IDENTIFICATION, DESCRIPTION, AND DAILY GROWTH OF PELAGIC LARVAL AND JUVENILE SQUARESPOT ROCKFISH, *SEBASTES HOPKINSI* (FAMILY SEBASTIDAE)

THOMAS E. LAIDIG, KEITH M. SAKUMA
NOAA Fisheries Service
Southwest Fisheries Science Center
Fisheries Ecology Division
110 Shaffer Road
Santa Cruz, California 95060
tom.laidig@noaa.gov

JOHN R. HYDE, WILLIAM WATSON
NOAA Fisheries Service
Southwest Fisheries Science Center
Fisheries Resources Division
8604 La Jolla Shores Drive
La Jolla, California 92037

CYNTHIA TAYLOR LAWLEY
Illumina, Inc.,
25861 Industrial Boulevard,
Hayward, California 94545

ABSTRACT

Identifying pelagic larval and juvenile *Sebastes* spp. is important for biomass estimates and recruitment studies. However, only about 50% of *Sebastes* spp. can be unambiguously identified. In this study, pelagic larval and juvenile squarespot rockfish (*Sebastes hopkinsi*) are described and a series of fish ranging from 3.5 mm notochord length to 52.3 mm standard length are illustrated. Species descriptions include pigmentation patterns, meristic characters, morphometric measurements, head spination, and otolith morphology. Species identification was confirmed using mitochondrial DNA sequence data. The growth rate for small larvae averaged 0.17 mm/day, while for late larvae and juveniles the average growth rate increased to 0.47 mm/day; both of these growth rates are typical for early life stages of *Sebastes* from California.

INTRODUCTION

Accurate identification of pelagic larval and juvenile *Sebastes* spp. is important for biomass estimates and recruitment studies (Hunter and Lo 1993; Ralston et al. 2003). Within the northeastern Pacific Ocean region, there are at least 72 species of rockfishes, *Sebastes s*pp. (Love et al. 2002). Differentiating them in the larval (and to a lesser extent the juvenile) stages is extremely difficult due to similar pigment patterns. Many species are even difficult to tell apart as adults (e.g., the subgenus *Sebastomus*). Complete descriptions of these early life stages exist for only 20 rockfishes; for 10 of these species only the juvenile stages are described, for seven species, only some larval stages are described; for the remainder we have few or no descriptions, and only a few images (see Laroche[1], Matarese et al. 1989; Moser 1996). Larval and juvenile *Sebastes* spp. have been identified through rearing studies and descriptions based on a size-series of field-caught specimens (Matarese et al. 1989; Moser 1996). Otolith characteristics have also proven useful in discerning some *Sebastes* spp. (Laidig and Ralston 1995), and genetic methods are another effective identification tool (Rocha-Olivares et al. 2000; Taylor et al. 2004; Pearse et al. 2007).

Sebastes spp. comprise a substantial portion of the groundfish fishery off the west coast of North America, although the abundances of several of these commercial species (e.g., canary rockfish, *S. pinniger*) have declined in recent years (PFMC 2004). With a decrease in abundance of large-sized rockfish, smaller-sized rockfish are appearing more frequently in landings. The small-sized squarespot rockfish (*Sebastes hopkinsi*; maximum size of 29 cm total length) is now the most commonly landed rockfish in the recreational catch from southern California (Love et al. 2002). *Sebastes hopkinsi* generally occur around rocky outcrops, boulder fields, and hard fractured substrata between 18–224 m depth. Although they may be solitary, *S. hopkinsi* are often found in large aggregations numbering in the thousands (Love et al. 2002). While *S. hopkinsi* has recreational value, it is also an ecologically important species, commonly found in the stomachs of pinnipeds (NMFS 1997).

In this study, we provide the means to identify the larvae and pelagic juveniles of *S. hopkinsi*, and also examine the age and growth from otoliths. We use mitochondrial DNA (mtDNA) sequence data to confirm the pelagic larval through juvenile *S. hopkinsi* specimens identified based on morphological, meristic, and pigmentation characters, and in order to assure that the assembled developmental series is monospecific.

METHODS

Specimen Collection

Specimens of pelagic larval and juvenile *S. hopkinsi* were obtained from research cruises aboard the NOAA RV *David Starr Jordan* off the coast of California. Preflexion, flexion, and postflexion larvae, up to 16.0 mm standard length (SL), were collected off southern California 1-15 April 1999 in oblique bongo net (0.505 mm mesh) tows through the upper 212 m of the water column during CalCOFI cruise 9904 and following standard CalCOFI procedures (Kramer et al. 1972). Sampling effort was distributed between CalCOFI lines 80 and 93, with larval *S. hopkinsi* collected at 19 of the 58 stations surveyed. Positive larval collections occurred at the more inshore stations of the survey (covering most of

[1]Laroche, W. A. 1987. Guide to larval and juvenile rockfishes (*Sebastes*) of North America. Box 216, Enosburg Falls, VT 05450. Unpubl. Manu. 311 pp.

the Southern California Bight). Samples from the starboard side of the bongo net were fixed in 5% sodium borate buffered formalin for later species identification, while those from the port side were fixed in 95% ethanol for use in molecular identification and larval aging studies. Late-stage larval and juvenile specimens were collected from central California from mid-May to mid-June during 1990–93 and 2001 and between Bodega Bay (north of San Francisco) and Cypress Point (south of Monterey Bay) using a modified Cobb midwater trawl with a 26 m headrope and a 9.5 mm stretched mesh codend liner. Specimens were frozen at sea for later analysis.

Meristics, Morphometrics, and Body Pigmentation

A total of 236 pelagic larval and juvenile *S. hopkinsi,* ranging in total length from 3.5 to 52.3 mm, were examined for pigmentation patterns and physical characteristics. Notochord length (NL) was measured on all flexion and preflexion larvae, and SL was measured for postflexion individuals. Counts of dorsal, anal, and pectoral fin rays, and the number of gill rakers on the first gill arch were recorded whenever possible, and subsequently used in identifications. Accurate gill raker counts were obtained only from fish larger than 15 mm SL. Specimens greater than 19.9 mm were identified using meristic characters (fin ray and gill raker counts) and head spination (Matarese et al. 1989; Moreland and Reilly 1991; Moser 1996; Laroche[1]), and pigment patterns were recorded.

Larval *S. hopkinsi* collected from the CalCOFI cruise (n = 85) were first identified to species genetically because they could not be identified through other means (fin ray and gill raker counts). Pigment patterns were recorded from these fish after positive genetic identifications. Due to the removal of tissue for genetic identification, complete pigment patterns for the caudal fin and peduncle could not be determined for each specimen.

Morphometric measurements recorded for 30 *S. hopkinsi*, ranging in size from 14.4–45.0 mm SL, included snout-to-anus length, head length, snout length, eye diameter, body depth at the pectoral fin base, body depth at anus, and pectoral fin length, and followed Richardson and Laroche's methods (1979). Head spination was examined on 22 specimens (6.0 to 45.0 mm SL) stained with Alizarin red S. Terminology for head spination follows Richardson and Laroche (1979).

Otolith Examination

Sagittal otoliths were removed from the rockfish and ages were obtained by increment counts beginning at the first growth increment after the extrusion check (a mark in the otolith that forms when the larva is initially released from the mother) using a compound microscope at 1000× magnification (Laidig et al. 1991). Growth increments were not validated during this study, and no other researchers have conducted validation studies on this species. However, these counts were assumed to correspond to daily ages because the formation of daily growth increments has been validated for other cooccurring rockfish species, including *S. jordani* (shortbelly rockfish) (Laidig et al. 1991), *S. paucispinis* (bocaccio), *S. goodei* (chilipepper), *S. entomelas* (widow rockfish), and *S. flavidus* (yellowtail rockfish) (Woodbury and Ralston 1991). The radius of each otolith was measured from the center to the postrostral edge of the extrusion check for comparison with similar measurements from other *Sebastes* spp. (Laidig and Ralston 1995). Transformation from the larval stage to the juvenile stage was ascertained by the occurrence of accessory growth primordia (Laidig et al. 1991). Accessory primordia are areas away from the otolith core from which new increment growth begins (also called secondary growth centers or primordia).

Molecular Identification

Genomic DNA was extracted from caudal fin, muscle, or eye tissue using a chelex extraction protocol (Walsh et al. 1991). The polymerase chain reaction (PCR) was used to amplify 862 bp of the mtDNA cytochrome-*b* gene in a 1× buffer containing 20 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 800 μM dNTPs, 0.3 μM of each primer, and 0.5 units *Taq* DNA polymerase (New England Biolabs). Primers included previously published GluRF and CB3RF (Rocha-Olivares et al. 1999) and internal custom primers (CB306F 5'-TTACTACGGCTCV-TACCT-3, Cb521R 5'-GTTGCATTGTCTACTGAG-3', and CB364F, 5'-CTAGTTATAATAACTGCTTT-3'). The PCR temperature profile was: 90°C for 2:00 min, followed by 36 cycles of 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 either EXOSAP-IT (USB Corp.) or a Qiaquick PCR purification kit (Qiagen) and according to the manufacturer's protocol. Cleaned PCR products were cycle-sequenced using BigDye v.3.1 (Applied Biosystems) using internal sequencing primers and analyzed on an ABI 3100 automated capillary sequencer (Applied Biosystems). Chromatogram data for sequenced DNA were aligned using the biosequence analysis and editor program, Sequencher (v. 4.1.1 Gene Codes).

Sequences were compared to DNA reference sequence data of 374 independent haplotypes that represented 67 species of identified adult *Sebastes* using an iterative approach within the software program Phylogenetic Analysis Using Parsimony (PAUP* 4b10; Swofford 2000) or Molecular Evolutionary Genetic Analysis (MEGA v2.1; Kumar et al. 2001) with the optimality criterion set to distance (number of bp differences divided by total bp

sequenced). A complete listing of species included in the PAUP reference file is included in Taylor et al. (2004). Nonparametric bootstrapping was used (1000 replications, MAXTREES set to 1000) to cluster each unknown haplotype within a database of consensus haplotypes (consensus = most common haplotype from a database of up to 17 known adults) for putative identification. The identification of the specimen was accepted if it clustered with a single haplotype of a reference species with a bootstrap value of at least 90%. The distance between reference haplotypes and the unknown haplotype was examined to confirm that the unknown fell within the expected intra-specific diversity based on the reference data. A first identification was accepted if a specimen's sequence clustered with a single haplotype of a species with a bootstrap of less than 90%. Secondary analysis was performed using all available haplotypes of at least the three nearest (in distance) species to the unknown haplotype. The species of the unknown haplotype was identified by comparing it to the sequences of the reference species. Additionally, sequences were screened for unique single nucleotide polymorphisms that differentiate *S. hopkinsi* from all other *Sebastes* spp. in the eastern Pacific Ocean (J. Hyde unpublished data). Intra-specific diversity for reference species in the northeast Pacific Ocean has a mean distance of 0.006 with a minimum of 0.0 (e.g., *S. jordani*) and a maximum of 0.041 (in *S. hopkinsi*).

RESULTS

General Development

The smallest *S. hopkinsi* larva collected was 3.5 mm NL. Flexion began at approximately 6.0 mm NL and

TABLE 1
Frequency of occurrence of soft dorsal, anal, and pectoral fin ray counts, and gill raker counts from 181 *Sebastes hopkinsi*, squarespot rockfish. Gill raker counts are from specimens 15 mm SL and larger.

	Dorsal fin count				Anal fin count			Pectoral fin count			Gill raker count							
	13	14	15	16	6	7	8	16	17	18	34	35	36	37	38	39	40	41
Frequency of Occurrence	3	82	85	11	14	162	5	10	155	10	2	7	11	21	29	27	6	4
Percent Occurrence	2	45	47	6	7	90	3	6	88	6	2	6	10	20	27	25	6	4

TABLE 2
Morphometric measurements (in mm) from individuals of *Sebastes hopkinsi*, squarespot rockfish. Number in parentheses represents the ratio of the measurements to the SL.

SL	Snout-anus length	Head length	Snout length	Eye diameter	Body depth at pectoral base	Body depth at anus	Pectoral fin length
14.4	8.5 (.59)	5.0 (.35)	1.4 (1.0)	1.9 (.13)	3.6 (.25)	2.1 (.15)	N/A
14.8	8.9 (.60)	4.7 (.32)	1.5 (.10)	2.0 (.14)	3.8 (.26)	3.0 (.20)	2.4 (.16)
15.0	9.0 (.60)	5.1 (.34)	1.5 (.10)	1.6 (.11)	4.0 (.27)	3.3 (.22)	2.6 (.17)
15.1	9.3 (.62)	5.0 (.33)	1.4 (.09)	2.0 (.13)	3.8 (.25)	3.1 (.21)	2.8 (.19)
15.7	9.4 (.60)	5.0 (.32)	1.6 (.10)	2.2 (.14)	4.5 (.29)	3.4 (.22)	2.9 (.18)
16.1	9.5 (.59)	5.1 (.32)	1.6 (.10)	2.1 (.13)	4.2 (.26)	3.3 (.20)	2.4 (.15)
16.5	9.7 (.59)	5.9 (.36)	1.9 (.11)	2.2 (.13)	4.3 (.26)	3.4 (.21)	3.6 (.22)
16.7	10.2 (.61)	5.9 (.35)	1.9 (.11)	2.4 (.14)	4.2 (.25)	3.4 (.20)	3.5 (.21)
18.1	11.0 (.61)	6.3 (.35)	1.9 (.10)	2.4 (.13)	4.8 (.27)	4.1 (.23)	3.8 (.21)
18.4	10.9 (.59)	6.1 (.33)	1.7 (.09)	2.2 (.12)	5.0 (.27)	4.2 (.23)	3.7 (.20)
18.5	10.3 (.56)	5.9 (.32)	1.4 (.08)	2.1 (.11)	4.5 (.24)	4.1 (.22)	3.4 (.18)
19.4	11.4 (.59)	6.1 (.31)	1.7 (.09)	2.2 (.11)	4.9 (.25)	3.9 (.20)	3.6 (.19)
19.8	12.1 (.61)	6.4 (.32)	1.8 (.09)	2.3 (.11)	4.9 (.25)	3.9 (.20)	4.3 (.22)
20.1	12.1 (.60)	6.7 (.33)	2.1 (.10)	2.5 (.12)	5.4 (.27)	4.4 (.22)	4.2 (.21)
20.9	11.7 (.56)	5.4 (.26)	1.7 (.08)	2.3 (.11)	4.4 (.21)	3.7 (.18)	3.6 (.17)
21.2	12.8 (.60)	6.7 (.32)	1.8 (.08)	2.5 (.12)	5.4 (.25)	4.4 (.21)	5.0 (.24)
22.5	13.8 (.61)	7.7 (.34)	2.1 (.09)	2.4 (.11)	5.5 (.24)	4.5 (.20)	4.6 (.20)
23.2	14.0 (.60)	7.9 (.34)	2.4 (.10)	2.7 (.12)	5.6 (.24)	5.0 (.22)	5.4 (.23)
25.2	14.9 (.59)	8.8 (.35)	2.3 (.09)	2.9 (.12)	5.8 (.23)	5.0 (.20)	5.4 (.21)
25.3	15.3 (.60)	8.9 (.35)	2.5 (.10)	2.9 (.11)	6.5 (.26)	5.5 (.22)	5.6 (.22)
25.9	15.7 (.61)	8.4 (.32)	2.3 (.09)	2.8 (.11)	5.8 (.22)	5.4 (.21)	5.1 (.20)
26.3	15.6 (.59)	8.2 (.31)	2.3 (.09)	2.9 (.11)	6.1 (.23)	5.5 (.21)	6.1 (.23)
26.5	16.2 (.61)	8.2 (.31)	2.4 (.09)	2.8 (.11)	5.6 (.21)	5.3 (.20)	5.4 (.20)
27.5	16.3 (.59)	9.9 (.36)	2.4 (.09)	3.2 (.12)	6.5 (.24)	5.7 (.21)	5.7 (.21)
27.9	17.2 (.62)	8.9 (.32)	2.1 (.08)	3.0 (.11)	6.2 (.22)	5.6 (.20)	6.2 (.22)
31.5	20.4 (.65)	10.0 (.32)	2.7 (.09)	3.4 (.11)	7.7 (.24)	6.5 (.21)	7.1 (.23)
33.0	20.7 (.63)	10.7 (.32)	3.0 (.09)	3.4 (.10)	7.6 (.23)	6.7 (.20)	7.4 (.22)
37.8	24.3 (.64)	12.7 (.34)	3.2 (.08)	4.0 (.11)	10.0 (.26)	9.1 (.24)	9.3 (.25)
41.7	26.3 (.63)	14.5 (.35)	3.7 (.09)	4.1 (.10)	10.7 (.26)	9.5 (.23)	11.0 (.26)
45.0	27.5 (.61)	15.4 (.34)	3.8 (.08)	4.4 (.10)	11.4 (.25)	9.9 (.22)	11.1 (.25)

TABLE 3
Development of head spines in individual *Sebastes hopkinsi*, squarespot rockfish. "0" means spine absent, "1" means spine developing, and "-" means spine overgrown by another spine.

	Standard length (mm)																				
Spines	6.0	7.9	8.3	9.5	10.0	10.5	14.4	15.7	16.1	17.3	18.9	19.6	20.4	22.0	24.1	26.5	28.3	32.8	34.2	37.8	41.7	45.0
Nasal	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Preocular	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Supraocular	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
Postocular	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coronal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tympanic	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
Parietal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Nuchal	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pterotic	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Posttemporals																						
Superior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Inferior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Supracleithral	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Opercular																						
Superior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Inferior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Preopercular																						
1st Anterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd Anterior	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3rd Anterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1st Posterior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd Posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3rd Posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4th Posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5th Posterior	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Infraorbitals																						
1st Inferior	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd Inferior	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3rd Inferior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1st Superior	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
2nd Superior	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3rd Superior	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
4th Superior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-

was completed at approximately 8.5 mm SL. Transformation to the juvenile stage occurred at about 20–25 mm SL. A full adult complement of dorsal, anal, and pectoral fin rays was present in fish over 9.5 mm SL. The modal counts of segmented fin rays were 15 dorsal, 7 anal, and 17 pectoral, although a dorsal ray count of 14 was almost equally common (tab. 1). Gill raker counts varied from 34 to 41, with a mode of 38. The ratios of morphometric measurements to SL were comparable in all sizes (tab. 2), indicating that body form was similar throughout the size range investigated (14.4–45 mm SL).

Head Spination

By 6.0 mm NL, the parietal, the first and third anterior preopercular, and second to fourth posterior preopercular head spines had formed on *S. hopkinsi* (tab. 3). By 7.9 mm SL, the postocular and pterotic spines were present. The fifth posterior preopercular spine was first observed at 8.3 mm SL. At 9.5 mm SL, the supracleithral, the first inferior infraorbital, and the first superior infraorbital spines were present. By 14.4 mm SL, the nasal, nuchal, post temporals, operculars, first posterior preopercular, third inferior infraorbital, and fourth superior infraorbital spines were present. At 17.3 mm SL, the preocular and third superior infraorbital were present. By 18.9 mm SL, the supraocular and tympanic spines had formed. The first superior infraorbital became overgrown by 37.8 mm SL and the fourth superior infraorbital became overgrown by 26.5 mm SL. The coronal, second anterior preopercular, second inferior infraorbital, and second superior infraorbital spines were not observed in any of our specimens.

Body Pigmentation

Prior to extrusion, *S. hopkinsi* possess pigment only along the dorsal and ventral body midlines, and ventrally and posteriorly on the gut (fig. 1A; tab. 4; Moser et al. 1977). All larvae less than 4.0 mm NL had pigment on the head and anterior of the eyes; therefore, these pigments must form soon after extrusion. It is also possible that the presence or absence of these pigments varied and the specimen shown in Moser et al. (1977) doesn't represent the full range of variation.

TABLE 4

Proportion of *Sebastes hopkinsi*, squarespot rockfish, with melanophores present at various areas averaged over 2.0 mm size bins (range of +/– 1.0 mm). SL = standard length (notochord length in preflexion specimens) in mm. Definitions of each area are given below.

SL	N	MAX	ULJ	LJ	EYE	SNOUT	OPER	CHK	NAPE	DORS	VENT	MID	HYP	DFIN	PECF	ANAL	PECB	VEB	AFB	PEDB
3	18	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	19	0.0	0.1	0.1	0.0	1.0	0.0	0.0	0.0	1.0	1.0	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
7	11	0.0	0.0	0.3	0.0	1.0	0.0	0.0	0.0	1.0	1.0	0.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
9	2	0.5	0.0	1.0	0.0	1.0	1.0	0.0	0.5	1.0	1.0	0.5	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
11	2	1.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	1.0	1.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	2	0.5	0.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	5	0.8	0.2	1.0	0.2	1.0	1.0	0.0	0.2	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	11	0.9	0.1	1.0	0.6	1.0	1.0	0.0	0.6	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	14	1.0	0.9	1.0	0.9	1.0	1.0	0.0	0.9	1.0	1.0	1.0	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
21	9	1.0	0.6	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	14	1.0	0.9	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0
25	12	1.0	0.9	1.0	1.0	1.0	1.0	0.1	1.0	1.0	1.0	1.0	1.0	0.5	0.0	0.0	0.4	0.0	0.0	0.2
27	9	1.0	1.0	1.0	1.0	1.0	1.0	0.2	1.0	1.0	1.0	1.0	1.0	0.9	0.0	0.0	1.0	0.2	0.3	0.8
29	11	1.0	0.8	1.0	1.0	1.0	1.0	0.2	1.0	1.0	1.0	1.0	1.0	0.8	0.1	0.0	0.6	0.3	0.3	0.5
31	10	1.0	1.0	1.0	1.0	1.0	1.0	0.1	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.8	0.3	0.5	0.7
33	4	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.8	0.5	1.0	1.0
35	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.0	1.0	0.5	0.5	0.5
37	4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	0.5	1.0	1.0	1.0	1.0
39	9	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0
41	8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0
43	16	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
45	10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
47	10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
49	15	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
51	9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

MAX=side of maxilla, ULJ=under lower jaw, LJ=anterior tip of lower jaw, EYE=posterior-ventral edge of eye orbit, SNOUT=dorsal surface anterior to eye, OPER=operculum, CHK=radiating cheek bars, NAPE=nape, DORS=dorsal body, VENT=ventral body, MID=along lateral midline, HYP=hypural region, DFIN=spinous dorsal fin, PECF=pectoral fin blade, ANAL=anal fin blade, PECB=body bar at pectoral fin, VEB=body bar at vent, AFB=body bar at anal fin, PEDB=body bar at peduncle.

Pigment on the tip of the lower jaw and along the lateral midline was first observed in fish approximately 5.0 mm NL with all fish having lower jaw pigment by 9.0 mm SL and lateral midline pigment by 11 mm SL (tab. 4). Pigment formed on the maxilla and operculum by 9.0 mm SL. Pigment along the bases of the dorsal-most pectoral fin rays was observed in six fish from 6–10 mm SL, but otherwise was absent before the juvenile stage. Among larvae less than 10 mm SL, only one specimen each had pigment under the lower jaw and along the nape.

By 10.5 mm SL, pigment on the head and anterior to the orbit had intensified and merged to form a continuous pigment patch (fig. 1B; tab. 4). Pigment along the tip of the lower jaw became denser and began to extend posteriorly. Maxillary pigment also was denser compared to smaller specimens. Dorsal and ventral midline melanophores increased in number and intensity and extended anteriorly. Dorsal midline pigment occurred from the caudal peduncle to the second anterior dorsal spine, while ventral midline pigment was present from the caudal fin to the base of the posterior-most anal fin rays. A few melanophores were present along the lateral midline on the caudal peduncle in all specimens. Hypural pigment had begun to form with a few melanophores along the edge of the lower hypural plate. Pigment was not observed on any of the fins, but pigment on the axillary surface of the pectoral fin base was present in all specimens.

At 14.4 mm SL, pigment along the tip of the lower jaw, maxilla, and anterior orbital area intensified and increased in density (fig. 1C; tab. 4). Head pigment intensified and covered the entire dorsal surface. Opercular pigments increased in number, sparsely covering the dorsal surface of the operculum. Dorsal midline pigment increased in density and spread forward to the first or second anterior dorsal spine. However, ventral midline pigment changed little in fish ranging from 12–16 mm SL, and even decreased in some individuals. A few melanophores were added anteriorly at the bases of some of the anal fin rays. Hypural pigment strengthened and was present in all specimens ≥13 mm SL. Lateral midline pigment intensified and progressed both anteriorly and posteriorly compared to smaller specimens, extending from the hypural edge to below the dorsal soft rays.

At 19.4 mm SL, pigment along both jaws increased in number and progressed posteriorly (fig. 1D; tab. 4). Pigment on the ventral surface of the lower jaw increased in number and covered a wider area. Pigment had formed along the nape and merged with the head, snout, upper jaw, and dorsal midline pigments to form a continuous

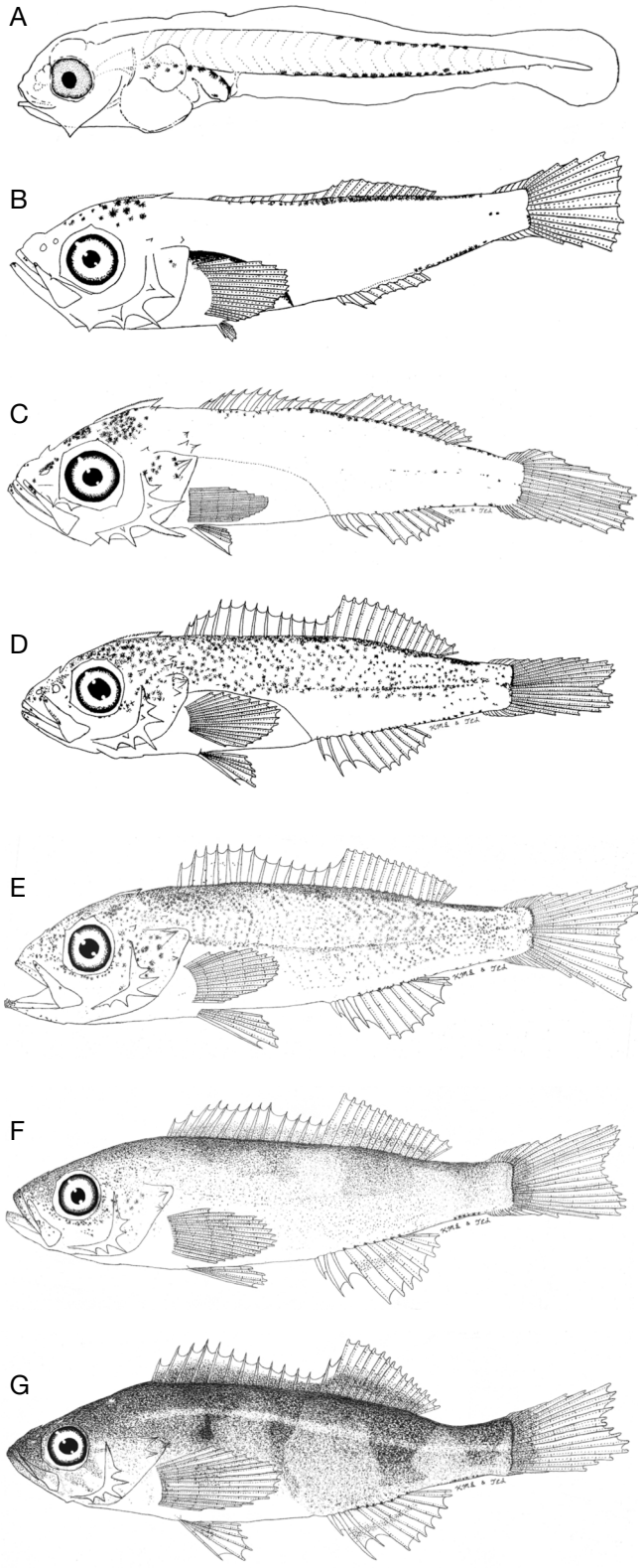

Figure 1. Developmental series of *Sebastes hopkinsi*, squarespot rockfish. (A) 4.7 mm SL preextrusion larva (from Moser et al. 1977); (B) 10.5 mm SL larva; (C)14.4 mm SL larva; (D) 19.4 mm SL pelagic juvenile; (E) 26.9 mm SL pelagic juvenile; (F) 36.9 mm SL pelagic juvenile; (G) 45.0 mm SL pelagic juvenile. Illustrations B-G drawn by authors. Note that not all head spines are included in the illustrations.

band from the tip of the jaw to the caudal fin. Head, nape, and opercular pigment intensified and merged to cover the upper half of the head (fig. 1D). Melanophores appeared along the posterioventral edge of the orbit. The dorsal half of the body was dotted with pigment, although no body bars had yet appeared. The lateral midline pigment stretched from the caudal fin to the gut, almost forming a continuous line. Hypural pigment continued to intensify, especially on the dorsal region. Ventral midline pigment stretched from the caudal fin to the first anal spine and increased in density. All fins remained unpigmented in specimens from 17–21 mm SL, except for one specimen (19.1 mm SL) that had a few melanophores in the spinous dorsal fin. The ventral half of the body remained only lightly pigmented.

By 27 mm SL, the dorsal half of the body was heavily pigmented, except for an unpigmented area below the dorsal midline of the caudal peduncle (fig. 1E; tab. 4). More of the ventral half of the body was pigmented than in smaller specimens. The spinous dorsal fin was pigmented in 90% of the specimens, with melanophores on the membranes between the anterior-most spines. All other fins remain unpigmented. Cheek bars had begun to form on a few specimens by 27 mm SL. Hypural pigment became a thick band along the distal margin. The ventral midline was much less pigmented than the dorsal midline. The dorsal half of the anterior-most body bar (at the pectoral fin) was evident in most fish. The other body bars began to form in a few specimens.

At 37 mm SL, juveniles had pigment covering most of the body (fig. 1F; tab. 4). The hypural, dorsal midline, nape, head, snout, and maxilla pigments were all well developed and formed a broad, dark band from the caudal fin to the mouth. The lower jaw and maxilla pigments formed a ring around the mouth. Circumorbital pigment had increased compared to smaller fish. Both cheek bars were present, but incomplete. The lower halves of both the spinous and soft dorsal fins were pigmented throughout the entire length of the fin. Pectoral fin pigment was present in 80% of the specimens between 35 and 39 mm SL, occurring from the base of the fin out about halfway to the distal edge. The anterior portion of the caudal fin was pigmented. The anal fin was pigmented in half of the specimens, with a pigment stripe occurring from about the midpoint of the fin (fig. 1F). The pelvic fins remained unpigmented. Body bars were forming at the caudal peduncle, under the soft dorsal fin, above the vent, and above the pectoral fin. The caudal and anal fin body bars were prominent in all specimens.

At 45.0 mm SL, *S. hopkinsi* juveniles remained heavily pigmented along the dorsal half of the body with the ventral region much less pigmented (fig. 1G; tab. 4). The dorsal midline, nape, head, snout, maxilla, lower jaw, ventral surface of the lower jaw, and hypural pigments were

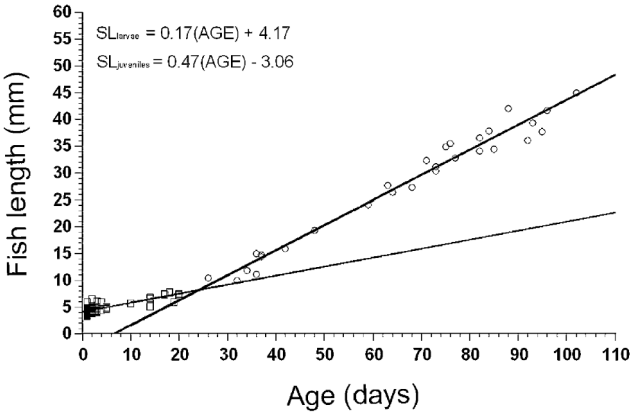

Figure 2. Relationship between fish standard length (notochord length below 9.0 mm) and age for *Sebastes hopkinsi*, squarespot rockfish (n = 116). Open squares represent larvae (n = 85), open circles represent juveniles (n = 31), and solid lines represent predicted values from each relationship.

all dense and surrounded much of the fish in a thick, dark band. The ventral midline pigment was much less dense than in smaller specimens and only reached from the caudal fin to the third anal spine. The area from the vent to the eye, including the gut area, was only lightly pigmented, with the heavier pigmentation on the operculum and in the two cheek bars, which were clearly visible by this size. All four body bars were well developed, with each stretching ventrad from the dorsal midline, but stopping short of the ventral midline. All of the fins were pigmented. The dorsal, caudal, and pelvic fins were pigmented proximally, leaving the tips clear of pigment. Pigment in the anal fin consisted of a horizontal stripe about midway through the fin, stretching from the first spine to the sixth ray. The pectoral fin was pigmented proximally and in an area in the middle of the fin.

Otolith Examination

A total of 116 otoliths were examined from larval and juvenile *S. hopkinsi* ranging in length from 3.5–45.0 mm SL and in age from 1 to 92 days. Two linear relationships were found to exist between SL and age (as estimated from otolith increment counts); one for preflexion and flexion larvae (slope = 0.17 mm/d; intercept = 4.17 mm; r^2 = 0.66; n = 85; fig. 2) and one for postflexion larvae and juveniles (slope = 0.47 mm/d; intercept = –3.06 mm; r^2 = 0.97; n = 31; fig. 2). The two linear relationships were considered the best model because of the differential growth between postflexion and preflexion larvae (Laidig et al. 1991; Sakuma and Laidig 1995; Laidig et al. 1996). The radius of the extrusion check ranged from 12.4 to 13.8 µm, averaging 13.2 µm (SD = 0.34; n = 23). Accessory primordia first appeared in the otolith of a 24.2 mm specimen and were present in all larger specimens. The average increment count at the beginning of the innermost accessory primordium was 53 (n = 20). This equates to a size of 21.8 mm SL using the above length-and-age relationship for fish that completed flexion. Based on these characters, transition from the larval to juvenile phase begins at approximately 22 mm SL.

Molecular Identification

Phylogenetic analysis of the cytochrome-*b* gene, comparing unknowns to sequences from all eastern Pacific *Sebastes* spp., grouped all juveniles identified by morphology as *S. hopkinsi* with reference samples of *S. hopkinsi* and *S. ovalis* (speckled rockfish; minimum evolution tree, Kimura 2 parameter model as implemented in MEGA v2.1 [Kumar et al. 2001], 1000 bootstrap replicates, 99% bootstrap support). Additionally, all samples shared a genetic polymorphism at position 246 of the cytochrome-*b* gene, which is unique to *S. hopkinsi* and *S. ovalis*. These two species are closely related and can only be reliably separated genetically by an autapomorphic nucleotide substitution for *S. ovalis* at position 543 of the cytochrome-*b* gene (J. Hyde unpublished data). There was a high degree of intra-specific variation at this gene (4.2%, n = 37) for *S. hopkinsi* while this variation was much reduced (0.26%, n = 15) for *S. ovalis* (J. Hyde unpublished data). Due to the high degree of intra-specific variation for *S. hopkinsi*, unless a sequence matched 100% with that of the reference sequences, the diagnostic nucleotide at base 543 was used to distinguish between *S. hopkinsi* (A) and *S. ovalis* (T). Three of the fish examined in this study (MI11, MI12, and MI17) matched 100% with the sequence from reference specimens, while the remainder differed from the closest reference specimen by one to seven nucleotides (0.13–0.90% sequence divergence; fig. 3).

DISCUSSION

Sebastes hopkinsi have distinctive pigment patterns at different sizes. Large juvenile *S. hopkinsi* had a four-bar pattern, while smaller juveniles had diffuse pigment over most of their bodies. Larvae tended to be pigmented mainly on the head and along the dorsal midline, with some lateral midline and pectoral fin pigment present in some individuals. Modal counts of segmented fin rays were seven for the anal fin, 17 for the pectoral fin, and 14 or 15 for the dorsal fin. By using a combination of pigment characters and fin ray counts, more accurate field and laboratory species identifications can be achieved.

By using fin ray and gill raker counts, only 10 *Sebastes* spp. could be confused with *S. hopkinsi* at sizes greater than 15 mm SL. Five of these (*S. flavidus*, *S. goodei*, *S. proriger* [redstripe rockfish], *S. wilsoni* [pygmy rockfish], and *S. zacentrus* [sharpchin rockfish]) can be separated from *S. hopkinsi* after about 18 mm SL by their lack of a supraocular spine. Five species (*S. semicinctus* [halfbanded

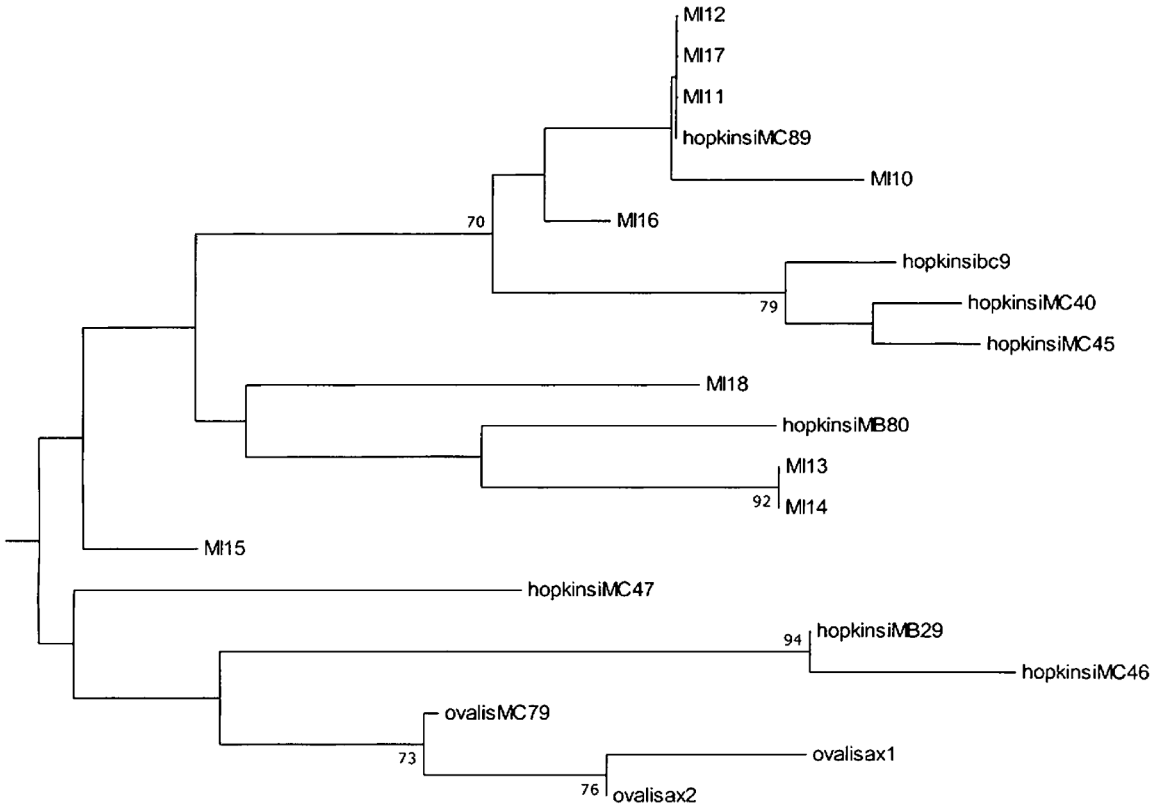

Figure 3. Sub-tree of a Minimum Evolution tree generated from the first 782bp of the mitochondrial cytochrome-*b* gene with the Kimura 2-parameter distance model MEGA v2.1, 1000 bootstrap replicates. Juvenile *Sebastes hopkinsi*, squarespot rockfish, described in this study are denoted as MI10-MI18. Haplotypes from reference specimens are denoted with the species name followed by unique 3- or 4-character identifiers (e.g., ovalisAX2 is *S. ovalis* sample # AX2). Branch nodes with greater than 70% bootstrap support values are labeled.

rockfish], *S. pinniger*, *S. miniatus* [vermilion rockfish], *S. entomelas*, and *S. alutus* [Pacific Ocean perch]) cannot be separated from *S. hopkinsi* by fin ray counts, gill raker counts, or by the presence of a supraocular spine. *Sebastes semicinctus* has only about a 15% overlap in dorsal fin ray counts, with a mode of 13 compared to counts of 14 and 15 for *S. hopkinsi* (Laroche[1]). Using this in combination with the distinctive barring patterns of both *S. semicinctus* and *S. hopkinsi*, the two species can be separated. *Sebastes pinniger* and *S. miniatus* can be distinguished by their distinctive pigmentation patterns and their distinctive body shapes (Matarese et al. 1989). Postflexion larvae of both species are distinctly deeper-bodied than *S. hopkinsi* and, up to 15 mm SL, both species have little dorsal body pigment, except where it has coalesced into dark patches near the dorsal midline (*S. pinniger*) or anteriorly into a broad saddle (*S. miniatus*). This contrasts with the thick band of dorsal body pigment that is present in late larval *S. hopkinsi*. By 25 mm SL, two saddles cover most of the body in *S. miniatus*, easily distinguishing it from *S. hopkinsi*. *Sebastes pinniger* remains lightly pigmented until the late juvenile stage after settlement to the benthos, and would therefore not be confused with *S. hopkinsi*. *Sebastes entomelas* has pigmented pectoral and pelvic fins, with more extensive pectoral pigmentation then those individuals of *S. hopkinsi* having pectoral pigment. Pelagic juveniles also have a distinctive saddling pattern on the dorsal half of the body, and a black spot in the spinous dorsal fin (Laroche and Richardson 1981). This pattern is easily discernable from the *S. hopkinsi* pigment pattern. The modal pectoral fin ray counts differ between *S. alutus* (18 fin rays) and *S. hopkinsi* (17 fin rays), with only a 4% overlap. There is only a 7% overlap in anal fin ray counts. Only two published illustrations exist for *S. alutus*; one at the preflexion stage and one for a 57 mm SL juvenile *S. alutus* (Laroche[1]), making it difficult to compare pigmentation with *S. hopkinsi*. However, because *S. alutus* has uniform mottling covering the body as a late-stage juvenile, and *S. hopkinsi* has a barred pattern in fish greater than 30 mm SL, the presence of bars should allow separation. Capture location may also aid in this differentiation, with *S. alutus* ranging from Baja California north (uncommon south of northern California) and *S. hopkinsi* ranging from southern Oregon south, but rare north of Monterey (Love et al. 2002). Caution must be used with species ranges because larvae and juveniles are pelagic and may have a broader geographic range than the adults.

Using fin ray and gill raker counts, supraocular spine presence, and general pigment patterns, *S. hopkinsi* can be separated from other species at sizes greater than 15 mm SL with a relatively high degree of certainty.

Compared to the few species similar to *S. hopkinsi* at larger sizes, many species can be confused with *S. hopkinsi* at sizes less than 15 mm SL. Fin ray counts can be useful at sizes greater than 8 mm SL, while head spine and gill raker counts are unreliable for most larvae. Based on fin ray counts alone, 27 species could be confused with *S. hopkinsi* at small sizes. Some of these species have distinctive pigmentation and can be separated from *S. hopkinsi*, leaving 11 species to consider. Two of these species primarily occur farther north, and five spawn later in the year (Love et al. 2002); only *S. maliger* (quillback rockfish), *S. nebulosus* (china rockfish), *S. ovalis*, *S. ruberrimus* (yelloweye rockfish), and *S. semicinctus* cannot reliably be separated from *S. hopkinsi* at 8–15 mm SL. Larval illustrations of these five species are not sufficient to distinguish them from *S. hopkinsi*, and each of these species has at least one overlapping fin ray count with *S. hopkinsi*.

At sizes less than 8 mm SL, pigmentation, timing of parturition, and location of capture can be used to differentiate between some, but not most, species. Many species are poorly illustrated at small sizes, and the distinctions between species are slight to nonexistent for many individuals. Small *S. hopkinsi* cannot be reliably separated from *Sebastes* spp. of the subgenus *Pteropodus* except by the use of DNA (Taylor and Watson 2004; Watson and Robertson 2004). Identifications of distinct species based on pigmentation may never be obtained at these small sizes.

The identification of larval and juvenile *S. hopkinsi* was confirmed using DNA sequence analyses. Only one species, *S. ovalis*, was grouped with *S. hopkinsi*. Among *Sebastes*, these species uniquely share a genetic polymorphism at position 246 of the cytochrome-*b* gene, and belong to the same subgenus, *Acutomentum*. They can be reliably separated by an autapomorphic nucleotide substitution for *S. ovalis* at position 543 of the cytochrome-*b* gene. At birth, *S. ovalis* closely resemble *S. hopkinsi*, but they may develop heavier pectoral fin pigmentation during the preflexion stage (Moser et al. 1977); however, the pigment pattern for most of the early life history of *S. ovalis* is presently unknown.

The high genetic diversity in *S. hopkinsi* and the geographic differences in collection sites for the larval and juvenile *S. hopkinsi* may confound identification of the species (and subsequent separations from other *Sebastes* spp.). These factors may also lead to changes in pigmentation in larval stages. Sakuma et al. (2005) reported variability in pigmentation in *S. jordani*, a species with low genetic diversity. However, the differences in pigmentation did not preclude the authors from distinguishing *S. jordani* from other species. Data from the current study cannot adequately address pigmentation differences in *S. hopkinsi*, and further study will be needed to determine if genetic or geographic differences will have an influence on the identification of *S. hopkinsi*.

The radius of the extrusion check can be a useful character for differentiating *Sebastes* spp. (Laidig and Ralston 1995). In this study, the radius of the extrusion check ranged from 12.4–13.8 μm, averaging 13.2 μm (SD = 0.34). Of the other sixteen *Sebastes* spp. with measurement information for the extrusion check radius (Laidig and Ralston 1995; Laidig et al. 1996; Laidig and Sakuma 1998; Laidig et al. 2004), three had radii much larger than *S. hopkinsi*, and eight had radii much smaller than *S. hopkinsi*. That leaves five species with similar radii: *S. auriculatus* (14.1 μm, SD = 1.5), *S. flavidus* (12.1 μm, SD = 0.7), *S. entomelas* (11.8 μm, SD = 0.9), *S. paucispinis* (12.2 μm, SD = 1.1), and *S. rastrelliger*, grass rockfish (14.0 μm, SD = 0.4). Among these, all but *S. entomelas* can be distinguished from *S. hopkinsi* using meristics and head spination, and all five species can be differentiated from *S. hopkinsi* using pigmentation at sizes greater than 8 mm SL. At smaller sizes, the extrusion check radius may be helpful in eliminating *S. hopkinsi* as a possibility in groups of unknown larvae, but it cannot be used to specifically identify a larva as *S. hopkinsi*, because the size of the extrusion check radius is not known for all *Sebastes* spp.

The daily growth rate of juvenile *S. hopkinsi* was 0.47 mm/day, which is similar to the growth rates of other juvenile *Sebastes* spp. The fastest growth rates were recorded for juvenile *S. paucispinis*, (0.56–0.97 mm/day) and *S. jordani* at (0.52–0.64 mm/day) (Laidig et al. 1991; Woodbury and Ralston 1991). Growth rates of *S. goodei* (0.40–0.56 mm/day) and *S. entomelas* (0.30–0.60 mm/day) are similar to *S. hopkinsi* (Woodbury and Ralston 1991). *Sebastes rastrelliger* (0.36 mm/day; Laidig and Sakuma 1998), *S. saxicola* (stripetail rockfish; 0.37 mm/day; Laidig et al. 1996), and *S. wilsoni* (0.28 mm/day; Laidig et al. 2004) all have slower growth rates. The slower rate (0.17 mm/day) in this study for larval *S. hopkinsi* that had not completed notochord flexion is similar to that in previous studies of *Sebastes* growth during their first month of life (Laidig et al. 1991; Sakuma and Laidig 1995; Laidig et al. 1996).

The ability to correctly identify rockfish larvae and juveniles is important for developing accurate species recruitment indices. Recruitment data can be used to determine population biomasses through larval production models (Ralston et al. 2003), and have proven useful in stock assessments (MacCall et al. 2002). Currently, there are pigment descriptions of larval and juvenile stages for only approximately 50% of the *Sebastes* spp. occurring

along the west coast of North America. Further work is needed to describe the remaining unidentified life history stages of over 30 species.

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