce only a single brood annually, though a few species, mainly within the Southern California Bight, show evidence for multiple annual broods (e.g., *S. paucispinis*) (Moser 1967; MacGregor 1970). Despite the combination of high fecundity and enhanced survivorship due to the inherent protection afforded to the developing larvae during the typical period of greatest mortality for oviparous species (i.e., egg and early larval stages), larvae of *Sebastes* spp. still suffer high levels of mortality and variability in recruitment success. This results in dominant year classes occurring approximately once per decade (Tolimieri & Levin 2005) when parturition coincides with favorable environmental parameters (sensu Cushing 1975). The genetic diversity of the subsequent year-class likely represents a disproportionate contribution from a very small subset of the spawning females (sensu Hedgecock 1994). This bias toward a reduction in genetic diversity is clearly greater in species that produce broods with a single sire as compared to multiple sires.

Of the 100+ *Sebastes* species, courtship behavior has been formally described for only two, *S. inermis* (Shinomiya & Ezaki 1991) and *S. mystinus* (Helvey 1982), demersal and semi-pelagic species, respectively. 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, females and males of both species have been observed to mate 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, there is an asynchrony between mating, maturation of oocytes, and the development of embryos. This as well as the finding of both free swimming and embedded spermatozoa in the ovaries, suggests that sperm are stored in some manner (Moser 1967; Eldridge et al. 1991; Takahashi et al. 1991; K. Clifford pers. obs.). Studies that examined both hormonal changes (Mori et al. 2003) and general gonad histology (Takahashi et al. 1991) have found that insemination can precede fertilization by up to six months.

Observation of females engaged in multiple mating events with different males leads to the question as to whether multiple males are able to sire offspring within the same brood? To address this question we examined broods from both captive and wild populations of several species. The captive populations represent two species that are important components of the US west coast commercial and recreational fishery. The first group was composed of 12 adult grass rockfish (*S. rastrelliger*) maintained at the Southwest Fisheries Science Center experimental aquarium (La Jolla, CA). The second group was composed of 80 adult yelloweye rockfish (*S. ruberrimus*) maintained in an exhibit at the Oregon Coast Aquarium (Newport, OR). Samples from wild populations were collected opportunistically from fishery caught animals and selected to represent a phylogenetically diverse sample of species (Hyde & Vetter 2007).

Methods:

Sample collection:

Aquarium studies:

All fish were captured, individually tagged (using either PIT or Floy T-Bar tags), measured, and tissue samples were taken. Tissue was taken from the caudal end of the anal fin and immediately stored in 95% undenatured ethanol. 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.

Wild caught fish:

Twenty-eight gravid females and associated larvae, representing 16 species, were collected opportunistically using various methods including; otter trawl, pole spear, and hook and line. Specimens were identified to species using and Love et al. (2002). A sample portion of fin tissue was preserved in 95% undenatured ethanol from the gravid female. 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 adult fish and larvae from wild caught fish, DNA was extracted from fin clips by a proteinase K digestion followed by nucleic acid separation and purification using a LiCl:chloroform protocol and subsequent ethanol precipitation (Gemmel & Akiyama 1996). In order to expedite the analyses for 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 (BioRad laboratories) based boiling protocol was employed using a single, whole larva for each extraction (Hyde et al. 2005). All DNA extractions were stored frozen at -20°C until needed.

PCR amplification of microsatellites:

All fish from the aquarium studies were genotyped at six (*S. rastrelliger*) or eight (*S. ruberrimus*) microsatellite loci. 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 five loci were surveyed. The microsatellite loci used were chosen as they work well across multiple species while also exhibiting moderate levels of polymorphism (Gomez-Uchida et al. 2003; Westerman et al. 2005; J. Hyde unpublished data). Neff & Pitcher (2002) showed that it should be possible to detect multiple paternity with >90% probability by using as few as two mildly polymorphic (e.g., five alleles) microsatellite loci on samples of greater than 20 larvae, therefore it is expected that 100 larvae and five loci are more than sufficient to survey for incidences of multiple paternity. All microsatellite 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 sized using either an ABI 377XL automated sequencer or an ABI 3130XL Genetic Analyzer with the ROX 500 size standard and scored using Genemapper v3.7 software (Applied Biosystems).

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 two or more possible sires for an individual offspring, 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.

For wild caught specimens, paternal alleles were deduced by exclusion of maternal alleles as before. The finding of three or more paternal alleles at any microsatellite locus was recorded 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 within a brood while only detecting two or fewer paternal alleles at any locus.

Modeling the effect of multiple paternity on F_{IS} 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, using Resampling Stats (Simon 1997) and used to construct composite offspring genotypes. The genotypes of these "pseudo-larvae" were then combined 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} (Figure 4-2) and total allele number across all loci (Figure 4-3) are presented for up to four (*S. rastrelliger*) or five (*S. ruberrimus*) sires.

Results:

Aquarium husbandry studies:

As seen in Table 4-1, more than one father was identified in broods three and four from *S. rastrelliger*. The finding of three fathers in brood three supports the assertion of Neff & Pitcher (2002) that multiple fathers can be detected with low sample size (n=11 of approximately 400,000 larvae). The levels of paternal contribution in brood three suggest a correlation with male size, perhaps larger size being a proxy for greater sperm capacity and subsequent transfer and competition. Unfortunately, few samples were available from this brood and represent an insufficient proportion of the total brood size to

accurately assess this hypothesis. In contrast, brood four does not seem to fit this pattern, as the smaller male seems to be the primary contributor of paternity.

Similarly, for the *S. ruberrimus* broods, it was found that multiple males contributed to the paternity of a single brood (see Table 4-1). In contrast to *S. rastrelliger*, ratios of paternity within a brood were strongly skewed towards a single individual. All sires were of similar size but the females were markedly smaller.

Wild-caught specimens:

All wild caught females and larvae were successfully genotyped at four or five 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 more than two paternal alleles were found at any of the five 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 4-1 to compare the number of deduced paternal alleles at each locus with the number of sires found in the aquarium studies). Of the 28 individuals, representing 16 species, broods from nine species showed evidence for multiple paternity (see Table 4-2).

Discussion:

Our results show occurrences of multiple paternity in 10 species of *Sebastes* (*S. atrovirens, S. brevispinis, S. diploproa, S. elongatus, S. goodei, S. jordani, S. proriger, S. rastrelliger, S. ruberrimus,* and *S. rufus*). The remaining nine 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 examined, using a fairly conservative approach, suggests that multiple paternity within the genus is common. Furthermore, 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 nearshore (*S. atrovirens*), and shallow high-relief reef (*S. rastrelliger*)), in the northeast Pacific (see Figure 4-1). Ng et al. (2003) found evidence for multiple paternity in *Sebastiscus marmoratus*, an appropriate outgroup for *Sebastes* (Hyde & Vetter 2007). As such, the most parsimonious hypothesis is that this is a plesiomorphic trait and examination of additional species within the genus and subfamily will likely yield additional occurrences of multiple paternity.

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 (Gray 1997; Lank et al. 2002), where females often gain material benefits (e.g., access to male's territory or shelter, food, protection) through mating.

Darwin (1871) proposed that sexual selection acts to disproportionately enhance the reproductive success of a few individuals that exhibit unique traits that are desirable to the opposite sex. These unique traits may or may not confer physical advantages, other than the increased probability of mating, and in some cases may be detrimental (e.g., conspicuous coloration or behavior increasing the risk of predation). As with most species, it would seem advantageous for a female to mate with a high quality male to help ensure that her offspring acquire evolutionarily beneficial traits. There is a great deal of literature on sexual selection and mating strategies but much of the literature may be less relevant to *Sebastes* spp. Much of the theory of sexual selection is based upon *K*-selected organisms (primarily birds and mammals) where offspring fitness based on mate choice outweighs chance as a predictor of successful recruitment. 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 1975, 1990) is thought to play a greater role in subsequent reproductive success. Under these environmental constraints, bet-hedging to match unpredictable environmental conditions may be an important component of reproductive success. Here 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, as a consequence of multiple paternity, has been proposed as theory to explain this behavior and this is particularly compelling for *r*-selected species. As phenotype and physiological variation are usually determined through the interaction of multiple genes acted upon by selection, the direct comparison of allelic diversity at selectively neutral loci does not represent the true extent of phenotypic variation possible within a population. However, estimates of genetic diversity using these markers may act as a proxy for assessing the potential for adaptive variability within a population (Carvalho et al. 2002). Using genotypic data from our aquarium studies, 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 Figure 4-3). This can have multiple advantages. Due to the potentially large dispersal distances for larvae of *Sebastes* spp. and the uncertainty of settling conditions, it would seem beneficial to maximize genetic diversity within a brood such that some larvae are genetically predisposed to survive a variety of potential challenges. Many species of *Sebastes* appear to recruit in a sweepstakes fashion, likely due to the timing of parturition coinciding with favorable environmental conditions (sensu Cushing 1975, 1990), with dominant year classes occurring approximately once a decade (Tolimieri & Levin 2005). The few successful spawners probably do not represent the genetic diversity of the population as a whole (Hedgecock 1994) but multiple paternity may help to ameliorate this bias. Beyond the direct benefit of increased diversity, several indirect benefits have been realized by other taxa. In the guppy, *Poecilia reticulata*, offspring from multiply sired broods show increased

schooling competence and greater predator avoidance when compared to single sired broods (Evans & Magurran 2000). Colonies of the bumblebee, *Bombus terrestris*, started by multiply inseminated queens had fewer parasites than colonies from singly inseminated queens (Baer & Schmid-Hempel 1999). Both of these examples result in decreased mortality from predation and disease, traits that are universally advantageous and directly applicable to *Sebastes* spp.

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 (see Edmands 2007). This reduction in genetic heterozygosity both increases the risk that detrimental recessive traits will be expressed, as well as reducing the overall genetic diversity of the population. When it does occur, inbreeding can be reduced by both sperm selection and the dilution of the less desirable gametes with those from different sources (Stockley et al. 1993). Resampling the genotypic data from our aquarium husbandry studies we modeled the change in the inbreeding coefficient of a brood (F_{IS}) when multiple sires were present (see Figure 4-2). The inbreeding coefficient of a brood increasingly tends toward zero with increasing number of sires. Given the remarkable site fidelity, lack of movement of adult rockfish, and low geographic dispersal of larvae in some demersal species (Mitamura et al. 2002; Starr et al. 2002; Buonaccorsi et al. 2005) there is a reasonable concern for inbreeding. However, the discovery of this mating behavior throughout the ecological (schooling vs. solitary, shallow vs. deep

dwelling, reef associated vs. midwater) as well as phylogenetic breadth (see Figure 4-1) of the genus suggests a more basic advantage.

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, over-lapping generations, and populations that experience a high variance in reproductive success). Hedgecock (1994) proposed that environmental match or mismatch (sensu Cushing 1975, 1990) acts to disproportionately favor the reproductive success of a subset of the population, which in effect wins the reproductive sweepstakes. This can result in a large discrepancy between N_E and N_C, in the case of the oysters in his study, the difference was five orders of magnitude. Recent studies of N_E in populations of several *r*-selected marine fishes including squirefish (*Chrysophrys auratus* (Hauser et al. 2002)), red drum (*Sciaenops ocellatus* (Turner et al. 2002)), canary (*S. 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 three to five orders of magnitude.

By analyzing historical samples (archived scales and otoliths) of squirefish (Hauser et al. 2002) and red drum (Hutchinson et al. 2003) it was found that heavy fishery exploitation over the past 50 years has caused a significant decline in both N_E and overall genetic diversity of these species. These results are especially significant as the census population sizes of the species from both of these studies are in the millions, a

number that was thought to be too high to experience a reduction in genetic diversity. Their reasoning for this disparity was that the large difference between N_E and N_C in these species requires very large census population sizes to maintain genetic diversity. Using widely accepted values for the effective number of individuals needed to maintain short-term heterozygosity (n=50) and long-term population adaptability (n=500), Hauser et al. (2002) calculated that populations of 5 million and 50 million individuals, respectively, would be required. This suggests that many of the world's exploited species have already undergone a reduction in genetic diversity and perhaps adaptability due to the intense levels of exploitation over the past 50-100 years.

Interestingly, in a study of the heavily over-fished canary rockfish (*S. pinniger*), Gomez-Uchida (2006) did not find a reduction in genetic diversity from samples spanning 31 years. The discordance between his finding and that in other species may be due to the lack of samples from the pre- or early exploitation period. Also, canary rockfish have a roughly 75% greater lifespan than the squirefish in Hauser et al.'s (2002) study, a life history trait that would act to temporarily dampen the effect of overfishing on genetic diversity. Though they were not assayed in this study, the finding of multiple paternity throughout the genus suggests that canary rockfish also exhibit this behavior.

Mating behavior that results in multiple paternity within a brood creates offspring with more possible genetic combinations than would be possible with single father parentage. This can have important implications in the maintenance of genetic diversity and preventing the loss of rare alleles (Robbins et al. 1987; 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 or adult populations of kelp rockfish (*Sebastes atrovirens*), a species shown in this study to practice multiple paternity. The authors attribute this to a lack of sweepstakes recruitment in this species, however this lack of heterogeneity may also be due to the increased genetic diversity afforded to multiply sired broods.

Concomitant with increased genetic diversity, multiple paternity carries with it an increase in the effective population size of spawning individuals (Waples 1987; Sugg & Chesser 1994; Martinez et al. 2000). Waples (1987) calculated that multiple paternity can almost double effective population size. Though a two-fold difference may seem minor, when coupled with the large discrepancies between census and effective population sizes this equates to a realized buffer against loss of genetic diversity of millions to 10's of millions of fish. This buffer should help recover and maximize genetic diversity during bottleneck (e.g., overfishing, severe environmental fluctuations, disease) and/or founding events. Such a behavioral trait, in conjunction with the potential for long distance dispersal and assortative mating, may be an important reason for the high species diversity of *Sebastes* (Hyde & Vetter 2007). The ability to carry a diverse genetic reservoir within the ovaries of a single female would help reduce the chance of a genetic bottleneck should only a single or a few females disperse to a novel geographic area (e.g., the colonization of the north Atlantic, Gulf of California and the southern hemisphere) or adopt assortative mating strategies (e.g., assortative mating presumably based on color in *S. carnatus* and *S. chrysomelas*, Narum et al. 2004).

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Table 4-1

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 denotes microsatellite loci that were not examined.

species	Mother's ID	n 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)
S. rastrelliger (brood 1)	MA2	24	2	2	2	2	2	2	N/E	N/E	N/E	N/E	MA9 (100)
S. rastrelliger (brood 2)	MA2	20	2	2	2	2	2	2	N/E	N/E	N/E	N/E	MA9 (100)
S. rastrelliger (brood 3)	MA2	11	2	2	3	4	2	4	N/E	N/E	N/E	N/E	MA6 (27), MA9 (9), MA10 (64)
S. rastrelliger (brood 4)	MA2	24	2	2	3	2	2	4	N/E	N/E	N/E	N/E	MA3 (21), MA6 (79)
S. ruberrimus	MQ19	48	N/E	1	2	3	N/E	1	2	1	3	2	MQ24 (94), MQ54 (2), MQ65 (4)
S. ruberrimus	MQ39	48	N/E	2	1	1	N/E	1	2	0	2	2	MQ6 (4), MQ31 (96)
S. ruberrimus	MQ9	48	N/E	2	2	4	N/E		4	1	0	1	

Table 4-2
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 indicates failed amplification of microsatellite locus.

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

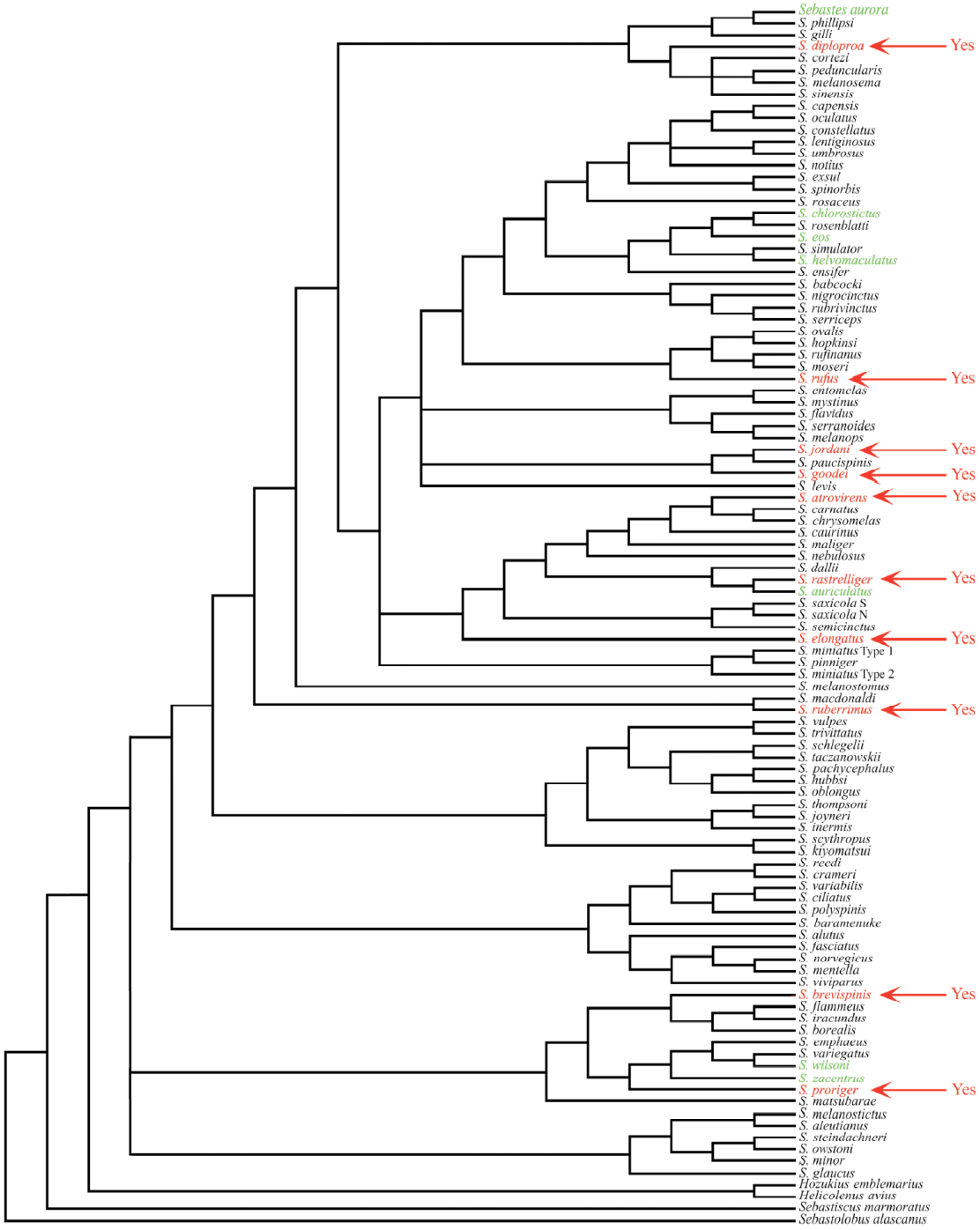

Figure 4-1
Bayesian derived phylogeny for *Sebastes* spp. and other members of the scorpaenid subfamily, Sebastinae (adapted from Hyde & Vetter 2007). Species names in red, with arrows were found to have multiple paternity within a single brood. Species names in green showed no evidence for multiple paternity in this study.

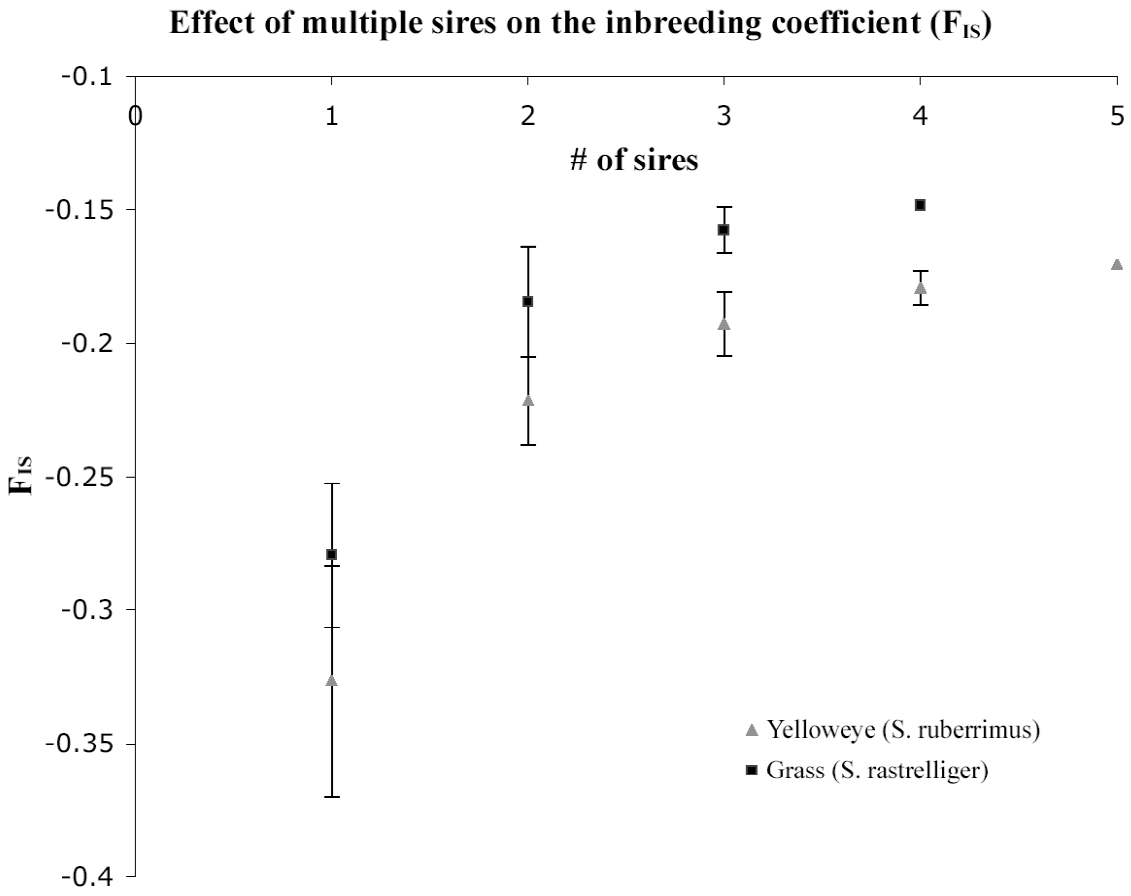

Figure 4-2
Genotypic data from all possible mate pairings were sampled with replacement for 1000 iterations, using Resampling Stats (Simon 1997) and used to construct composite offspring genotypes. Means and standard deviations of the inbreeding coefficient, F_{IS} (Weir & Cockerham 1984) are plotted for both *S. rastrelliger* and *S. ruberrimus*

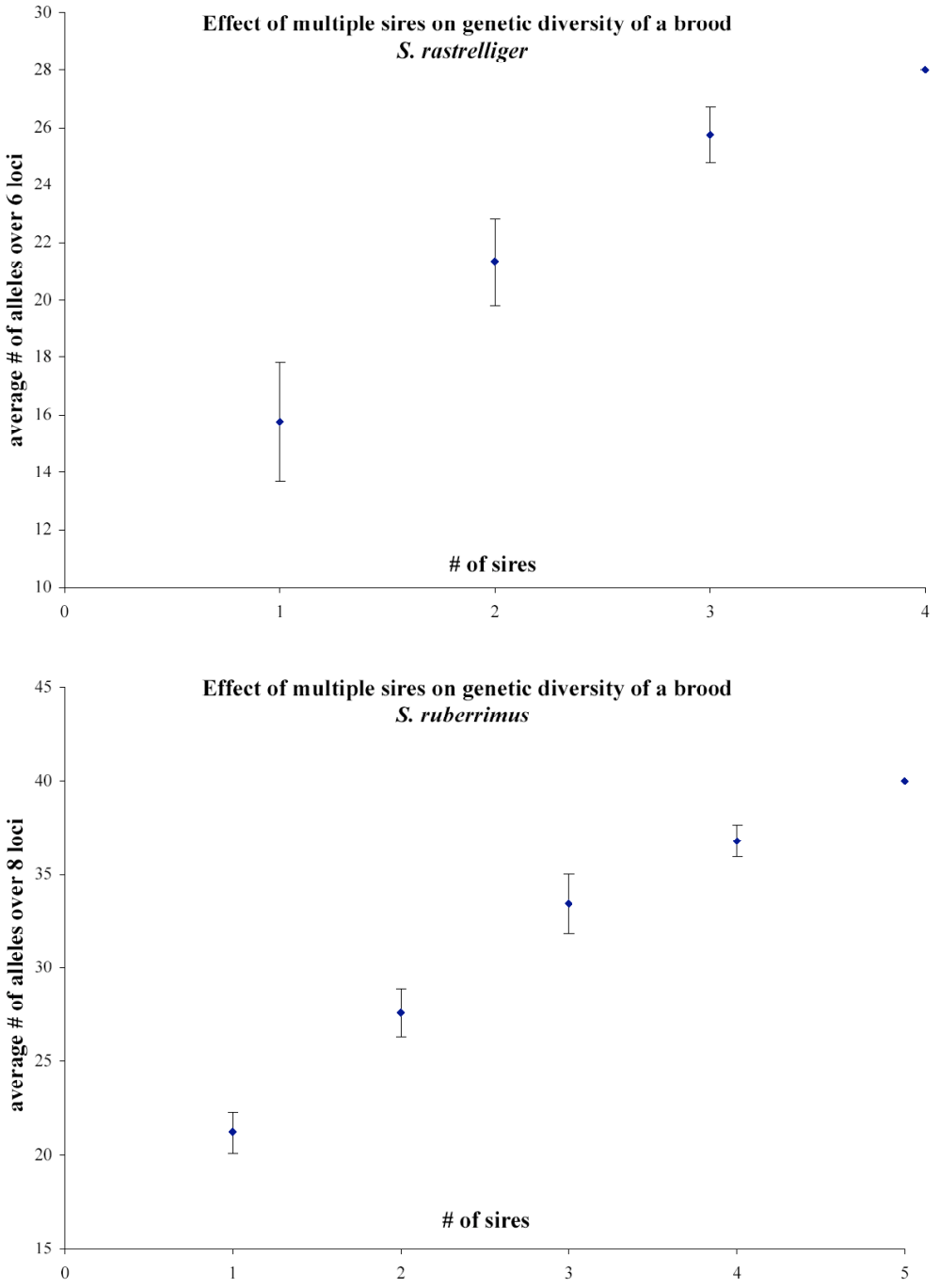

Figure 4-3
Genotypic data from all possible mate pairings were sampled with replacement for 1000 iterations, using Resampling Stats (Simon 1997) and used to construct composite offspring genotypes. Means and standard deviations of the average number of alleles per brood, across all examined loci, are plotted for both *S. rastrelliger* and *S. ruberrimus.*

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