RETENTION OF LARVAL ROCKFISHES, *SEBASTES*, NEAR NATAL HABITAT IN THE SOUTHERN CALIFORNIA BIGHT, AS INDICATED BY MOLECULAR IDENTIFICATION METHODS

CYNTHIA A. TAYLOR[1]
Scripps Institution of Oceanography
University of California, San Diego
9500 Gilman Drive
La Jolla, California 92093-0203
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, California 92037-1508
ctaylor@illumina.com

WILLIAM WATSON
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, California 92037-1508

TERESA CHERESKIN
Scripps Institution of Oceanography
University of California, San Diego,
9500 Gilman Drive
La Jolla, California 92093-0203

JOHN HYDE
Scripps Institution of Oceanography
University of California, San Diego
9500 Gilman Drive
La Jolla, California 92093-0203
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, California 92037-1508

RUSSELL VETTER
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, California 92037-1508

ABSTRACT

Larval (<16 mm SL) *Sebastes* spp. that could not be identified visually were identified with molecular genetic methods from plankton samples collected during a California Cooperative Oceanic Fisheries Investigations (CalCOFI) cruise in April 1999. Our goals were to characterize natal habitat and to determine the abundances of individual rockfish species in CalCOFI ichthyoplankton samples. This is the first time the entire complex of *Sebastes* larvae collected during a CalCOFI cruise has been identified to species. For three abundant species, we found a significant association between abundance and stations within the Southern California Eddy (SCE), and a significant relationship between the distribution of 1-d-old larvae and potential adult habitat for the most narrowly distributed of the three species. These results suggest that the interplay of natal spawning habitat overlaid by the persistent SCE contributes 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 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. Because net tows are easier and cheaper than sampling benthic or patchily distributed adults, surveys of planktonic larvae can be an efficient means to assess populations of marine fishes (Lasker 1985). 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 taken in commercial and sport fisheries (Miller and Lea 1972; Eschmeyer et al. 1983). Adult rockfishes commonly are benthic and often show high fidelity to a single site (Love 1979; Love et al. 1990).

The CalCOFI collections are a rich data source for constructing temporal abundance trends in many species; however, larvae of only seven northeastern Pacific *Sebastes* species can currently be positively identified by means of pigmentation and morphological criteria (Moser et al. 1977; Moser 1996). The remainder, grouped as unidentified *Sebastes* spp., ranked fourth in abundance and second in frequency of occurrence over the history of CalCOFI sampling from 1951 through 1998 (Moser et al. 2001).

Molecular genetic data, a constant at all life stages, provide a method to assign species identifications to unidentified larvae by comparison to reference data of known adults. This study is the first in which the entire complex of *Sebastes* larvae collected during any cruise has been identified to species through a combination of visual and molecular methods. We present species-specific larval distributions mapped onto the velocity flow field during the April 1999 CalCOFI cruise to examine patterns over the course of the early (<40 days) pelagic period, and to examine the hypothesis that larvae are equally distributed inside and outside of the Southern California Eddy (SCE), a persistent feature with a low velocity center in the spring in the SCB. We further examined distributions of 1-d-old larvae for the

[Manuscript received 8 April 2004]

[1]Current address: 9885 Towne Center Drive, San Diego, CA 92121-1975

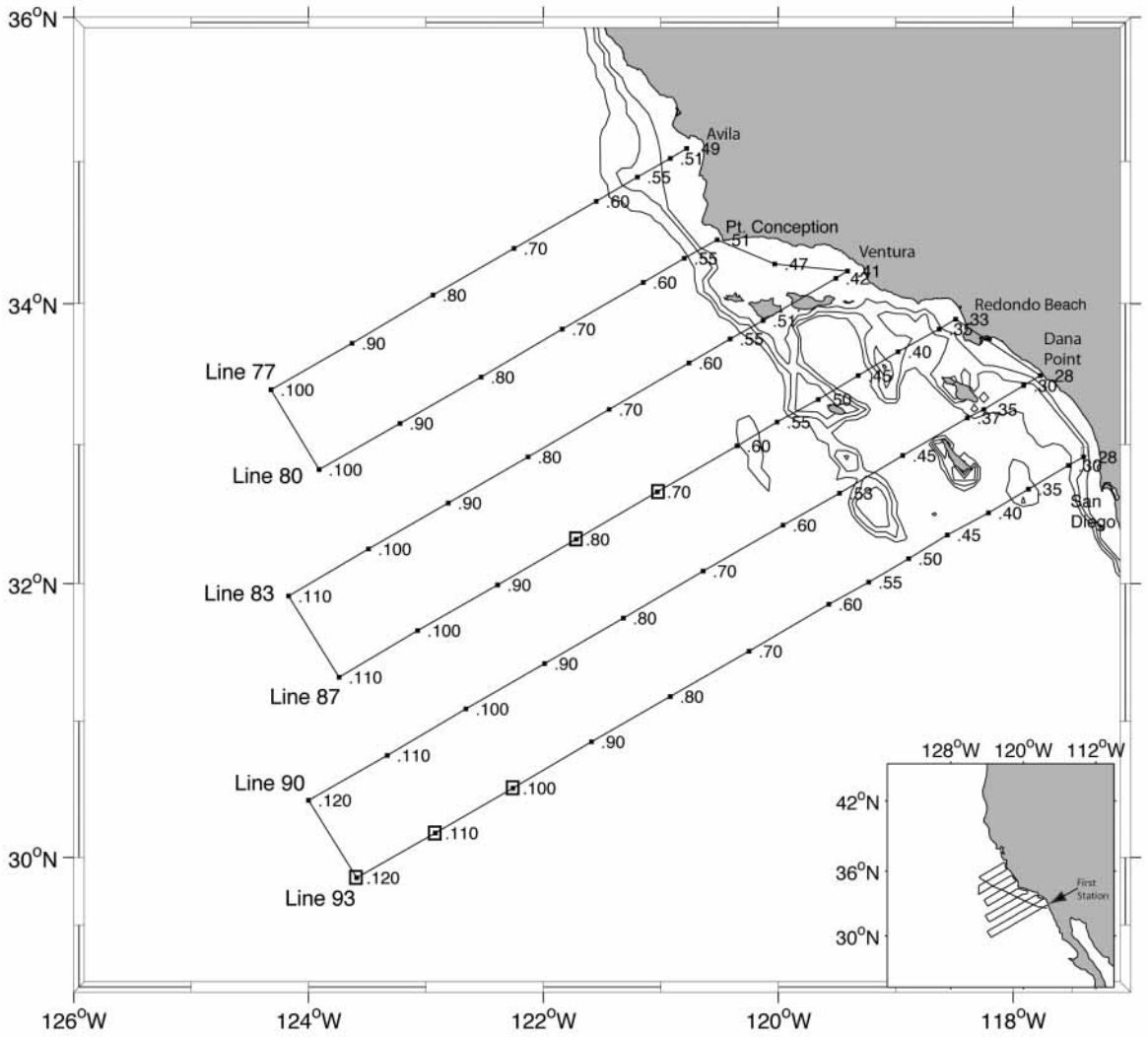

Figure 1. Standard CalCOFI stations. Squares indicate stations that were not sampled during the April 1999 cruise (from Ambrose et al. 2001). Ethanol-fixed samples were collected seaward to station 80 on all lines. Isobaths are 300, 500, and 700 m. Underway acoustic measurements were made up to Monterey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

most abundant species relative to adult depth ranges, which we define as potential adult habitat (PAH), to evaluate the association of larval occurrence with PAH. These data are useful for identifying the natal habitat of target species—critical information for fisheries management, including the siting of marine protected areas.

MATERIALS AND METHODS

Sample Collection

Parturition in *Sebastes* takes place 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 1 to 15 April 1999 aboard the *RV David Starr Jordan*. 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 net with 0.505 mm mesh and towed at a ship speed of about 1 m s^{-1} at each of 61 standard stations from Avila Beach to San Diego (fig. 1). The sample from one side of the bongo net was fixed in 5% sodium-borate-buffered formalin at all stations. At stations shoreward from station 80, the other sample 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 station 80 (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 counts 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 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. moseri,* and *S. paucispinis*) were presorted from the ethanol-fixed samples, and all the remaining *Sebastes* spp. were identified using molecular methods. Larvae that were positively identified to species based on pigmentation and morphology were not reidentified with 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 1× buffer containing 20 mM Tris HCl, 50 mM KCl, and 1.5 mM $MgCl_2$ 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 protocol: 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 with Qiaquick kits (Qiagen, Inc.) and cycle-sequenced according to manufacturer protocols with an ABI 3100 automated sequencer. Chromatogram data for sequenced DNA were aligned by means of the biosequence analysis and editor program Sequencher (ver. 4.1.1 Gene Codes, Inc.).

Larval sequences were compared to DNA reference sequence data of 374 independent haplotypes representing 67 species of identified adult *Sebastes* with 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 the appendix.

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 up to 17 known adults, see appendix) from known adults for putative identification. If a larva clustered with the single haplotype of a reference species with a bootstrap value ≥ 90%, this was accepted as the identification of the larva. Distance between reference haplotypes and the unknown was examined to confirm that the unknown fell within the expected intraspecific diversity based on the reference data. If a larva clustered with a single haplotype of a species with a bootstrap < 90%, this was accepted as a first-pass identification; 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 direct comparison to the reference species. Intraspecific diversity for reference species in the NE Pacific has a mean distance 0.002 with a minimum of 0 (e.g., *S. jordani*) and a maximum of 0.01 (in *S. aleutianus*).

ADCP and Circulation

Upper ocean currents were measured continuously along the ship track from a hull-mounted RD Instruments 150 kHz narrowband acoustic Doppler current profiler (ADCP). The ADCP was configured to transmit an 8 m pulse every second along 4 beams directed downwards at 30 degrees from 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 by means of GPS measurements. The absolute currents were then averaged over hourly intervals, reducing 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 with 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 near the surface. 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 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). We used a quasi-geostrophic streamfunction objective analysis (Chereskin and Trunnell 1996) to map the averaged and de-tided ADCP velocities. The smoothing and nondivergence enforced by the mapping further reduces errors in the large-scale velocity field.

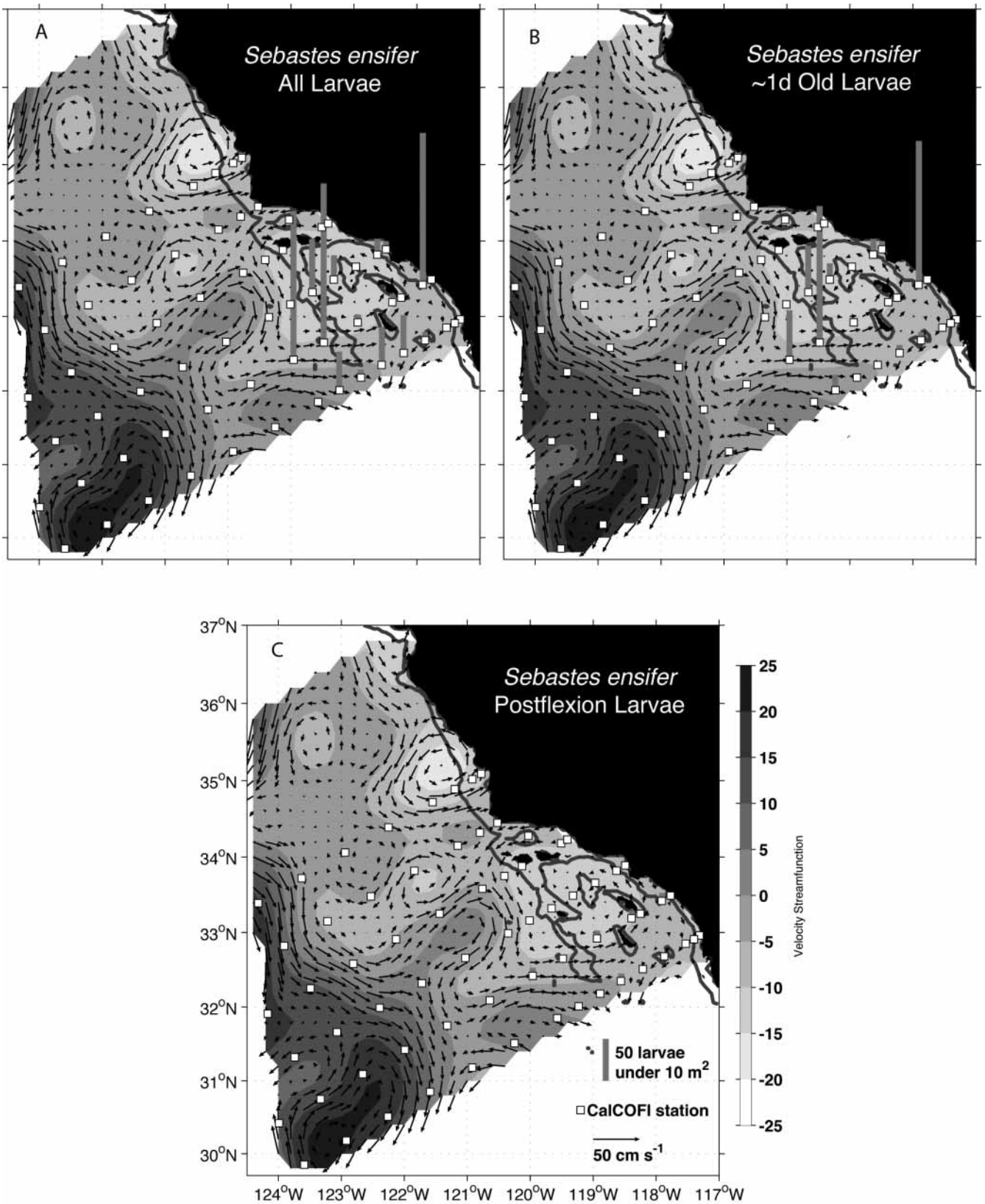

Figure 2. Distributions of larval *Sebastes ensifer* off southern California in April 1999. (*A*), all larvae; (*B*), larvae estimated to be ≤ 1-d-old; (*C*), postflexion-stage larvae. The 500 m isobath, indicating potential adult habitat, is shown as a dashed line. The ADCP-derived velocity stream-function data are shown with shading, and current vectors are shown as arrows.

TABLE 1
Rockfish Species with Fewer than Two Occurrences During the April 1999 CalCOFI Cruise

Sebastes spp.	Observed station(s)	Abundance(per 10 m^2)	Length (mm)	Flexion
S. constellatus	87.50, 83.55	23.67,16.35	3.0–3.8	Preflexion
S. elongatus	90.53, 87.35	18.09, 9.20	2.9–4.2	Preflexion
S. semicinctus	90.45, 87.35	9.32, 9.20	4.0 and 17.4	Pre- and postflexion
S. chlorosticutus	87.35	18.39	3.5 and 3.6	Preflexion
S. rosaceus	90.60, 87.50	9.86, 7.89	3.2 and 4.6	Preflexion
S. atrovirens	83.55	16.34	3.4 and 4.1	Preflexion
S. goodei	90.35	10.4	7.3	Flexion
S. rufinanus	90.60	9.86	4.5	Preflexion
S. carnatus	90.45	9.32	7.5	Flexion
S. rosenblatti	93.55	9.15	5.4	Preflexion
S. levis	90.53	9.05	4.2	Preflexion
S. caurinus	93.28	8.73	9.5	Postflexion
S. diploproa	87.50	7.89	4.2	Preflexion

Eddy and Potential Adult Habitat Association

We used an analysis of variance (ANOVA) to examine the null hypothesis that each species is distributed equally 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. We used the high-frequency array of surface currents collected in April 1999 and used CODAR (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 15 eddy-positive stations included line 90, stations 28–53; line 86, stations 33–55; line 83, stations 51–55; and line 82, station 47 (e.g., fig. 2). All abundance data (A) were converted to ln(A+1) for the analyses, which could be performed only on the most abundant species (*S. hopkinsi, S. ensifer, S. rufus,* and *S. jordani*). Distributions of species with more than three occurrences were mapped (and examined) relative to the ADCP (and CODAR) data. Observations for species with fewer occurrences are listed in Table 1 as are corresponding data on locality, abundance, and size.

We also used ANOVA to examine the null hypothesis that 1-d-old larvae are randomly distributed and no more likely to be found in the vicinity of PAH (potential adult habitat) than non-PAH. Stations were categorized as within 20 km of being vertically over PAH, or beyond 20 km (non-PAH). Generally we refer to any habitat deeper than 501 m as "basin." We defined 1-d-old larvae by otolith-based aging (*S. hopkinsi*),[2] or by using length (mm) as a proxy for age (*S. ensifer* and *S. rufus*), where otolith 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.

[2]Taylor, C. A., W. W. Watson, T. Chereskin, A. Henry, and R. D. Vetter. Age-specific dispersal patterns of larval (1–42-d-old) squarespot rockfish, *Sebastes hopkinsi,* in the Southern California Bight identified using molecular methods (manuscript).

RESULTS

Five *Sebastes* species were identified in the formalin-fixed samples (tab. 2), with 94% of the total *Sebastes* larvae classified as "*Sebastes* spp." In the ethanol-fixed samples an additional 18 species were identified through molecular methods; only 0.64% of the total *Sebastes* larvae remained unidentified (tab. 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 23 species. *Sebastes hopkinsi* occurred in highest abundance and most frequently, followed by *S. ensifer*, *S. rufus*, and *S. jordani* (tab. 2). For *S. "wilsoni"* it was possible only to narrow the identification to three (*S. variegatus*, *S. wilsoni*, and *S. zacentrus*); for *S. carnatus*," to two species (*S. carnatus* and *S. chrysomelas*). For cytochrome *b*, there is incomplete lineage sorting among some closely related *Sebastes* sister species. *Sebastes variegatus* does not occur off southern California, and although *S. zacentrus* does, it is much less common than *S. wilsoni* (Love et al. 2002; J. Butler, pers. comm.). Thus, *S. wilsoni* is the most likely identification among the three, but we use quotations to indicate the ambiguity. For the *S. "carnatus,"* although the haplotype was most closely related to an adult *S. carnatus* haplotype, both *S. carnatus* and *S. chrysomelas* are common in the SCB, and either species is a likely identification for this larva.

Most larvae identified visually as *S. "moseri"* were presorted from the ethanol-fixed samples and not sequenced. There was one exception: a larva that was missed in the presort was subsequently identified as *S. rufinanus*. In an earlier study of larval *S. moseri* based on other ethanol-preserved CalCOFI samples, while most matched the putative *S. moseri* haplotype, 22% had a haplotype that differed by 2% from the *S. moseri* reference. These were

TABLE 2
Rockfish Species from the April 1999 CalCOFI Cruise

Sebastes spp.	Total abundance	Positive stations	Larval counts	Mean abundance	
				All tows	Positive tows
S. hopkinsi	979.74	22	116	21.07	44.07
S. ensifer	843.06	14	91	18.33	60.22
S. rufus	140.91	9	15	3.06	15.66
S. jordani	204.92	9	23	4.45	22.77
S. melanostomus	103.59	5	11	2.25	20.72
S. "wilsoni"	92.76	7	11	2.02	13.25
S. "moseri"	89.67	8	18	1.94	11.21
S. ovalis	68.22	3	8	1.48	22.74
S. jordani[a]	64.84	3	13	1.41	21.62
S. aurora[a]	62.56	5	12	1.36	12.51
S. "moseri"[a]	57.80	6	8	1.26	9.63
S. paucispinis[a]	52.37	5	12	1.14	10.47
S. saxicola	45.63	3	5	0.99	15.21
S. constellatus	40.02	2	5	0.87	20.01
S. simulator	28.59	3	3	0.62	9.53
S. elongatus	27.29	2	3	0.59	13.64
S. semicinctus	18.51	2	2	0.40	9.25
S. chlorosticutus	18.39	2	2	0.40	18.39
Sebastes spp.	26.14	3	2	0.57	8.71
S. rosaceus	17.75	2	3	0.39	8.87
S. atrovirens	16.35	1	2	0.36	2.38
S. goodei	10.40	1	1	0.23	10.39
S. rufinanus	9.86	1	1	0.21	9.86
S. "carnatus"	9.32	1	1	0.20	9.31
S. rosenblatti	9.15	1	1	0.20	9.15
S. levis	9.05	1	1	0.20	9.05
S. caurinus	8.73	1	1	0.19	8.73
S. diploproa	7.89	1	1	0.17	7.89
S. levis[a]	4.58	1	1	0.10	4.58

Note: Species in quotation marks are the most likely of two possible identifications.
[a]Species from the formalin-fixed side of the bongo net.

TABLE 3
Comparison of the Ethanol-fixed Versus the Formalin-fixed Sides of the Bongo Net for CalCOFI Cruise 9904

Side of bongo net	Species identified	Total abundance	Positive tows	Larval counts
Formalin-fixed	5 + 94% in *Sebastes* spp.	2,521.16	28	577
Ethanol-fixed	23 + 0.64% in *Sebastes* spp.	2,916.03	26	327

Note: An OPC adjustment based on larval counts was used to make formalin- and ethanol-fixed sides of the bongo net comparable.

later found to match the *S. rufinanus* haplotype. Thus, both species are likely to be included in the presorted group listed here as *S. "moseri"* (tab. 2), although the larvae are probably predominantly *S. moseri*. Observations from submersibles suggest that *S. moseri* is more abundant than *S. rufinanus* off southern California (J. Butler, pers. comm.).

The California Current appears 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., fig. 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^{-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 they have the potential to transport anomalies (and potentially larvae) offshore (Chereskin et al. 2000; Cornuelle et al. 2000). In April 1999 there were 3 cyclonic and 2 anticyclonic eddies in the SCB. A cyclone/anticyclone pair occurred between lines 80 and 87 near station 70. A second anticyclone was located near line 93, station 120. A second cyclone was off Avila, and a

third was consistent with the SCE, a persistent feature in the SCB northwest of San Clemente Island (fig. 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). Thus, some of the weakest currents observed during April 1999 were within the SCB, and the area of highest potential larval retention was over PAH.

Sebastes hopkinsi larvae were the most abundant of the rockfish larvae. Adults have been reported to 150 m depth (Love et al. 2002) but have been observed on ROV transects to 200 m (J. Wagner, pers. comm.). This currently is the most commonly taken rockfish in the southern California recreational fishery, primarily because of the declining abundances of larger species (Love et al. 2002). We mapped distributions of the 1–42-day-old larvae relative to the 200 m depth contour.[3] 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 = 0.0001). All 1-d-old larvae were near PAH, with no observations farther than 20 km away.

Sebastes ensifer, a small species sold primarily in the Asian fish market, is only a moderate part of the recreational catch, probably because of its small size (Love et al. 2002). It ranges deeper (to 433 m) than *S. hopkinsi*, more like other species in the survey. Larval distributions were mapped relative to a 500 m PAH (fig. 2). Total larval abundance was significantly higher at eddy stations (mean[A] = 1.868) than at non-eddy stations (mean[A] = 0.691; F_1 = 4.977, p = 0.031). Although a statistically significant association of 1-d-old larvae with PAH was not detected (F_1 = 1.302, p = 0.26), mean abundance over PAH was more than twice that at stations away from PAH (mean[A] = 0.415).

Sebastes rufus is often seen in ROV surveys off southern California (J. Butler, pers. comm.) and frequently is caught by recreational fishers (Love et al. 2002). It ranges to a maximum reported depth of 454 m. We mapped larval distributions over a 500 m PAH (fig. 3) and found that larval occurrence was not concordant with the SCE (F_1 = 0.23, p = 0.64), and 1-d-old larvae were not more abundant over PAH (F_1 = 0.493, p = 0.49).

Sebastes jordani has been thought to have the highest biomass of any rockfish off California (e.g., Ralston et al. 2003), but it has little direct commercial or recreational fishery value (Love et al. 2002). It ranges to 491 m, and we mapped larval distributions relative to a 500 m PAH (fig. 4). Total larvae were significantly more abundant within eddies (mean[A] = 1.15) than outside eddies (mean[A] = 0.243; F_1 = 7.03, p = 0.01). This was the only *Sebastes* species found in the Santa Barbara Channel. *Sebastes jordani* offered an opportunity to compare catches between the ethanol- and formalin-fixed sides of the bongo net (fig. 4A,B). The highest abundances were concordant between nets and near adult habitat. However, some positive stations in the ethanol-fixed samples lacked matching occurrences in the formalin-fixed samples. The majority of these (4 of 6 stations) reflect a lack of *S. jordani* in the formalin-preserved samples; at one of the remaining two stations, two larvae identified as *Sebastes* spp. were similar to *S. jordani* and might represent a previously unrecognized pigment polymorphism. In the ethanol-fixed samples, 56% of the *S. jordani* larvae were too damaged or distorted to be positively identified by sight as *S. jordani* but could be positively identified with molecular methods.

Sebastes melanostomus, one of the deeper-dwelling species, with a reported maximum depth of 768 m, is important in Asian fish markets of southern California and is occasionally landed in the recreational fishery (Love et al. 2002). The larval distribution was largely concordant with PAH, and most occurrences were within the SCE, with the highest abundances within the gyre (fig. 5A).

Sebastes wilsoni ranges to 383 m depth, and we mapped the larval distribution of *S. "wilsoni"* relative to a 500 m PAH. There was no clear association of preflexion larvae with either PAH or the SCE (fig. 5B).

Sebastes ovalis is a part of the commercial catch off southern California. Adults range to 366 m (Love et al. 2002), and larval distributions were mapped relative to a 500 m PAH. Abundances were concordant with both PAH and the SCE (fig. 5C).

Sebastes aurora has been important in artisanal fisheries off Newport Beach, California, and is landed recreationally off southern California. It ranges to 768 m (Love et al. 2002) and we mapped larval distributions relative to a 1,000 m PAH. Larval abundance was concordant with PAH, the SCE, and with a coastal eddy off Avila (fig. 5D).

Sebastes paucispinis is an important sport and commercial fishery species currently considered depleted and subject to a stock rebuilding plan (MacCall et al. 1999). Adults range to 478 m (Love et al. 2002, and the larval distribution was mapped relative to a 500 m PAH (Love et al. 2002). With the exception of nearshore stations off San Diego and Dana Point, collections were concordant with the SCE (fig. 6A). All larval occurrences were concordant with PAH.

[3]Taylor et al., Age-specific dispersal patterns (manuscript).

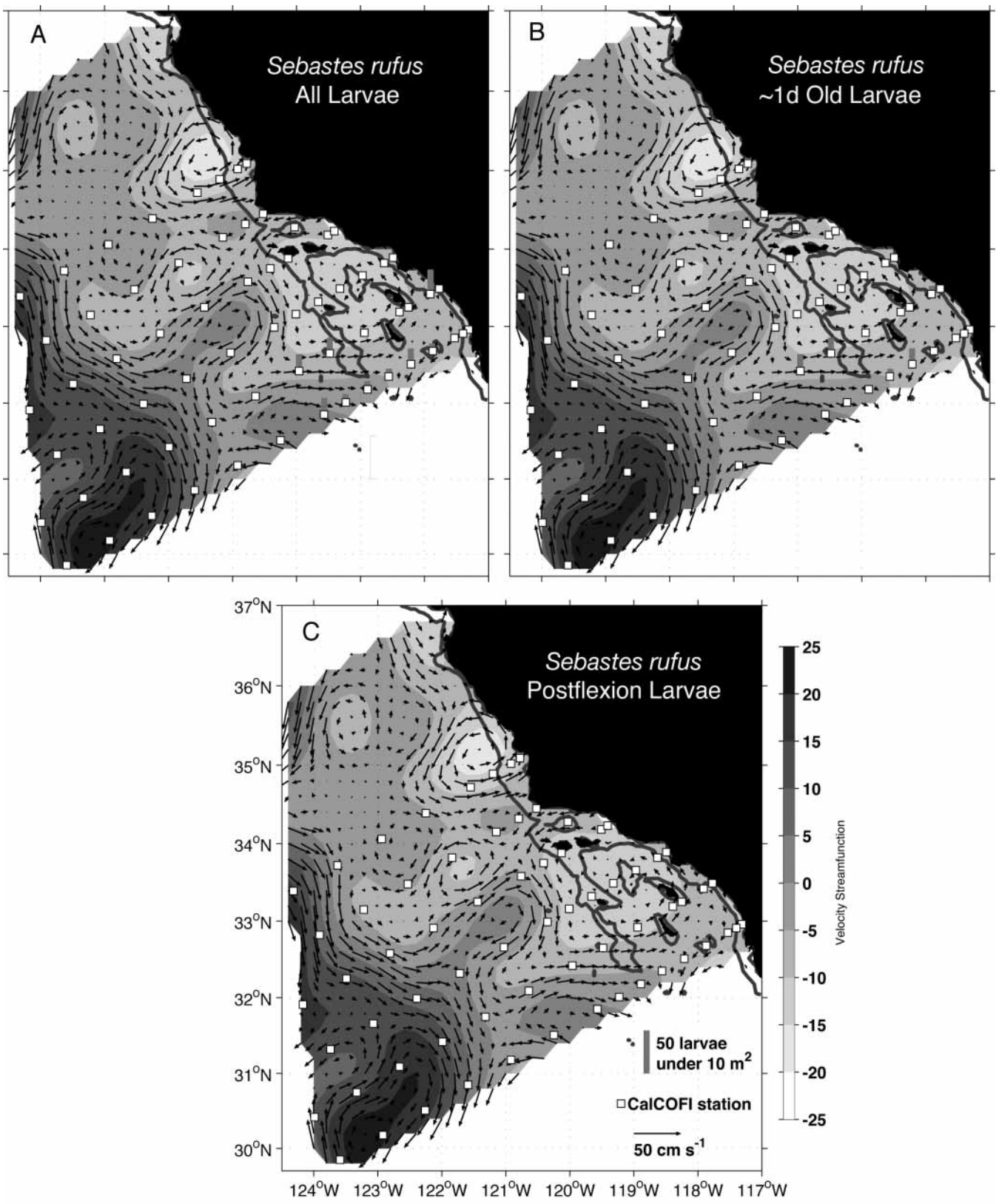

Figure 3. Distributions of larval *Sebastes rufus* off southern California in April 1999. (*A*), all larvae; (*B*), larvae estimated to be ≤ 1-d-old ; (*C*), postflexion-stage larvae. The 500 m isobath, indicating potential adult habitat, is shown as a dashed line. The ADCP-derived velocity stream-function data are shown with shading, and current vectors are shown as arrows.

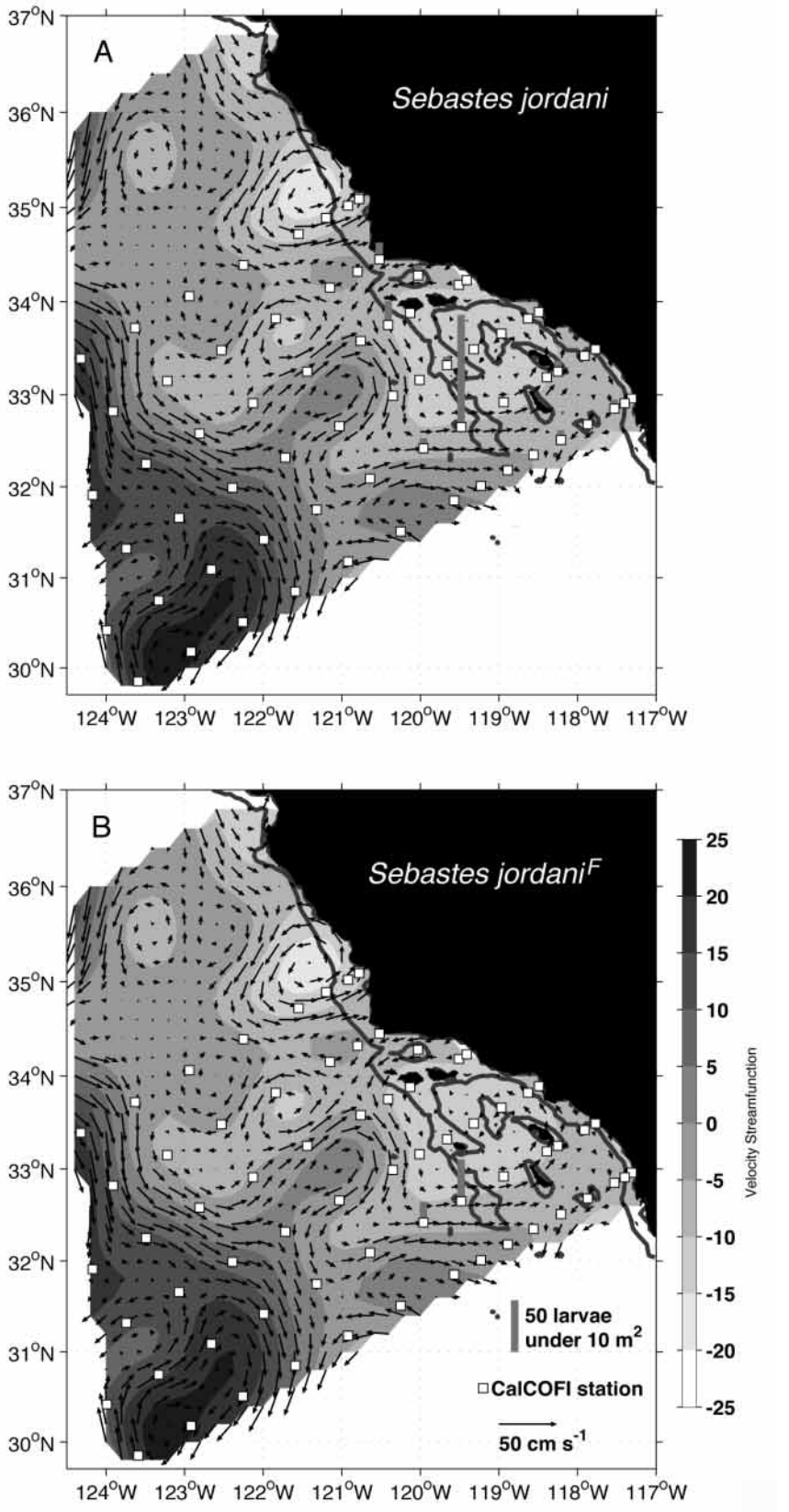

Figure 4. Distributions of larval *Sebastes jordani* off southern California in April 1999. (*A*) all larvae from ethanol-preserved samples; (*B*) all larvae from formalin-preserved samples. The 500 m isobath, indicating potential adult habitat, is shown as a dashed line. The ADCP-derived velocity stream-function data are shown with shading, and current vectors are shown as arrows.

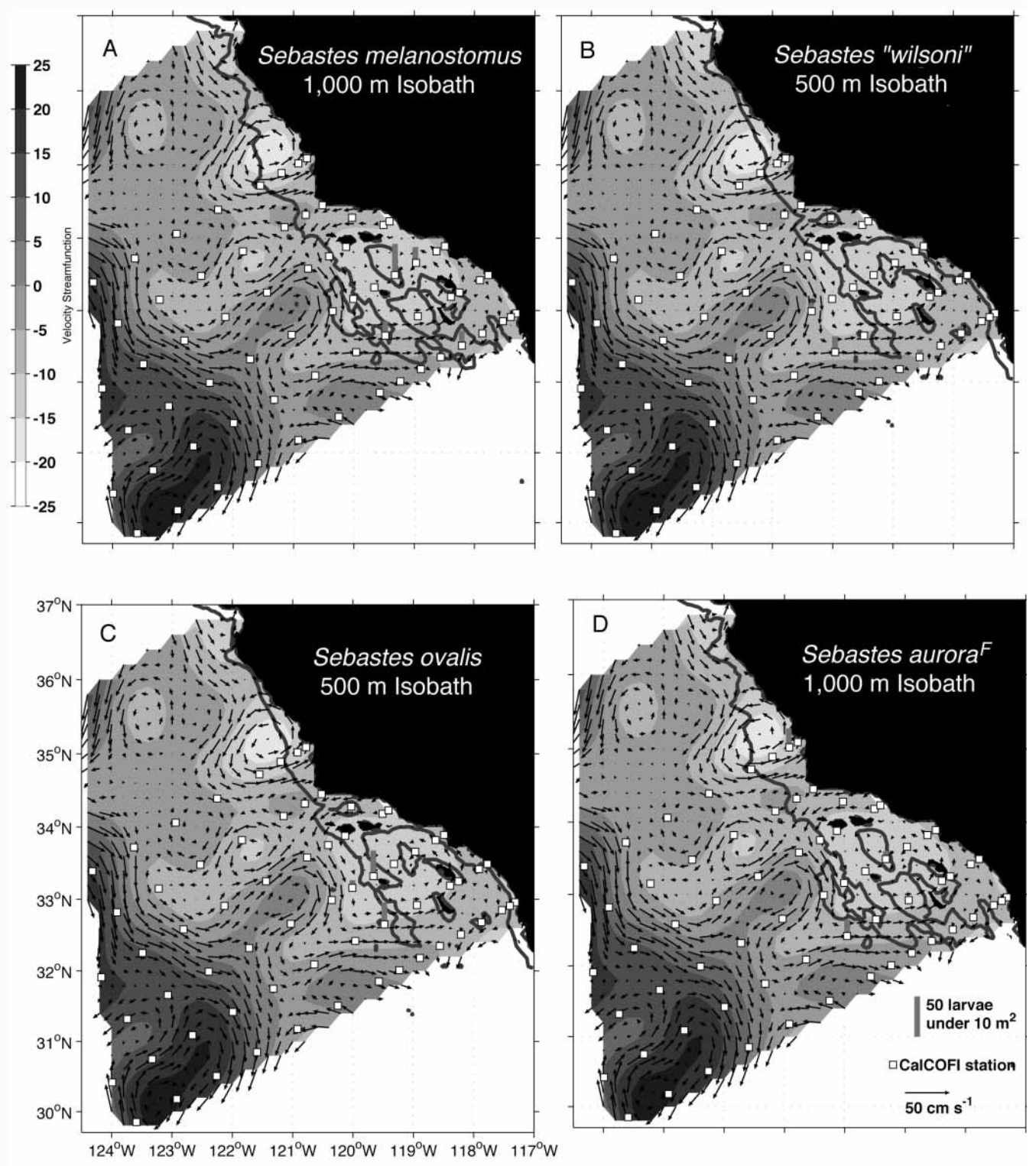

Figure 5. Distributions of larval *Sebastes* off southern California in April 1999. (*A*) *S. melanostomus*, all larvae; (*B*) *S. "wilsoni,"* all larvae; (*C*) *S. ovalis*, all larvae; (*D*) *S. aurora*, all larvae from formalin-preserved samples. Potential adult habitat is denoted by the 500 m (*B*, *C*) or 1,000 m (*A*, *D*) isobaths, shown as a dashed line. The ADCP-derived velocity stream-function data are shown with shading, and current vectors are shown as arrows.

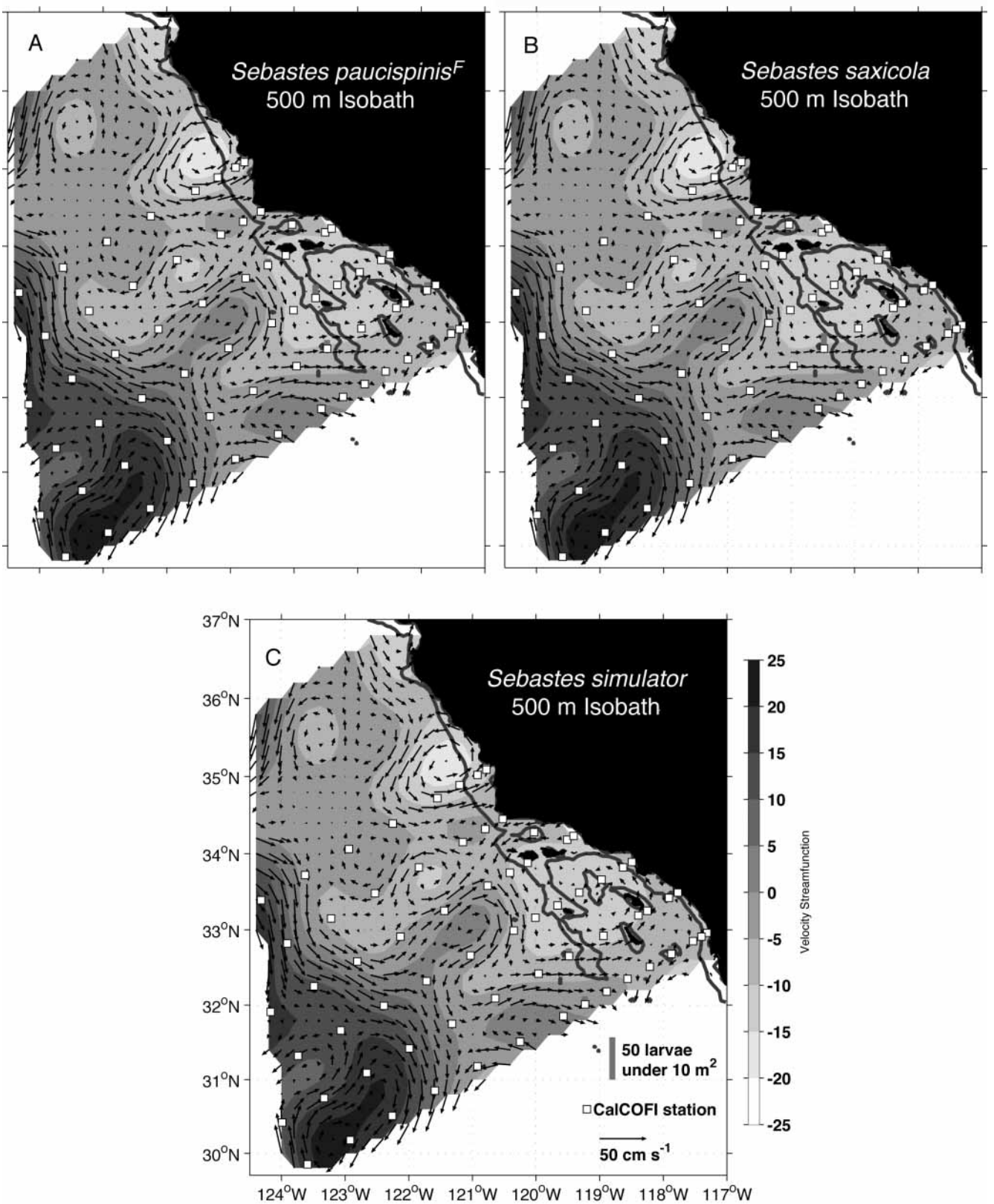

Figure 6. Distributions of larval *Sebastes* off southern California in April 1999. (*A*) *S. paucispinis*, all larvae in formalin-preserved samples; (*B*) *S. saxicola*, all larvae; (*C*) *S. simulator*, all larvae. The 500 m isobath, indicating potential adult habitat, is shown as a dashed line. The ADCP-derived velocity stream-function data are shown with shading, and current vectors are shown as arrows.

Sebastes saxicola is a small species not used 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 500 m PAH. Postflexion larvae were found at a nearshore station (93.30); 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 (fig. 6B).

Little is known about *Sebastes simulator*, but it is thought to occur at depths to 265 m. We used a 500 m PAH and found the larvae to be near PAH but well outside the SCE (fig. 6C).

DISCUSSION

This is the first time that the entire complex of *Sebastes* larvae collected during a CalCOFI cruise has been identified to species; this was made possible by the use of a combination of visual and molecular methods. In doing so, we confirmed seasonal spawning incidence of 22 species and identified the extent of their larval dispersal pattern in the SCB during April 1999. We also present the first attempt at reconciling larval *Sebastes* distributions with concurrent ADCP data.

Identifying larval vertical distributions 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 some undergo an ontogenetic shift in vertical distribution during the pelagic period (Tully and Oceidigh 1989; Doyle 1992). Early larvae typically occur above the thermocline (Ahlstrom 1959; Boehlert et al. 1985; Moser and Boehlert 1991), but not as shallow as the neuston (Tully and Oceidigh 1989; Doyle 1992). Later-stage larvae and juveniles are rarely found deeper than 100 m in depth-stratified samples in the SCB (Nishimoto 2000; Watson and Taylor, unpub. data). Thus 25–75 m was a reasonable depth interval over which to integrate velocity data in this study. We also observed consistent patterns of flow 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.

By mapping ADCP data and treating it as a synoptic view of the velocity field experienced by the larvae, we demonstrated concordance of the two most abundant species with the SCE. Our data suggest that the SCE is a retention mechanism, overlaying some 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 locations. Distributions of 1-d-old larvae probably give the best estimate of rockfish natal habitat. For species with natal habitat overlaid by the SCE during the April 1999 cruise (e.g., *S. hopkinsi*, not shown, and *S. ensifer*, fig. 2B), total larval abundance was significantly associated with the SCE. We surmise that those larvae are retained in the vicinity of their natal habitat.

Some other species did not fit this pattern, however. For example *S. rufus* (fig. 3A) occurred largely outside the SCE, and presumed 1-d-old larvae (fig. 3B) were not concordant with expected PAH. It could be that *S. rufus* has a deeper than reported distribution, or that this species, often observed in schools (Love et al. 2002; J. Butler, pers. comm.), might release larvae away from expected PAH. We suggest that if larvae are not released within the SCE, they cannot be retained there, although they might later return as pelagic juveniles.

For demersal species with habitat fidelity and live-born larvae, the interplay of two key but independent features—PAH overlain by a persistent SCE in the season of parturition—might contribute retention in these species off southern California. The degree to which behavioral factors, including swimming ability, might contribute to larval retention is unknown; these factors probably become important only as larvae develop into the postflexion stage. Observations of postflexion larvae for *S. hopkinsi* (not shown), *S. ensifer* (fig. 2C) and *S. rufus* (fig. 3C) show close proximity to PAH, but we did not statistically test the significance of this association, because of the high effect of mortality on these abundance data. The relatively brief sampling of this survey limits our ability to generalize, but the hypothesis of SCE retention might be explored by examining ADCP data and larval distributions from additional cruises when the SCE is weaker or absent.

Both the CalCOFI grid and our definition of PAH are coarse. Reported *Sebastes* depth data are largely from fishers and may be adjusted in the future with ROV and submersible surveys when we can observe rockfishes in their natural habitat. Our plan in this study was to resolve mesoscale features of the natal habitat for individual rockfish species. This information would make it possible to prioritize areas of species habitat as targets for marine reserves and more focused sampling (e.g., the Cowcod Closure Area off southern California is currently the subject of high-resolution ichthyoplankton surveys). This information would also facilitate characterizing relative production from various regions in order to prioritize real estate in the marine environment. Collecting these data over time during the principal *Sebastes* parturition season (winter–spring) will bring us closer to these goals, but data based on the current CalCOFI grid apparently will be useful primarily for offshore species (e.g., abundant species identified here) and not for 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*) rockfish (Taylor 1998). Few larvae of this subgenus were collected, and only at nearshore stations (tab. 1). The most likely explanation is that CalCOFI stations are too far offshore to assess these kelp forest and nearshore 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 natal habitat and characterizing abundance trends for 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. For example, *S. jordani* on cruise 9904 might have been under-reported by >50% without molecular identification (fig. 4), and a specimen of *S. levis* identified by molecular methods was not visually identifiable in the presort of ethanol-fixed samples because of severe distortion of the pectoral fins, a key diagnostic character (tab. 1).

In the past decade, molecular identification methods have become easier to use, and costs have decreased enough to allow quick sequencing of many individuals. As the number of genetic markers and information on intraspecific polymorphism increases, apparently fixed differences between species in the form of single nucleotide polymorphisms will be useful for designating some species and species complexes within *Sebastes*. A reasonable outcome from this is the development of microarrays and microbeads multiplex assays that will allow automated reading of fluorescent-labeled probes after a series of species-specific enzyme-ligation steps. These methods have sufficiently high throughput and are rapid enough to be promising for collection of real-time data aboard ship.

ACKNOWLEDGMENTS

Samples were obtained through CalCOFI in cooperation with the California Department of Fish and Game, the Scripps Institution of Oceanography, NOAA Fisheries and scientists and crew on the NOAA ship RV *David Starr Jordan*. We are especially indebted to A. Hays, D. Griffith, and R. Charter for their help in obtaining samples, and A. Brooks and H. Timms for their assistance in the laboratory. We also thank the Oceanids Foundation, the Frieda Daum Urey Scholarship Committee, and the Biological Oceanography Fellowship Committee. TKC was supported through NSF grant OCE-0324360. 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.

LITERATURE CITED

Ahlstrom, E. H. 1959. Vertical distribution of pelagic fish eggs and larvae off California and Baja California. Fish. Bull. 60:107–145.

Ambrose, D. A., R. L. Charter, and H. G. Moser. 2001. Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations Survey Cruises in 1999. NOAA Tech. Memo. NOAA-TM-NMFS-SWFSC-311. 69 pp.

Boehlert, G. W., D. M. Gadomski, and B. C. Mundy. 1985. Vertical distribution of ichthyoplankton off the Oregon coast in spring and summer months. Fish. Bull. 79:789–794.

Chereskin, T., and M. Trunnell. 1996. Correlation scales, objective mapping, and absolute geostrophic flow in the California Current. J. Geophys. Res. 101:22619–22629.

Chereskin, T. K., M. Y. Morris, P. P. Niiler, P. M. Kosro, R. L. Smith, S. R. Ramp, C. A. Collins, and D. L. Musgrave. 2000. Spatial and temporal characteristics of the mesoscale circulation of the California Current from eddy-resolving moored and shipboard measurements. J. Geophys. Res. 105:1245–1269.

Cornuelle, B. D., T. K. Chereskin, P. P. Niiler, M. Y. Morris, and D. L. Musgrave. 2000. Observations and modeling of a California undercurrent eddy. J. Geophys. Res. 105:1227–1243.

Doyle, M. J. 1992. Neustonic ichthyoplankton in the northern region of the California Current ecosystem. Calif. Coop. Oceanic Fish. Invest. Rep. 33:141–161.

Egbert, G. D., A. G. Bennett, and M. G. G. Foreman. 1994. TOPEX/POSEIDON tides estimated using a global inverse model. J. Geophys. Res. 99:24821–24852.

Eschmeyer, W. N., and E. S. Herald. 1983. A field guide to Pacific Coast fishes of North America. Houghton Mifflin, Boston.

Kramer, D., M. Kalin, E. G. Stevens, J. R. Thrailkill, and J. R. Zweifel. 1972. Collecting and processing data on fish eggs and larvae in the California Current region. NOAA Tech. Rep. NMFS Circ. 370. 38 pp.

Lasker, R. 1985. An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy *Engraulis mordax*. U.S. Dep. Commer. NOAA Tech. Rep. NMFS 36.

Logerwell, E., and P. E. Smith. 2001. Mesoscale eddies and survival of late stage Pacific sardine (*Sardinops sagax*) larvae. Fish. Oceanogr. 10:13–25.

Love, M. S. 1979. Isolation of olive rockfish, Sebastes serranoides, populations off Southern California. Fish. Bull. 77:975–983.

Love, M. S., P. Morris, M. McCrae, and R. Collins. 1990. Life history aspects of 19 rockfish species (Scorpaenidae: *Sebastes*) from the Southern California Bight. NOAA Tech. Rep. NMFS 87. 38 pp.

Love, M. S., M. Yoklavich, and L. Thorsteinson. 2002. The rockfishes of the Northeast Pacific. Univ. of Calif. Press, Berkeley.

Lynn, R. J., and J. J. Simpson. 1987. The California Current system: the seasonal variability of its physical characteristics. J. Geophys. Res. 92:12947–12966.

MacCall, A. D., S. Ralston, D. Pearson, and E. Williams. 1999. Status of the bocaccio off California in 1999 and outlook for the next millennium. *In* Appendix to the status of the Pacific Coast groundfish fishery through 1999 and recommended acceptable biological catches for 2000. Pacific Fisheries Management Council, 2130 SW Fifth Ave., Suite 224, Portland, Ore. 97201.

Miller, D. J., and R. N. Lea. 1972. Guide to the coastal marine fishes of California. Calif. Dep. Fish Game, Fish. Bull. 157. 235 pp.

Moser, H. G. 1996. The early stages of fishes in the California Current region. Calif. Coop. Oceanic Fish. Invest. Atlas 33. Allen Press, Lawrence, Kans.

Moser, H. G., and G. W. Boehlert. 1991. Ecology of pelagic larvae and juveniles of the genus *Sebastes*. Environ. Biol. Fish. 30:203–224.

Moser, H. G., and T. Pommeranz. 1998. Vertical distribution of eggs and larvae of northern anchovy, *Engraulis mordax*, and the larvae of associated fishes at two sites in the southern California Bight Fish. Bull. 97:920–943.

Moser, H. G., E. H. Ahlstrom, and E. M. Sandknop. 1977. Guide to the identification of scorpionfish larvae (family Scorpaenidae) in the eastern Pacific with comparative notes on species of *Sebastes* and *Helicolenus* from other oceans. U.S. Dep. Commer. NOAA Tech. Rep. NMFS Circ. 402. 71 pp.

Moser, H. G., R. L. Charter, P. E. Smith, D. A. Ambrose, S. R. Charter, C. A. Meyer, E. M. Sandknop, and W. Watson. 1993. Distributional atlas of fish larvae and eggs in the California Current region: taxa with 1000 or more total larvae, 1951 through 1984. Calif. Coop. Oceanic Fish. Invest. Atlas 31. 233 pp.

Moser, H. G., R. L. Charter, W. Watson, D. A. Ambrose, J. L. Butler, S. R. Charter, and E. M. Sandknop. 2000. Abundance and distribution of rockfish (*Sebastes*) larvae in the Southern California Bight in relation to environmental conditions and fishery exploitation. Calif. Coop. Oceanic Fish. Invest. Rep. 41:132–148.

Moser, H. G., R. L. Charter, P. E. Smith, D. A. Ambrose, W. Watson, S. R. Charter, and E. M. Sandknop. 2001. Distributional atlas of fish larvae and eggs in the California Current region: 1951 through 1998. Calif. Coop. Oceanic Fish. Invest. Atlas 34. 166 pp.

Nishimoto, M. M. 2000. Distributions of late-larval and pelagic juvenile rockfishes in relation to water masses around the Santa Barbara Channel Islands in early summer, 1996. Proceedings of the Fifth California Islands Symposium, Santa Barbara, Calif.

Ohman, M. D., and P. E. Smith. 1995. A comparison of zooplankton sampling methods in the CalCOFI time series. Calif. Coop. Oceanic Fish. Invest. Rep. 36:153–158.

Pollard, J., and J. Read. 1989. A method for calibrating ship-mounted acoustic Doppler profilers and the limitations of gyro compasses. J. Atmos. Oceanic Technol. 6:859–865.

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

Rocha-Olivares, A., C. A. Kimbrell, B. J. Eitner, and R. Vetter. 1999. Evolution of a mitochondrial cytochrome *b* gene sequence in the species-rich genus *Sebastes* (Teleostei, Scorpaenidae) and its utility in testing the monophyly of the subgenus *Sebastomus*. Mol. Phylog. Evol. 11:426–440.

Sakuma, K. M., S. Ralston, and D. A. Roberts. 1999. Diel vertical distribution of postflexion larval *Citharichthys* spp. and *Sebastes* spp. off central California. Fish. Oceanogr. 8:68–76.

SIO. Scripps Institution of Oceanography. 2000. Physical, chemical, and biological data, CalCOFI Cruise 9901, CalCOFI Cruise 9904. Scripps Institution of Oceanography Ref. 00-6. Scripps Institution of Oceanography, La Jolla, Calif. 99 pp.

Smith, P. E., and S. L. Richardson. 1977. Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175. 100 pp.

Swofford, D. L. 2000. PAUP.* Phylogenetic analysis using parsimony (*and other methods). Ver. 4. Sinauer Associates, Sunderland, Mass.

Taylor, C. A. 1998. Phylogenetics and identification or rockfishes (genus *Sebastes*) using mitochondrial DNA techniques. Biol. Sci., San Diego State Univ., pp. 113.

Tully, O., and P. Oceidigh. 1989. The ichthyoneuston of Galway Bay (Ireland). I. The seasonal, diel, and spatial distribution of larval, postlarval, and juvenile fish. Mar. Biol. 101:27–41.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTech 10:506–513.

Walters, K. 2001. Live fish. *In* Review of some California fisheries for 1999: market squid, Dungeness crab, sea urchin, prawn, abalone, Pacific sardine, Pacific herring, Pacific mackerel, reduction, white seabass, and recreational, L. Rogers-Bennett, ed. Calif. Coop. Ocean Fish. Invest. Rep. 41:22–24.

APPENDIX
Locations of Adults Used for Reference to Identify Larvae

Sebastes spp.	Location	Latitude, longitude	Total haplotypes
S. aleutianus	Point Reyes, Calif.	37.93°N, 123.46°W	3
S. alutus	Cape Mendocino, Calif.	40.92°N, 124.42°W	2
S. atrovirens	La Jolla, Calif.	32.83°N, 117.25°W	9
S. auriculatus	Monterey Bay, Calif.	36.98°N, 122.20°W	6
S. aurora	Monterey Bay, Calif.	36.79°N, 122.13°W	7
S. babcocki	San Francisco, Calif.	37.24°N, 122.72°W	2
S. borealis	Trinidad Head, Calif.	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, Calif.	35.14°N, 120.72°W	6
S. caurinus	Punta Baja, Baja California, Mex.	29.89°N, 115.82°W	6
S. chlorostictus	Richardson Rock, Calif.	34.12°N, 120.55°W	11
S. chrysomelas	Monterey Bay, Calif.	36.75°N, 122.00°W	1
S. ciliatus	S. Baranof Island, Alaska	56.08°N, 134.92°W	6
S. constellatus	Cordell Bank, Calif.	38.00°N, 123.04°W	11
S. crameri	San Francisco, Calif.	37.58°N, 122.05°W	5
S. dallii	San Luis Obispo, Calif.	35.14°N, 120.72°W	5
S. diploproa	Monterey Bay, Calif.	36.77°N, 122.20°W	7
S. elongatus	S. San Clemente Island, Calif.	32.74°N, 118.41°W	6
S. emphaeus	Seattle Aquarium, Hood Canal, Wash.	47.92°N, 122.58°W	1
S. ensifer	60-mile Bank, Calif.	32.08°N, 118.25°W	7
S. entomelas	Ascension Canyon, Calif.	36.98°N, 122.58°W	7
S. eos	Palos Verdes, Calif.	33.70°N, 118.36°W	16
S. exsul	N. Gulf of Calif., Mex.	28.59°N, 113.43°W	5
S. flavidus	Ascension Canyon, Calif.	36.98°N, 122.58°W	6
S. gilli	Cortes Bank, Calif.	32.57°N, 119.25°W	2
S. goodei	S. San Francisco, Calif.	37.25°N, 122.85°W	6
S. helvomaculatus	N. San Francisco, Calif.	38.26°N, 123.49°W	9
S. hopkinsi	Offshore of Hood Canal, Wash.	32.57°N, 119.25°W	17
S. jordani	Ascension Canyon, Calif.	36.98°N, 122.58°W	11
S. lentiginosus	La Jolla, Calif.	32.83°N, 117.25°W	2
S. levis	60-mile Bank, Calif.	32.08°N, 118.25°W	5
S. macdonaldi	Bahia de Los Angeles, Mex.	28.98°N, 113.43°W	9
S. maliger	San Francisco Fish Market	N/A	6
S. melanostomus	Offshore of San Diego, Calif.	32.75°N, 117.75°W	5
S. melanops	Davenport port sample	37.02°N, 122.17°W	8
S. melanosema	San Pablo Point, Baja California, Mex.	N/A	1
S. miniatus	San Clemente Island, Calif.	37.74°N, 118.41°W	6
S. moseri	San Clemente Isle, Calif.	32.63°N, 117.96°W	1
S. mystinus	Point Sur, Calif.	36.28°N, 121.97°W	8
S. nebulosus	San Francisco Fish Market	N/A	6
S. nigrocinctus	Fairweather Grounds, O'Connell, Alaska	58.25°N, 139.00°W	3
S. notius	Uncle Sam Bank, Mex.	25.59°N, 113.37°W	2
S. ovalis	S. San Clemente Island, Calif.	32.74°N, 118.40°W	8
S. paucispinis	S. of Monterey, Calif.	36.82°N, 122.10°W	2
S. phillipsi	Seattle, Wash.	N/A	4
S. pinniger	Humboldt, Calif.	41.75°N, 124.08°W	5
S. polyspinis	Off Kodiak Isle, Alaska	58.40°N, 153.65°W	5
S. proriger	Cape Mendocino, Calif.	40.56°N, 124.50°W	4
S. rastrelliger	Bodega Bay, Calif.	38.30°N, 123.10°W	5
S. reedi	Point Arena, Calif.	38.64°N, 123.74°W	1
S. rosaceous	Ascension Canyon, Calif.	36.98°N, 122.58°W	7
S. rosenblatti	Monterey Bay, Calif.	36.83°N, 122.16°W	12
S. ruberrimus	Off Inverness, Marin County, Calif.	38.56°N, 123.62°W	5
S. rubrivinctus	San Clemente Island, Calif.	32.74°N, 118.41°W	5
S. rufinanus	San Clemente Island, Calif.	32.79°N, 118.33°W	1
S. rufus	Lincoln City, Ore.	45.74°N, 124.69°W	4
S. saxicola	Point Sur, Calif.	36.83°N, 122.16°W	1
S. semicinctus	Santa Cruz, Calif.	37.41°N, 122.91°W	6
S. serranoides	Richardson Rock, Calif.	34.12°N, 120.55°W	6
S. serriceps	Santa Catalina Island, Calif.	33.28°N, 118.34°W	5
S. simulator	Baja California Norte, Mex.	32.87°N, 117.87°W	10
S. spinorbis	Bahia de Los Angeles, Mex.	28.98°N, 113.43°W	3
S. umbrosus	Between Cortes and 60-mile Bank	32.43°N, 119.11°W	6
S. variegatus	Alaska fisheries	N/A	2
S. wilsoni	Santa Cruz, Calif.	37.24°N, 122.77°W	5
S. zacentrus	Santa Cruz, Calif.	37.25°N, 122.85°W	2

Note: GenBank accession numbers available on request.