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PATTERNS OF EARLY-STAGE PELAGIC DISPERSAL AND GENE FLOW IN ROCKFISH SPECIES FROM THE SOUTHERN CALIFORNIA BIGHT

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in

Oceanography

by

Cynthia A. Taylor

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2004

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University of California, San Diego

2004

DEDICATION

M^3: To Mom. To Mark. To Micky.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

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ABSTRACT OF THE DISSERTATION

PATTERNS OF EARLY-STAGE PELAGIC DISPERSAL AND GENE FLOW IN ROCKFISH SPECIES FROM THE SOUTHERN CALIFORNIA BIGHT

by

Cynthia A. Taylor

Doctor of Philosophy in Oceanography

University of California, San Diego, 2004

Ron Burton, Co-Chair

John Hunter, Co-Chair

Connectivity at relevant scales for management and conservation of many marine species occurs through dispersal of larvae. The goal of this study was to assess dispersal and connectivity among populations of depth-limited rockfish using a combination of indirect and direct methods. In one component of the dissertation, kelp rockfish populations were examined for evidence of genetic structure using 8 coastal and 5 island locations from central California to Baja using seven microsatellite loci. Although no significant population structure was detected with all populations treated separately, significant differences were found when populations were pooled based on hydrographic regions identified using average sea surface temperature as a proxy for hydrography at the time of spawning and pelagic dispersal (Fst = 0.002). This structure appeared to be driven by an isolation by distance pattern of gene flow among coastal and nearshore island comparisons (Mantel Test, p = 0.01).

An estimate of average dispersal was calculated using the relationship between the the isolation by distance regression and linearized density (Rousset 1997) as 9-13 km for coastal and nearshore island populations in kelp rockfish.

In another component of the thesis, larval distributions were examined directly by collecting early-stage (3-16 mm) rockfish samples through the California Cooperative Oceanic Fisheries Investigation (CalCOFI) surveys, completing species-specific identifications on larvae and ageing the larvae using otolith-based methods on the squarespot rockfish, the most abundant species in the survey. A significant pattern of higher abundance and younger larvae (mean = 9.2 days) within eddies in the SCB and lower abundance and older larvae (mean = 15.6 days) outside eddies ($F_1 = 26.13$, $p < 0.001$) was found.

The results suggest an overall pattern of retention of these early-stage larvae within the SCB and limitation to dispersal beyond that expected for the time in the pelagic for both the nearshore kelp rockfish and the more offshore but also depth-limited squarespot rockfish. These data, collected using integrated methods support previous work on many species suggesting that pelagic fish larvae have a lower realized dispersal than expected considering the length of their pelagic dispersal period.

Chapter 1

Introduction

Rockfishes in the genus *Sebastes* comprise one of the most species-rich genera of marine fishes and primarily inhabit temperate and subarctic waters of the north Pacific. In all, over 100 species have been described, with new species (Eitner et al. 1999) and cryptic species (Rocha-Olivares et al. 1999) still being recognized. The center of *Sebastes* abundance is in the northeast (NE) Pacific but species are also commonly found in coastal waters of the northwest (NW) Pacific (Cramer 1895), the north Atlantic (Templeman 1959), Gulf of California (Chen 1971; Eschmeyer et al. 1983), and the southern hemisphere (DeBuen 1960; Chen 1971; Kong Urbina 1985). Rockfishes exhibit a wide range of color patterns, behaviors, and preferred habitats that range from solitary, territorial, benthic nearshore predators (e.g., *Sebastes rastrelliger*), to pelagic, schooling fishes that form large midwater shoals (e.g., *Sebastes jordani, S. mystinus).* Despite the range of diversity in habitat, all species can be considered inhabitants of the continental shelf and upper slope (Miller and Lea 1972; Eschmeyer et al. 1983; Vetter and Lynn 1997). Like many marine fishes, *Sebastes* have high fecundity and an extended pelagic larval and juvenile phase (Moser 1967).

In most marine fish, females and males independently release eggs and sperm into the water in broadcast spawning events. Mating criteria and parental care are minimal and spawning opportunities can occur frequently throughout a spawning season or throughout the year. Fertilized eggs drift passively until hatching with high variability in the planktonic phases among fish taxa. In contrast, rockfishes have paired mating and internal fertilization. This is followed by an extended internal brooding period over many months (DeLacy et al. 1964) that includes maternal nutritional investment (matrotrophy; Boehlert and Yoklavich 1984). The length of pelagic phase for individual species has been described primarily from underwater observations of nearshore species (e.g., Love and Johnson 1998) and from piecing together observations of recreational anglers with surveys of juveniles for deeper-dwelling species (Love et al. 2002). In contrast to many marine species, rockfishes in the genus *Sebastes* release developed swimming larvae that have potential to disperse widely during an extended (1-4) month pelagic period. Most adult *Sebastes* display little migratory behavior; therefore the early life history phase is the period during which dispersal among populations most likely occurs.

This dissertation was proposed to mitigate the paucity of information available to early life history stages of *Sebastes* in an effort to better understand dispersal potential in the group. Although over 50 years of sampling data from California Cooperative Oceanic Fisheries Investigations (CalCOFI) have included *Sebastes*, and they are abundant and frequently occur off southern California, species-specific distribution data of larvae are lacking for all but a handful of these species. Chapter 2 of the dissertation will address how molecular and morphological methods were combined in an effort to identify the previously unidentifiable *Sebastes* spp. component from the April, 1999 CalCOFI cruise. Chapter 3 will address an extended use of these data: Larvae of the most abundant species in the survey were aged and mapped relative to acoustic Doppler current profile data (velocity streamfunction) to examine the trajectories of larvae as they progressed over the course of their pelagic period. Chapter 4 examines an indirect examination of gene flow within *Sebastes atrovirens,* the kelp rockfish.

Sebastes atrovirens is one of nine species within *Sebastes* that forms a monophyletic subgenus, *Pteropodus* (see Figure 1.1; Taylor 1998). These species inhabit the continental shelf with a maximum depth to 200 meters and display a minimum of movement in adults (Love 1979; Lea *et al.* 1999). Five are targets of heavy fishing pressure by the live finfish fishery in California (Barnes *et al.* 1995; Walters 2001) and all nine species are targets or bycatch of commercial and sportfishing efforts throughout their respective ranges. Genetic methods provide tools to address questions about dispersal using quickly evolving markers to test for significant differences among populations within species. If populations share migrants, statistical tests across loci will show genetic homogeneity among populations. If not, and if they have not shared migrants for a reasonable period of time (~on the order of hundreds of generations, depending upon the species), then populations will be likely to diverge due to genetic drift at neutral markers such as microsatellite loci.

Evidence for population differentiation or stock structure has been found on both large and small scales in *Sebastes*. Multiple stocks have been found in Pacific ocean perch, *Sebastes alutus* (Seeb and Gunderson 1988; Withler et al. 2001), rosethorn rockfish, *Sebastes helvomaculatus* (Rocha-Olivares and Vetter 1999), blue rockfish, *S. mystinus* (Cope, In Review), and copper rockfish, *S. caurinus* (Buonaccorsi *et al.* 2002). The kelp rockfish, *S. atrovirens*, has a narrow coastal range extending from

Fort Bragg in northern California to Baja California, Mexico but it has a range that spans the Oregonian (generally considered north of Point Conception) and Californian (south of Point Conception) biogeographic provinces (Briggs 1974) and has abundant populations at many of the Channel Islands. Chapter 4 examines hypotheses of gene flow among coastal and island populations within the Southern California Bight.

These chapters have been prepared for submission for publication, and as written, could stand alone. As a result, some of the introductory material in each chapter may be repetitive. Chapter 5 provides a synthesis of the earlier chapters and addresses directions for future research.

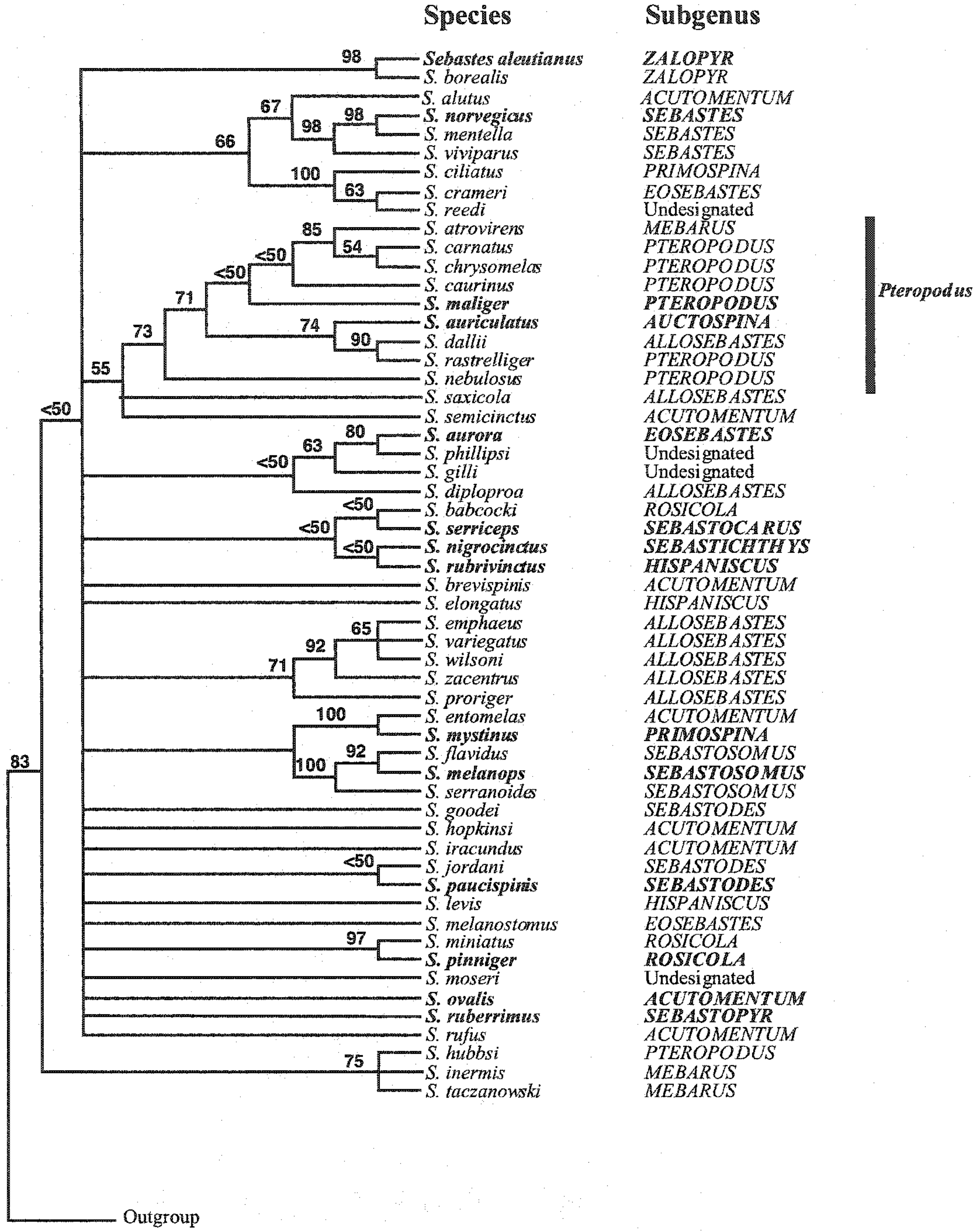

Figure 1.1. Strict consensus tree of 5,825 equally-shortest maximum parsimony trees based on an analysis of 782 base pairs of mitochondrial DNA cytochrome *b* sequence data. This figure was pared post analysis to exclude 14 species from the Subgenus *Sebasomus*; monophyly of this subgenus is covered in Rocha-Olivares et al. 1999) from the basal polytomy for brevity. Bootstrap support values are shown above clades nodes and Bremer support indices are shown below the nodes. Outgroup species included species from *Helicolenus*, *Sebastiscus*, and *Sebastolobus*.

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Chapter 2

Distributions of larval rockfishes, *Sebastes*, identified using molecular methods, suggest a pattern of retention near natal habitat in the Southern California Bight

Abstract

Early-stage larval (<16 mm SL) *Sebastes* spp. were identified using molecular genetic methods from plankton samples collected during a California Cooperative Oceanic Fisheries Investigations (CalCOFI) cruise in April, 1999. These identifications permitted characterization of natal habitat and for the first time allowed estimates of abundance for individual rockfish species in CalCOFI ichthyoplankton samples. For three abundant species, a significant association was found between log abundance and eddies within the Southern California Bight (SCB). A significant relationship between the distribution of 1 day old larvae and potential adult habitat for the most narrowly distributed of the three species was also found. These results suggest that the interplay of natal spawning habitat overlaid persistent eddy features might contribute to larval retention in this region. We also found low abundance and number of occurrences for larvae of the nearshore subgenus *Pteropodus*, suggesting that CalCOFI stations are too far offshore to evaluate distributions of this group.

Introduction

Early life history stages (ELH) of fishes collected during California Cooperative Oceanic Fisheries Investigations (CalCOFI) surveys are a primary source of fishery-independent information used to track abundances of marine fishes off southern California and to examine hypotheses about pelagic dispersal for rockfishes. Larval rockfishes (*Sebastes)* are common and abundant in CalCOFI plankton collections (Moser et al. 2000); more than 50 species occur in the Southern California Bight (SCB) and the majority are utilized in commercial and sport fisheries (Miller and Lea 1972; Eschmeyer et al. 1983). Although there is diversity within the genus, as a group these species are benthic as adults, with high site fidelity described for many (Love 1979; Love et al. 1990). The CalCOFI collections provide a rich source of data for constructing temporal abundance trends in many species; however, larvae of only seven northeastern Pacific *Sebastes* species currently can be identified with confidence using pigmentation and morphological criteria (Moser et al. 1977; Moser 1996). The remainder, grouped as unidentified *Sebastes* spp., ranked 4th in abundance and 2nd in frequency of occurrence over the history of CalCOFI sampling from 1951 through 1998 (Moser et al. 2001).

Quantifying and describing distributions of ELH stages of marine species are goals for fisheries oceanographers, primarily because net tows are easier and cheaper than sampling benthic and/or patchily distributed adults (Lasker 1985). Bottom trawl surveys have been conducted from Washington to California every three years by NOAA Fisheries, but usually are not done south of Point Conception (Rogers et al.

1996; Millar and Methot 2002).

Molecular genetic data, a constant at all life history stages, provides a method to determine species identifications to unidentified larvae by comparison to reference data of known adults. This study presents the first data where the entire complex of *Sebastes* larvae collected during any cruise has been identified to species using a combination of visual and molecular methods. We present larval distribution data at the species level using genetic identifications of the unknown *Sebastes* spp. collected during the April, 1999 CalCOFI cruise and map distributions onto the velocity flow field to examine larval distributions over the course of the early (<42 days) pelagic period. This allowed us to examine the hypothesis that larval abundance is equally distributed inside and outside of the Southern California Eddy (SCE), a persistent feature with a center of low velocity at this time of year in the SCB. We further examined the 1 day old larval distributions of the most abundant species relative to published records of adult depth ranges, which we define as potential adult habitat (PAH), to examine the significance of the association between positive stations and of these larvae with expected PAH.

Materials and methods

Sample Collection

Parturition in *Sebastes* occurs during winter and spring for most species (e.g., Moser et al. 2000); the April, 1999 CalCOFI cruise was selected for analysis because larval *Sebastes* were relatively abundant at that time (Ambrose et al. 2001). Oblique bongo net tows were made following standard CalCOFI protocols (Kramer et al. 1972;

Ohman and Smith 1995) from April 1-15, 1999 aboard the *R/V David Starr Jordan* as part of the regular CalCOFI sampling program. Briefly, a tow was made through the upper 212 m (or from 15 m above the bottom in shallower water) using a flowmetered 71 cm bongo equipped with 0.505 mm mesh nets and towed at a ship speed of about 1 m s^{-1} at each of 61 standard stations (Ambrose et al. 2001). These samples were collected over 15 days at stations along six parallel lines spaced at approximately 70 km intervals and oriented approximately perpendicular to the coast from Avila Beach to San Diego (Figure 2.1). The sample from one side of the bongo net was fixed in 5% sodium borate buffered formalin and the other was fixed and preserved in 95% tris-buffered ethanol that was changed within 24 hours after fixation. Ethanol-preserved samples were collected seaward to station 80; nearly all *Sebastes* larvae occur shoreward of that station (e.g., Moser et al. 2001).

Fish larvae were sorted from the macrozooplankton in the laboratory, identified to the lowest possible taxon, and enumerated. The count data were converted to abundance (number of larvae under 10 m^2 sea surface) by multiplying the larval count by the standard haul factor (SHF = [10• (tow depth/volume of water filtered)]) for each tow (Kramer et al. 1972; Smith and Richardson 1977; Moser et al. 1993). An "OPC adjustment" was made to account for the effect of an optical plankton counter that partially obstructed the opening of the net used for ethanol-fixed samples. This adjustment was calculated by use of a least squares regression between counts of *Sebastes* larvae collected in the obstructed side of the bongo and counts from the unobstructed side ($R^2 = 0.90$, [Formalin = (Ethanol * 1.51) + 1.86]). Morphologically identifiable *Sebastes* larvae (*S. aurora, S. diploproa, S. goodei, S. jordani, S. levis, S.*

paucispinis, and *S. macdonaldi*) were presorted from the ethanol-fixed samples, and the "*Sebastes* spp." component was identified using molecular methods. Larvae from the formalin-fixed side of the bongo were visually identified to the lowest possible taxon.

DNA Extraction and Data Collection

Genomic DNA was extracted from caudal fin or muscle tissue of larvae using a chelex extraction protocol (Walsh et al. 1991). PCR was used to amplify 782 bp of the mtDNA cytochrome *b* gene in a 1X buffer containing 20mM Tris HCl, 50mM KCl, and 1.5mM $MgCl_2$ with 0.3µM of each primer. Primers included previously published GluRF and CB3RF (Rocha-Olivares et al. 1999) and internal custom primers (CB306F 5'-TTACTACGGCTCVTACCT-3, Cb521R 5'-GTTGCATTGTCTACTGAG-3', and CB364F, 5'-CTAGTTATAATAACTGCTTT-3'). The following protocol was used: Hotstart at 90°C for 2:00 min, followed by 36 cycles with denaturing at 92°C for 0:45 min, annealing at 50 °C for 1:00 min and elongation at 72°C for 1:30 min. PCR products were cleaned using Qiaquick kits (Qiagen, Inc.) and cycle-sequenced according to manufacturer protocols using an ABI 3100 automated sequencer. Chromatogram data for sequenced DNA were aligned using the biosequence analysis and editor program Sequencher (v. 4.1.1 Gene Codes, Inc.).

Larval sequences were compared to DNA reference sequence data of 374 independent haplotype sequences representing 67 species of identified adult *Sebastes* using an iterative approach within the software program Phylogenetic Analysis Using

Parsimony (PAUP* 4b10; Swofford 2000) with the optimality criterion set to distance (number of bp differences divided by total bp sequenced). Species included in the PAUP reference file are listed in Appendix 1.

Nonparametric bootstrapping was used (100 replications, MAXTREES set to 1000) to cluster each unknown larval haplotype within a database of consensus haplotypes (consensus = most common haplotype from a database of 1 to 17 known adults, see Appendix I) from known adults for putative identification. If a larva clustered with the single haplotype of a known reference species with a bootstrap value ≥90%, then this was accepted as the identification of the larva. Distance between known reference haplotypes to the unknown was examined to confirm that the unknown fell within the intraspecific diversity expected for the putative identification species as a secondary confirmation of the identification. If a larva clustered with a single haplotype of a species with a bootstrap < 90%, then this was accepted as a first-pass identification and a secondary analysis was performed that included all available haplotypes of at least the three nearest (in distance) species to the unknown larval haplotype, and the haplotype was identified by comparison of sequence to the intraspecific diversity of the putative species. Intraspecific diversity for reference species in the NE Pacific has a mean distance 0.002 (this corresponds to 2 bp difference in 782 bp sequenced) with a minimum of 0 (e.g., *S. jordani*) and a maximum of 0.01 (in *S. aleutianus*). Divergence between species within the 782 bp sequence has been reported between .0013 (e.g., between *S. chlorostictus* and *S. rosenblatti*) and 0.107 (e.g., between *S. helvomaculatus* and *S. inermis*) with a mean and mode of .045 (Taylor 1998).

ADCP and Circulation

Upper ocean currents were measured continuously along the ship track from a hull-mounted acoustic Doppler current profiler (ADCP). The ADCP was an RD Instruments 150 kHz narrowband model, and it was configured to transmit an 8 m pulse every second along 4 beams directed downwards at 30 deg with respect to vertical and equally spaced in azimuth. The recorded data were 3-min vector-averages. The estimates were binned vertically every 8 m. The shallowest depth with good data was 24 m, and the maximum depth range of the profiler was about 350 m. Velocities were calibrated for transducer misalignment and in situ temperature (Pollard and Read 1989) and converted from ship-relative to absolute currents using GPS measurements. The absolute currents were then averaged over hourly intervals, which reduces the errors due to position uncertainty to about 2 cm s^{-1}. Additional errors arise from the aliasing of unresolved short period motions such as tides and near-inertial waves. The barotropic tide was estimated using the OSU global tide model TPXO6.2 (Egbert et al. 1994) and subtracted from collocated ADCP current observations. The barotropic tide predicted by the model was a fairly small signal, 1-3 cm s^{-1}. Near-inertial motions typically have high vertical wavenumber and are largest within the surface layer. To reduce noise from these motions, we averaged the currents vertically from 25 m to 75 m. This layer was below the mixed layer in the SCB during April, 1999 (SIO Ref. 00-6 2000) and corresponded to the upper part of the bongo tow depth where *Sebastes* larvae are often most abundant (e.g. Ahlstrom 1959; Moser and Pommeranz 1998). The averaged and de-tided ADCP velocities were then mapped using a quasigeostrophic streamfunction objective analysis (Chereskin and Trunnell 1996). The smoothing and nondivergence enforced by the mapping further reduces errors in the large-scale velocity field.

Eddy and Potential Adult Habitat Association

We used an analysis of variance to examine the null hypothesis that each species is distributed with an equal abundance inside and outside of eddies within the SCB. We used the map of the ADCP data to categorize stations as inside or outside the SCE and we used the high frequency array of surface currents collected using CODAR over the period of the cruise, available from the Institute for Computational Earth System Science research at the University of California, Santa Barbara, to identify a gyre within the Santa Barbara Channel (www.icess.ucsb.edu/iog/codar_realtime.htm). The fifteen eddy-positive stations included Line 90, stations 28 through 53, Line 86, stations 33 through 55, Line 83, stations 51 and 55 and Line 82, station 47 (e.g., Figure 2.2 between –10 and –15 velocity streamfunction in the SCE). All abundance data (A) were converted to ln(A+1) for the analysis; and it was only possible to test the hypothesis with the most abundant species (*S. hopkinsi*, *S. ensifer*, *S. rufus*, and *S. jordani*). Distributions of the species with at least three occurrences were mapped (and examined) relative to the ADCP (and CODAR) data. The only species with occurrences within the eddy within the Santa Barbara Channel eddy was S. jordani. For other species, eddy positive stations were confined solely to the SCE. Observations for species with fewer thank three occurrences are listed in Table 2.1 along with corresponding locality, abundance and size data.

An analysis of variance also was used to examine the null hypothesis that 1 day old larvae are randomly distributed among stations and no more likely to be found in the vicinity of PAH than over basins (basins are over water between 500 m to > 4,000 m and considered unsuitable habitat). Stations were categorized as to whether they were within 20 km of PAH of being vertically over PAH (based on an approximation of average dispersal distance for coastal rockfish species, Buonaccorsi et al. in prep; Taylor and Vetter in prep). We defined 1 day old larvae by otolith based aging (*S. hopkinsi*: Taylor et al. in prep), or by using length (mm) as a proxy for age (*S. ensifer* and *S. rufus)*, where larval ages were not available. We used published average length at parturition for *S. ensifer* (4.2 mm) and *S. rufus* (4.5 mm), and mapped distributions for larvae within 0.5 mm of this size.

Results

Four *Sebastes* species were identifiable in the formalin-fixed samples with 94% of the remaining larvae classified as "*Sebastes* spp." In the ethanol-fixed samples, an additional 22 species were identified with only 0.64% of the total remaining in the unidentified *Sebastes* spp. category (Table 2.3). For the few ethanol-preserved larvae in the *Sebastes* spp. category, PCR amplification was unsuccessful and identification was not possible; we suspect poor DNA quality, perhaps because the larvae were from an earlier haul that was incompletely washed down, delaying fixation.

Molecular identification resulted in unambiguous identifications for 20 of the 22 species from ethanol-fixed samples. *Sebastes hopkinsi* occurred in highest abundance and most frequently followed by *S. ensifer*, *S. rufus*, and *S. jordani* (Table 2.2). For *S.*

"wilsoni" and *S. "carnatus"*, it was only possible to narrow the identification to three species (*S. variegatus, S. wilsoni and S. zacentrus*) and two species (*S. carnatus and S. chrysomelas*) respectively based on the molecular data available. For cytochrome *b*, it appears that there is incomplete lineage sorting among some closely related sister taxa including these species. Although *S. zacentrus* occurs off southern California, it is much less common than *S. wilsoni* (Love et al. 2002; J. Wagner pers. comm.). Thus, we considered *S. wilsoni* the most likely identification for these larvae, but use quotations to indicate the ambiguity. For the unknown haplotype most closely related to one adult *S. carnatus* haplotype, both *S. carnatus* and *S. chrysomelas* are common in the SCB and either species is a likely identification for these nearshore sister taxa.

The California Current can be seen in objective maps of velocity and velocity streamfunction at 50 m depth as a meander offshore of Station 80, seaward of the continental shelf break (e.g., Figure 2.2). Velocity streamfunction is analogous to dynamic height, with positive streamfunction corresponding to dynamic highs and negative streamfunction corresponding to dynamic lows. The California Current flows south as a core of high velocity at the boundary between dynamically lower coastal waters and the offshore high of the North Pacific subtropical gyre. The change in streamfunction across the core of about 25 m km s^{-1} over roughly 100 km corresponds to currents of magnitude 25 cm s^{-1} that flow parallel to the mapped streamlines. Between the California Current core and the coast there are numerous mesoscale eddies and meanders, with cyclonic circulation corresponding to cold anomalies and anticyclonic circulation corresponding to warm anomalies. These eddies are nonlinear, with amplitudes on the same order as the total height increase across the

California Current, and thus have the potential to transport anomalies (and potentially larvae) offshore (Chereskin et al. 2000; Cornuelle et al. 2000). The census of eddies in the SCB for April 1999 included 3 cyclones and 2 anticyclones. A cyclone/anticyclone pair was observed between lines 80 and 87 near Station 70. A second anticyclone was located at the southwest edge of the study region, centered near line 93 Station 120. A second cyclone was located off Avila, and a third was consistent with the SCE, a persistent feature in the SCB northwest of San Clemente Island (e.g., Figure 2.2). The SCE is weakest in spring; it strengthens with the seasonal appearance of the Inshore Countercurrent and shoaling of the California Undercurrent in summer and fall (Lynn and Simpson 1987; Chereskin and Trunnell 1996). Hence some of the weakest currents observed during April 1999 were within the SCB, and the area of the highest potential retention was over potential adult rockfish habitat.

Larvae of the squarespot rockfish, *Sebastes hopkinsi*, were the most abundant species seen in the survey. Adults have a reported depth to 150 m (Love et al. 2002) but have been observed on ROV transects to 200 m (J. Butler pers. comm.). Squarespot rockfish currently is the most commonly taken rockfish in the southern California recreational fishery, primarily because of the decline in abundance of larger species (Love et al. 2002). We mapped distributions of the 1-42 day old larvae found during the survey relative to the 200 m depth contour to represent PAH. Age-specific larval distributions allowed "tracking" of larvae through the early pelagic period, assuming constant larval input. The data suggested that although larvae were quickly swept away from the immediate vicinity of PAH, the youngest larvae and total larvae were retained in the proximity of the SCE in significantly higher abundance (mean[A]

= 0.16 larvae under 10 m^2) than outside (mean[A] = 0.02) (F_1= 26.13, p = .0001). All 1-d-old larvae were near PAH, with no observations farther than 20 km away.

Sebastes ensifer, the swordspine rockfish, is a small species sold primarily in the Asian fish market and is only a moderate part of the recreational catch, probably because of its small size (Pearson 2000; Love et al. 2002). It has a deeper reported depth range (to 433 m) than *S. hopkinsi*, more similar with other species in the survey. Distribution of larval swordspine rockfish were mapped relative to the 500 m isobath for total larvae, larvae estimated to be 1day old, and postflexion larvae (Figure 2.2). Total larval abundance was significantly higher at eddy stations (mean = 1.868) than at non-eddy stations (mean = 0.691; F_1 = 4.977, p = 0.031). Although a statistically significant association of 1 day old larvae with our definition of PAH was not detected (F_1 = 1.302, p = 0.26), mean abundance over PAH was more than twice (mean[A] = 0.941) that at stations away from PAH (mean[A] = 0.415).

The bank rockfish, *S. rufus*, is often seen in ROV surveys off southern California (J. Butler, pers. comm.), and frequently caught by recreational fishers (Love et al. 2002). It is reported to range to a maximum depth of 454 m. We mapped distributions over a PAH of 500 m (Figure 2.3) and found that larval occurrence was not concordant with the SCE (F_1 = 0.23, p = 0.64), and 1 day old larvae were not more abundant over PAH (F_1 = 0.493, p = 0.49).

Shortbelly rockfish, *Sebastes jordani* often has been considered to have the highest biomass of any rockfish off California (e.g. Ralston et al. 2003). It has little, if any, direct commercial or recreational fishery value (Love et al. 2002). It ranges to a maximum reported depth of 491 m and we mapped larval distributions relative to a

PAH of 500 m (Figure 2.4). Total larvae were significantly more abundant within eddies (mean = 1.15) than outside eddies (mean = 0.243; $F_1 = 7.03$, $p = 0.11$). *Sebastes jordani* was the only *Sebastes* species found in the Santa Barbara Channel. *Sebastes jordani* offered an opportunity to compare catches between the ethanol- (Figure 2.4A) and formalin-fixed sides of the bongo (Figure 2.4B). The highest abundances were concordant between nets and near adult habitat. However, there were positive stations in the ethanol-fixed samples that lacked matching occurrences in the formalin-fixed samples. For the majority of these (4 of 6 stations), this reflects a real lack of *S. jordani* in the formalin-preserved samples; at one of the remaining two stations, two larvae identified as *Sebastes* spp. were quite similar to *S. jordani* and might represent previously unrecognized pigment polymorphism for the species. In the ethanol-fixed samples, 56% of the *S. jordani* larvae were too damaged or distorted to positively attribute to *S. jordani* using visual methods; these could be positively identified only by using molecular methods. The ethanol-fixed larvae ranged from 3.7-6.7 mm and both the ethanol- and formalin-fixed larvae all were preflexion stage.

The blackgill rockfish, *S. melanostomus*, one of the deeper-dwelling species of the survey with a reported maximum depth of 768 m, is important in the Asian fish markets of southern California and is occasionally landed in the recreational fishery. We mapped larval distributions over the 1,000 m depth contour (Figure 2.5A). The larval distribution was largely concordant with PAH and most occurrences were within the SCE, with the highest abundances within the gyre.

The dwarf rockfish, *S. "wilsoni"*, is reported to range to 383 m depth and we mapped the distribution of presumed larval *S. "wilsoni"* relative to a PAH of 500 m.

There was no clear association of preflexion larvae with either PAH or the SCE. Two flexion-stage larvae at CalCOFI stations 87.60 and 90.37, and a postflexion larva at station 93.35 were in the gyre (Figure 2.5B).

Sebastes ovalis, the speckled rockfish, is a part of the commercial catch off southern California. Adults have been found to 366 m (Love et al. 2002) and larval distributions were mapped relative to a PAH of 500 m. Abundances were concordant with both PAH and the SCE (Figure 2.5C).

The aurora rockfish, *S. aurora*, has been important in artisanal fisheries off Newport Beach, California and is landed recreationally off southern California. Its reported depth ranges extends to 768 m (Love et al. 2002). We mapped larval distributions relative to a PAH of 1,000 m and abundance data were concordant with adult habitat, the SCE and with a coastal eddy off Avila, California (Figure 2.5D).

The bocaccio, *S. paucispinis*, is an important sport and commercial fishery species currently considered depleted and subject to a stock rebuilding plan (MacCall et al. 1999). Bocaccio is reported to range to a depth of 478 m (Love et al. 2002) and the larval distribution was mapped relative to a PAH of 500 m (Love et al. 2002). With the exception of nearshore stations off San Diego and Dana Point, California, collections were concordant with the SCE (Figure 2.6A). All larval occurrences were concordant with PAH.

Sebastes saxicola, the stripetail rockfish, is a small species not utilized in sport or commercial fisheries (Love et al. 2002). It can be found to 547 m and we mapped the larval distribution relative to a PAH of 500 m. The postflexion larvae were found at a nearshore station (93.30) while the preflexion larvae were found in association with the SCE, near adult habitat, and at a station too close to the edge of the CalCOFI pattern to determine the closest adult habitat (Figure 2.6B).

Little is known about the pinkrose rockfish, *Sebastes simulator*, but it is thought to occur at depths to 265 m. We used a PAH of 500 m and found the larval distribution to be near PAH but well outside the SCE (Figure 2.6C).

Discussion

This is the first time that the entire complex of *Sebastes* larvae collected during a CalCOFI cruise has been identified to species using a combination of visual and molecular methods. In doing so, we have confirmed seasonal spawning incidence of 22 species and identified the extent of their pelagic larval dispersal pattern in the Southern California Bight during April, 1999. We also present the first attempt at reconciling larval *Sebastes* distribution data with ADCP data collected concurrently.

Identifying the vertical distribution of larvae is important when attempting to understand the degree to which larvae might be vulnerable to Ekman transport and resulting onshore-offshore advection, and when putting larval distributions into the context of physical oceanography at depth. Larval *Sebastes* might show species-specific vertical distributions (Ahlstrom 1959; Sakuma et al. 1999; Nishimoto 2000) and to experience an ontogenetic shift during the pelagic period (Tully and Oceidigh 1989; Doyle 1992). Early larvae, typically occur above the thermocline (Ahlstrom1959; Boehlert et al. 1985; Moser and Boehlert 1991), but not as shallow as the neuston (Tully and Oceidigh 1989; Doyle 1992). Later larvae and juveniles are rarely found deeper than 100 m in depth stratified samples in the SCB (Nishimoto

2000, Watson and Taylor, unpublished data). Thus 25-75 meters was a reasonable depth interval over which to integrate velocity data in this study, but we also examined currents through the water column and observed consistent patterns of flow (i.e., weakly sheared flow) to 100 m depth at positive stations. Larvae would have gained little additional horizontal transport from vertically migrating at this time of year.

Distributions of the two most abundant species were concordant with the SCE. Our data suggest the SCE is a retention mechanism, overlaying some or most of PAH for species in the study. Unlike planktonic spawners that are pelagic as adults (e.g, Pacific sardine, Logerwell and Smith 2001), rockfishes typically release their larvae from demersal source locations. We propose that distributions of 1 day old larvae give the best estimate of rockfish natal habitat. For some species where natal habitat is overlaid by the SCE (e.g., *S. hopkinsi*, not shown, and *S. ensifer,* Figure 2.2B), we found total larval abundance of significantly associated with the SCE and surmise that those larvae are retained in the vicinity of their natal habitat. However, some other species clearly are not retained. For example, presumed 1 d old *S. rufus* (Figure 2.3B) occurred largely outside the SCE and were not concordant with what we expected to be PAH within the SCB. It could be that the *S. rufus* has a deeper than reported depth distribution and that the distribution of smallest larvae do reflect location of natal habitat at this time of year. This suggests that if the earliest larvae are not released within the SCE, then they cannot be retained there. The temporal nature of sampling of the survey limits the generality of our suggestions, but the hypothesis of SCE retention might be further explored by examining ADCP data on additional cruises at this time of year and comparing distributions at times when the SCE is absent. For demersal species with habitat fidelity and liveborn larvae, that the interplay of two key but independent features, PAH overlain by a persistent SCE at the time of year of parturition, might contribute to successful year classes of many species off southern California. The degree to which behavioral factors, swimming ability of these liveborn larvae, might contribute to their retention is still unknown, but probably only becomes important as larvae develop into the flexion stage in the final two months of their pelagic period.

The CalCOFI grid is coarse and our definition of PAH is coarse. Much of the reported depth data for *Sebastes* are from fishermen and may be adjusted as we learn more from ROV and submersible surveys where we can observe rockfishes in their natural habitat. Our plan in this study was to resolve mesoscale features of the essential natal habitat for individual rockfish species. Knowing this, it would be possible to prioritize areas of natal habitat for species of particular interest as targets for marine reserves and more focused sampling (e.g., the Cowcod Closure Area off southern California currently is the subject of high resolution ichthyoplankton surveys). This also would facilitate the characterization of relative production from various regions for purposes of prioritizing real estate in the marine environment. Collecting these data over time during the principal *Sebastes* parturition season (winter and spring) will bring us closer to these goals, but data based on the current CalCOFI grid apparently will be useful primarily for species of rockfish centered offshore and not for those species with more restricted nearshore distributions.

At the outset of the study we expected to find larvae of the subgenus *Sebastes Pteropodus,* a complex of nearshore species heavily targeted by the live-fish fishery

(Walters 2001). This group includes the grass (*Sebastes rastrelliger*), black-and-yellow (*S. chrysomelas*), gopher (*S. carnatus*), copper (*S. caurinus*), quillback (*S. maliger*), China (*S. nebulosus*), calico (*S. dallii*), brown (*S. auriculatus*), and kelp (*S. atrovirens*) rockfishes (Taylor 1998). Few larvae of this subgenus were collected, and the occurrence only at nearshore stations (Table 2.1); the most likely explanation is that CalCOFI stations are too far offshore to assess these nearshore kelp forest species. Nearshore, long-term monitoring projects, like those conducted by the Channel Islands National Park and nearshore ichthyoplankton surveys such as those proposed as part of the Southern California Coastal Ocean Observing System (SCCOOS) are better suited for identifying essential natal habitat for these nearshore species.

Molecular methods are useful for identifying both previously unidentifiable species and damaged or distorted specimens of the visually identifiable species that previously would not have been included in counts of those species. For example, *S. jordani* on cruise 9904 might have been under-reported by more than 50% without molecular identification due to severe distortion of the pectoral fins, a key diagnostic character (see Figure 2.4) and a specimen of *S levis* identified by molecular methods was not visually identifiable in the presort of ethanol-fixed samples (Table 2.1).

During the course of this study, molecular methods of identification improved significantly and costs have decreased sufficiently to allow sequencing to be completed quickly on many individuals. As we increase our database on the intraspecific polymorphism for some species complexes, we are able to use apparently fixed differences between species in the form of single nucleotide polymorphisms to design microarrays (with species-specific binding sites), and microbeads which allow the automated reading of a fluorescent label following species-specific enzyme-ligation. These methods have sufficiently high throughput and are rapid enough to be promising for application aboard ship to collect real-time data.

Acknowledgements

Samples were obtained through CalCOFI in cooperation with California Department of Fish and Game, Scripps Institution of Oceanography, NOAA Fisheries and scientists and crew on the NOAA ship R/V *David Starr Jordan*. I am especially indebted to A. Hayes, D. Griffith, and R. Charter for their assistance in obtaining samples, and A. Brooks and H. Timms for their assistance in the laboratory. I also thank the Oceanids Foundation, the Frieda Daum Urey Scholarship Committee, and the Biological Oceanography Fellowship Committee. The genetic data collection was funded by NOAA Fisheries, the California Department of Fish and Game, California Sea Grant and the Marine Ecological Research Reserves Program 4-M-N. This chapter has been submitted for publication in CalCOFI Reports (2004). Taylor, C. A., W. Watson, T. Chereskin, J. Hyde, R. Vetter. The dissertation author was the primary investigator on this paper.

Table 2.1. Rockfish species for larvae with fewer than 2 positive stations in the survey along with stations, abundance, length, and stage of flexion.

Sebastes sp.	**Observed Station(s)**	**Abundance(s)**	**Length (mm)**	**Flexion**
constellatus	87.50, 83.55	23.67,16.35	3.0-3.8	Preflexion
elongatus	90.53, 87.35	18.09, 9.20	2.9-4.2	Preflexion
semicinctus	90.45, 87.35	9.32, 9.20	4.0 and 17.4	Pre/Postflexion
chlorosticutus	87.35	18.39	3.5 and 3.6	Preflexion
rosaceus	90.60, 87.50	9.86, 7.89	3.2 and 4.6	Preflexion
atrovirens	83.55	16.34	3.4 and 4.1	Preflexion
goodei	90.35	10.4	7.3	Flexion
rufinanus	90.60	9.86	4.5	Preflexion
carnatus	90.45	9.32	7.5	Flexion
rosenblatti	93.55	9.15	5.4	Preflexion
levis	90.53	9.05	4.2	Preflexion
caurinus	93.28	8.73	9.5	Postflexion
diplopora	87.50	7.89	4.2	Preflexion

Table 2.2. List of rockfish species, their abundance under 10 m^2, number of positive stations, larval counts and the mean abundance over all stations and over positive stations from the April, 1999 CalCOFI cruise. Species with the superscript "F" were from the formalin-fixed side of the bongo net.

Sebastes spp.	Total Abundance	Positive Stations	Larval Counts	Mean Ab All Tows	Mean Ab Positive Tows
hopkinsi	979.74	22	116	21.07	44.07
ensifer	843.06	14	91	18.33	60.22
rufus	140.91	9	15	3.06	15.66
jordani	204.92	9	23	4.45	22.77
melanostomus	103.59	5	11	2.25	20.72
"wilsoni"	92.76	7	11	2.02	13.25
ovalis	68.22	3	8	1.48	22.74
jordani^F	64.84	3	13	1.41	21.62
aurora^F	62.56	5	12	1.36	12.51
paucispinis^F	52.37	5	12	1.14	10.47
saxicola	45.63	3	5	0.99	15.21
constellatus	40.02	2	5	0.87	20.01
simulator	28.59	3	3	0.62	9.53
elongatus	27.29	2	3	0.59	13.64
semicinctus	18.51	2	2	0.40	9.25
chlorosticutus	18.39	2	2	0.40	18.39
Sebastes spp.	26.14	3	2	0.57	8.71
rosaceus	17.75	2	3	0.39	8.87
atrovirens	16.35	1	2	0.36	2.38

goodei	10.40	1	1	0.23	10.39
rufinanus	9.86	1	1	0.21	9.86
"carnatus"	9.32	1	1	0.20	9.31
rosenblatti	9.15	1	1	0.20	9.15
levis	9.05	1	1	0.20	9.05
caurinus	8.73	1	1	0.19	8.73
diploproa	7.89	1	1	0.17	7.89
levis^F	4.58	1	1	0.10	4.58

Table 2.3. Comparison of number of species identified, total abundance, number of positive tows and larval counts in the ethanol versus formalin-fixed sides of the bongo for CalCOFI 9904 cruise. Larval counts were used to determine the need for an OPC adjustment to make formalin and ethanol-fixed sides of the bongo comparable.

	Species Identified:	**Total Abundance:**	**Positive Tows:**	**Larval Counts:**
Formalin-Fixed	4+ 94% in *Sebastes* spp.	2521.16	28	577
Ethanol-Fixed	22 + 0.64% in *Sebastes* spp.	2736.26	26	309

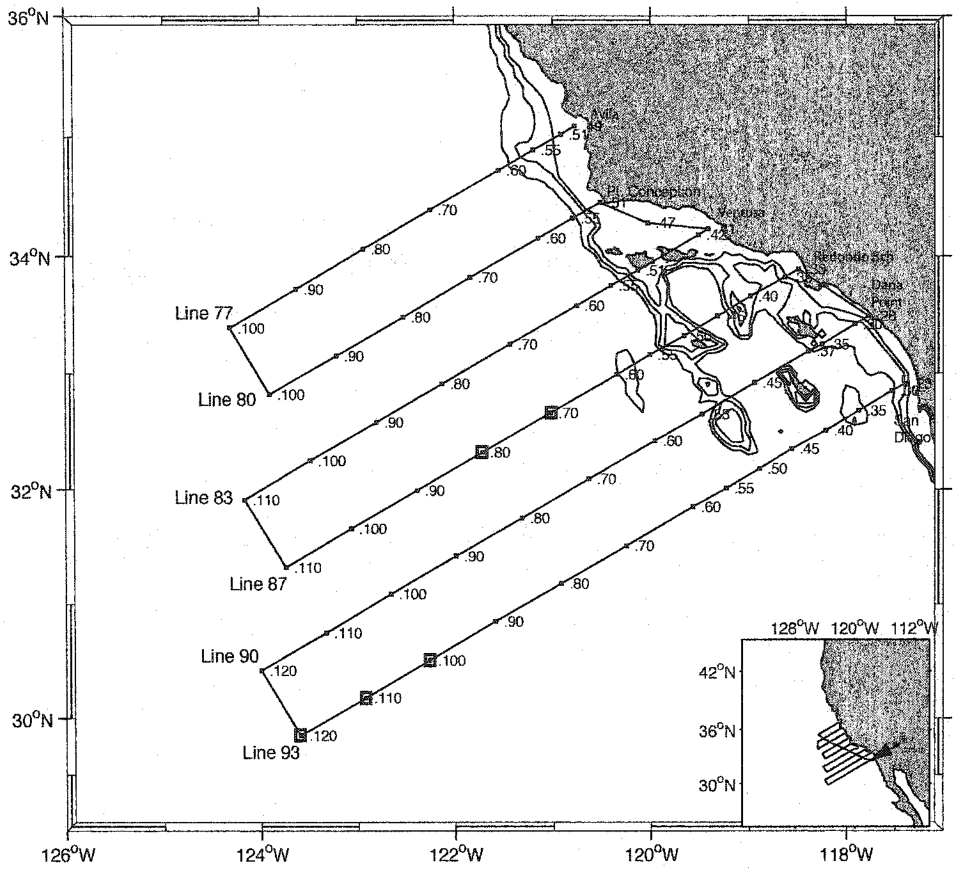

Figure 2.1. Standard CalCOFI stations. Squares indicate stations that were not sampled during the April, 1999 cruise (from Ambrose et al. 2001). Ethanol-fixed samples were collected seaward to station 80 on all lines. Isobaths are 300, 500 and 700 m. Underway acoustic measurements were made up to Monterey (inset). The first station occupancy was 1-Apr-1999 at station 93.30 off San Diego.

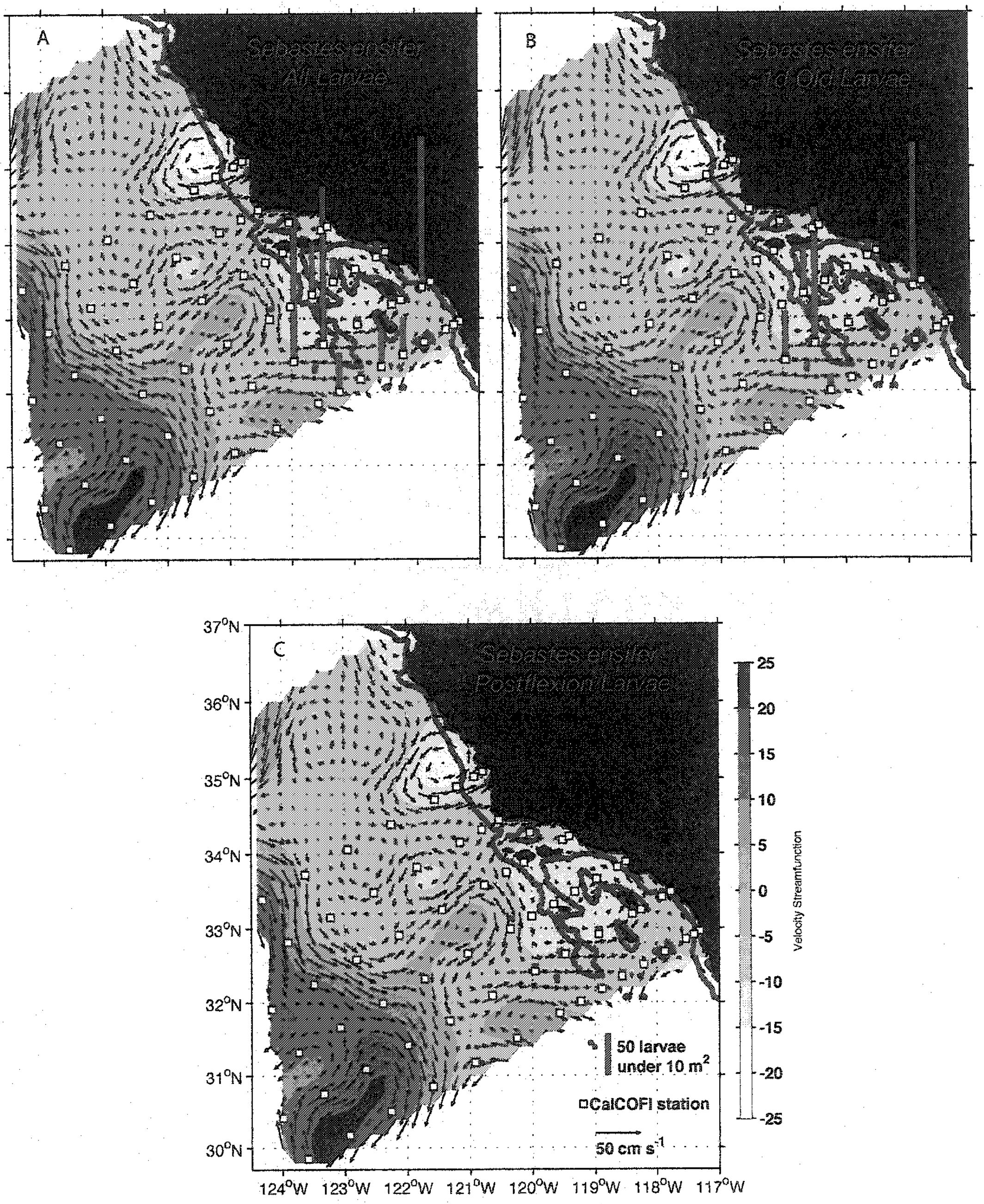

Figure 2.2 Distributions of larval *Sebastes ensifer* off southern California in April, 1999. A, all larvae; B, larvae estimated to be ≤ 1 day old; C, postflexion-stage larvae. The 500 m isobath, indicating potential adult habitat, is shown in blue. The ADCP-derived velocity streamfunction data are shown with shading and current vectors are shown as arrows.

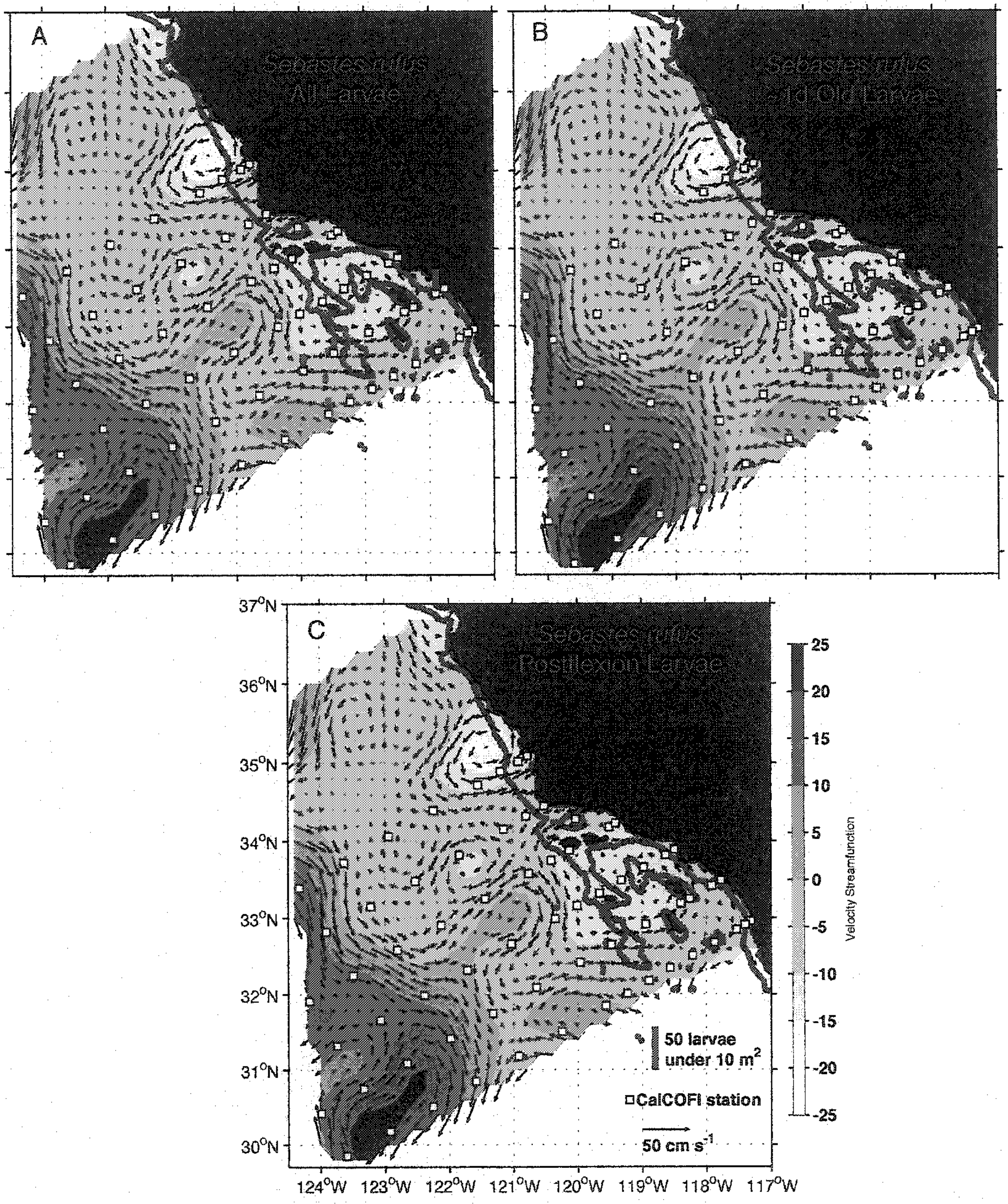

Figure 2.3. Distributions of larval *Sebastes rufus* off southern California in April, 1999. A, all larvae; B, larvae estimated to be ≤ 1 day old; C, postflexion-stage larvae. The 500 m isobath, indicating potential adult habitat, is shown in blue. The ADCP-derived velocity streamfunction data are shown with shading and current vectors are shown as arrows.

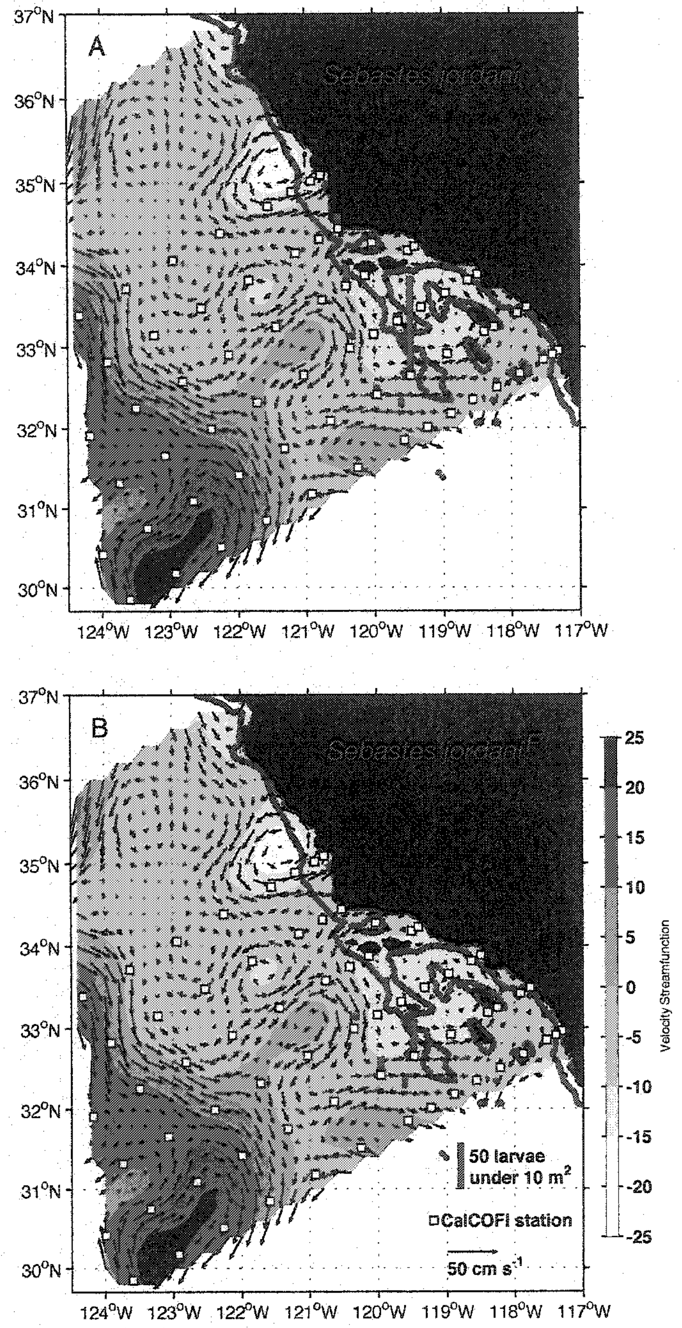

Figure 2.4. Distributions of larval *Sebastes jordani* off southern California in April, 1999. A, all larvae from ethanol-preserved samples; B, all larvae from formalin-preserved samples. The 500 m isobath, indicating potential adult habitat, is shown in blue. The ADCP-derived velocity streamfunction data are shown with shading and current vectors are shown as arrows.

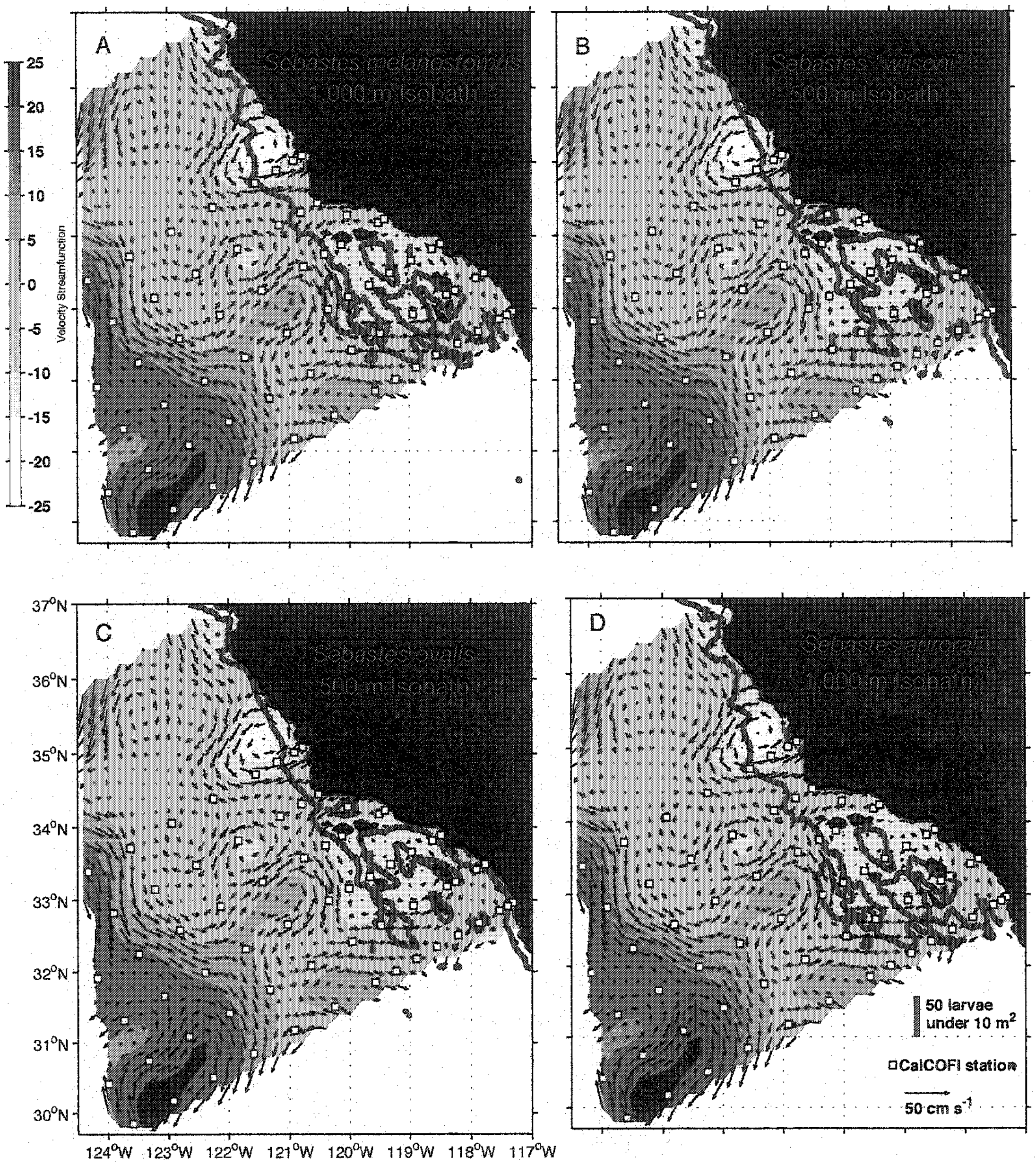

Figure 2.5. Distributions of larval *Sebastes* off southern California in April, 1999. A, *S. melanostomus*, all larvae; B, *S. "wilsoni"*, all larvae; C, *S. ovalis,* all larvae; D, *S. aurora*, all larvae from formalin-preserved samples. Potential adult habitat is denoted by the 500 m (B, C) or 1000 m (A, D) isobaths, shown in blue. The ADCP-derived velocity streamfunction data are shown with shading and current vectors are shown as arrows

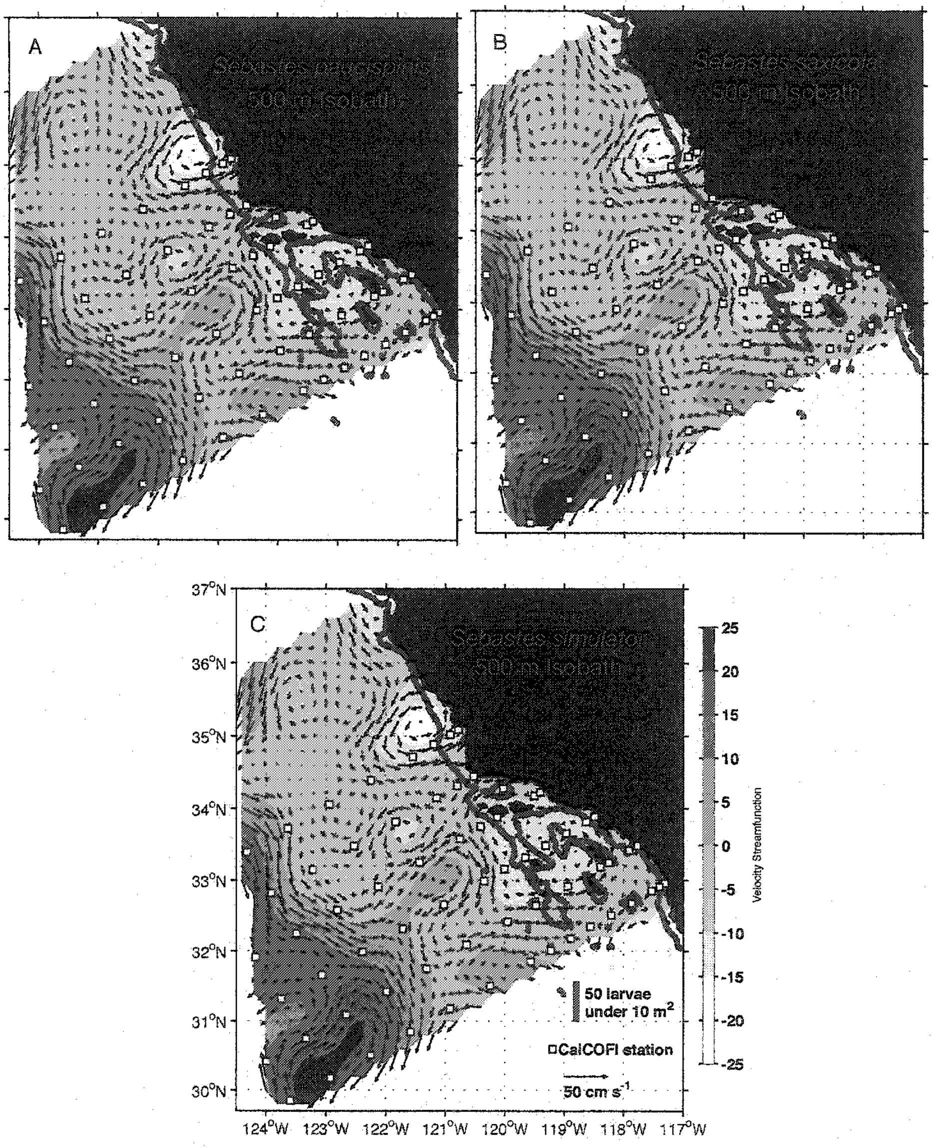

Figure 2.6. Distributions of larval *Sebastes* off southern California in April, 1999. A, *S. paucispinis*, all larvae in formalin-preserved samples; B, *S. saxicola*, all larvae; C, *S. simulator*, all larvae. The 500 m isobath, indicating potential adult habitat, is shown in blue. The ADCP-derived velocity streamfunction data are shown with shading and current vectors are shown as arrows.

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Appendix 2.1. Locality Data for reference adults used in identification of larvae.

Genus Species	**Location**	**Latitude, Longitude**	**Total Haplotypes**
Sebastes aleutianus	Pt. Reyes, CA	37.93°N, 123.46°W	3
S. alutus	Cape Mendocino, CA	40.92°N, 124.42°W	2
S. atrovirens	La Jolla, CA	32.83°N, 117.25°W	9
S. auriculatus	Monterey Bay, CA	36.98°N, 122.20°W	6
S. aurora	Monterey Bay, CA	36.79°N, 122.13°W	7
S. babcocki	San Francisco, CA	37.24°N, 122.72°W	2
S. borealis	Trinidad Head, CA	41.23°N, 124.42°W	2
S. brevispinis	Alaska	49.20°N, 126.74°W	4
S. capensis	off South Africa	N/A	9
S. carnatus	San Luis Obispo Bay, CA	35.14°N, 120.72°W	6
S. caurinus	Punta Baja, Baja California, Mexico	29.89°N, 115.82°W	6
S. chlorostictus	Richardson Rock, CA	34.12°N, 120.55°W	11
S. chrysomelas	Monterey Bay, CA	36.75°N, 122.00°W	1
S. ciliatus	S. Baranof Isl., Alaska	56.08°N, 134.92°W	6
S. constellatus	Cordell Bank, CA	38.00°N, 123.04°W	11
S. crameri	San Francisco, CA	37.58°N, 122.05°W	5
S. dallii	San Luis Obispo, CA	35.14°N, 120.72°W	5
S. diploproa	Monterey Bay, CA	36.77°N, 122.20°W	7
S. elongatus	S. San Clemente Isl., CA	32.74°N, 118.41°W	6

S. emphaeus	Seattle Aquarium, Hood Canal, WA	47.92°N, 122.58°W	1
S. ensifer	60-mile Bank, CA	32.08°N, 118.25°W	7
S. entomelas	Ascension Canyon, CA	36.98°N, 122.58°W	7
S. eos	Palos Verdes, CA	33.70°N, 118.36°W	16
S. exsul	N. Gulf of CA, MX	28.59°N, 113.43°W	5
S. flavidus	Ascension Canyon, CA	36.98°N, 122.58°W	6
S. gilli	Cortes Bank, CA	32.57°N, 119.25°W	2
S. goodei	S. San Francisco, CA	37.25°N, 122.85°W	6
S. helvomaculatus	N. San Francisco, CA	38.26°N, 123.49°W	9
S. hopkinsi	Offshore of The Hood	32.57°N, 119.25°W	17
S. jordani	Ascension Canyon, CA	36.98°N, 122.58°W	11
S. lentiginosus	La Jolla, CA	32.83°N, 117.25°W	2
S. levis	60-mile Bank, CA	32.08°N, 118.25°W	5
S. macdonaldi	Bahia de Los Angeles, Mexico	28.98°N, 113.43°W	9
S. maliger	San Francisco Fish Market	N/A	6
S. melanostomus		32.75°N, 117.75°W	5
S. melanops	Davenport Port Sample	37.02°N, 122.17°W	8
S. melanosema	San Pablo Pt., Baja California, Mexico	N/A	1
S. miniatus	San Clemente Isl., CA	37.74°N, 118.41°W	6

S. moseri	San Clemente Isle, CA	32.63°N, 117.96°W	1
S. mystinus	Pt. Sur, CA	36.28°N, 121.97°W	8
S. nebulosus	San Francisco Fish Market	N/A	6
S. nigrocinctus	Fairweather Grounds, O'Connell AK	58.25°N, 139.00°W	3
S. notius	Uncle Sam Bank, Mexico	25.59°N, 113.37°W	2
S. ovalis	S. San Clemente Isl., CA	32.74°N, 118.40°W	8
S. paucispinis	S. of Monterey, CA	36.82°N, 122.10°W	2
S. phillipsi	Seattle, WA	N/A	4
S. pinniger	Humboldt, CA	41.75°N, 124.08°W	5
S. polyspinis	Off Kodiak Isle, AK	58.40°N, 153.65°W	5
S. proriger	Cape Mendocino, CA	40.56°N, 124.50°W	4
S. rastrelliger	Bodega Bay, CA	38.30°N, 123.10°W	5
S. reedi	Pt. Arena, CA	38.64°N, 123.74°W	1
S. rosaceous	Ascension Canyon, CA	36.98°N, 122.58°W	7
S. rosenblatti	Monterey Bay, CA	36.83°N, 122.16°W	12
S. ruberrimus	Off Inverness, Marin County, CA	38.56°N, 123.62°W	5
S. rubrivinctus	San Clemente Isl., CA	32.74°N, 118.41°W	5
S. rufinanus	San Clemente Isl, CA	32.79°N, 118.33°W	1
S. rufus	Lincoln City, OR	45.74°N, 124.69°W	4
S. saxicola	Pt. Sur, CA	36.83°N, 122.16°W	1

S. semicinctus	Santa Cruz, CA	37.41°N, 122.91°W	6
S. serranoides	Richardson Rock, CA	34.12°N, 120.55°W	6
S. serriceps	Santa Catalina Island, CA	33.28°N, 118.34°W	5
S. simulator	Baja California Norte, Mexico	32.87°N, 117.87°W	10
S. spinorbis	Bahia de Los Angeles, Mexico	28.98°N, 113.43°W	3
S. umbrosus	Between Cortez and 60-mile Banks	32.43°N, 119.11°W	6
S. variegatus	Personnel, Alaska Fisheries	N/A	2
S. wilsoni	Santa Cruz, CA	37.24°N, 122.77°W	5
S. zacentrus	Santa Cruz, CA	37.25°N, 122.85°W	2

Chapter 3

Age-specific Dispersal Patterns of Larval Squarespot Rockfish, *Sebastes hopkinsi*, in the Southern California Bight Identified Using Molecular Methods

Abstract

Early-stage larval (<16 mm SL) *Sebastes hopkinsi* were identified from ethanol-fixed samples collected in the April, 1999 California Cooperative Oceanic Fisheries Investigations quarterly cruise by generating 782 bp of the mtDNA cytochrome *b* for unknown larvae and comparing these data to a reference sequence database of 372 haplotypes representing 65 Northeast Pacific species of *Sebastes*. Larval distributions examined relative to ocean currents suggest an overall pattern of retention in a low velocity zone at the center of the southern California eddy. Distributions of 1 day old larvae show adult natal habitat. These larvae were quickly advected away from natal habitat while still in the preflexion stage, but still showed a significant pattern of younger larvae over shelf and older larvae over basins. Otolith-based methods of ageing were used to backcalculate birthdates and estimate peak parturition prior to and during the survey for larvae up to 42 days of age. An integrated instantaneous mortality (Z) was estimated as 0.054 ± 0.03 for aged larvae. When mortality was modeled into two life stages (preflexion, 0 to 14 days and flexion to postflexion, 15 to 42 days), instantaneous mortality for each of these phases was estimated at 0.20 and 0.021 respectively. Although we did not find statistically significant patterns of cohesion (larvae of the same age and birthdate in the same haul), we found a

significant association of total abundance at positive stations within the Southern California Eddy showing a significantly younger age distributions at stations inside the eddy.

Introduction

The only fishery-independent data collected from southern California in current fisheries assessments for rockfishes is from early life history stages (ELH) collected during California Cooperative Oceanic Fisheries Investigations (CalCOFI) surveys. The CalCOFI program is a cooperative effort among California Department of Fish and Game, National Marine Fisheries Service and Scripps Institution of Oceanography, and is the longest-running fisheries oceanography monitoring program in the world. Rockfishes of the genus *Sebastes* are common and abundant in CalCOFI plankton collections (Moser et al. 2000); more than 50 *Sebastes* species occur in the Southern California Bight (SCB) and the majority are utilized in commercial and sport fisheries (Miller and Lea 1972; Eschmeyer et al. 1983). The CalCOFI collections potentially provide a rich source of data for constructing temporal abundance trends in many species with habitat fidelity off southern California; however, only seven *Sebastes* species can currently be identified with confidence from CalCOFI samples using pigmentation and morphological criteria (Moser et al. 1977; Moser 1996). The remainder, grouped as unidentified *Sebastes* spp., ranked 4th in abundance and 2nd in frequency of occurrence over the history of sampling from 1951 through 1998 (Moser et al. 2001).

Quantifying and describing distributions of ELH stages of marine species are goals for fisheries oceanographers, primarily because net tows are easier and cheaper than sampling benthic and/or patchily distributed adults (Lasker 1985). Bottom trawl surveys are conducted from Washington to California every three years by NOAA

Fisheries, but such surveys are not carried out south of Point Conception (Rogers et al. 1996; Millar and Methot 2002).

Sebastes hopkinsi, the squarespot rockfish, reaches a maximum size of 29 cm and ranges from southern Oregon to central Baja California, and is common in the SCB (Love et al. 2002). They have a maximum reported depth of 150 m, but have been observed to 200 m on ROV surveys off southern California (J. Butler, pers. comm.). The pelagic stage usually lasts 100-120 days, after which juveniles settle over nearshore rocks to depths as shallow as 27 m. Adults are found near rocky habitat where crevices are abundant (Love et al. 2002). With recent declines in abundance of larger rockfish species, squarespots have become one of the most common species on heavily fished reefs where the larger species are absent (Love et al. 2002) and they are becoming an increasingly important component of the southern California recreational fishery. It is also one of the ten most abundant species taken in the annual midwater trawl juvenile-rockfish survey conducted by the Santa Cruz Laboratory of the NOAA Fisheries and is incorporated into an overall rockfish recruitment index. Information on the early life history is totally lacking since early-stage larvae cannot be identified using morphological characteristics. One objective of this study was to provide baseline information on the early life history of squarespot rockfishes using molecular methods to identify this species in the CalCOFI collections. This information includes size specific larval distributions, stage-specific larval distributions and identification of natal or "essential fish habitat" of the adults.

Molecular genetic data, a constant at all life history stages, provides a method through which we may assign species identifications by comparison to reference data of known adults. We present here the first larval distribution data of *squarespot rockfish* using genetic identifications of the larvae from the April, 1999 CalCOFI cruise. We map age-specific distributions of the larvae and overlay these onto the velocity flow field to examine larval dispersal over the course of the pelagic period. We also use the age-specific data, back-calculated birthdates, and data on larval distribution to examine potential evidence for cohort cohesion and retention during the early pelagic period.

Materials and methods

Sample collection

Oblique bongo net tows were made following standard CalCOFI protocols (Kramer et al. 1972; Ohman and Smith 1995) from April 1-15, 1999 aboard the *R/V David Starr Jordan* as part of the regular CalCOFI sampling program. Briefly, a tow was made through the upper 212 m (or from 15 m above the bottom in shallower water) using a flowmetered 71 cm bongo equipped with 0.505 mm mesh nets and towed at a ship speed of about 1 m s^{-1} at each of 61 standard stations (Ambrose et al. 2001). These samples were collected over 15 days along six parallel lines spaced at approximately 70 km intervals and oriented approximately perpendicular to the coast from Avila Beach to San Diego (Figure 3.1). The sample from one side of the bongo net was fixed in 5% sodium borate buffered formalin and the other was fixed and preserved in 95% tris-buffered ethanol that was changed within 24 hours after fixation.

Fish larvae were sorted from the macrozooplankton in the Ichthyoplankton Sorting Laboratory at the Southwest Fisheries Science Center, La Jolla. Subsequently, larvae were identified to the lowest possible taxon and enumerated. The count data were converted to abundance (number of larvae under 10 m^2 sea surface) by multiplying the larval count by the standard haul factor (SHF = [10• (tow depth/volume of water filtered)]) for each tow (Kramer et al. 1972; Smith and Richardson 1977; Moser et al. 1993). In addition an "OPC adjustment" was necessary to account for the effect of an optical plankton counter that partially obstructed the opening of the bongo net used for ethanol-fixed samples. This adjustment was calculated by use of a least square regression between counts of rockfish larvae collected in the obstructed side of the bongo and counts from the unobstructed side ($R^2 = 0.90$, [Formalin = (Ethanol * 1.51) + 1.86]).

Larval Sorting and Morphological Descriptions

One of the initial objectives of this study was to test the hypothesis that larval rockfishes of the subgenus *Sebastes Pteropodus*, which currently cannot be identified from plankton samples, are identifiable at the level of subgenus using traditional morphological and pigmentation characters. Larval *Pteropodus* are typically relatively large at birth (ca. 4.5-5.0 mm body length), slender, and have characteristic pigmentation that seems to distinguish them from other rockfishes, especially during the preflexion stage. The main elements of this apparently unique *Pteropodus* pigment pattern are a long dorsal row of melanophores on the tail, often extending forward onto the trunk, a long ventral row of melanophores on the tail that originates at the last preanal or first postanal myomere, and little or no pigment on the pectoral fins (Watson and Robertson, in press). Thus, during the larval fish identification and enumeration process, unidentified rockfish larvae were further sorted into several categories that can generally be grouped as "*Pteropodus*-like" and "other." The "*Pteropodus*-like" larvae all had a moderately long to long dorsal row of melanophores on the tail, sometimes extending onto the trunk, all had a long ventral row of melanophores on the tail, but in almost all cases originating at postanal myomere 2-6 (usually 3-4) rather than 1, and all had little or no pigment on the pectoral fins. Interestingly, molecular identification revealed that the majority of the "*Pteropodus*-like" larvae were *S. hopkinsi*, not a member of *Pteropodus* (Taylor and Watson in prep).

Otolith-based Ageing

One or both sagittal otoliths were removed from each larva using a dissecting microscope with a polarizing filter between the light source and the slide. A fine probe and hog eyelash were used to clean and manipulate otoliths. Otoliths were attached with an acrylic adhesive to microscope slides and, if necessary, ground against a sintered glass plate using carborundum aluminum oxide lapping film (30 and 9μm). Otoliths were visualized using a Leica compound light microscope under a 400 and 630x magnification (40x and 63X objective with a 10X ocular) and images were created using ImagePro Plus software (v. 4.0, Media Cybernetics). Daily otolith ring counts were made by two independent observers and usually agreed, but in a few cases their counts differed by ≤1 day and were decided upon by a third count.

We used an analysis of covariance to examine the null hypothesis that age distributions of *Sebastes hopkinsi*, is not significantly different for positive stations inside and outside of eddies and over shelf (PAH) and basin (off PAH) habitat in the SCB. We used the map of the ADCP data to categorize stations as to whether they occurred inside or outside the SCE and we used the high frequency array of surface currents collected using CODAR over the period of the cruise, available through the Institute for Computational Earth System Science research at the University of California, Santa Barbara to identify the eddy within the Santa Barbara Channel (www.icess.ucsb.edu/iog/codar_realtime.htm). The fifteen eddy positive stations included Line 90, stations 28 through 53, Line 86, stations 33 through 55, Line 83, stations 51 and 55 and Line 82, station 47 (e.g., Figure 2.2 between –10 and –15 velocity streamfunction in the SCE). Stations were categorized as in the vicinity of PAH if they were within 20 km of being vertically over the 200 m depth contour mapped in Figure 3.2. All abundance data were converted to lognormal abundance for the analysis.

We estimated instantaneous mortality with the caveat that these data should be compiled over multiple cruises for a reasonable estimate of mortality over the course of parturition for the species. We based this estimate on age data (A) and stage data (S) to compare the instantaneous mortality of the lower resolution data (Z_S) to that of the higher resolution data set (Z_A). We also parsed the mortality into life-stage specific instantaneous mortality (Lo 1986) and reported mortality for through the preflexion (P, Z_P) stage and from flexion through to 42 days of age (F, Z_F).

DNA extraction and data collection

Genomic DNA was extracted from tail or muscle tissue of larvae using a chelex extraction protocol (Walsh et al. 1991). PCR was used to amplify mtDNA in a 1X buffer containing 20mM Tris HCl, 50mM KCl, and 1.5mM $MgCl_2$ with 0.3μM of each primer. Primers included previously published GluRF and CB3RF (Rocha-Olivares et al. 1999) and internal custom primers (CB306F 5'-TTACTACGGCTCVTACCT-3, Cb521R 5'-GTTGCATTGTCTACTGAG-3', and CB364F, 5'-CTAGTTATAATAACTGCTTT-3'). The following protocol was used: Hotstart at 90°C for 2:00 min, followed by 36 cycles with denaturing at 92°C for 0:45 min, annealing at 50 °C for 1:00 min and elongation at 72 °C for 1:30 min. PCR products were cleaned using Qiaquick kits (Qiagen, Inc.) and cycle-sequenced according to manufacturer protocols using an ABI 3100 automated sequencer. Chromatogram data for sequenced DNA were aligned using the biosequence analysis and editor program Sequencher (v. 4.1.1 Gene Codes, Inc.).

Larval sequences were compared to DNA reference sequence data of 372 independent haplotype sequences representing 65 species of identified adult *Sebastes* using an iterative approach within the software program Phylogenetic Analysis Using Parsimony (PAUP* 4b10; Swofford 2000) with the optimality criterion set to distance (uncorrected "p" or number of bp differences divided by total bp sequenced). Species included in the PAUP reference file are listed in Appendix 2.1.

Nonparametric bootstrapping was used (100 replications, MAXTREES set to 1000) to cluster each unknown larval haplotype within a database of consensus haplotypes (consensus = most common haplotype from a database of 5 to 20 known adults) from known adults for putative identification. If a larva clustered with the single haplotype of a known reference species with a bootstrap value ≥90%, then this was accepted as the identification of the larva. Distance between known reference haplotypes to the unknown was examined to confirm that the unknown fell within the intraspecific diversity expected for the putative identification species (where possible) as a secondary confirmation of the identification. If a larva clustered with a single haplotype of a species with a bootstrap < 90%, then this was accepted as a first-pass identification and a secondary analysis was performed that included all available haplotypes of at least the three nearest (in uncorrected 'p') species to the unknown larval haplotype. Intraspecific diversity for sampled reference species in the NE Pacific shows a mean distance 0.002 (this corresponds to 2 bp difference in 782 bp sequenced) with a minimum of 0 (e.g., *S. jordani*) and a maximum of 0.01 (in *S. aleutianus*). Intraspecific distance within adult *S. hopkinsi* is 0.0096. Divergence between species within the 782 bp sequence is reported to vary from between .0013 (e.g., between *S. chlorostictus* and *S. rosenblatti*) and 0.107 (e.g., between *S. helvomaculatus* and *S. inermis*) with a mean and mode of .045 (Taylor 1998). The overlap between inter- and intraspecific distances suggests that the ability to differentiate between some closely related sister taxon pairs like *S. chlorostictus* and *S. rosenblatti* using this coding gene depends not only upon examination of overall distance, but direct examination of sequence data or single nucleotide polymophisms contributing to the distance. Even then, closely related sister taxa may not be reciprocally monophyletic.

Larval haplotypes were examined for evidence of cohorts moving or being retained together by constructing a hypothesis of phylogenetic relationships using PAUP to examinine whether most-closely related haplotypes were found in the same haul. Larval haplotypes (N = 118) consisting of 782 bp of the mtDNA cytochome *b* gene were compared to construct a strict consensus tree that essentially clusters closest haplotypes together. Haul data from larvae at various ages were mapped onto the tree to examine the hypotheses 1) that individuals with identical haplotypes (from the same mother) remain together, and 2) that individuals of the same age and birthdate move together (collected in the same haul) and to what age this might be occurring. We considered the tests of the second hypothesis one of cohort cohesion and defined cohort cohesion as the incidence of larvae of the same age and birthdate occurring more often in the same haul than would be expected by chance during the preflexion stage. We tested the null hypothesis by tallying total larvae versus co-occurring larvae across all 22 positive stations in each age/birthdate category from 1 day to 14 days of age within the preflexion stage. We then created an expected distribution of co-occurring larvae by using the counts of total larvae in each age/birthdate category and randomly assigning individuals to hauls over 100 replicates to create a mean number of co-occurring larvae expected at random for each of the age/birthdate categories. We used a nonparametric Kolmogorov-Smirnov comparison to examine the significance of the relationship between expected and observed distributions of co-occurring larvae.

ADCP and Circulation

Upper ocean currents were measured continuously along the ship track from a hull-mounted acoustic Doppler current profiler (ADCP). The ADCP was an RD Instruments 150 kHz narrowband model, and it was configured to transmit an 8 m pulse every second along 4 beams directed downwards at 30 deg with respect to vertical and equally spaced in azimuth. The recorded data were 3-min vector-averages. The estimates were binned vertically every 8 m. The shallowest depth with good data was 24 m, and the maximum depth range of the profiler was about 350 m. Velocities were calibrated for transducer misalignment and in situ temperature (Pollard and Read 1989) and converted from ship-relative to absolute currents using GPS measurements. The absolute currents were then averaged over hourly intervals, which reduces errors due to position uncertainty to about 2 $cm \cdot s^{-1}$. Additional errors arise from the aliasing of unresolved short period motions such as tides and near-inertial waves. The barotropic tide was estimated using the OSU global tide model TPXO6.2 (Egbert et al. 1994) and subtracted from collocated ADCP observations. The barotropic tide predicted by the model was a fairly small signal, 1-3 $cm \cdot s^{-1}$. Near-inertial motions typically have high vertical wavenumber and are largest within the surface layer. To reduce noise from these motions, we averaged the currents vertically from 25 m to 75 m. This layer was below the mixed layer in the SCB during April, 1999 (SIO Ref. 00-6 2000) and corresponded to the upper part of the bongo tow depth where *Sebastes* larvae are often most abundant (e.g. Ahlstrom 1959; Moser and Pommeranz 1998). The averaged and de-tided ADCP velocities were then mapped using a quasigeostrophic streamfunction objective analysis (Chereskin and Trunnell 1996). The smoothing and nondivergence enforced by the mapping further reduces errors in the large-scale velocity field.

Results

Areal Distribution

The data presented here are from plankton collections taken at CalCOFI station 80 and all stations shoreward of station 80 along the 6 lines that constitute the present survey pattern (Figure 3.1). The overall mean abundance was 21.72 larvae under 10 m^2 (SD= 55.81) with a mean abundance at positive stations of 44.07 (N=22, SD=74.93) and *S. hopkinsi* represented the most abundant of 22 species identified in the survey, comprising almost one-third of abundance for total *Sebastes* observed (980 of 2916 total larvae per $10m^2$ sea surface). The inner third of the CalCOFI survey pattern is situated over the continental shelf and slope, whereas the outer two-thirds (stations 70-120, Figure 3.1) is over deep basin (to 4,000 m) and the unidentifiable component *Sebastes* spp. are not often found in this region (Moser et al. 1993; Moser et al. 2000).

The mean age of larvae inside of eddies was significantly lower (mean = 9.24) than outside of eddies (mean = 15.62; $F_1 = 5.81$, $p= 0.02$), although *S. hopkinsi* was found strictly within the SCE in the SCB with no positive tows within the Santa Barbara Basin. There was no significant difference in age distributions over PAH (i.e., younger larvae were not more inclined to be found over PAH than older larvae; $F_1=1.46$, $p = 0.23$), nor was there an overall association of total abundance with PAH at the 0.05 significance level ($F_1 = 3.10$, $p = 0.07$). There was an overall effect of the eddy with overall distributions of *S. hopkinsi*, in that they were more likely to be found in association with the eddies over all age groups than over basins (non PAH; $F_1 = 40.50$, $p < 0.0001$).

Modeling mortality integrated from parturition to 42 days resulted in instantaneous mortalities for staged ($Z_S = 0.11 \pm 0.08$, Figure 3.2A) and aged larvae ($Z_A = 0.054 \pm 0.03$, Figure 3.2B) that were within the confidence interval of the slope of each regression. Slopes of the regression corresponding to estimates of larval abundance at parturition (y-intercept) of 223 (staged) and 46 (aged) larvae per 10 m^2 sea surface at positive stations. The 95% confidence intervals for these estimates are much larger for the staged data (24-2,208 larvae per 10m^2 sea surface; Figure 3.2A) than for aged data (27-81 larvae; Figure 3.2B) due to the lower resolution of the staged data. A two life-stage model of mortality appears to better fit the age data ($R^2 = 0.65$ vs $R^2 = 0.36$ for the single stage model; Lo 1986). Estimated instantaneous mortality through the preflexion stage (14 days of age) was $Z_P = 0.20$ ($\pm$ 0.16) and much lower, $Z_F = 0.021$ ($\pm$ 0.03) from flexion through to 42 days of age. The estimate of larval abundance at parturition corresponding to the two-stage-specific mortality was 135 larvae with 95% confidence interval of 36-504 larvae per 10 m^2 sea surface. Estimates of mortality are only possible with the data presented here by making a series of assumptions including: 1) the simplifying assumption about the larval input (constant over the period of sampling and estimation of birthdates) catch curve in Figure 3.4A representing a larval release (“spawning”) curve; 2) that early stage larvae are not more likely to be extruded from the net than later stages; and 3) that older larvae are not showing higher net avoidance over younger larvae. Because of the issue of net avoidance with increasing age, estimates that include older stages are likely to overestimate instantaneous mortality that includes these stages. We also have a single cruise and are able to represent mortality only through 42 days of age in the pelagic period yet *Sebastes hopkinsi* are pelagic over 100 days (Love et al. 2002; Sakuma and Laidig unpublished data), and the presettlement juvenile phase can be important in identifying patterns in subsequent recruitment to the fishery (Ralston and Howard 1995; Nishimoto 2000; Ralston et al. 2003).

Larval Distributions Relative to Physical Oceanography

The California Current can be seen in objective maps of velocity and velocity streamfunction at 50 m depth as a meander offshore of Station 80, seaward of the continental shelf break (Figure 3.3 A-D). Velocity streamfunction is analogous to dynamic height, with positive streamfunction corresponding to dynamic highs and negative streamfunction corresponding to dynamic lows. The California Current flows south as a core of high velocity at the boundary between lower coastal waters and the offshore high of the North Pacific subtropical gyre. The change in streamfunction across the core of about 25 $m{\bullet}km{\bullet}s^{-1}$ over roughly 100 km corresponds to currents of magnitude 25 $cm{\bullet}s^{-1}$ that flow parallel to the mapped streamlines. The census of eddies in the SCB for April 1999 included 3 cyclones and 2 anticyclones. A cyclone/anticyclone pair was observed between lines 80 and 87 near Station 70. A second anticyclone was located at the southwest edge of the study region, centered near line 93 Station 120. A second cyclone was located off Avila, and a third was consistent with the southern California eddy (SCE), a persistent feature in the SCB northwest of San Clemente Island (Figure 3.3A-D). The SCE is weakest in spring; it strengthens with the seasonal appearance of the Inshore Countercurrent and shoaling of the California Undercurrent in summer and fall (Lynn and Simpson 1987; Chereskin and Trunnell 1996). Hence some of the weakest currents observed during April 1999 were within the SCB.

The preflexion category contained larvae that had not begun notocord flexion and included 1 day old larvae (Figure 3.3A) as well as others with a mean age of 9.6 days and maximum age of 14 days (Figure 3.3B). Flexion-stage larvae had a mean age of 16.8 days and ranged in age from 9-25 days. Postflexion-stage larvae averaged 31.8 days of age with a range of 18-42 days.

There were 29 birthdates represented by larvae collected over the cruise, with the maximum abundances of 204 and 181 larvae under $10m^2$ corresponding to parturition dates of 6-Apr-1999 and 7-Apr-1999 respectively (Figure 3.4A). This peak in parturition followed the full moon event on 31-Mar-1999 (Figure 3.4A). The analysis of potential minimum number of maternal "spawners" consisted of examining single larval counts (not abundance data), overlaying station data where each larva was found along with estimated age of the larva, and comparing these data with other larvae sharing the same estimated birthdate (e.g., Figure 3.3). Larval haplotypes were used to construct a strict consensus tree of 1,000 equally-shortest minimum evolution trees to examine the relatedness of larvae released on estimated birthdates and to test the hypothesis of cohesion.

The dates within our data with the highest larval release activity were 6- and 7-Apr-1999. The clearest pattern of females releasing young on the same day was on 7-Apr near San Nicolas Island and, although there were examples of potential siblings in that collection, there was evidence for larvae from a minimum of 18 maternal haplotypes at Station 87.50 (Figure 3.4C). Multiple females releasing young on the same day was also evident on 6-Apr, where the release of young occurred nearshore, with a minimum of 12 females identified (Figure 3.4B, Station 87.33). Two day old larvae with this birthdate were collected at the offshore stations 87.50 and 87.55, suggesting synchronous larval release (within one day) during the 6-7 Apr peak at both onshore and offshore stations. Simultaneous release was also seen on 11-Apr near Point Conception (Station 80.51) and near Santa Cruz Island (Station 83.42).

There were three instances on 6-Apr-1999 where 2 day old larvae with identical haplotypes were found at the same station (Figure 3.4B, Station 87.50). We found no evidence that larvae with identical haplotypes were found together beyond 2 days of age. There were instances of cohesion of larvae, where larvae of the same age and birthdate were found together. For example, this was seen for 1 and 2 day old larvae already discussed with estimated birthdates of 6 and 7-Apr-1999 (Figure 3.4B and C), 1, 2 and 3 day old larvae born on 5-Apr-1999 (not shown), 2 and 3 day old larvae born on 4-Apr-1999 (not shown), 3 d old larvae born on 3 Apr-1999 (Figure 3.5H) and 8-Apr-1999 (Figure 3.5I), 5 d old larvae born on 1-Apr-1999 (Figure 3.5F), and 14 day old larvae born on 25-Mar-1999 (Figure 3.5D). We used a Kolmogorov-Smirnov comparison and found no significant difference between expected (based on random assignment over 100 replications) and observed counts of larvae caught in hauls with other larvae with the same age and birthdate (P = 0.861; Table 3.1).

Discussion

The overall distribution pattern of larvae observed in CalCOFI 9904 showed a significant association of *Sebastes hopkinsi* with the SCE identified during the cruise. Between the California Current core and the coast there are numerous mesoscale eddies and meanders, with cyclonic circulation corresponding to cold anomalies and anticyclonic circulation corresponding to warm anomalies. These eddies are nonlinear, with amplitudes on the same order as the total height increase across the California Current, and thus have the potential to transport anomalies (and potentially larvae; Chereskin et al. 2000; Cornuelle et al. 2000). The age of larvae found within the eddy were significantly younger and more abundant, but these stages were not found in significant association with the shelf habitat where they were released.

Distributions of 1 day old larvae (Figure 3.3A) suggest natal habitat for *S. hopkinsi*. Positive stations include those nearest adult habitat off Point Conception, Santa Rosa Island, Ventura, Redondo Beach, Tanner and Cortez Banks, and Dana Point with the highest abundances near San Nicolas Island, and offshore of Redondo Beach. Distributions of preflexion-stage larvae are less reflective of adult natal habitat because they include larvae that have been in the plankton long enough to be advected from natal source locations. Distributions of preflexion larvae include stations along the southernmost CalCOFI line and near Tanner and Cortez Banks (Figure 3.3B), along with those of 1 day olds. Preflexion-stage larvae appear to be correlated with the SCE and are primarily over or near adult habitat. Larvae undergoing flexion showed distributions primarily in the southern region of the SCB, with the highest abundance offshore near Tanner and Cortez Banks, also associated with the SCE. Postflexion larvae are expected to be the most competent swimmers, increasingly with development to the pelagic juvenile stage. Postflexion-stage larvae were found primarily in the vicinity of potential adult habitat. Positive stations included those near San Nicolas and Santa Catalina Islands, offshore between Malibu and Paradise Cove, and offshore of La Jolla. An anomalous occurrence offshore at station 80.80 was well off continental shelf habitat and might represent an individual entrained in the offshore flow around the anticyclonic gyre.

The purpose of ageing and mapping larval distributions is to gain insight into ELH stages. One is to allow for parsing the youngest larvae, 1 day old larvae, to use their distributions as a proxy for natal habitat, critical species-specific information. The significant association of younger larvae with eddies suggests that these young stage larvae, although capable of some swimming behavior, are probably not controlling their placement over adult habitat. The lack of a significant difference in age distributions over PAH versus non-PAH in not unexpected. As larvae progress to postflexion, their ability to swim improves and the degree to which behavioral factors might be important in controlling their placement can be surprising (Leis and Carson-Ewart 1997, 1998). The distribution of postflexion *Sebastes hopkinsi* shows a proximity to adult habitat despite another 70+ anticipated days in the planktonic phase beyond the oldest larva found in our data. This would suggest a inverted dome-shaped relationship of age with PAH in *S. hopkinsi* with early-stage and late stage larvae correlated with PAH and middle-aged larvae (flexion stage) being advected away from PAH. This would suggest that larvae, rather might have both a mechanism of retention in the vicinity and within that retention zone they might be able to then navigate to PAH for future settlement.

Mortality is expected to have an important impact on larvae moving off of adult habitat and it is difficult to separate the factors of expatriation from PAH (e.g., due to advection) from disappearance of larvae due to mortality. We estimated mortality three ways in order to demonstrate the potential, as a cost-effective measure and in an attempt to gain some life history data where so little are available for *Sebastes*, to estimate mortality using stages rather than ages once the baseline data on the parameters of stages is available for individual species. Data on number of days spent in preflexion, flexion or postflexion stages is expected to be somewhat temperature dependent and between quarterly cruises and over many years would require obtaining age data to corroborate the parameters (number of days comprised in each stage). Fisheries management currently works with little early life history data on *Sebastes* and this is suggested as a method of obtaining an indication of mortality across species while we pool resources to obtain the best available data for fisheries management. The two-stage model of mortality that provides a $Z_P = 0.20$ ($\pm$ 0.16) is probably the best estimate of mortality presented here. It excludes late stage larvae probably capable of net avoidance and incorporates the high-resolution age data. Staged data provided an estimate of mortality of $Z_S = 0.11$ ($\pm$ 0.08) that may be an underestimate, but may provide the best available data for species where we may obtain identifications but not have the resources to do age data on all larvae.

The concordance to the SCE of young and overall larvae (Figures 3.3A-D) showed an overall pattern that suggests larvae are retained in the SCE. There were few positive stations in the data seen seaward of Station 50, and only a single positive station offshore of Station 60. Similar patterns are seen in other rockfish species (e.g. cowcod and bocaccio) that share similar adult site fidelity to *S. hopkinsi* (Moser et al. 2001), although a deeper overall preferred adult habitat (Love et al. 2002). There were also few stations in the northern region of the SCB and none north of Point Conception. With the exception of the single larva collected at station 80.80, all positive stations were either nearshore and/or within the SCE, or at a station with currents showing zero or onshore trajectories. This suggests a mechanism for retention, but for rockfish this mechanism is probably coupled with some behavioral adaptation on the part of the larvae (Nishimoto 2000). The *S. hopkinsi* distributions should be compared to other species-specific distributions that share similar adult habitat to determine the extent to which retention within the SCE appears to be consistent across species within this cruise. Unfortunately, there were no other species with a distribution limited to 200 m with more than 2 positive station in the CalCOFI 9904 cruise with which to compare these data.

Another line of evidence suggesting retention in the SCB is a repeated pattern of larvae sharing the same age and birthdate within a single line or station from one another. This suggests that larvae, while not remaining together on a scale of meters, are retained through some mechanism on a scale of kilometers of one another from the time of their estimated birthdate. We were unable to demonstrate statistically that larvae with identical age and birthdates occur together through the preflexion stage in a single haul more often than expected by chance with our limited data set, however with more data, this pattern might hold through to 5 day old larvae. The mechanism expected for such cohesion is advection of larvae together in parcels of water, where dispersal might be highly dependent upon local hydrography.

Identifying the vertical distribution of larvae is important when attempting to understand the degree to which larvae might be vulnerable to Ekman transport and resulting onshore-offshore advection, and when putting larval distributions into the context of physical oceanography at depth. Larval *Sebastes* might show species-specific vertical distributions (Ahlstrom 1959; Sakuma et al. 1999; Nishimoto 2000) and experience an ontogenetic shift with development during the pelagic period (Tully and Oceidigh 1989; Doyle 1992). For early larvae, distributions have been found primarily above the thermocline (Ahlstrom1959; Boehlert et al. 1985; Moser and Boehlert 1991). However, as a complex they do not appear to preferentially occupy the neuston (Tully and Oceidigh (1989; Doyle 1992) and are bounded at depth, as larval and juvenile *Sebastes* are rarely found deeper than 100 m in depth stratified samples in the SCB (Nishimoto 2000, Watson and Taylor unpublished data). It appears that 25-75 meters was a reasonable depth interval over which to integrate velocity data for species within the study, but we also examined currents through the water column and observed consistent patterns of flow (i.e., weakly sheared flow) to 100 m depth at positive stations. Larvae thus would have gained little additional horizontal transport from migrating vertically at this time of year.

Sebastes hopkinsi can spend over 100 days in the pelagic zone (Sakuma and Laidig unpublished data) and although postflexion larvae appear to be near potential adult habitat where settlement is possible, it may be up to two months before the late stage larvae transform to the benthic juvenile stage, when year class strength likely is determined (Bradford 1992, Ralston and Howard 1995). As the number of preferred sportfishing species, (e.g., cowcod and bocaccio) have dwindled at the offshore banks, species like *S. hopkinsi* are increasing in frequency in the sportfishing catch off southern California (Love et al. 2002). The CalCOFI quarterly sampling program offers an ideal context to identify these transitions at annual and decadal scales not possible for rockfish species prior to the advent of molecular methods of identification. To identify trends in mortality and abundance, and important early life history factors such as frequency and time larval release for many species such as *S. hopkinsi*, it is clear that methods of molecular identification of larvae and otolith-based ageing integrated with the physical oceanography of the region will be critical for understanding changes over time.

Acknowledgements

Samples were obtained through CalCOFI in cooperation with California Department of Fish and Game, Scripps Institution of Oceanography, colleagues in the NOAA Fisheries and scientists and crew on the NOAA ship the R/V *David Starr Jordan*. I am especially indebted to A. Hayes, D. Griffith, and R. Charter, for their assistance in obtaining samples and data to support this work, and to J. Butler and J. Hyde for the use of valuable resources and feedback in the laboratory, respectively. I also wish to thank the Oceanids Foundation and the Frieda Daum Urey foundation for their generous endowment. The genetic data collection was funded through NOAA Fisheries base funds to the Fisheries Resources Division, through support from Scripps Institution of Oceanography, and through the Marine Ecological Research Reserves Program 4-M-N. And finally, I wish to thank my committee for their thoughtful comments and helpful suggestions on all aspects of the work. This chapter is intended to be submitted in full as a collaborative publication. Taylor, C. A., W. Watson, T. Chereskin, A. Henry, R. Vetter. The dissertation author was the primary investigator on this paper.

Table 3.1. Total counts of larvae, and the counts of observed and expected larvae sharing the same age and birthdate in a haul. Results of Kolmogorov-Smirnov comparison showing observed and expected distributions.

	Total Counts	**Observed**	**Expected**
1 day	45	40	39.31
2 day	23	19	14.85
3 day	12	11	4.65
4 day	2	0	0.14
5 day	4	2	0.57
6 day	0	0	0
7 day	1	0	0
8 day	0	0	0
9 day	2	0	0.14
10 day	2	0	0.06
11 day	1	0	0
12 day	1	0	0
13 day	0	0	0
14 day	5	2	1.04
K-S Test	D = .2143	p=.862	

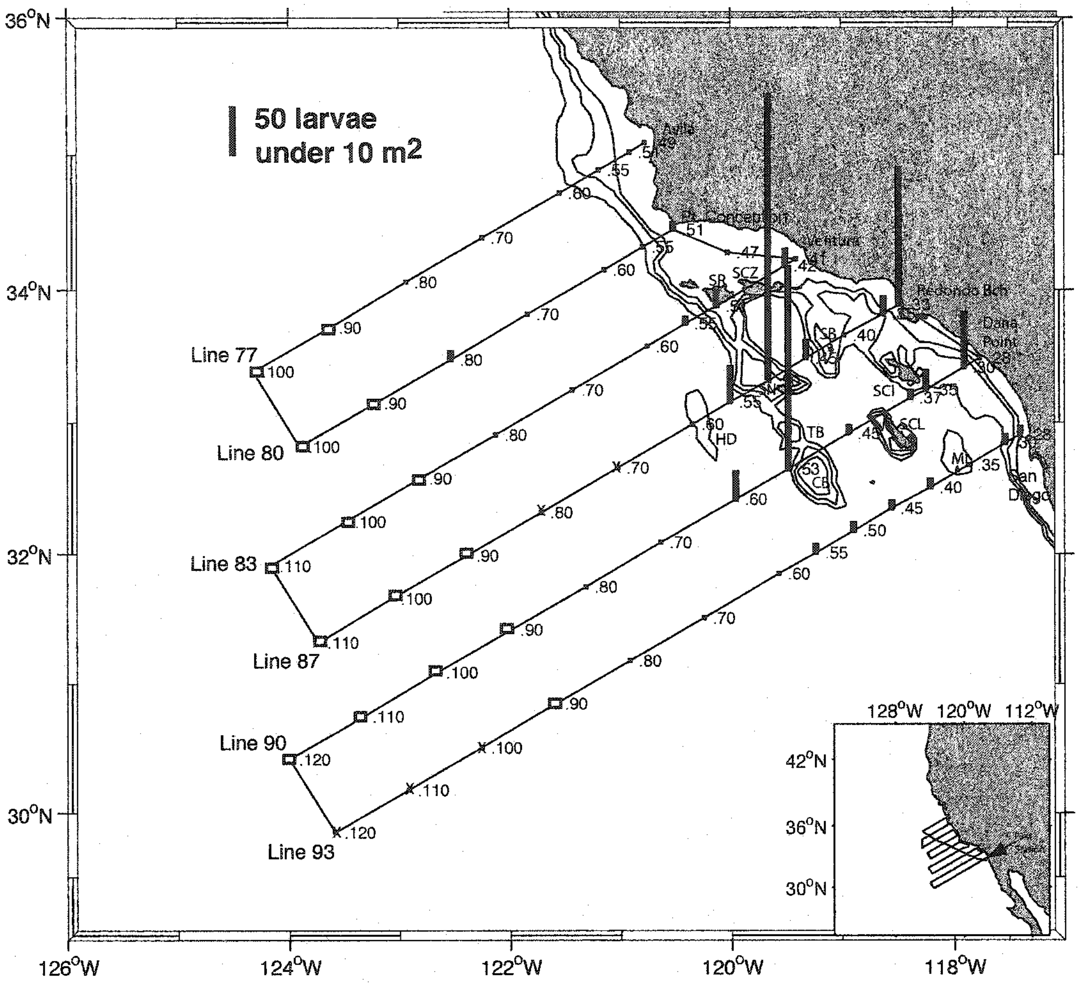

Figure 3.1. Abundance of all larval *Sebastes hopkinsi* from ethanol-fixed samples collected on the April-1999 CalCOFI cruise; these samples were collected seaward to station 80 on all lines (closed squares). An "X" indicates stations that were not sampled, primarily due to poor sea conditions, during the survey. The 300, 500 and 700 m isobaths also are shown. Abbreviations indicate locations discussed relevant to larval distributions: SR, Santa Rosa Island; SCZ, Santa Cruz Island; SN, San Nicolas Island; SB, Santa Barbara Island; HD, Hood Bank; TB, Tanner Bank; SCI, Santa Catalina Island; CB, Cortez Bank; SCL, San Clemente Island; MI, 41 Mile Bank. Underway measurements were made up to Monterey and the inset shows the shiptrack. The first station of the pattern was conducted 1-Apr-1999 at 93.30 off San Diego.

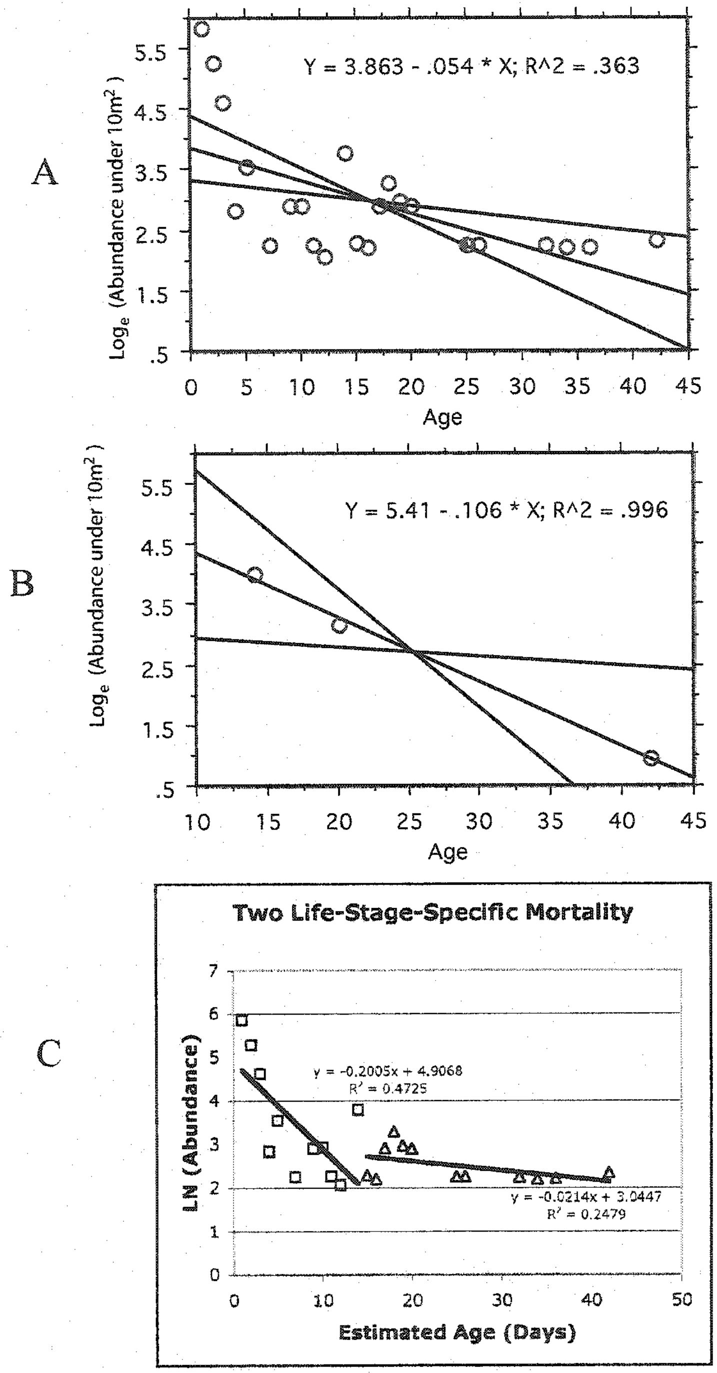

Figure 3.2. Mortality curves used to estimate instantaneous mortality rates during the early stage of the pelagic period for *S. hopkinsi* assuming equal sampling across life stages and constant larval input. A, Integrated mortality curve for age data, B, Integrated mortality for stage data corresponding to preflexion, flexion and postflexion stages, and C, life-stage specific mortality parsed into preflexion and flexion+postflexion mortality components.

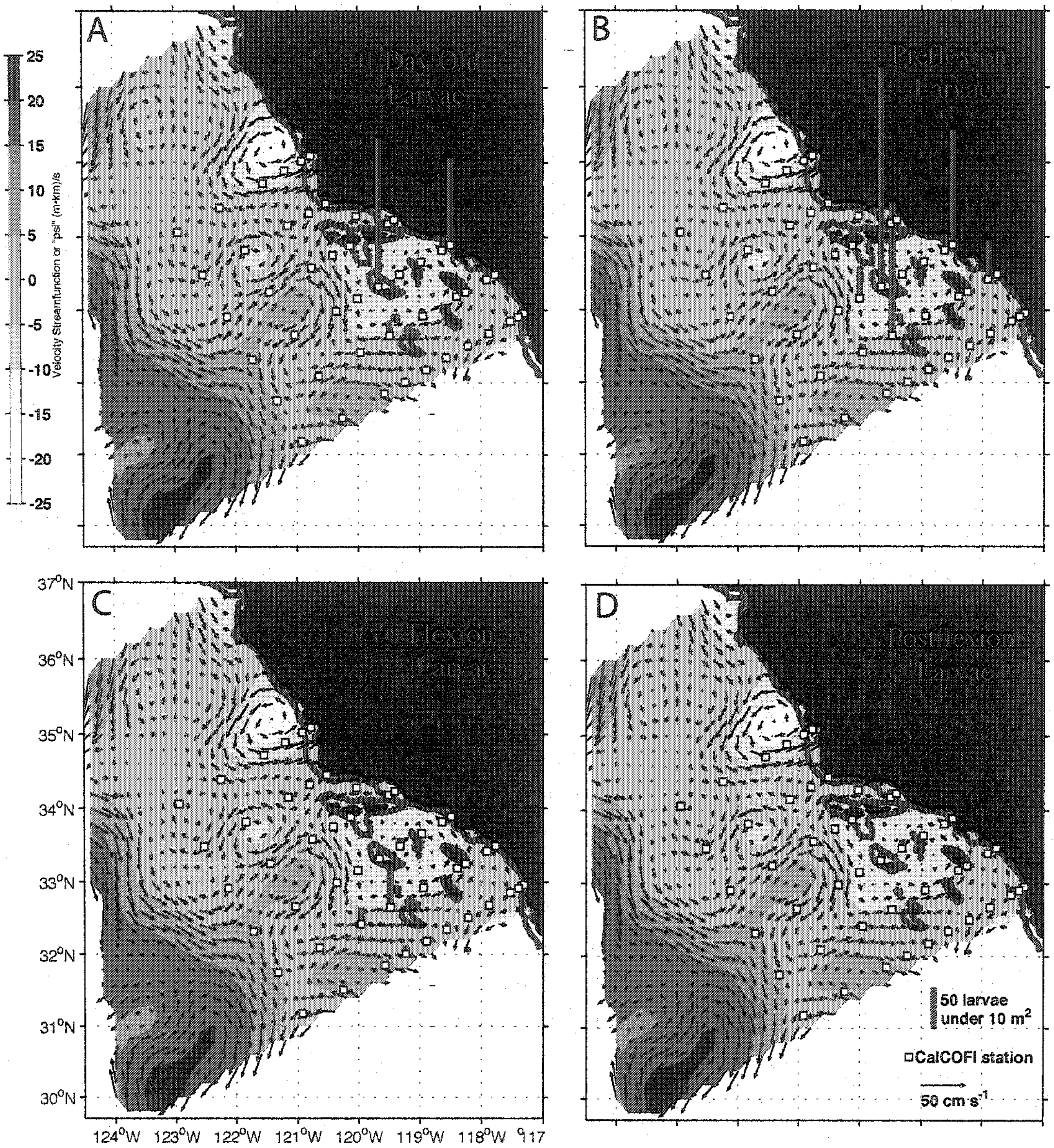

Figure 3.3. Abundance of *Sebastes hopkinsi* larvae during the April, 1999 CalCOFI cruise overlaid onto vector velocities (arrows) and velocity streamfunction (shading) from ADCP data averaged over the 25-75 m depth interval. The 200 m contour representing deepest known adult habitat for *Sebastes hopkinsi* (Eschmeyer et al. 1983) is shown in blue. A, 1 day old larvae; B, Preflexion larvae; C, Flexion larvae; D, Postflexion larvae. Open squares depict stations where ethanol-fixed samples were taken.

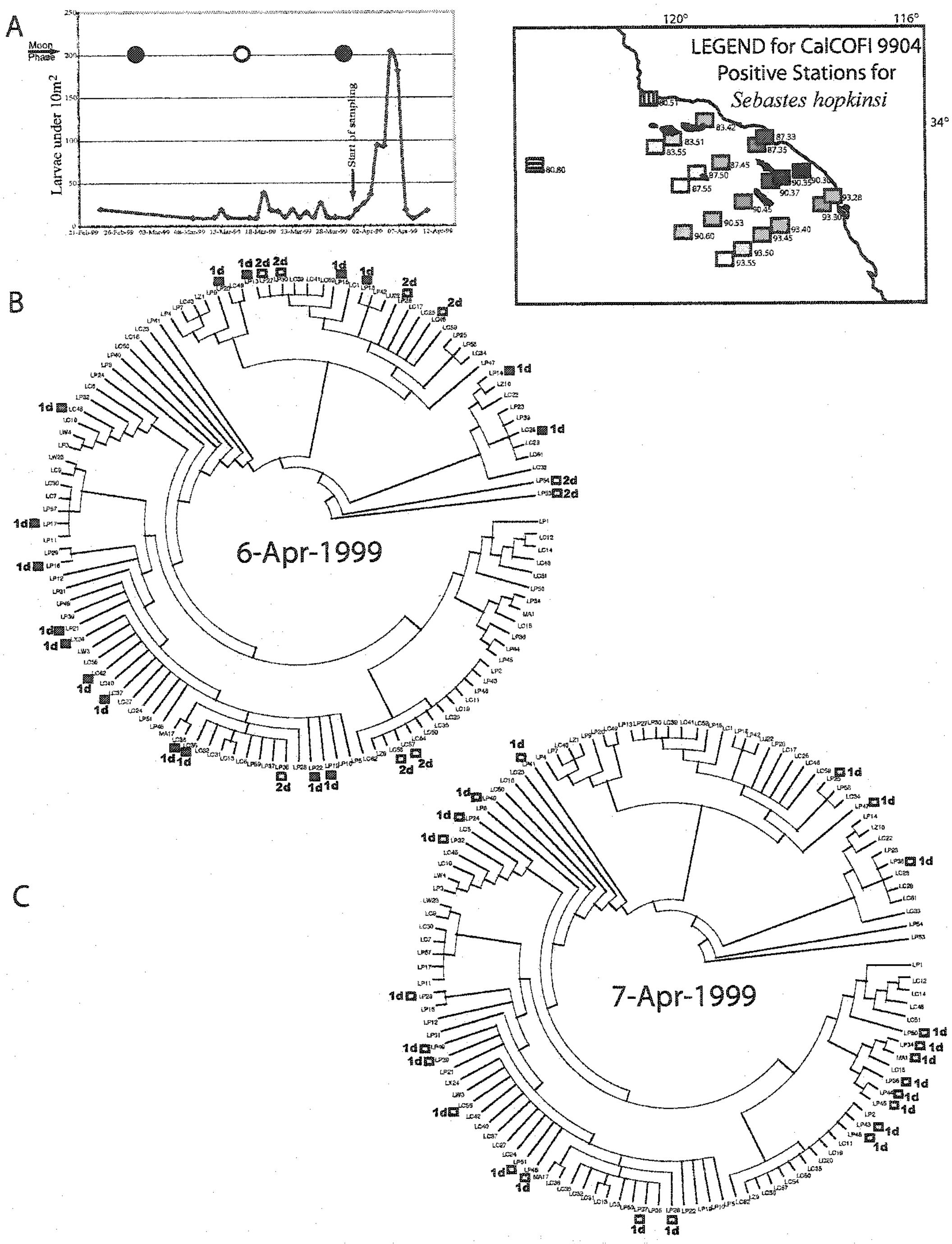

Figure 3.4. Representation of relationships among larval *Sebastes hopkinsi* during 6-7 April 1999. A, Estimated abundance of larvae (number under 10 m^2 sea surface) for each estimated birthdate over the period of time covered by larval sampling. Phases of the moon are shown relative to spawning events with filled circles representing the full moon and open circles, the new moon; B, Age and station data for all larvae with an estimated birthdate of 6-April, shown overlaid on a strict consensus tree constructed from all available larval haplotypes of *S. hopkinsi* from the 9904 CalCOFI cruise; C, Ages and location data of all larvae with an estimated birthdate on April-7-1999 overlaid on the strict consensus tree for *S. hopkinsi*.

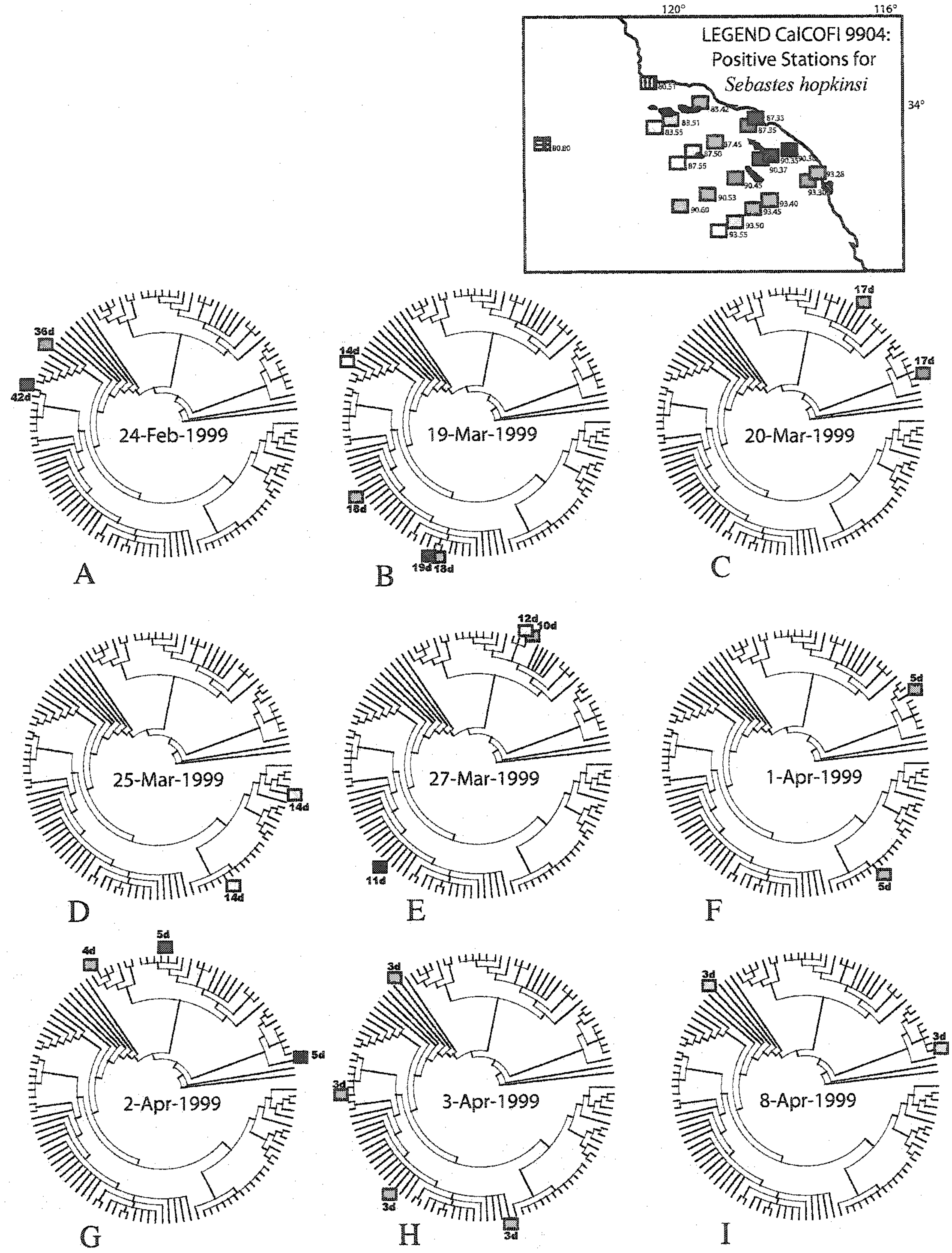

Figure 3.5. Ages and location data of larvae collected with selected estimated birthdates. A, 24-Feb-1999; B, 19-Mar-1999; C, 20-Mar-1999; D, 25-Mar-1999; E, 27-Mar-1999; F, 1-Apr-1999; G, 2-Apr-1999; H, 3-Apr-1999; I, 8-Apr-1999.

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Chapter 4

Gene flow of coastal and island populations of California kelp rockfish (*Sebastes atrovirens*) with implications for the design of marine protected areas

Abstract

As management strategies move toward including ecosystem-based approaches (e.g. Marine Protected Areas), conservation of many harvested groundfish and other habitat-associated marine species depends increasingly upon our understanding of processes that influence dispersal in pelagic early life history stages. The goal of the current study was to assess connectivity among populations of the depth-limited (<200m) kelp rockfish, *Sebastes atrovirens,* along the California Coast and among the Channel Islands of the Southern California Bight. Adults of the species show little to no movement but larvae have an extended pelagic period during which dispersal potential is high. Genetic diversity was examined for populations from seven coastal and five island locations using seven microsatellite loci to estimate dispersal from central California to Baja California, Mexico. No significant population structure was detected among all collections treated separately but significant differences were found when populations were pooled based on common hydrography at the time of spawning and pelagic dispersal reflecting the shifting geographic extension of Californian and Oregonian zoogeographic provinces. ($\Phi_{st} = 0.002$). A significant isolation-by-distance (IBD) signal was found among coastal populations (Mantel test,

$p = 0.03$) and when pairwise comparisons of nearshore islands were included in the analysis ($p = 0.011$). The IBD signal was severely depressed to borderline significance ($p = 0.043$) when the furthest offshore islands of San Nicolas and San Clemente were included. We attribute higher scales of dispersal offshore to persistent mesoscale eddies that can effectively bring recruits to offshore islands from a number of distant and neighboring habitats. In contrast, coastal and nearshore island populations do not appear to receive recruits from long distances, and once larvae are advected offshore it appears unlikely that they successfully recruit back to coastal populations distant from natal spawning grounds. These results provide important information for the processes of designing marine reserve networks within the Channel Islands National Marine Sanctuary and throughout the Southern California Bight. The Reserves associated with the four northern islands are not isolated from the mainland but are more likely to provide larvae to nearby habitat rather than add to a Bight-wide larval pool. Because kelp rockfish have been exploited by the live finfish fishery and are almost exclusively associated with fragile kelp forest habitat, understanding the connectivity of their populations is a critical step to incorporating ecosystem-based strategies for management and in describing the functioning of marine reserve networks.

Introduction

Management strategies that incorporate Marine Protected Areas (MPAs) for successful conservation of harvested groundfish and habitat-associated marine species will depend increasingly upon our understanding of processes that influence dispersal in pelagic early life history stages (Botsford et al. 2001; Palumbi 2003). Yet larval dispersal distances are known for relatively few species (for a review see Shanks et al. 2003) and much remains to be learned about factors that act as barriers to dispersal for species in the marine environment.

For marine species, the time a newly released larva spends in its dispersing phase can vary from a few minutes (e.g., 3.6 min in the tunicate *Diplosoma similis*; Stoner 1992) to several months (e.g., 293 days in the mollusk, *Cymatium parthenopeum*; Scheltema 1971) and although the predictive relation between larval duration and realized dispersal distance does not appear to be straightforward (Shanks et al. 2003), species with shorter larval duration are expected to be more limited in dispersal than those that have an extended planktonic period. Given their small size and high mortality rates, the direct measurement of larval dispersal is almost impossible in natural environments. However, measurements of genetic differences among populations examined in a geographic context can be used to infer dispersal patterns in species with a sedentary adult stage (Palumbi 1995). Where genetic structure in reef fishes off coastal California has been examined, heterogeneity has been found in reef fishes that lack a pelagic stage (e.g., surfperch, *Embiotoca jacksoni*; Bernardi 2000) and in fish that brood eggs in burrows in seasonally closed estuaries and lagoons with a dispersal period that lasts for only a few days (e.g., tidewater goby, *Eucyclogobius newberryi*; Dawson et al. 2001). For offshore species, habitat plays a role: Deepwater species with rather continuous habitat along the continental slope have shown little genetic structure (Stepien 1999; Stepien et al. 2000) whereas offshore species limited to patchy habitat have been shown to have a surprising amount of structure and heterogeneity (e.g., Seeb and Gunderson 1988; Rocha-Olivares and Vetter 1999).

Studies in eastern Pacific rockfishes have documented limits to dispersal at a variety of scales. Broad-scale differences (>1000 km) in mtDNA haplotype frequencies were described in the rosethorn rockfish, *S. helvomaculatus* (Rocha-Olivares and Vetter 1999), among coastal populations in microsatellite frequencies in copper rockfish, *S. caurinus* (Buonaccorsi et al. 2002), grass rockfish, *S. rastrelliger* (Buonaccorsi et al. In Prep) and brown rockfish *S. auriculatus* (Buonaccorsi et al. In Review) and in allozyme data for Pacific Ocean Perch, *S. alutus* (Seeb and Gunderson 1988). Differentiation at a more restricted spatial scale (<100 km) was detected between Puget Sound proper and populations in the Canadian Gulf Islands in *S. caurinus* (Buonaccorsi et al. 2002) and in sympatric populations of *S. alutus* off British Columbia (Withler et al. 2001).

The kelp rockfish, *Sebastes atrovirens*, is an abundant fish in nearshore California waters that is a good candidate species for examining heterogeneity, realized dispersal and phylogeography. Kelp rockfish are in the monophyletic subgenus of nearshore species, *Sebastes Pteropodus*, within which the highest limitations to gene flow are expected to exist within the genus. Along with the kelp rockfish, the subgenus includes the brown (*S. auriculatus*), gopher (*S. carnatus)*, copper (*S. caurinus*), black-and-yellow (*S. chrysomelas)*, calico (*S. dallii),* quillback (*S. maliger),* China (*S. nebulosus)* and grass (*S. rastrelliger*) rockfishes and form a monophyletic clade in the subgenus *Pteropodus* that has been confirmed using molecular data (Taylor 1998). They share a complex array of ecological features that make predicting their population connectivity elusive. Their apparent low (seasonal or annual) frequency of spawning (Moser 1967; Love et al. 1990), high variance in reproductive success (Ralston and Howard 1995), narrow depth distribution for pelagic larvae (Moser and Smith 1993; Sakuma et al. 1999) and nearshore settlement habitat (Miller and Geibel 1973; Dewees and Gotshall 1974; Love et al. 2002; for a review on features contributing to genetic structure in reef fishes see Shulman 1998) suggest mechanisms for genetic structuring among populations. In addition, rockfishes bear swimming larvae (Moser 1967). By circumventing the pelagic egg stage, their potential for behavioral retention exceeds that of other species with a pelagic phase (Burton and Feldman 1982). Many species within *Sebastes*, like the kelp rockfish, have distributions that span the biogeographic boundary at Point Conception (Addicott 1966; Briggs 1974), adding an additional dimension—one of hydrography—to the potential challenges to dispersal.

The current study examines kelp rockfish populations for evidence of limited gene flow. The kelp rockfish is common at the Channel Islands and its range spans from Fort Bragg in northern California to central Baja California, Mexico (Miller and Lea 1972). The species distribution offers an opportunity to look at fine scales of dispersal among island and coastal populations throughout its range including the

Southern California Bight (SCB). Previous work by Love and Larson (1978) showed significant geographic variation in tympanic spine frequencies between central and southern California populations, suggesting potential stock structure within the species that is important to confirm genetically.

This study used a novel hydrographic approach for *a priori* pooling of populations to test hypotheses of population genetic structure at the geographic scale detected by Love and Larson (1978). We examined the geographic extent of the Oregonian and Californian provinces relative to sampled populations in order to test the hypothesis that properties of the water masses in which kelp rockfish larvae were spawned might limit their potential to disperse.

We tested for significant differences in gene flow by pooling populations north of San Nicolas Island versus all populations south and east of San Clemente Island (La Jolla, Point Loma and Mexico) and maintained populations at the offshore islands of San Clemente and San Nicolas as separate groupings. These north and south groups have persistent current regimes (e.g., eddies) within which larval retention might occur (Nishimoto 2000, Logerwell and Smith 2001) and between which we hypothesize barriers to gene flow to exist. Further, we examined the hypothesis that isolation by distance, or a stepping stone pattern of gene flow might be responsible for significant differences in north to south among group variance. We use the relationship of isolation by distance coupled with data on density to calculate an estimate of average dispersal distance for the species.

Materials and methods

Sample collection

A total of 624 adult fish were sampled from eight coastal and five island locations from 1995 to 2002 (Table 4.1). Seven of the eight sampled coastal locations were used in the analysis (Ft. Bragg was excluded due to insufficient sample size). Sampling locations included Monterey (MO), Big Creek (BC), San Miguel Island (SM), Santa Cruz Island (SCZ), Anacapa Island (AN), Santa Barbara (SB), Big Sycamore Canyon (BS), San Clemente Island (SCL), San Nicolas Island (SN), La Jolla (LJ), Point Loma (PL) and Mexico (MX) (Figure 4.1). Sampling sources included scientific cruises, sportfishing competitions, fishery-independent abundance surveys, and commercial live finfish fisheries. All identifications were made by experts in the field, but rockfishes are notoriously difficult to identify due to high phenotypic polymorphism and every effort was made to obtain whole fish specimens for future reference. After collection of genotype data, we used an assignment testing method to test closely related candidate species genotypes (e.g., *S. auriculatus;* V. Buonaccorsi, unpublished data) to ensure against misidentifications. Fin clips were stored in 95% ethanol for extraction and whole bodies were kept frozen at –20°C.

Standard length data (where available) were converted to total length data (according to the regression reported in Lea et al. 1999), and despite a high variance associated with this regression, we considered this a reasonable method to estimate the range of year classes sampled from each locality (Table 4.2). An analysis of variance was performed on these data to compare the relative age/size distributions among sampling locations to determine whether we needed to be concerned with a temporal sampling bias of a single year class at any sampling location (Allendorf and Phelps 1981) and a post-hoc test was used to compare means of size frequency distributions across sampling locations.

DNA extraction and microsatellite data collection

Genomic DNA was extracted from fin clip or white muscle tissue using standard phenol/cholorform protocols (Sambrook et al. 1989) or a modified version of a salting out protocol (Gemmell and Akiyama 1996). DNA was quantified using a spectrophotometer and diluted for consistent concentrations across samples. Polymerase chain reaction (PCR) was used to amplify microsatellite loci originally developed from the grass rockfish *Sebastes rastrelliger* (Westerman et al. in prep; GenBank submission numbers AF269052-AT269061). PCRs were completed in a 15 uL volume with 150 ng of DNA template and final concentrations of 20mM Tris HCl, 50mM KCl, and 1.5mM $MgCl_2$ with 0.3μM of each primer. Forward primers were fluorescently labeled with HEX or FAM and are listed along with reverse primers, annealing temperatures and flanking region sizes in Table 4.3. Typical PCR cycling conditions included a 2 min hot start at 90°C, then 35 cycles at 95°C for 0:45, annealing for 1:00, and 72° for 1:00. Fluorescently-labeled PCR fragments were analyzed using Genescan software on an ABI 377 automated sequencer (PE Applied Biosystems, Inc.) according to manufacturer's protocols.

Statistical Analyses

Unbiased F statistics were used to analyze patterns of genetic diversity within and among samples according to Weir and Cockerham (1984) and as implemented in GENEPOP (v. 3.4; Raymond and Rousset 1995a,b). Exact probability methods (Louis and Dempster 1987; Guo and Thompson 1992) were used in comparing allelic counts to test for population differentiation, deviations from Hardy-Weinberg equilibrium and linkage equilibrium. Where temporal replicates of a sampling location were available (Table 4.1), the null hypothesis of homogeneity in temporal allele frequency distributions was tested prior to pooling to test for spatial population differentiation. Fisher's method, as implemented in GENEPOP, was used to combine independent probabilities across multiple loci (Fisher 1950; Sokal and Rohlf 1995).

Among-group, among populations and among populations within group estimations of Φ statistics (referred to in tables and text as F_{ct}, F_{st} and F_{sc} respectively), a set of hierarchical F_{st} analogs that measure the proportion of molecular genetic variation attributable to different hierarchical levels (Excoffier et al. 1992), were calculated using an analysis of molecular variance using the software program ARLEQUIN (Schneider et al. 1997). Structure used for hierarchical analyses was based on common SST at spawning. Pathfinder and Coastwatch AVHRR satellite data were examined during this inclusive pelagic larval period (generally the larvae are pelagic March through June), and years of extreme SST during which these data were available (1996-2002) are shown in Figure 4.1A (February-July, 1998) and Figure 4.1B (February-July, 1999). Populations were pooled for the AMOVA by including all populations north of SN as the northern pooled group (MO, BC, SM, SB, SCZ, AN, and BS) versus the southern pooled group that included PL, LJ, and MX (Figure 4.1A). The populations at offshore islands of SN and SCL were each left as a single "groups" in the AMOVA due to their uncertain placement within northern or southern provinces. Where appropriate, a Bonferroni correction is presented explicitly in the text where multiple tests were performed (Rice 1989).

Genetic diversity within each population was quantified using average number of alleles observed per population (A) and heterozygosity. Because the measure of A observed is dependent upon sampled individuals across populations, a standardized sample size was determined by constructing allele discovery curves using Matlab (Mathworks 5.2) for each locus. Across loci and populations, samples of $2N = 40$ offered an either asymptotic (for 4 loci) or linear relationship (for the remaining 3 loci) between new alleles discovered and sample size. All populations were then normalized to this sample size by random sampling of 100 pseudoreplicates and these were used to calculate normalized average alleles (A_n). Differences among populations in A, A_n and heterozygosity were tested using a Friedman nonparametric test (Sokal and Rohlf 1995).

We used the program BOTTLENECK (Piry et al. 1999) to examine microsatellite loci for conformance to the infinite allele (IAM) relative to stepwise mutation models (SMM) of mutation (Cornuet and Luikart 1996). This program was also used to examine allele frequency data for evidence of heterozygosity excess (HE; Cornuet and Luikart 1996; Luikart and Cornuet 1998), which might detect evidence of a recent bottleneck in our sampled populations. This was further investigated using the M statistic calculated using the software program of Garza and Williamson (2001).

Relationships among populations were illustrated by constructing a matrix of pairwise F_{st} values within GENEPOP. We used these data to generate a midpoint rooted neighbor-joining (NJ) phenogram using the software program Molecular Evolutionary Genetic Analysis (MEGA v. 2.1; Kumar et al. 1994) to examine phylogeographic relationships.

The strength of a potential correlation between geographic distance (in linear kilometers) and genetic distance ($F_{st}/1\text{-}F_{st}$) was tested overall loci using a Spearman's nonparametric correlation coefficient. The significance of this correlation was determined using a Mantel test with 100,000 permutations as implemented in GENEPOP (Mantel 1967).

We used the model of Rousset (1997) to relate the linear regression between geographic and genetic distance and estimate average dispersal distance using census data on population density of kelp rockfish among the Channel Islands. This model relates $4D\sigma^2$ to the inverse of the slope of regression between genetic [Fst/(1-Fst)] and geographic distance, where D is an estimate of population density per 1000km of linearized coastline and σ^2 is the variance in parent to offspring distance. To attain variance for the non-axial or one-sided (Rousset's "unsigned dispersal distance"; Rousset 2003), one must divide this σ^2 by half. This accounts for the elevated variance due to the use of absolute values rather than signed values in the two-sided distribution of calculating parent to offspring trajectories. Mean dispersal distance ("d" or Rousset's "$1/\alpha$") is calculated as the standard deviation or square root of this variance ($\sigma^2/2$).

Visual transects on SCUBA have been collected for 12 sites on 5 of the Channel Islands in the Kelp Forest Monitoring Program since 1985 to characterize whole habitat changes over time in this region (Davis et al. 1997). We incorporated these data into estimates of density on adult kelp rockfish with the following assumptions: all animals seen on transect surveys were representative parents in Rousset's parent to offspring relationship; band transects were linearized (consistent with Rousset 1997); transects were extrapolated from 50 m to 1000m, a 50 m and 100 m wide kelp bed with continuous habitat at the site of sampling were assumed; and that island density data are representative of linear coastal density data at the time of genetic sampling.

Results

Year Class Data on Sampled Adults

Standard length data for whole fish were used to estimate the range of year classes sampled from five localities using von Bertalanffy growth curves (Lea et al. 1999). The analysis of variance (ANOVA) showed a significant difference in standard length across sampled populations ($F = 27.99$, $p<0.001$). SM showed a significantly higher (324.36 mm TL) and SCL significantly lower (223.63 mm TL) mean size from other populations in the post hoc examination, but all populations showed multiple year classes (Table 4.1) based on the assumption of using the regression on northern animals to estimate age.

Size frequency distribution data from the SCL population revealed a group of animals (N = 32 of 93) of approximately 3 years of age (mean=3.31, SD=0.13) and a mean standard length of 185 mm (SD = 3.63 mm). The animals were collected in March, 2001, suggesting a potential recruitment in 1998 during the El Niño year. These animals (termed SCL^{98}) were treated as a "temporal replicate" (to examine the possibility of temporal bias) and compared against the remaining animals from SCL prior to pooling SCL population data into a single population sample.

Hardy Weinberg and Linkage Equilibrium

Locus *Sra* 5-32, displayed a deficit in heterozygotes across multiple populations despite a narrow range in size calls for the PCR products (99bp to 111bp) and relatively few detected alleles for the locus (A = 6). This locus also appeared to be in linkage disequilibrium with locus *Sra* 5-9 and we suspected a potential overlap in amplification regions or proximity between the two amplified regions. We removed this locus from the final analysis and completed population genetic analyses based on seven polymorphic loci. Locus *Sra* 7-2 was monomorphic and was not screened for more than 10 individuals per population after the initial 100 individuals sampled (from BC and PL).

We tested the sampled populations, the subset population SCL^{98}, and pooled north and south populations for conformance to Hardy-Weinberg expectations (HWE), calculated F_{is} for seven microsatellite loci and estimated significance of the deviation of F_{is} from zero across seven microsatellite loci (Table 4.4). The only significant departure from HWE was seen in the San Miguel Island (SM) population (F_{is} =0. 011,

$p = 0.045$) due to a deficiency in heterozygotes. Pooled populations that included SM also had significant deviations from HWE driven by the inclusion of SM in the pooled sample population (Table 4.4).

Differentiation Among Temporal Replicates

Multiple sampling trips resulted in temporal subsampling at four locations (see Table 4.1), and increased the likelihood that single year classes were not overrepresented. Temporal replicates were examined for BC (N=20 from September, 1998 and N=24 from June, 2000), BS (N=15 from June, 1998 and N=18 from February through June, 1999), SM (N=36 from February through June, 1998 and N=33 from March, 2001) and SN (N=15 from August, 2000 and N=19 from March, 2001). In addition we used the N=32 individuals that made up SCL^{98} versus N=61 in the remainder of SCL. Tests for allele frequency differences among temporal replicates showed no significant differences across seven loci and SCL was pooled as a population sample in tests for spatial population differentiation.

Spatial Population Differentiation and Demographic Bottlenecks

When all populations were treated separately, no significant differentiation was seen over all loci (X^2=15.35, df=14, p=0.355), although significant pairwise F_{st} values were observed between SM/MX (0.007, p=0.043), SM /SCL (0.003, p=0.021) and AN/MX (0.008, p=0.033). A hierarchical AMOVA that structured the northern group, the southern group and the offshore islands into four groupings revealed that all but a small fraction of the percent variance was attributable to that within populations. The remainder (0.15%) was attributable to among group variance and was significant (F_{ct} = 0.002, p=0.011). The among-populations F_{st} was not significantly different from zero (this was equivalent to testing overall population structure described above, F_{st} = 0.0012, p = 0.241) and essentially zero variance (-0.024%) was found to be attributable to that of populations within the groups (F_{sc} = -0.00025, p= 0.192).

We considered it most conservative to maintain offshore islands as separate groups rather than placing them into northern or southern hierarchical groupings, but the hydrography during the pelagic period of the kelp rockfish suggested placement of SN into the north and we tested this hypothesis (Figure 4.1). With this structure, we observed significant among group variance (F_{ct}= 0.002, p= 0.005, with a Bonferroni correction, p = 0.05/3 = 0.0167). The percent among-group variance with SN pooled in the north (and the southern and SCL populations each grouped separately) was 0.15% of the total variance.

When we removed the SCL sample and replaced it with a subset, the SCL^{98} sample to test the hypothesis of homogeneity including this apparent year class with other included populations, no overall genic differentiation was found (X^2=14.63, df= 14p =0.404), nor were there any significant pairwise differences with other sampled populations in the analysis. Tests for population bottlenecks based on excess heterozygosity (Luikart and Cornuet 1998) and allele distribution pattern (Garza and Williamson 2001) revealed no evidence for recent demographic reductions in sample size at any of our sample sites. An examination of loci based on observed and expected heterozygosities under the stepwise mutation model (SMM) and the infinite allele model (IAM) indicated an overall two phase model (TPM) of mutation in the microsatellite loci examined that is consistent with a 64% SMM.

Isolation by Distance

Significant IBD was seen in individual analyses when we used the Mantel test to examine pairwise coastal populations excluding both nearshore and offshore island populations (p=0.030, Figure 4.2A), although with all populations included, only 12% of the variance in genetic distance is explained by geographic distance. The significance of this regression is driven primarily by the pairwise comparisons between coastal and nearshore island populations. Pairwise comparisons between offshore island and nearshore islands (SN-AN, SCL-AN, SN-SM, SCL-SM) have elevated genetic distances over those expected by an IBD model and pairwise coastal to offshore island comparisons show low zero or low Fst values at low and high geographic distances, suggesting admixture at these populations. When island-island and coastal to offshore islands are removed, the geographic distance explains 29% of variance in genetic distance (Figure 4.2B). A test for IBD with pairwise comparisons of nearshore island and coastal comparisons only (excluding offshore islands of SCL and SN) in an analysis showed higher significance (Mantel Test, p = 0.011). With a Bonferroni correction, the overall IBD analyses (with offshore islands included) would be considered non-significant (p = 0.05/2 or 0.025).

We used the slope of the IBD regression between genetic and geographic distance overall populations, density data and the model of Rousset (1997) to calculate an estimate of average dispersal distance between 9 and 13 km (for a 100-50 m crossshelf wide kelp bed, respectively). Density data were estimated at 810 animals per linear kilometer of coastline per 100 m wide kelp bed. The regression used for the calculation was Figure 4.2A (Coastal and nearshore island populations only, excluding offshore island populations of SCL and SN).

Phylogeography

A NJ phenogram constructed using pairwise F_{st} values (Figure 4.1B) for sampled populations depicted differentiation between northern and southern populations (Figure 4.3). Due to the small pairwise F_{st} values used in constructing the phenogram, especially in the northern portion of the tree, rather than showing a clear north to south pattern in the northern topology, the populations of BC and MO were mixed among populations of SCZ, SB, BS, AN clustering with the northern populations of BC and MO. The offshore island populations of SN and SCL clustered with southern populations of LJ, PL and MX.

Discussion

Gene Flow at the Coast, Nearshore and at Offshore Islands

Kelp rockfish experience restricted gene flow, but this appears to be focused primarily along the coast and at nearshore islands. The data do not discount an abrupt barrier to gene flow, although the signal appears, based on the resolution of our data, to be driven by isolation due to stepping-stone dispersal along the coast and along the nearshore northern Channel Islands. The overall low pairwise F_{st} values (Figure 4.3), the affinity of SN with southern (in phylogeography) as well as northern (in the hierarchical analysis) populations and the loss of IBD with the inclusion of offshore islands all suggest limitations to gene flow along the coast with higher levels of realized gene flow at offshore islands. The IBD result suggests that the most likely source of a larval immigrant to a coastal population is from the adjacent populations. This pattern has also been observed in coastal populations of copper *S. caurinus* (Buonaccorsi et al. 2002), brown, *S. auriculatus* (Buonaccorsi et al. in prep), and grass, *S. rastrelliger* rockfishes (Buonaccorsi et al. in review), but it had not been examined in a reef fish abundant at the Channel Islands. All of these species live in the near shore as adults with a dispersing pelagic phase that can last several months. When pairwise comparisons between nearshore islands and coastal population were sequentially added to coastal pairwise comparisons alone (San Miguel, Santa Cruz, Anacapa, regressions not shown), each overlaid the coastal regression. This suggests that the nearshore islands that are part of the Channel Islands National Marine Sanctuary (CINMS) are behaving as narrowly dispersing populations not unlike coastal populations.

It is difficult to assess from the genetic microsatellite data to what degree the offshore SN and SCL islands are self-seeding, but it is clear that they are not solely self-seeding. If so, we would see high pairwise genetic distances from coastal populations, showing these populations to be on independent trajectories due to genetic drift from whatever isolating factors might limit them from dispersing to neighboring populations. Instead we see small genetic distances between these island populations and nearby populations (like PL versus SCL) as well as for distant populations comparisons (like between MO versus SCL). Coast and offshore island comparisons can be seen in Figure 4.2 as closed circles with most comparisons showing zero or near zero genetic distance.

The pattern of gene flow seen in our data agrees well with the circulation off central and southern California. In the study area from Monterey, CA to Ensenada, Mexico the absolute temperature may vary widely, particularly in the 1998-1999 El Nino-La Nina transition shown in Figure 4.1, but the structure is still apparent. The cool waters of the California Current flow south along the northeast Pacific, leaving the coast at Point Conception and following the Santa Rosa Ridge, a submerged bank that peaks at San Nicholas Island. Waters within the SCB are formed from a meander of the California Current and waters of subtropical origin (Hickey 1992), and the distinct provincial differences between these Californian provincial biogeographic waters and Oregonian waters further north is due to seasonal warming of the water in the Bight due to atmospheric heating. Here surface waters generally flow northward along the Southern California coast. The warm poleward southern California Counter Current together with eddy driven flow resolved from moored data in the Santa Barbara Channel, suggest an overall pattern of equatorward flow in the spring and poleward flow from summer through winter (Harms and Winant 1998). Surface circulation in the Santa Barbara Basin that influence dispersal among the four northern Channel Islands and from islands to the nearby mainland are complex (Harms and Winant,1998, Hendershott and Winant 1996). Despite the complexity, flow regimes in the eastern mouth reverses seasonally, moving generally equatorward (out of the channel) from February through June when kelp rockfish larvae are expected to be pelagic, and reversing to a poleward flow from July through January (Hendershott and

Winant 1996). The western mouth of the channel is dominated by annual mean surface flow that is westward along the north shore of the channel and eastward along the north side of the northern Channel Islands. Thus it is not surprising that populations at the northern Channel Islands reflect a northern affinity (Figure 4.3).

Comparative underwater flora and faunal surveys of species with pelagic dispersing stages suggest that dispersal corridors might exist between these nearshore island populations and central California coastal populations. Such affinities have also been suggested among San Miguel Island, areas of San Nicolas Island and central California, and among the inshore islands of Santa Cruz, Anacapa and Santa Catalina (Ebeling et al. 1980; Engle 1993; Seapy and Littler 1980; Murray et al. 1980). Such survey data support a strategy of pooling offshore samples based on common hydrography during the pelagic period rather than pooling populations based on geographic proximity along the coast and support the placement of SN with the north in the hierarchical among-group AMOVA. However, the overall low pairwise Fst seen in the NJ tree with the placement of SN among southern samples and the suggestion from the IBD analysis that it receives recruits from multiple coastal sources suggests that SN is probably not solely populated from the north but may be seeded intermittently from the north and the south. The contribution of common eddies in the region (probably have important consequences for recruitment of kelp rockfish, certainly at this offshore island (SN) and the offshore island population at SCL as well.

The IBD pattern at offshore islands of SN and SCL, based on pairwise comparisons of these island populations, showing zero or near zero genetic distance at both low and high geographic distances suggests that these island populations are as likely to receive recruits from the geographically closest coastal populations as ones more distant. The loss of the IBD signal when off shore islands were included indicates that these islands appear to be experiencing gene flow among a variety of adjacent and non-adjacent locations akin to the island model of dispersal. These eddies can provide a mechanism by which larvae are mechanically aggregated and maintained in an area of increased local production (Owen 1980). Patterns of recruitment have been seen in sheephead at San Nicolas Island, where year-to-year variations in the northward flow of the Southern California Eddy (Cowen 1985) accounted for anomalies in recruitment. Recruitment in kelp rockfish from northern populations could similarly be explained during anomalously cold years by displaced eddies known to entrain larvae and eggs of anchovy (Fiedler 1984) and sardine (Logerwell and Smith 2001). These mesoscale features were found by Logerwell and Smith (2001) to provide habitat to spatially aggregated larvae ('survivors'greater than or equal to 18d old) of sardine.

Variable Year Classes and Variable Recruitment?

Ralston and Howard (1995) described variable year classes in rockfish species and how, due to their high fecundity, long lives and slow growth, populations can be sustained by a few successful year classes. For island populations or those limited to offshore banks where there is a high degree of annual to decadal variability in oceanographic conditions (Moser et al. 2000), the effect of variable recruitment can result in high extinction risk. Our data, though addressing gene flow in a single species within the nearshore group, have application for understanding recruitment in habitat-associated rockfishes. Many rockfish species essentially live on islands by using a preferred depth stratum among offshore banks and islands in the complex basin and sill bathymetry off southern California. Kelp rockfishes are further tied to kelp habitat that fluctuates in abundance based on local ecosystem dynamics (Dayton et al. 1984; Dayton et al. 1998).

The backcalculation of the age of the subset SCL^{98} of individuals from the SCL population suggests that kelp rockfish had good recruitment during the 1998 El Niño at San Clemente Island. This agrees well with data from central California midwater trawl surveys that catch pre-recruiting rockfishes where nearshore *Pteropodus* rockfishes have been well represented in warm, El Niño years, but poorly represented in colder La Niña years (Lenarz et al. 1995), although the degree to which paradigms hold for southern versus the central coast is difficult to ascertain. The conformance to HWE and homogeneity of SCL^{98} with the remainder of the SCL sample suggests that it was an admixture of multiple cohorts arriving at the island in that year ("many winners" of the survival sweepstakes; Flowers et al. 2002) or that this was an example local retention of SCL island spawners.

These data indicate reasonable gene flow for offshore islands, an important finding for dispersal in this species. It suggests that they might be found in net surveys taken through the California Cooperative Oceanic Fisheries Investigations Surveys (CalCOFI), a fifty-three year fisheries observation program conducted off the California coast that includes early life history stages (5-12mm length with estimated ages of 1 to 32 days for nearshore rockfish larvae) (Moser et al. 2001). However, only a single count of a kelp rockfish larva was obtained, standardized to 16 larvae under 10 m^2 sea surface approximately 70 km from potential adult kelp habitat at Santa Cruz Island (Figure 4.4). Only two other species of *Pteropodus* were encountered during this survey. Larval distribution data suggest larvae can disperse from nearshore islands like San Cruz Island up to 70 km, if we assume this to be the source population for these CalCOFI kelp rockfish larvae. The Rousset (1997) model estimated a realized average dispersal between 9 and 13 km. The estimate from genetic data of limited gene flow in coastal populations emphasizes the need for more nearshore sampling to complement the offshore array in CalCOFI to characterize distributions and abundance of these nearshore species. If Lenarz et al. (1995) are correct, and nearshore *Pteropodus* are poorly represented in La Niña years, then contrasting the distributions of these CalCOFI 9904 data with those of 9804 is an important goal. Genetic identification of long term monitoring data such as those obtained in CalCOFI will be critical for documenting dispersal patterns, the variance associated with dispersal patterns and physical and primary production that might contribute to dynamics in rockfish recruitment over scales of their dispersal.

Conservation and Management Goals

Currently MPAs are being considered as a major tool for the conservation of marine biodiversity in the United States. In this regard, SCL and SN would work well due to their remote location and diversity of habitat. As an MPA to support fisheries in a larval export capacity, one but not both of these locations might be useful. These islands appear to be acting more as sink than source populations relative to other populations in our study and we therefore suggest that they would not be a major contributor to the replenishment of nearshore coastal populations, nor do they appear to be largely self-seeding. They appear reliant upon coastal source populations, with evidence of slightly higher (although not significant) Fst values compared to the northern Channel Islands. The caution is that if we place stock in offshore island reserves without protecting coastal sources that might act to re-seed them, we may doom these offshore island populations to extinction.

The hypothesis-testing framework used in the current study is useful to define management units, although it has limitations for continuously distributed species. Significant differences were found between northern and southern pooled populations, however, these differences are most likely driven by IBD and the statistical artifact of hypothesis-testing between the most disparate of the isolates. The weakness of this approach is that by pooling populations, the power to detect allele frequency differences improves, but the power to detect more than two fishery management units or stepping stone gene flow is reduced (Taylor and Dizon 1999; Martien and Taylor in press). The demonstration of clinal gene flow might not be seen as useful from a traditional management perspective, but it can be useful in postulating the construction of MPAs within the SCB. Our mean estimate of dispersal distance seems to confirm this scale of limited gene flow. If we accept the heterogeneity as observed differences between the two hydrographic domains, whether driven by IBD or not, then this suggests that a comprehensive rangewide reserve network would require reserves within both hydrographic domains. While these hydrographic domains are similar to biogeographic provinces, they are not strictly confined to north and south of Point

Conception. We have also shown that despite limited realized dispersal the four northern islands, (within the CINMS MPA network) are not isolated from local coastal populations and it is reasonable to expect limited short-range dispersal between island and coastal populations, although long-range dispersal in demographically significant numbers is unlikely. The outer islands of San Nicholas and San Clemente were not genetically isolated and also did not conform to the isolation by distance model and appear to receive enough migrants from a variety of local and distant sources to have a well mixed gene pool.

Conclusion

These data indicate reasonable gene flow for offshore islands on a background pattern of restricted gene flow for coastal populations, an important finding for dispersal in this species. The results suggest that it is possible for kelp rockfish to survive long distance dispersal, but that coastal populations do not appear to be realizing their dispersal potential except in a one way dispersal to island populations. Our study uses an explicit method of examining averaged sea surface temperature data during the pelagic early life history period to pool populations to test for heterogeneity. We believe this to be a useful approach, especially when considered in the context of basic natural history data published on the biodiversity and ecosystem dynamics of the Channel Islands. Pooling population genetic data within an *a priori* framework to examine population genetic structure is critical in species where dispersal may be limited within a short geographic range and the signal difficult to detect, like that of the kelp rockfish, where if populations are treated separately the signal is too weak even with reasonable sample sizes (separating the "wheat from the chaff" discussed by Waples 1998). Within this framework, we were able to elucidate differential scales of dispersal at coastal versus island populations and these data will be important for the design of MPAs in groundfish species within the SCB.

The loss of IBD at offshore island populations relative to the stronger signal in coastal and nearshore island populations is an important finding for this species in the effort to assess dispersal at scales that are effective for fisheries as well as conservation management. This is the first study to examine a fish species at this scale in the SCB—one abundant at both coastal and offshore islands with an extended pelagic life history phase using genetic data examined kelp rockfish using morphological data. In light of current pressure from the live finfish and other fisheries, we might want to avoid putting our MPA effort into the placement of offshore island reserves at locations such San Nicolas and San Clemente Islands without simultaneously protecting coastal areas. Locations of MPAs are critical for species protection whether it be for conservation, protection of adult spawning stock for larval export or for aesthetic goals.

Acknowledgements

The collaborative sampling effort for this study was enormous and I am extremely grateful, but those to thank are too numerous to mention here. Briefly, much of the genetic sampling effort was assisted and organized by C. Kimbrell, H. G. Moser, and W. Watson. Samples were obtained through cooperation with California Department of Fish and Game, colleagues in the NOAA Fisheries and scientists and crew on the NOAA ships the R/V *David Starr Jordan* and the *MacArthur* and the SIO ship the *Sproul.* I am also indebted to D. Kushner, R. Galipeau, and the National Park Service for making available data obtained from the Kelp Forest Monitoring Program. The importance of continuing the collection of these data to document changes in the SCB cannot be overstated. I am also indebted to R. Larson and M. Love for the availability of unpublished data and D. Foley, M. Laurs, Coastwatch, and the NASA Physical Oceanography Distributed Active Archive Center at the Jet Propulsion Laboratory, California Institute of Technology for availability of Pathfinder sea surface temperature data. I acknowledge R. Burton, V. Buonaccorsi, J. Cope, B. Javor, R. LeDuc, A. Henry, H. Lee, K. Martien, P. Morin, and T. Olive for their time and thoughtful comments on earlier drafts of this manuscript and A. Brooks for help in the laboratory. I also wish to thank the Oceanids Foundation and the Frieda Daum Urey scholarship committee for their generous endowment. The genetic data collection was funded through California Sea Grant (R/F-175 and 4-M-N), a NOAA Saltonstall-Kennedy Internal Grant, the California Department of Fish and Game (P0070013), NOAA Fisheries base funds and support from Scripps Institution of Oceanography. And finally, I wish to thank my committee for their thoughtful

comments and helpful suggestions on all aspects of the work. This chapter is intended to be submitted in full as a collaborative publication. Taylor, C. A., R. Vetter. The dissertation author was the primary investigator on this paper.

Table 4.1. Sampling locations in order from north to south.

Sample Location	Latitude/ Longitude (or range)	Tissue Collected	Date Collected	Number (N)
Fort Bragg				
FB	39.34/-123.83	Fin Clip	April, 1999	5
	39.43/-123.82	Fin Clip	February, 2000	1
	39.28/-123.81	Fin Clip	May, 2000	3
	39.45/-123.80	Fin Clip	June, 2000	1
	39.13/-123.72	Fin Clip	July, 2000	3
				N = 13
Monterey				
MO	36.58/-121.98	Fin Clip	July, 1995	48
	36.58/-121.98	Fin Clip	January, 2000	7
				N = 55
Big Creek				
BC	36.07to36.12/-121.83to-121.66	Fin Clip	Sept, 1998	20
	35.98/-121.49	Fin Clip	April, 2000	2
	35.97/-121.48	Fin Clip	May, 2000	12
	35.92to36.01/-121.47to-121.52	Fin Clip	June, 2000	24
				N = 58
Big Sycamore				
BS	34.05/-119.00	Fin Clip	June, 1998	19
	34.05/-119.00	Fin Clip	June, 1999	14
	34.05/-118.97	Whole Fish	March, 2001	4
				N = 37
Santa Barbara				
SB	34.39/-119.70	Fin Clip	August, 1998	5
	34.39/-119.70	Fin Clip	October, 1998	1
	34.41/-119.88	Fin Clip	April, 2000	26
	34.41/-119.88	Whole Fish	October, 2001	3
				N = 35
San Miguel				
Island	34.01/-120.34to-120.37	Fin Clip	February, 1998	27
SM	34.01/-120.37	Fin Clip	June, 1998	9
	34.01/-120.37	Fin Clip	July, 1999	5
	34.02/-120.39	Whole Fish	March, 2001	33
				N = 74
Santa Cruz				
Island	33.96/-119.71	Whole Fish	March, 2001	70
SCZ				N = 70
Anacapa				
Island	34.01/-119.39	Fin Clip	November, 1997	5
AN	34.01/-119.39	Fin Clip	June, 1998	1
	34.01/-119.39	Fin Clip	July, 1999	4
	34.01/-119.39	Fin Clip	February, 1999	3
	34.01/-119.39	Whole Fish	July, 2001	17
				N = 30

Table 4.1 (cont'd). Sampling locations from north to south.

San Nicolas				
Island	33.26/-119.46	Fin Clip	August, 2000	15
SN	33.22/-119.50	Whole Fish	March, 2001	19
				N = 34
San Clemente				
Island	33.01/-118.56	Whole Fish	August, 2000	8
SCL	33.03/-118.56	Whole Fish	March, 2001	82
	32.82/-118.35	Whole Fish	July, 2001	3
				N = 93
La Jolla				
LJ	La Jolla Kelp Beds	Whole Fish	July, 1997	4
	32.83/-117.25	Whole Fish	August, 1998	12
	32.83/-117.25	Whole Fish	October, 1998	7
	Scripps Canyon, La Jolla	Fin Clip	Nov-Dec, 1999	2
	Scripps Canyon, La Jolla	Whole Fish	November, 1999	3
	32.83/117.25	Fin Clip	April-June, 2000	3
	32.83/117.25	Whole Fish	October/Nov, 2000	4
				N = 35
Point Loma				
PL	32.67/-117.25	Fin Clip	June, 1996	4
	32.67/-117.25	Whole Fish	April, 1997	8
	South Point Loma Kelp Beds	Whole Fish	August, 1997	7
	32.67/-117.25	Fin Clip	August, 1997	29
	32.67/-117.25	Whole Fish	September, 1997	11
	32.67/-117.25	Fin Clip	October, 1998	8
	32.67/-117.25	Fin Clip	December, 1998	1
	32.67/-117.25	Fin Clip	June, 2000	1
				N = 69
Mexico				
MX	31.80/-116.82	Fin Clip	May, 2000	2
	30.48/-116.10	Whole Fish	December, 2001	8
	30.48/-116.10	Fin Clip	December, 2001	11
				N = 21

Table 4.2. Mean standard length data for whole body samples of *S. atrovirens* collected. Approximate mean minimum ages for each sampling site are based on von Bertalanffy growth reported in Lea et al. (1999).

Sample Location	Number (N)	Mean TL(mm)	Standard Deviation TL	Approximate Mean Age (Yrs)	Approximate Age Range (Yrs)
San Miguel Island (SM)	N = 31	324	32.57	7.76	3-12
Santa Cruz Island (SCZ)	N = 55	242	29.83	3.72	2-6
Anacapa Island (AN)	N = 17	227	30.87	3.28	2-5
San Nicolas Island (SN)	N = 33	258	31.52	4.29	2-7
San Clemente (SCL)	N = 91	223	23.86	3.18	1-5
La Jolla (LJ)	N = 30	230	28.52	3.38	2-5
Point Loma (PL)	N = 26	247	36.45	3.92	2-7
ALL	N = 275	247	41.7	3.91	1-12

Table 4.3. Primer sequences, fluorescent labels, nucleotide repeat type, flanking region size and anneal temperatures for microsatellites screened to examine population structure in *S. atrovirens*.

Locus	Primer Sequence	5' Label	Repeat (bp)	Flank Region (bp)	Anneal Temp (°C)
5-9 REVERSE	5'-CCTCATAATAGAGCTTGTAATAACG-3'				
FORWARD	5'-CTTGCTACTGCAGAGTGACTAC-3'	FAM	di	85	57
7-2 REVERSE	5'-GTCAAACAACTGCAGAATGTTCG-3'				
FORWARD	5'-GAACATCCCTCCTTCCGACGC-3'	FAM	di	120	57
7-7 REVERSE	5'-CATGTGATTCTGTGTCTAACTGAG-3'				
FORWARD	5'-GCATGAAAGTGTATGAAAGGC-3'	FAM	di	158	52
7-25 REVERSE	5'-CAAGAGGCGGTGGTGCTGATGG-3'				
FORWARD	5'-GACCTTTCCCTGAACACACCG-3'	HEX	di	145	52
5-32 REVERSE	5'-AGCACACACGTCTAAAACACT-3'				
FORWARD	5'-GTGAGGAGGTTAAGATGACCG-3'	HEX	di	89	50
11-103 REVERSE	5'-GGCTGATGACATTGCAACCTTG-3'				
FORWARD	5'-CTTGCAGGTAACGGAAGG-3'	FAM	tri	203	52
15-23 REVERSE	5'-CCGTCTTGATCCAGATGGTACATGTC-3'				
FORWARD	5'-CCCCAAATACTGTCTTGCCAG-3'	FAM	tetra	90	45
16-5 REVERSE	5'-GAGAAGAGGCCTACAAGTACC-3'	HEX or			
FORWARD	5'-CCATCTGTGCTGAGCTGTCACTG-3'	FAM	tetra	131	50
15-8 REVERSE	5'-GGGTTTACTCATTGTAGAC-3'				
FORWARD	5'-GGAGATGTGCGTGGCTCGTCTGG-3'	FAM	tetra	267	52

Table 4.4. Average alleles detected overall loci (A), average alleles normalized by sample size of 2N=40 (A_n), observed (H_o) and expected (H_e) heterozygosities, deviation from Hardy Weinberg equilibrium (F_{is}) and estimated significance of the deviation from zero (*<0.05, *<0.01) for seven microsatellite loci.

Abbreviation	N	A	A_n	Ho	He	Fis	P
MO	55	11.3	8.4	0.6536	0.6810	0.0402	0.7271
BC	58	12.0	8.6	0.6633	0.6986	0.0505	0.1073
BS	37	10.0	7.7	0.6587	0.6701	0.0170	0.4318
SB	35	11.0	8.4	0.8000	0.7143	-0.1199	0.8633
SM	74	12.3	8.3	0.6673	0.6747	0.011*	0.0445
SCZ	55	12.6	8.3	0.8000	0.7766	-0.0301	0.2572
AN	30	10.1	8.0	0.7931	0.7851	-0.0102	0.5648
SN	34	9.6	7.6	0.6753	0.6897	0.0209	0.5919
SCL	93	13.3	8.6	0.7026	0.7010	-0.0023	0.0708
LJ	35	9.6	7.7	0.6619	0.6991	0.0533	0.0935
PL	69	13.0	8.7	0.6912	0.6804	-0.0159	0.2057
MX	21	9.4	7.8	0.7762	0.7159	-0.0842	0.9996
NORTH	344	15.7	8.8	0.6714	0.6836	0.0179	0.0150
SOUTH	125	11.7	8.9	0.6984	0.6909	-.0108	0.4346

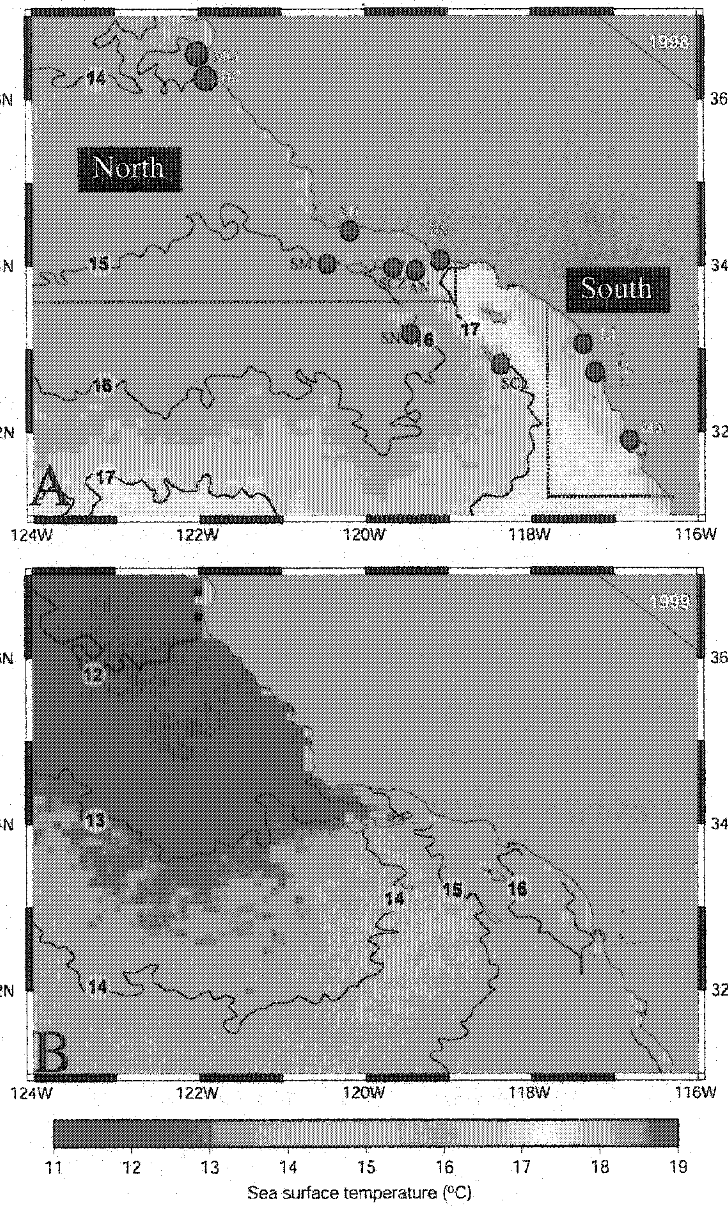

Figure 4.1. A, Map showing sampling locations throughout the range of kelp rockfish with groupings used in hierarchical hypothesis tests for homogeneity in allele frequencies overlaid on SST map representing composite for February through July, 1998 obtained by using AVHRR Pathfinder data to include the period of time during which larvae are expected to be pelagic. B, SST map that represents a composite for February through July, 1999. SST data from 1998 and 1999 characterize the extremes in the transition in water masses experienced by pelagic rockfish larvae spawned in respective Oregonian and Californian provinces in this region.

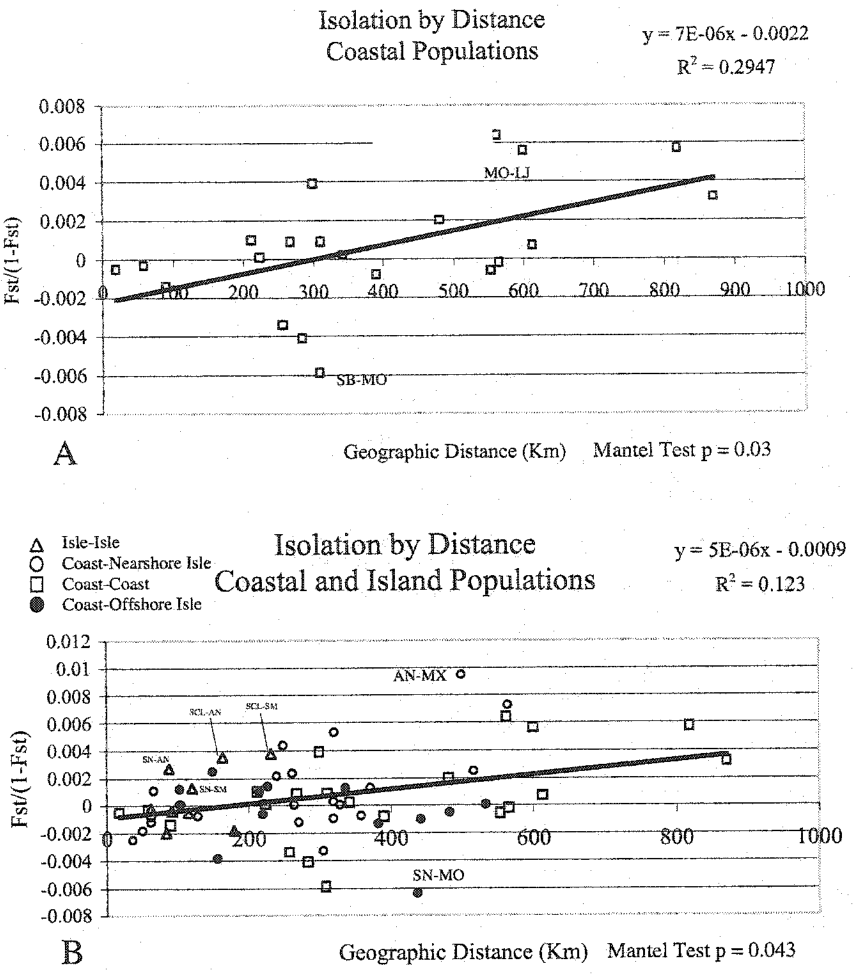

Figure 4.2. Isolation by distance relationships are shown for all coastal pairwise comparisons (A), and all pairwise comparisons (B). Open triangles represent pairwise comparisons between islands, open circles are pairwise comparisons between the coast and nearshore islands of SM, SCZ, and AN, open squares are coastal pairwise comparisons and closed circles are pairwise comparisons between offshore islands of SN and SCL and coastal populations.

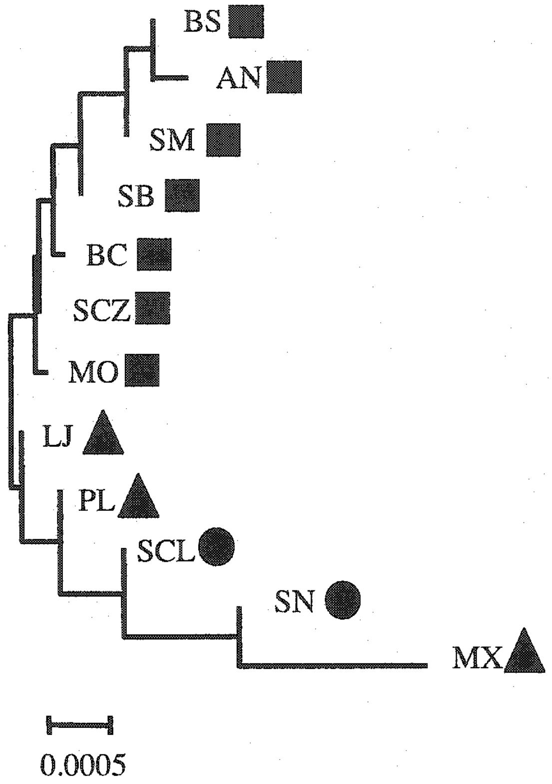

Figure 4.3. A, Midpoint rooted NJ phenogram using pairwise F_{st} are shown among all populations and B, with the subset SCL^{98} replacing the SCL population in the phylogeographic analysis. Symbols are used to represent general locations of populations relative to the linear coastal range of kelp rockfish. Squares are used for populations placed in the northern hierarchical analysis, circles for offshore island populations, and triangles for the southern populations.

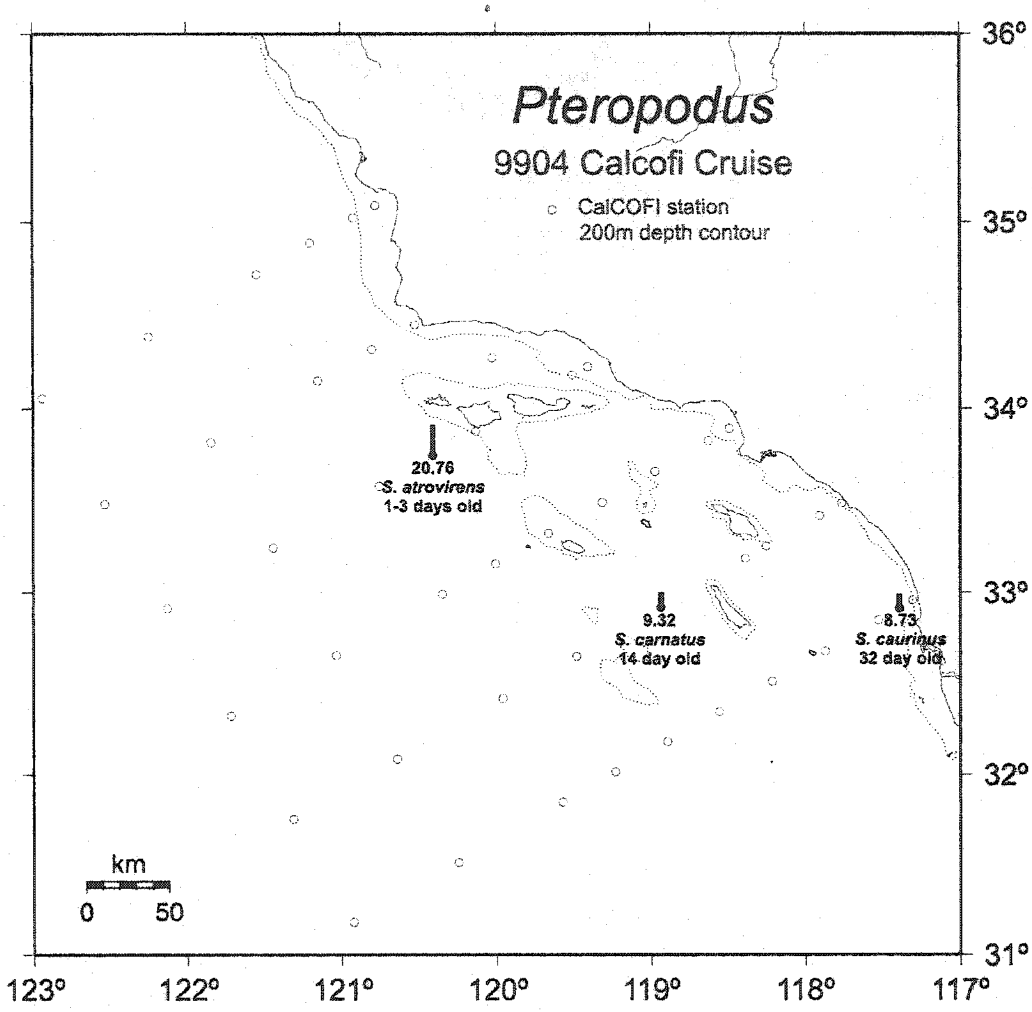

Figure 4.4. A map of the SCB showing inshore stations occupied for quarterly in California Cooperative Oceanic Fisheries Investigations (CalCOFI) grid in the SCB. Overlaid are positive stations for the April CalCOFI with nearshore *Pteropodus* rockfishes standardized based on the CalCOFI protocols to number of larvae under 10 m^2 sea surface (Taylor In Prep).

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Chapter 5

Conclusions and Future Directions

The goal of the work presented in the preceding chapters was to assess connectivity among populations of the depth-limited (<200m) squarespot, *Sebastes hopkinsi*, and kelp rockfish, *Sebastes atrovirens,* along the California Coast and among the basins and sills including the Channel Islands of the Southern California Bight. Adults of the two species show little to no movement while larvae have an extended pelagic period during which dispersal potential is high (Love et al. 2002). One of the main strengths of this dissertation work is that it integrates methods of ichthyoplankton sampling, acoustic Doppler (ADCP) velocity data, otolith ageing, genetic methods of larval identification and microsatellite data analysis to converge on a conclusion of overall larval retention in two species within the study area off southern California. Mounting evidence from a variety of methods targeting questions about dispersal in early life history in reef fishes has suggested higher degrees of self-recruitment (Jones et al. 1999, Swearer et al. 1999), and higher behavioral retention (Leis and Carson-Ewart 1997, 1998) than expected based on time in the pelagic zone.

The results from this dissertation provide important information for the processes of designing marine reserve networks within the Channel Islands National Marine Sanctuary and the Cowcod Closure Area in the southern area of the Bight. The reserves associated with the four northern islands are not isolated from the mainland but are more likely to provide larvae to nearby habitat rather than add to a

Bight-wide larval pool. Because kelp rockfish have been exploited by the live finfish fishery and are almost exclusively associated with kelp forest habitat, understanding the connectivity of their populations is a critical step to incorporating ecosystem-based strategies for management and in describing the functioning of marine reserve networks. The scale of Bight-wide regional retention seen in *Sebastes hopkinsi* suggests that future studies might examine the presence of mesoscale eddies to correlate these features to CalCOFI larval rockfish distributions and subsequent high recruitment in species that might be retained in these eddies (that are found over potential adult habitat). Generally the work advocates the examination of hypotheses of larval dispersal within the context of natural history and ecology of each species rather than the genus as a whole. Larval identification methods presented herein, and future methods streamlining larval identification within *Sebastes*, will allow the testing of such hypotheses.

Distributions of Rockfish in April, 1999

The study on distributions of rockfish (Chapter 2) represents the first time that the entire complex of *Sebastes* larvae collected during a CalCOFI cruise has been identified to species using a combination of visual and molecular methods. This work identified the incidence of larval release for 22 species never before identified from CalCOFI samples and characterized the extent of their pelagic larval dispersal pattern in the Southern California Bight during April, 1999. This work also presents the first attempt at reconciling larval *Sebastes* distribution data with ADCP data collected concurrently and showed that distributions of the two most abundant species were concordant with the Southern California Eddy (SCE). The data suggest the SCE might be a retention mechanism overlaying some or most of Potential Adult Habitat (PAH) for species in the study and that distributions of 1-day old larvae give the best estimate of rockfish natal habitat. Larvae were aged for the most abundant species in the survey (more about this in the next section; regarding Chapter 3) and larval distributions mapped, which allowed for testing of hypotheses not possible without age data. For demersal species with habitat fidelity and liveborn larvae, the interplay of two key but independent features, PAH overlain by a persistent SCE at the time of year of parturition, might contribute to successful year classes of many species off southern California.

Much of the reported depth data for *Sebastes* may be adjusted as we learn more from ROV and submersible surveys where rockfishes can be observed in their natural habitat. By conducting quarterly surveys over multiple years, we can collect data on natal habitat and characterize and prioritize areas of natal habitat for species of particular interest as targets for marine reserves and more focused sampling. This also will facilitate the characterization of relative production from various regions for purposes of prioritizing real estate in the marine environment. More nearshore data to complement those collected from CalCOFI will be required for rockfish species with nearshore and kelp forest natal habitat.

Molecular methods proved useful for identifying both morphologically unidentifiable species and damaged or distorted specimens of the visually identifiable species that previously would not have been included in species counts. Future identifications will include these morphologically identifiable species and a survey of the utility in comparing formalin- and ethanol-fixed samples to help determine the degree of damaged specimens contributing to CalCOFI samples.

During the course of this study, molecular methods of identification improved significantly and costs have decreased to allow sequencing to be completed quickly on many individuals. As the database of genetic markers and intraspecific polymorphism for individual species improves, apparently fixed differences between species in the form of single nucleotide polymorphisms might be used to design microarrays (with species-specific binding sites), and microbeads that allow the automated reading of a fluorescent label following species-specific enzyme-ligation. These methods have sufficiently high throughput and are rapid enough to be promising for application aboard ship to collect real-time data.

Dispersal of *Sebastes hopkinsi* from Ichthyoplankton Data

The overall distribution pattern of larvae observed in CalCOFI 9904 showed a significant association of *Sebastes hopkinsi,* the most abundant species in the cruise, with the SCE identified during the cruise (Chapter 3). Between the California Current core and the coast there were mesoscale eddies with amplitudes on the same order as the total height increase across the California Current (Chereskin et al. 2000; Cornuelle et al. 2000). The age of *S. hopkinsi* found within the largest of these eddies, the SCE, were significantly younger and therefore more abundant, but these stages were not found in significant association with the shelf habitat where they were released. Distributions of preflexion-stage larvae were less reflective of adult spawning habitat because they include larvae that have been in the plankton long enough to be advected from natal source locations. Postflexion larvae are expected to be the most competent swimmers, increasingly so with development to the pelagic juvenile stage. Postflexion-stage larvae were found primarily in the vicinity of potential adult habitat.

The significant association of younger larvae with eddies suggests that these young stage larvae, although capable of some swimming behavior, are probably not controlling their placement over adult habitat. The lack of a significant difference in age distributions over PAH versus non-PAH was not unexpected. As larvae progress to postflexion, their ability to swim improves and the degree to which behavioral factors might be important in controlling their placement might rival the average (4.1 cm s^{-1}) and maximum (37.5 cm s^{-1}) current velocities in the ADCP data. Leis and Carson-Ewart (1997, 1998) found this to be true in reef fish of many fish families off the Great Barrier Reef where they were able to follow late stage larvae during the pelagic period.

Mortality is expected to have an important impact on larvae moving off of adult habitat and it is difficult to separate the factors of expatriation from PAH (e.g., due to advection) from disappearance of larvae due to mortality. The two-stage model of mortality is probably the best estimate of instantaneous mortality because the late stage larvae capable of net avoidance can be ignored.

The concordance to the SCE of young and overall larvae (Figures 3.3A-D), showed a pattern that suggests larvae might be retained in this feature. Similar patterns are seen in other rockfish species (e.g. cowcod and bocaccio) that share similar adult site fidelity to *S. hopkinsi* (Moser et al. 2001), but occupy a deeper overall preferred adult habitat (Love et al. 2002). The data support the emphasis of continued CalCOFI samples inshore of Station 60 in the ethanol-fixed side of the bongo. These data will be critical for the construction of trends over time in individual rockfish species.

Sebastes hopkinsi can spend over 100 days in the pelagic zone (Sakuma and Laidig unpublished data) and although postflexion larvae from this study appear to be near potential adult habitat where settlement is possible, it may be up to two months before the late stage larvae transform to the benthic juvenile stage, when year class strength likely is determined (Bradford 1992, Ralston and Howard 1995). As the number of preferred sportfishing species (e.g., cowcod and bocaccio) have dwindled at the offshore banks, species like *S. hopkinsi* are increasing in frequency in the sportfishing catch off southern California (Love et al. 2002). The CalCOFI quarterly sampling program offers an ideal context to identify these transitions at annual and decadal scales not possible for rockfish species prior to the advent of molecular methods of identification. To identify trends in mortality and abundance, and important early life history factors such as frequency and time of spawning for many species such as *S. hopkinsi*, it is clear that methods of molecular identification of larval and otolith-based ageing integrated with the physical oceanography of the region will be critical for understanding changes over time.

Kelp Rockfish Genetics and Dispersal

Kelp rockfish data show restricted gene flow, but this appears to be focused primarily along the coast and at nearshore island populations. The data do not discount an abrupt barrier to gene flow, although the signal appears to be driven by an isolation by distance (IBD) signal along the coast and at nearshore northern Channel Islands. This suggests that the nearshore islands that are part of the Channel Islands National Marine Sanctuary (CINMS) are behaving as narrowly dispersing populations not unlike coastal populations.

The pattern of gene flow seen in our data agrees well with the circulation off central and southern California. The contribution of common eddies in the region probably have important consequences for recruitment of kelp rockfish, certainly at this offshore islands of San Nicolas (SN) and San Clemente (SCL). The IBD pattern at offshore islands of SN and SCL, based on pairwise comparisons of these island populations, showing zero or near zero genetic distance at both low and high geographic distances suggest that these island populations are as likely to receive recruits from the geographically closest coastal populations as ones more distant. The loss of the IBD signal when offshore islands were included indicates that these islands appear to be experiencing gene flow among a variety of adjacent and non-adjacent locations akin to the island model of dispersal. Understanding the degree of connectivity among these differing populations is critical considering that kelp habitat with which they are associated can also have large fluctuations in abundance based on local ecosystem dynamics (Dayton et al. 1984; Dayton et al. 1998). In kelp rockfish, larvae appear to get offshore from coastal populations as evidenced by the pairwise comparisons of these coastal populations with offshore islands. Larvae do not appear to move far alongshore or realize much dispersal distance alongshore. Instead, larvae that move offshore may be caught up in eddy flow, if it is present, or be lost through mortality. If eddies were depositing larvae at long distances from source populations along the coast, we would not expect to find an IBD signal in the data. Instead it is more likely that eddies contribute to deposition of larvae at offshore islands, whereas flow into kelp beds might be dampened nearshore due to a coastal boundary layer (Largier 2003) and fluid dynamics within the kelp bed (Jackson 1983; Jackson and Winant 1984). The degree to which these factors might be important is a potential avenue of future study.

The backcalculation of the age of the subset SCL^{98} of individuals from the SCL population suggests another avenue for future work in examining the differential recruitment between two groups of nearshore subgenera of rockfishes tested for monophyly in Taylor (1998). Data from central California midwater trawl surveys suggest that pre-recruiting late-larval stage rockfishes within the subgenus *Pteropodus* have been well represented in warm, El Niño years, but poorly represented in colder La Niña years. In contrast, the subgenus of structure schooling nearshore species within the subgenus *Sebastosomus* appeared to be well represented in colder La Niña years and well-represented in warm, El Niño years (Lenarz et al. 1995). If Lenarz et al. (1995) are correct, and nearshore *Pteropodus* are poorly represented in La Niña years, then contrasting the distributions of the CalCOFI 9904 data (Chapter 2) with those of 9804 is an important future goal to make the distinction between whether CalCOFI is too offshore to obtain these *Pteropodus* larvae or whether they were just poorly represented in the cruise represented here. The absence of members from the subgenus *Sebastosomus* in the CalCOFI 9904 samples supports the former explanation (that the CalCOFI sampling grid is too far offshore to represent these nearshore species). Genetic identification of long term monitoring data such as those obtained in CalCOFI and complementary nearshore stations will be critical for documenting dispersal patterns, the variance associated with dispersal patterns and physical and primary production that might contribute to dynamics in rockfish recruitment over scales of their dispersal.

Currently MPAs are being considered as a potential complementary tool (in addition to traditional management efforts e.g., limits on size, catch, effort, season) for the conservation of marine biodiversity in the United States. In this regard, the work on kelp rockfish suggests that offshore islands of SCL and SN would work well due to their remote location and diversity of habitat. As an MPA to support fisheries in a larval export capacity, one but not both of these locations might be useful. These islands appear to be acting more as sink than source populations relative to other populations in the study and they do not appear to be a major contributor to the replenishment of nearshore coastal populations, nor do they appear to be exclusively self-seeding. The caution is that if we place stock in offshore island reserves without protecting coastal sources that might act to re-seed them, we may doom these offshore island populations to extinction.

The hypothesis-testing framework used in the population genetic study for kelp rockfish is useful to define management units, although it has limitations for continuously distributed species. Significant differences were found between northern and southern pooled populations, however, these differences are most likely driven by IBD and the statistical artifact of hypothesis-testing between the most disparate of the isolates. The demonstration of clinal gene flow can be useful in postulating the construction of MPAs within the SCB or heterogeneity detected might be accepted as a strict barrier to gene flow, whether driven by IBD or not, as observed differences between the two hydrographic domains. Such an acceptance would suggest that a comprehensive region-wide reserve network would require reserves within the northern region including the northern Channel Islands, the southern region including Point Loma, La Jolla and Mexico and one or both of the offshore islands, although the outer islands of San Nicholas and San Clemente were not genetically isolated, did not conform to the isolation by distance model, and appear to receive enough migrants from a variety of local and distant sources to have a well-mixed gene pool.

The loss of IBD at offshore island populations relative to the strong signal in coastal populations is an important finding for this species in the effort to assess dispersal at scales that are effective for fisheries as well as conservation management. This is the first study to examine a fish species at this scale in the SCB—one abundant at both coastal and offshore islands with an extended pelagic life history phase using genetic data examined kelp rockfish using morphological data. In light of current pressure from the live finfish and other fisheries, we might want to avoid putting our MPA effort into the placement of offshore island reserves at locations such San Nicolas and San Clemente Islands without simultaneously protecting coastal areas. Locations of MPAs are critical for species protection whether it be for conservation, protection of adult spawning stock for larval export or for aesthetic goals.

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