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Early larvae of the swordspine rockfish (*Sebastes ensifer*) identified by molecular methods

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Abstract—About 56 rockfish (*Sebastes*) species occur in Southern California, but the larvae of most of them are undescribed. Larval rockfishes collected off Southern California during the California Cooperative Oceanic Fisheries Investigations (CalCOFI) cruise in April 1999 and the Baseline Cowcod Conservation Area (CCA) cruise in February 2002 were identified by using mitochondrial cytochrome *b* genomic DNA to determine the abundances of individual species. About 27% of the larvae from the CalCOFI cruise and 16% of the larvae from the CCA cruise were *Sebastes ensifer*. Larval *S. ensifer* were undescribed for most of the size range identified here (2.6–8.4 mm, early preflexion through early postflexion stage). Larval S. ensifer are moderately deep-bodied and robust and have melanophores dorsally and ventrally on the gut, in a single ventral row on the tail, and on the pectoral fins. Starting at about 3.8 mm, melanophores form on the anterior part of the mandible. Larvae ≥6 mm have pigment in the mid- and hindbrain areas. Preflexion-stage larval *S. ensifer* are indistinguishable from the described larvae of sympatric species in the *Sebastes* subgenus *Sebastomus*, except perhaps *Sebastes rosaceus* and *S. umbrosus*. In later stages, *S. ensifer* may be distinguishable from *S. constellatus* and *S. helvomaculatus*.

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The rockfish genus *Sebastes* includes about 72 species in the eastern North Pacific Ocean; approximately 56 of these species occur in the Southern California Bight (Eschmeyer et al., 1983; Kendall, 1991), and many are important in sport and commercial fisheries (e.g., Lenarz, 1987; Leet et al., 1992, 2001). The swordspine rockfish, *Sebastes ensifer* Chen, 1971, is an abundant, small species (total length to about 25–30 cm) that ranges from northcentral California to the central Baja California Peninsula, Mexico (Love et al., 2002). Although not a preferred fishery species owing to its small size, *S. ensifer* contributes moderately to recreational fishery catches because of its abundance, and it is sold in Asian fish markets (Love et al., 2002).

Sebastes are live-bearers, and larvae are readily obtained from pregnant females and occur commonly in plankton samples, particularly during late winter and spring (Moser et al., 1993). Owing to this availability and to the use of larvae in providing fishery-independent estimates of spawning biomass (e.g., Moser and Butler, 1987; Moser et al., 2000; Ralston et al., 2003; Ralston and MacFarlane, 2010), considerable effort has been directed toward improving our ability to identify *Sebastes* larvae (Kendall, 1991; Moser, 1996b). However, the larvae are difficult to rear (e.g., Kendall, 1991), and there is considerable overlap among species in meristic, morphological, and pigment characters (Moser, 1996b). Therefore, to date, field-collected larvae of only 7 of the species that occur off Southern California have been visually identified with certainty (Moser et al., 2000).

Larvae of *S. ensifer,* a member of the Sebastes subgenus *Sebastomus*, are not readily identifiable. Differences among larvae of *Sebastomus* species are quite small (e.g., Moser, 1996b; Rocha-Olivares, 1998; Rocha-Olivares et al., 2000), and currently larvae of none of these species can be reliably identified with traditional morphological and pigment characters. Complete larval development for any *Sebastomus* species is still unknown; to date, extrusion larvae are known for 7 of the 15 species, and some later stages are known for 4 species, including the

cape redfish (*S. capensis*) of the Southern Hemisphere (e.g., Westrheim et al.[1]; Moser, 1967; Moser et al., 1977; Richardson and Laroche, 1979; Moser and Butler, 1987; Matarese et al., 1989; Sabatés and Olivar, 1990; Moser, 1996b; Rocha-Olivares et al., 2000). Larvae of another species, probably buccaneer rockfish (*S. exsul*) or spinyeye rockfish (*S. spinorbis*), have been described through the postflexion stage (Moser et al., 1977). Early development of *S. ensifer* is known only from newly extruded larvae (Moser, 1967) and from large, transforming larvae (≥19.8 mm standard length) and pelagic juveniles (Rocha-Olivares et al., 2000). The purpose of this article is to describe external morphology and pigmentation of larval *S. ensifer* from the early preflexion stage through the early postflexion stage, nearly the full developmental range not previously described in the literature and a range that encompasses most of the rockfish larvae routinely collected in standard plankton tows (e.g., Moser, 1996b).

Materials and methods

This study was based on plankton samples collected with a bongo sampler that was equipped with 0.505-mm mesh nets and a flowmeter and towed obliquely through the upper 212 m of the water column during the quarterly California Cooperative Oceanic Fisheries Investigations (CalCOFI) survey in April 1999 (Ambrose et al., 2001) and during the Baseline Cowcod Conservation Area (CCA) survey in February 2002, both conducted in the Southern California Bight. Details of the CalCOFI sampling area, gear, and methods are available in the literature (Kramer et al., 1972; Ohman and Smith, 1995). Standard CalCOFI protocol was followed for collection of plankton in the CCA with the bongo sampler. A minor protocol revision, instituted in 1997, was the preservation of the sample from the port net of the bongo sampler in 95% tris-buffered ethanol to facilitate aging and genetics studies of selected species. All fish larvae were sorted from the ethanol-preserved samples, retained in 95% ethanol, and archived pending analysis.

Eight ethanol-preserved larval specimens of the genus *Sebastes* (4.1–8.4 mm, preflexion through early postflexion stage) from the CalCOFI survey were assigned tentatively to a unique unidentified type on the basis of similarities in morphological features and pigmentation pattern. All specimens were relatively robust; in specimens ≥6 mm long, there were large parietal and preopercular spines and melanophores on the lower jaw, on the margins of the pectoral fins, and on the ventral margin of the tail but they were absent on the upper jaw and snout and dorsally and laterally on the trunk and tail. Before molecular analysis, all 8 larvae were examined with a Wild M5 Stereomicroscope[2] (Wild Stereo Microscopes, now manufactured by Leica Microsystems Inc., Buffalo Grove, IL) equipped with an ocular micrometer, and the larvae best representative of a developmental sequence (Fig. 1) were drawn with the use of a camera lucida and the Wild microscope.

Pigmentation patterns, counts of myomeres, head spines, and fin rays, and various measurements were recorded. These measurements, including body length (BL), preanal length, head length (HL), snout length, length of the pectoral-fin blade, eye diameter, and body depth, are defined in Moser (1996a). Descriptions of body proportions (e.g., "short preanal length") follow those of Leis and Carson-Ewart (2000). Spines measured include the preopercular (APO1–4 and PPO2–4), parietal, nuchal, pterotic, postorbital, first lower infraorbital, and posttemporal. Spine terms follow those of Moser and Ahlstrom (1978). Larval lengths refer to BL of ethanol-preserved larvae, and descriptions of pigmentation refer to melanistic pigment. No adjustments were made in the measurements to account for shrinkage of the preserved larvae; larval fish shrinkage in ethanol, typically in the range of about 3–6%, has been reported in the literature (e.g., Theilacker, 1980; Porter et al., 2001; Moku et al., 2004; Fey and Hare, 2005).

All unidentified ethanol-preserved larvae of *Sebastes* from the CalCOFI cruise, including the descriptive series, were identified by using mitochondrial cytochrome *b* genomic DNA extracted from caudal-fin or muscle tissue as described by Taylor et al. (2004). Primers included GluRF and CB3RF (from Rocha-Olivares et al., 1999) and internal custom primers (CB306F 5′-TTACTACGGCTCVTACCT-3, Cb521R 5′-GTTGCATTGTCTACTGAG-3′ and CB364F, 5′-CTAGTTATAATAACTGCTTT-3′).

After molecular identifications of the *Sebastes* larvae from the CalCOFI survey were completed, identifications of larvae from the CCA cruise began, and it soon became apparent that an appreciable fraction of those larvae were *S. ensifer*. We decided to include specimens from both surveys to base the description of larval development on a larger sample size: the 8 CalCOFI larvae plus 41 larvae from the CCA survey were used for the full description, and another 36 larvae from the CCA survey supplemented the description of larval pigmentation. During the interval between completion of analysis of the CalCOFI sample set and the beginning of analysis of the CCA sample set, improvements were made to the chelex extraction and PCR protocols. Therefore, for larvae collected during the CCA survey, the revised protocols described by Hyde et al. (2005) were used to extract mitochondrial cytochrome *b* genomic DNA from an eyeball or caudal muscle tissue. Primers included GluRF2 5′ AAC CAT CGT TGT TAT TCA ACT ACA AGA ACC and CB3RF2

[1] Westrheim, S. J., W. R. Harling, D. Davenport, and M. S. Smith. 1968. Preliminary report on maturity, spawning season and larval identification of rockfishes (Sebastodes) collected off British Columbia in 1968, 28p. Fish. Res. Board Can., Manuscr. Rep. Ser. 1005.

[2] Mention of trades names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

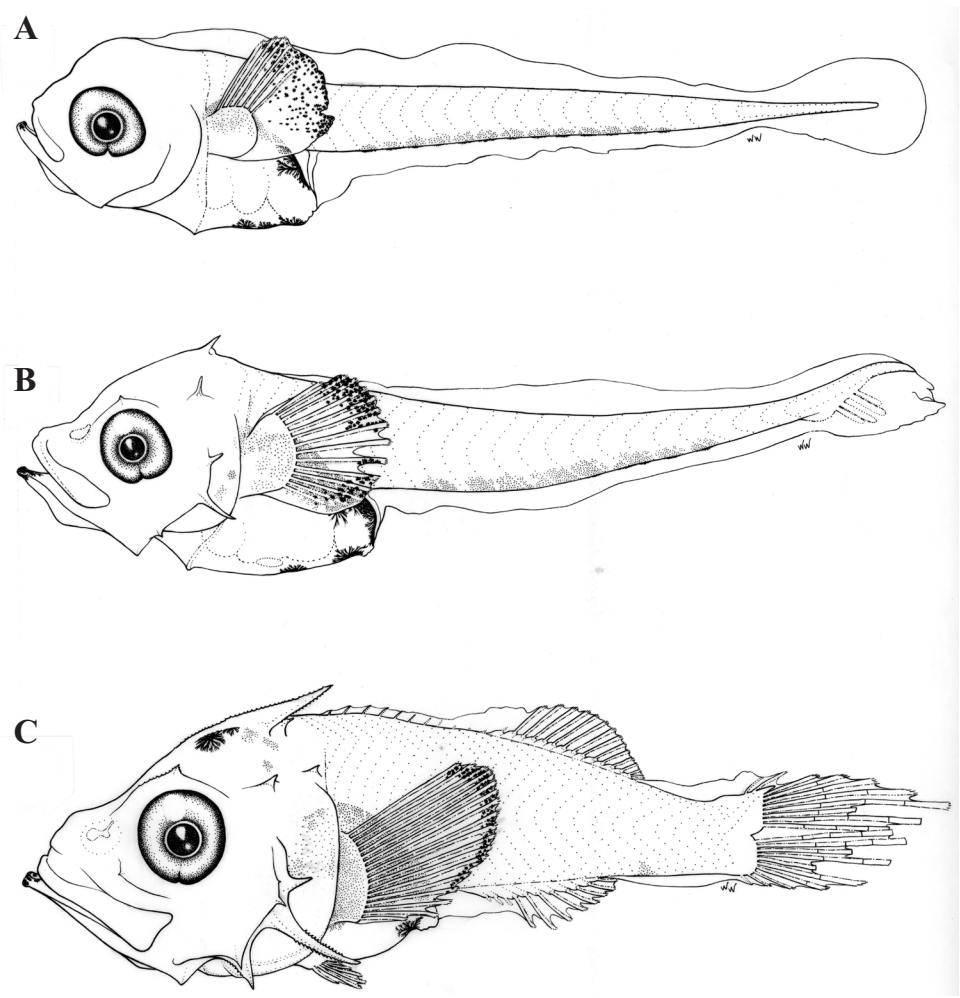

Figure 1

Larval swordspine rockfish (*Sebastes ensifer*) collected in the Southern California Bight during the California Cooperative Oceanic Fisheries Investigations survey during April 1999: (**A**) 4.4 mm (cruise 9904JD, station 90.30); (**B**) 6.2 mm (cruise 9904JD, station 90.53); (**C**) 7.7 mm (cruise 9904JD, station 90.37).

5′ CGA ACA GGA ART ATC AYT CTG G (Hyde and Vetter, 2009). In contrast to the protocols used for the CalCOFI specimens, this revised procedure provided a more robust amplification and saved both time and expense. Additionally, the use of only an eyeball, when possible, left the specimen otherwise intact for use in other studies.

Haplotype sequences from both the CalCOFI and CCA sample sets were compared with reference data of 374 independent haplotype sequences representing 67 *Sebastes* species (Hyde and Vetter, 2007) by using the software program PAUP*, vers. 4.0b10 (Sinauer Associates, Sunderland, MA; Swofford, 2000) with the optimality criterion set to distance (number of base-pair [bp] differences divided by total number of bp sequenced). Nonparametric bootstrapping was used (100 replications, MAXTREES set to 1000) to cluster each larval haplotype within a database of consensus haplotypes (i.e., most common haplotype from a database of known adults) for putative identification.

If the haplotype of a larva clustered with the single haplotype of a reference species with a bootstrap value ≥90%, the larva was identified as that species. Distances between reference haplotypes and the unknown were examined to confirm that the unknown fell within the intraspecific diversity as a secondary confirmation of identification. If a larva clustered with a single reference haplotype but the bootstrap value was <90%, a secondary analysis was performed that included all available haplotypes of at least the 3 nearest (in distance) species to the larval haplotype; the haplotype was identified by comparison with those of the reference species. Haplotype diversity for the reference species has a mean genetic distance of 0.0026 (correspond-

Table 1

Measurements of larval swordspine rockfish (*Sebastes ensifer*) collected in the Southern California Bight during the California Cooperative Oceanic Fisheries Investigations survey in April 1999 and Baseline Cowcod Conservation Area survey in February 2002, given as percentages of body length (BL): preanal length (PAL), body depth (BD), head length (HL), head width (HW), snout length (SnL), eye diameter (ED), length of the pectoral-fin blade (P_1L), and length of the pelvic fin (P_2L). For one specimen, an ED was not collected because the eyes were missing. *N*=number of specimens examined.

Stage	*N*	BL (mm)	PAL	BD	HL	HW	SnL	ED	P_1L	P_2L
Preflexion	2	2.6	39.0	20.6	21.9	9.2	3.6	9.6	6.9	0
Preflexion	3	2.8	39.5	19.9	21.7	8.6	5.0	9.3	7.1	0
Preflexion	2	2.9	39.5	18.4	19.5	9.0	5.5	8.6	5.5	0
Preflexion	3	3.0	36.4	18.6	18.3	7.4	5.2	9.9	5.6	0
Preflexion	3	3.1	34.4	17.8	21.3	10.0	6.5	9.5	8.8	0
Preflexion	3	3.2	35.2	18.1	21.8	8.2	5.6	8.8	6.7	0
Preflexion	3	3.3	34.3	19.7	21.8	7.3	4.9	8.2	6.7	0
Preflexion	2	3.4	28.5	17.9	19.3	6.9	4.9	7.9	5.9	0
Preflexion	2	3.5	38.6	16.7	17.7	9.9	5.7	8.9	8.4	0
Preflexion	3	3.6	35.0	18.9	17.1	8.9	6.5	9.4	6.9	0
Preflexion	1	3.8	41.3	21.1	27.6	11.3	8.2	–	9.7	0
Preflexion	2	3.9	39.5	19.7	19.7	8.7	7.9	9.0	9.5	0
Preflexion	1	4.1	33.0	15.0	13.6	7.3	2.9	6.8	5.8	0
Preflexion	2	4.2	33.6	18.3	21.1	11.3	5.7	8.1	8.9	0
Preflexion	3	4.3	41.9	24.9	25.3	11.5	6.5	9.5	12.2	0
Preflexion	1	4.4	36.4	20.9	20.0	12.7	4.5	8.6	10.0	0
Preflexion	1	4.5	45.1	21.8	19.1	8.9	5.6	8.2	10.9	0
Preflexion	3	4.6	34.8	20.3	25.4	8.5	6.7	8.7	9.7	0
Preflexion	1	4.9	35.9	18.2	21.4	8.8	6.9	7.6	13.9	0
Preflexion	1	5.1	34.5	23.5	20.6	9.6	4.9	9.0	13.3	0
Preflexion	1	5.2	36.4	21.7	22.5	10.9	5.4	7.8	7.8	0
Preflexion	1	5.4	38.7	20.6	25.0	9.1	5.7	8.0	14.8	0
Preflexion	1	6.0	37.4	23.8	25.2	15.2	7.3	8.8	11.3	0
Preflexion	1	6.2	41.6	23.7	25.0	13.6	8.4	8.4	11.0	0
Postflexion	2	7.7	52.5	32.9	38.2	22.8	13.5	11.6	17.9	6.0
Postflexion	1	8.4	53.6	33.8	33.8	20.0	8.6	11.2	18.6	6.2

ing to a 2-bp difference in the 782-bp sequenced) with a minimum of 0.0000 (e.g., *S. jordani*) and a maximum of 0.0066 (in *S. aleutianus*). Divergence among reference haplotypes for species of *Sebastomus* ranged from 0.0102 to 0.0204 (Hyde and Vetter, 2007).

Results

Identification of larvae

Respectively, 91 of 339 and 112 of 846 *Sebastes* larvae collected in the CalCOFI and CCA surveys were *S. ensifer*. Among the larval *S. ensifer* sequenced for this study, divergence from reference haplotypes ranged from 0.0000 to 0.0080. Of the 76 sequences submitted to GenBank (accession numbers KM974185–KM974260), 22 sequences were identical to haplotype references in the GenBank database, 20 sequences differed by 1 bp, 14 sequences differed by 2 bp, 18 sequences differed by 3 bp, 1 sequence differed by 4 bp; and 1 sequence differed by 5 bp.

Description

Moser (1967) illustrated a 4.2-mm early-stage larva as *Sebastodes rhodochloris*. The smallest larva described in our study, 2.6 mm, had no yolk or oil globule remaining. Notochord flexion begins soon after larvae reach 6.2 mm and is completed by about 7.7 mm. Transformation to the pelagic juvenile stage begins near 20 mm and is complete by about 27 mm (Rocha-Olivares et al., 2000).

Larvae are moderately deep-bodied and robust through much of their development, although they are initially somewhat elongate, have a rounded head and short snout, a short preanal length, and large, and slightly oval to round eyes (Fig. 1A, Table 1). Most body proportions increase gradually during development; more rapid increases in relative preanal length, body depth, HL, and eye diameter occur between preflexion and postflexion stages (Table 1). All larvae have 26 myomeres (6–8 preanal myomeres during the preflexion stage, shifting to 10–11 during the postflexion stage).

Table 2

Measurements of head and pectoral girdle spines of larval swordspine rockfish (*Sebastes ensifer*) collected in the Southern California Bight during the California Cooperative Oceanic Fisheries Investigations survey in April 1999 and the Baseline Cowcod Conservation Area (CCA) survey in February 2002, and given as percentages of head length (HL): posterior preopercular (PP01, PP03, PP04) and anterior preopercular (APO1 and AP04), parietal (PA), nuchal (NU), pterotic (PT), posttemporal (PST), postorbital (PSO), and first lower infraorbital (LIO1) spines. Lengths of larvae on which measurements were made are given as body length (BL).

BL (mm)	HL (mm)	Stage	PPO1	PPO3	PPO4	APO1	APO4	PA	NU	PT	PST	PSO	LIO1
4.2	0.6	Preflexion	0	0	0	0	0	0	0	0	0	0	0
4.4	0.9	Preflexion	0	0	0	0	0	0	0	0	0	0	0
5.2	1.2	Preflexion	0	0	0	0	0	0	0	0	0	0	0
6.0	1.5	Preflexion	9.2	30.3	5.3	0	0	17.1	0	10.5	0	2.6	0
6.2	1.6	Preflexion	7.8	20.8	0	0	0	13.0	0	7.8	0	3.9	0
7.7	2.8	Preflexion	14.1	29.6	15.5	2.8	8.5	26.8	0	7.0	1.8	3.5	0
7.7	3.0	Postflexion	13.2	27.6	14.5	5.3	5.9	26.3	3.9	2.6	2.6	3.9	2.0
8.4	2.8	Postflexion	14.1	35.9	18.3	5.6	7.0	32.4	2.1	8.5	7.7	4.9	0

Head and pectoral girdle spine formation begins during the preflexion stage between 5.2–6.0 mm, with the posterior preopercular, parietal, pterotic, and postocular spines. Posterior preopercular spine PPO3 and the parietal spine are large (Table 2). Anterior preopercular spines and a posttemporal spine are added by the early postflexion stage (~7.7 mm), and the nuccal and first lower infraorbital spine form soon after (Table 2). The margins of all spines and ridges are smooth through the preflexion stage, but by the early postflexion stage the parietal and supraocular ridges and their spines and preopercular spines PPO3, PPO4, and APO4 are serrate. By 8.4 mm, the posttemporal spine also is weakly serrate.

The first fin rays to form are the upper pectoral-fin rays, by 4.4 mm (Fig. 1A, Table 3). Rays are added ventrally, and the full complement of 16–18 rays is present by the early postflexion stage. Hypural development begins during the preflexion stage, by 5.2 mm, and principal caudal-fin rays begin to form late during this stage, by 6 mm. The full complement of 8+7 principal rays is present, and procurrent rays begin to form by the early postflexion stage (Table 3). Soft rays of the dorsal and anal fins are forming by early postflexion stage, and all (12–14 and 5–7, respectively) are present by ~7.7 mm. Pterygiophores of the dorsal- and anal-fin spines are discernible early during the postflexion stage. Dorsal-fin spines begin to form slightly later, and anal-fin spines form later still (Table 3); full complements of dorsal-fin and anal-fin spines were not present in the largest specimen (8.4 mm).

The principal elements of the larval pigment pattern are the presence of melanophores situated anteriorly on the lower jaw, dorsally and ventrally on the gut, in a single row on the ventral margin of the tail, and

Table 3

Counts of fin rays of larval swordspine rockfish (*Sebastes ensifer*) collected in the Southern California Bight during the California Cooperative Oceanic Fisheries Investigations survey in April 1999 and Baseline Cowcod Conservation Area (CCA) survey in February 2002. BL=body length. D=dorsal, A=anal, P_1=pectoral, P_2=pelvic, and C=caudal.

Stage	BL (mm)	D	A	P_1	P_2	Principal C	Procurrent C
Preflexion	4.1	0	0	0	0	0	0
Preflexion	4.4	0	0	6	0	0	0
Preflexion	5.2	0	0	4	0	(hypurals)	0
Preflexion	6.0	0	0	6	0	3 + 3	0
Preflexion	6.2	0	0	15	(buds)	2 + 2	0
Postflexion	7.7	11	8	18	I,5	8 + 7	1 + 1
Postflexion	7.7	VI,13	7	17	I,5	8 + 7	2 + 2
Postflexion	8.4	XI,13	I,7	16	I,5	8 + 7	2 + 2

Table 4

Pigmentation of larval swordspine rockfish (*Sebastes ensifer*) collected in the Southern California Bight during the California Cooperative Oceanic Fisheries Investigations survey in April 1999 and the Baseline Cowcod Conservation Area (CCA) survey in February 2002, given as percentages of the number of larvae examined that displayed the pigment character. Larvae ≤6.2 mm were in the preflexion stage; larvae ≥7.7 mm were in the postflexion stage. BL=body length, LJ=lower jaw, V margin=ventral margin of the tail, and P_1=pectoral fin. *N*=number of specimens examined.

BL (mm)	*N*	LJ	Brain	Nape	V margin	Blade (P_1)	Base (P_1)
2.6	3	0	0	0	100.0	0	0
2.7	1	0	0	0	100.0	0	0
2.8	8	0	0	0	100.0	0	0
2.9	3	0	0	0	100.0	0	0
3	8	37.5	0	0	100.0	0	0
3.1	3	33.3	0	0	100.0	0	0
3.2	10	10.0	0	0	100.0	0	0
3.3	12	25.0	0	0	100.0	8.3	0
3.4	5	20.0	0	0	100.0	0	0
3.5	3	66.7	0	0	100.0	66.7	33.3
3.6	4	50.0	0	0	100.0	37.5	37.5
3.8	1	100.0	0	0	100.0	100.0	0
3.9	2	100.0	50.0	50.0	100.0	100.0	0
4	1	0	0	0	100.0	0	0
4.1	1	100.0	0	0	100.0	100.0	0
4.2	2	50.0	0	0	100.0	50.0	0
4.3	4	100.0	25.0	25.0	100.0	100.0	25.0
4.4	1	100.0	0	0	100.0	100.0	0
4.5	1	100.0	100.0	100.0	100.0	100.0	100.0
4.6	3	100.0	33.3	0	100.0	100.0	66.7
4.9	1	100.0	100.0	0	100.0	100.0	100.0
5.1	1	100.0	100.0	0	100.0	100.0	100.0
5.2	1	100.0	0	0	100.0	100.0	0
5.4	1	100.0	100.0	0	100.0	100.0	100.0
6.0	1	100.0	100.0	0	100.0	100.0	100.0
6.2	1	100.0	0	0	100.0	100.0	100.0
7.7	2	100.0	100.0	100.0	100.0	100.0	100.0
8.4	1	100.0	100.0	100.0	100.0	100.0	100.0

on the pectoral fins, especially distally (Table 4, Fig. 1). Larvae ≥6 mm also have pigment in the mid- and hindbrain areas.

Pigmentation on the head initially is limited to 3–8 or more melanophores anteriorly on the lower jaw, primarily on the inner margin of the dentaries. This pigment increases and spreads posteriorly during the postflexion stage. Melanophores form over the midbrain area as early as 3.9 mm and always are present by 7.7 mm, covering much of the area dorsally and dorsolaterally. At the same time, 2–4 melanophores form internally on the hindbrain, usually anterolaterally on the cerebellum. There is no other pigmentation on the head through 8 mm.

Pigmentation on the gut in the smallest larva was largely limited to the dorsum and ventrum but melanophores expanded into the hindgut area to nearly surround it. Melanophores spread ventrally from the dorsum of the gut, beginning posteriorly at about 5 mm, and cover the upper 50–90% by postflexion stage. A melanophore forms internally on the peritoneum at the anterior margin of the liver by 4.4 mm, and more are added to cover much of the upper 50–90% by postflexion stage. There are about 1–5 ventral melanophores on the gut, commonly along the midline between the anterior midgut to near the anus.

Pigmentation of the trunk and tail is limited to the ventral margin of the tail. During the preflexion stage, a single row of 8–14 melanophores (mean: 11), usually elongate, extends from myomeres 10–11 to 20–24 (modal number: 10–21), at a spacing of about one melanophore per myomere, and appears as an almost continuous line of pigment. Most melanophores are primarily shallowly internal; the last 1–2 are primarily external. During the postflexion stage, the number of ventral melanophores decreases as the series becomes more deeply internal and begins to disappear from anterior to posterior.

The pectoral fins are pigmented in nearly all larvae, most densely at the margin and progressively more lightly toward the fin base. Early in the preflexion stage, melanophores occur on the distal ~50–60% of the pectoral fin but they become more concentrated near the margin with development and are only on the distal 10–20% of the fin during the postflexion stage. The pectoral fin base is unpigmented before 4.5 mm; afterward, melanophores form on its inner surface and densely cover it during the postflexion stage. Melanophores form on the distal ~25% of the pelvic fins during the postflexion stage, and pigmentation is densest at the margin between rays 1–2, sparser between rays 3–4, and little or none at ray 5. No other fin pigmentation was observed, except that the 8.4-mm specimen had one small melanophore each at the bases of anal-fin rays 2 and 3.

Discussion

Identification of larvae

It has only relatively recently become possible to identify to species all the *Sebastes* larvae that are collected during plankton surveys conducted in the vicinity of the California Current during peak parturition season (e.g., Chen, 1971; Wyllie Echeverria, 1987). Among the larval *S. ensifer* sequenced for this study, divergence from reference haplotypes (0.0000 to 0.0080) was well below the divergence between *Sebastomus* species (0.0102–0.0204 [Hyde and Vetter, 2007]). Haplotype diversity within *Sebastomus* species has a mean distance of 0.0046 (ranging from 0.000 in *S. oculatus* and *S. spinorbis* to 0.0115 in *S. rosaceus* [Hyde and Vetter, 2007]), comparable to the values found in this study for *S. ensifer*.

Distribution

Sebastes ensifer ranges along the Pacific coast from the central Baja California Peninsula, México, to central California; the highest abundance occurs off Southern California and northern Baja California (Love et al., 2002). Within the current CalCOFI domain (e.g., McClatchie, 2014), larval *S. ensifer* is the second most abundant rockfish species, accounting for about one-fifth of the total rockfish larvae collected in 1999 and 2002. Larval *S. ensifer* were collected primarily in the vicinity of the Southern California Eddy (SCE) (Taylor et al., 2004), a persistent feature in the Southern California Bight northwest of San Clemente Island.

Comparisons

Larval *Sebastomus* are more or less readily identifiable as a group on the basis of several shared characters, including a relatively robust body, with large parietal and preopercular spines present by about mid-preflexion stage and with melanophores usually present on the lower jaw, always present on the pectoral fins and ventral margin of the tail, absent on the upper jaw and snout, and absent dorsally and laterally on the trunk and tail until the latter part of the postflexion stage when a bar begins to form on the caudal peduncle. A dorsal saddle may form on the trunk as well, late during the postflexion stage or during transformation to the pelagic juvenile stage (e.g., Rocha-Olivares et al., 1999). On the basis of these characters, larval *Sebastomus* can be distinguished from larvae of most, but not all, *Sebastes* species described to date.

Within *Sebastomus* in the North Pacific, early-preflexion-stage larvae of all 6 species for which larvae are known share the pattern of melanophores dorsally and ventrally on the gut, on the ventral margin of much of the tail (but none dorsally on the trunk or tail), and melanophores present at birth or soon thereafter on the pectoral fins. Pectoral-fin melanophores are exclusively or most concentrated near the margin, but are more evenly distributed in the honeycomb rockfish (*Sebastes umbrosus*) (Moser et al., 1977; Moser, 1996b). All but the rosy rockfish (*S. rosaceus*) have pigment at the tip of the lower jaw (Moser et al., 1977; Matarese et al., 1989; Moser, 1996b). On the basis of morphological features and pigmentation early during the preflexion stage, larval *S. ensifer* are essentially indistinguishable from the other *Sebastomus* larvae, except perhaps *Sebastes rosaceus* and *S. umbrosus*.

Larvae older than the early preflexion stage are known for only 3 *Sebastomus* species in the North Pacific: the starry rockfish (*Sebastes constellatus*), known to the mid-flexion stage (Moser et al., 1977; Moser and Butler, 1987; Moser, 1996b) and the late postflexion to pelagic juvenile stages (Rocha-Olivares et al., 2000); the rosethorn rockfish (*S. helvomaculatus*), known from the late flexion to pelagic juvenile stages (Richardson and Laroche, 1979); and *S. ensifer*, illustrated at extrusion stage by Moser (1967) and described here through the early postflexion stage and by Rocha-Olivares et al. (2000) from the late postflexion to pelagic juvenile stages.

During the preflexion stage, *S. constellatus* and *S. ensifer* are nearly indistinguishable, although the pectoral fins may become more heavily pigmented in *S. constellatus* and *S. ensifer* may be somewhat deeper-bodied than *S. constellatus* (mean: 21% BL, range: 15–24% BL in *S. ensifer* versus mean: 16% BL, range: 15–17% BL in *S. constellatus*). Note that, although this comparison is between ethanol-preserved *S. ensifer* and formalin-preserved *S. constellatus*, shrinkage does not differ greatly between the 2 preservatives (typically about 3–6% in 80–95% ethanol and about 2–10% in 5% formalin solution).

By the late preflexion stage, *S. constellatus* has distinctly more evenly and heavily pigmented pectoral fins than *S. ensifer*, and it has melanophores on the snout and upper jaw, on the isthmus, and on the hypural margin, which are all lacking in *S. ensifer*. It should be noted that these comparisons are between field-collected *S. ensifer* and laboratory-reared *S. constellatus*; it is yet to be determined whether the differences

will be apparent in field-collected *S. constellatus*. By the late postflexion stage, field-collected specimens of both *S. constellatus* and *S. ensifer* are nearly identical in pigmentation and morphological features but can be distinguished by gill raker counts (34–40 on the first arch in *S. ensifer* versus 25–30 in *S. constellatus*), possibly by small differences in head spination, and by the longer dorsal- and anal-fin rays of *S. ensifer* (Rocha-Olivares et al., 2000).

Larval *S. ensifer* and *S. helvomaculatus* also are pigmented similarly, but *S. ensifer* apparently retains ventral pigment on the tail longer than ventral pigment on *S. helvomaculatus* (≥1 melanophore on the caudal peduncle to at least 8.4 mm versus none by 7.7 mm, respectively). The pectoral fin base apparently is more heavily pigmented in *S. helvomaculatus*: Richardson and Laroche (1979) reported (but did not show in their illustrations) that both the inner and outer surfaces of the fin base are pigmented by 7.7 mm in *S. helvomaculatus*. In contrast, *S. ensifer* has pigment only on the inner surface of the fin base through at least 8.4 mm. Larval *S. ensifer* may be slightly more slender than *S. helvomaculatus* (mean: 33% BL, range: 32–34% BL for *S. ensifer* 7.7–8.4 mm versus mean: 35% BL, range: 33–40% BL for *S. helvomaculatus* 7.7–8.8 mm), and have a slightly longer preopercular spine PPO3 (28–36% HL versus 27–31% HL for larvae within the size range of 7.7–8.8 mm).

Larval *Sebastes* of the subgenus *Sebastomus* are more or less readily identifiable as a group on the basis of traditional morphological and pigmentation characters, but the species within that group are not, perhaps reflecting the relatively recent origin and rapid radiation of this subgenus (Hyde and Vetter, 2007). Molecular techniques do allow for species identification and, coupled with visual pre-sorting to subgenus, do provide an efficient method for obtaining quantitative, species-specific data for these otherwise unidentifiable larvae. Development of microarrays and microbeads that allow automated reading of a fluorescent label after species-specific enzyme ligation may provide collection of real-time, species-specific abundance data aboard a ship during a survey.

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