Description and growth of larval and pelagic juvenile pygmy rockfish (*Sebastes wilsoni*) (family Sebastidae)

Thomas E. Laidig

Keith M. Sakuma

Santa Cruz Laboratory
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
110 Shaffer Rd.
Santa Cruz, California 95060
E-mail address: tom.laidig@noaa.gov

Jason A. Stannard

La Jolla Laboratory
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
P. O. Box 271
La Jolla, California 92038

Abstract—A developmental series of larval and pelagic juvenile pygmy rockfish (*Sebastes wilsoni*) from central California is illustrated and described. *Sebastes wilsoni* is a noncommercially, but ecologically, important rockfish, and the ability to differentiate its young stages will aid researchers in population abundance studies. Pigment patterns, meristic characters, morphometric measurements, and head spination were recorded from specimens that ranged from 8.1 to 34.4 mm in standard length. Larvae were identified initially by meristic characters and the absence of ventral and lateral midline pigment. Pelagic juveniles developed a prominent pigment pattern of three body bars that did not extend to the ventral surface. Species identification was confirmed subsequently by using mitochondrial sequence data of four representative specimens of various sizes. As determined from the examination of otoliths, the growth rate of larval and pelagic juvenile pygmy rockfish was 0.28 mm/day, which is relatively slow in comparison to the growth rate of other species of *Sebastes*. These data will aid researchers in determining species abundance.

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Rockfishes (genus *Sebastes*) form a diverse group comprising at least 72 species occurring in the northeastern Pacific (Love et al., 2002). Many of these species represent a substantial portion of the groundfish fishery off the west coast of North America, accounting for 20% of the groundfish landings in California in 2000 (Pacific Fishery Management Council, 2000). A few species are relatively abundant but are not harvested because of their small size. These species play vital roles in the community ecology, including providing prey for the larger, commercially important species. The pygmy rockfish (*Sebastes wilsoni*) having a maximum size of 23 cm total length, is among these small species (Love et al., 2002). Pygmy rockfish are common over sediment and rocky seafloor habitats at a depth of 30–274 m (Stein et al., 1992; Yoklavich et al., 2000). Stein et al. (1992) observed that pygmy rockfish were by far the most abundant fish species off Heceta Bank, Oregon, and Love et al. (1996) reported "clouds" of pygmy rockfish mixed with two other small species, squarespot rockfish (*S. hopkinsi*) and halfbanded rockfish (*S. semicinctus*) off southern California. In Soquel Canyon in central California, pygmy rockfish dominated fish assemblages in rock-boulder habitat at 75–175 m (Yoklavich et al., 2000).

Accurate identification of larval stages is critical. Biomass of rockfish populations can be estimated from larval production (Ralston et al., 2003) and larval and juvenile abundance studies (Moser and Butler, 1987; Hunter and Lo, 1993). If the larval and juvenile rockfish analyzed in these studies are not correctly identified, it could lead to either over- or underestimates of biomass or recruitment potential of a population. Identification of young stages of *Sebastes* has been accomplished through rearing studies and through descriptions based on developmental series of field-caught specimens of various sizes (Matarese et al., 1989; Moser, 1996). Otolith morphologies have been useful in discerning some *Sebastes* species (Laidig and Ralston, 1995; Stransky, 2001). Recently, molecular methods have proven to be an effective tool for the identification of *Sebastes* larvae (Seeb and Kendall, 1991; Rocha-Olivares, 1998; Rocha-Olivares et al., 2000).

In this study, we identify and describe the larvae and pelagic juveniles of pygmy rockfish based on morphometrics and pigmentation patterns, and estimate age and growth at two

developmental stages. Further, we examine otolith radius at time of larval extrusion to separate pygmy rockfish from other similarly pigmented *Sebastes* specimens. We also use mitochondrial DNA (mtDNA) sequence data to identify four putative pygmy rockfish specimens representing a continuum of late-larval through pelagic juvenile stages. The molecular results are used to confirm identifications based on morphological, meristic, and pigmentation characters and to assure that the assembled developmental series is monospecific.

Methods

Specimen collection

Specimens of larval and pelagic juvenile pygmy rockfish were obtained from research cruises conducted aboard the NOAA RV *David Starr Jordan* off central California. Specimens were collected in midwater (5–30 m) from mid-May to mid-June, 1990–92, between Bodega Bay (north of San Francisco) and Cypress Point (south of Monterey Bay) by using a 26 m×26 m modified Cobb midwater trawl (12.7-mm stretched-mesh codend liner). Specimens also were collected during early March, 1992–93, between Salt Point (north of San Francisco) and Cypress Point with a 5 m×5 m modified Isaacs-Kidd (MIK) frame trawl with 2-mm net mesh and 0.505-mm mesh codend. Specimens from the Cobb trawl were frozen and specimens from the MIK frame trawl were preserved in 95% ethanol for later analysis.

Meristics, morphometrics, and body pigmentation

We examined pigmentation patterns and physical characteristics of 122 pygmy rockfish larvae and pelagic juveniles. Standard length (SL) was measured for each individual and sizes ranged from 8.1 to 34.4 mm. Specimens greater than 19.9 mm were identified by using meristic characters (Chen, 1986; Matarese et al., 1989; Moreland and Reilly, 1991; and Laroche[1]), and pigment patterns were recorded. Specimens less than 20 mm were identified initially from pigment patterns from a series starting with the smallest (8.1 mm SL) identifiable individuals with complete fin-ray counts. Counts of dorsal-, anal-, and pectoral-fin rays, and the number of gill rakers on the first arch were recorded whenever possible and subsequently used in identifications. Gill raker counts were obtained only from fish larger than 15 mm SL.

We measured 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 on 16 specimens ranging from 8.1 to 29.6 mm SL, following Richardson and Laroche (1979). Head spination was examined on thirty-three specimens (8.1 to 29.6 mm SL) that were stained with alizarin red-s. Terminology for head spination follows Richardson and Laroche (1979). In the following descriptions, larval and juvenile lengths always refer to SL and pigmentation always refers to melanin.

Otolith examination

Sagittal otoliths were removed from 61 larval and pelagic juvenile pygmy rockfish (8.1–34.4 mm SL), and growth increments were counted beginning at the first increment after the extrusion check (the mark in the otolith formed when the larvae are released from their mother) by using a compound microscope at 1000× magnification (see Laidig et al., 1991). No validation of the these growth increments was performed during the present study, and none has been conducted by other researchers. However, we assumed that these growth increment counts corresponded to daily ages based on validation of daily growth increments in other co-occurring rockfishes, namely shortbelly rockfish, *S. jordani* (Laidig et al., 1991), black rockfish, *S. melanops* (Yoklavich and Boehlert, 1987), bocaccio, *S. paucispinis*, chilipepper, *S. goodei*, widow rockfish, *S. entomelas*, and yellowtail rockfish, *S. flavidus* (Woodbury and Ralston, 1991). The radius of the otolith was measured from the primordium to the postrostral edge of the extrusion check for comparison with similar measurements from other *Sebastes* spp. (as reported in Laidig and Ralston, 1995). Transformation from the larval stage to the pelagic juvenile stage was ascertained by the presence of accessory primordia (Laidig et al., 1991; Lee and Kim, 2000).

Molecular confirmation

Total genomic DNA was isolated from skeletal muscle tissue of four larval and juvenile putative pygmy rockfish specimens by using a CTAB and phenol-chloroform-isoamyl alcohol protocol (Winnepenninckx et al., 1993; Hillis et al., 1996). These four specimens ranged in length from 15 to 27 mm and had pigment patterns similar to the fish identified as pygmy rockfish in the present tudy. Polymerase chain reaction (PCR) amplifications and sequencing of partial mitochondrial DNA regions (cytochrome *b* [cyt-*b*] and control region [CR]) followed the methods of Rocha-Olivares et al. (1999a, 1999b). PCR products were verified on 2% agarose gels and purified by using a QIAquick™ PCR Cleanup Kit (Qiagen, Inc., Valencia, CA) following manufacturer protocols. Complementary strand sequence data were generated by using ABI PRISM™ DyeDeoxy™ terminator cycle sequence chemistry on an automated sequencer (Applied Biosystems, Model 377, Foster City, CA).

Cytochrome *b* sequence data (750 base pairs) from the four specimens were aligned with (previously generated) orthologous sequences from 119 individuals representing 61 species of *Sebastes* (Rocha-Olivares et al., 1999b). Species identifications, based on cyt-*b* data, were made by using distance-based cluster analyses in PAUP v4.0b2 (Phylogenetic Analysis Using Parsimony,

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

version 4, Sunderland, MA) and pairwise comparisons of sequence divergence (i.e., the number of nucleotide differences between two individuals expressed as a percentage). A secondary data set, which included an additional 450 base pairs of control region sequence, was generated for the four undetermined specimens and for a subgroup of known reference species with low levels of sequence divergence from the four putative pygmy rockfish specimens (Puget Sound rockfish [*S. emphaeus*], redstripe rockfish [*S. proriger*], harlequin rockfish [*S. variegatus*], sharpchin rockfish [*S. zacentrus*], and pygmy rockfish). Species identifications, based on this extended (cyt-*b* + CR) data subset, followed analyses described above.

Table 1

Frequency of occurrence (number of fish) of dorsal-, anal-, and pectoral-fin ray counts, and gill raker counts from 122 pygmy rockfish (*Sebastes wilsoni*).

Character	Count	Frequency of occurrence	Percent occurrence
Dorsal-fin rays	12	8	7
	13	110	91
	14	3	2
Anal-fin rays	5	2	2
	6	116	95
	7	4	3
Pectoral-fin rays	16	5	5
	17	92	90
	18	5	5
Gill rakers	36	11	14
	37	15	18
	38	22	28
	39	20	25
	40	10	13
	41	1	1
	42	1	1

Results

General development

All 122 fish had completed notochord flexion and possessed a full complement of segmented fin rays by 8.1 mm. The mode for dorsal-fin ray counts was 13, for anal-fin rays 6, and for pectoral-fin rays 17 (Table 1). The mode for gill raker counts was 38, and the range was 36–42. Anal- and dorsal-fin spines began to develop between 9.1 and 14.0 mm. Lateral line pores began to develop at 29 mm, although a full complement (37 to 46 pores) was not reached in our specimens. Morphometric measurements were taken from 16 individual pygmy rockfish of 8.1–29.6 mm (Table 2).

Head spination

At 8.1 mm, the postocular, parietal, nuchal, inferior posttemporal, supracleithral, superior opercular, preopercular (with the exception of the 2nd anterior), and 1st and

Table 2

Morphometric measurements (in mm) from 16 individuals of pygmy rockfish (*Sebastes wilsoni*).

SL	Snout–anus length	Head length	Snout length	Eye diameter	Body depth at pectoral base	Body depth at anus	Pectoral-fin length
8.1	5.2	3.3	0.8	1.3	2.8	2.3	1.5
9.0	5.3	3.0	1.0	1.5	3.0	2.3	1.7
10.8	6.5	4.2	1.2	1.7	3.5	2.8	2.3
12.1	7.0	4.3	1.3	2.0	3.8	2.8	2.5
12.8	7.7	4.8	1.3	2.2	3.7	3.0	2.5
14.2	8.3	4.7	1.3	2.2	4.3	3.5	3.3
15.2	9.2	5.7	1.7	2.0	4.2	3.5	3.5
16.2	9.7	5.2	1.5	2.0	4.5	3.8	3.8
17.5	10.8	6.2	2.0	2.3	5.0	3.8	4.2
18.6	11.5	6.0	2.0	2.7	5.5	4.3	5.0
20.7	12.7	6.2	2.0	2.7	6.2	5.2	5.0
22.3	14.2	6.7	2.2	2.8	6.8	5.7	6.2
23.8	14.5	6.7	2.0	3.2	7.3	6.3	5.9
24.3	14.2	7.0	2.2	2.7	7.0	5.8	6.3
28.9	17.0	8.0	2.6	3.3	8.5	7.3	7.0
29.6	16.9	7.8	2.6	3.5	8.7	7.5	6.9

2nd inferior and 1st superior infraorbital spines were present (Table 3). The nasal, pterotic, and 4th superior infraorbital first appeared at 10.8 mm. At 12.1 mm, the inferior opercular spine became evident. Between 14.2 and 17.5 mm, the preocular, tympanic, superior postemporal, 2nd anterior preopercular, and 3rd superior infraorbital spines formed. After 17.5 mm, no further changes in head spination were noted. The supraocular, coronal, 3rd inferior infraorbital, and 2nd superior infraorbital spines did not occur on any of the fish examined.

Body pigmentation

At 9.1 mm, pygmy rockfish had no pigment along the lateral and ventral body surfaces (Fig. 1A, Table 4). Pigment was heavy on the top of the head and present on the operculum. The dorsal midline surface had a few melanophores under the soft dorsal fin. The anterior lower jaw was pigmented on the tip and one to two melanophores were present on each side of the snout near the tip. Pigment also was present at the base and on the distal half of the pectoral fin.

By 14.0 mm, dense pigment along the dorsal midline stretched from the caudal peduncle to the first dorsal-fin spine (Fig. 1B, Table 4). The only lateral pigment on the body consisted of a few melanophores along the midline near the caudal peduncle. The ventral surface, including the anal and pelvic fins, remained unpigmented. Pigment on the pectoral fins had mostly disappeared by 11.0 mm and was rarely observed at 14.0 mm. Opercular and head pigment increased in density by 14.0 mm. Pigment along the lower edge of the orbit began to

Table 3

Development of head spines in individual pygmy rockfish (*Sebastes wilsoni*). "1" means spines present and "0" means spines absent.

	Standard length (mm)															
Spines	8.1	9.0	10.8	12.1	12.8	14.2	15.2	16.2	17.5	18.6	20.7	22.3	23.8	24.3	28.9	29.6
Nasal	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Preocular	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Supraocular	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Postocular	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
Tympanic	0	0	0	0	0	0	0	0	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
Nuchal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pterotic	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Posttemporal																
Superior	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Inferior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Supracleithral	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Opercular																
Superior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Inferior	0	0	0	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
2nd anterior	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
3rd anterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1st posterior	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
3rd posterior	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
5th posterior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Infraorbital																
1st inferior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2nd inferior	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3rd inferior	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1st superior	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
3rd superior	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
4th superior	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1

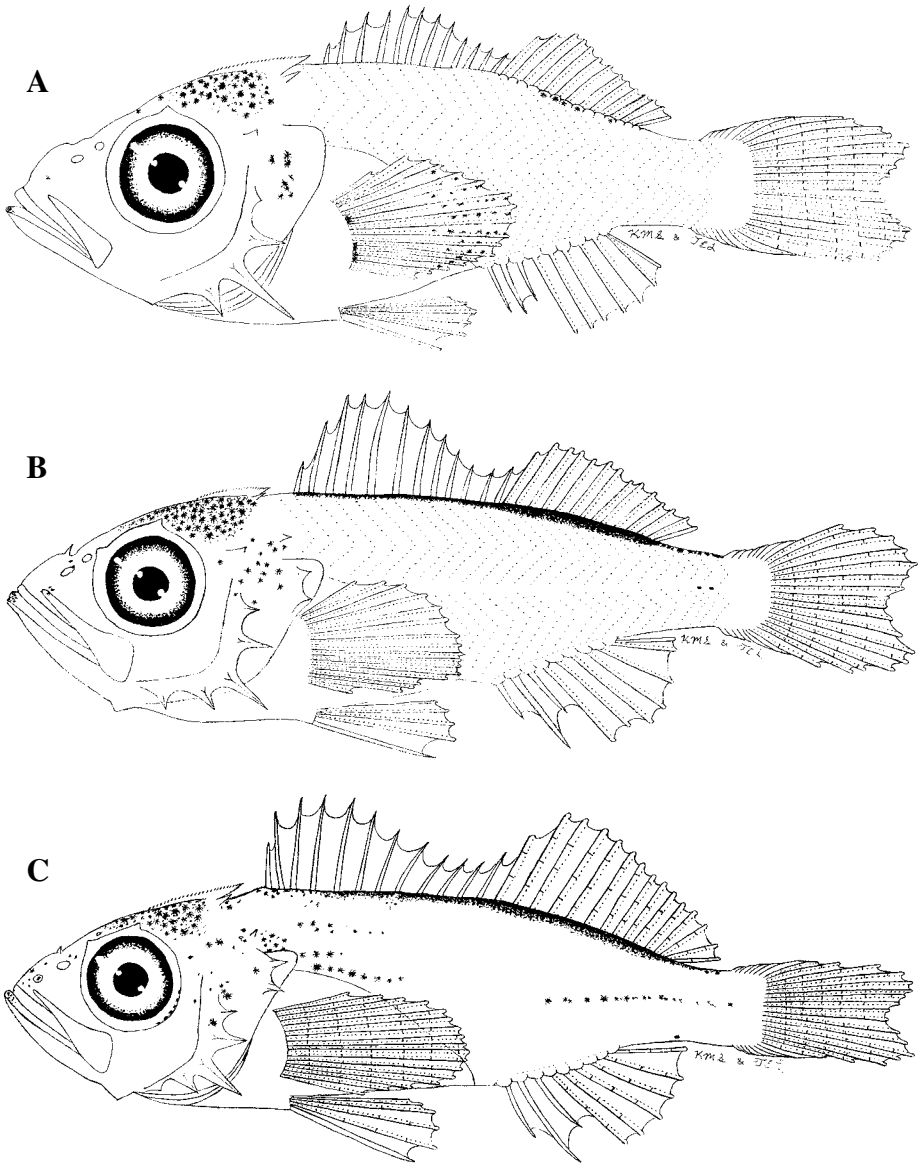

Figure 1

Developmental series of pygmy rockfish (*Sebastes wilsoni*) (drawn by authors). (**A**) 9.1-mm-SL larva; (**B**) 14.0-mm-SL larva; (**C**) 17.5-mm-SL larva; (**D**) 23.0-mm-SL pelagic juvenile; (**E**) 28.6-mm-SL pelagic juvenile. Arrow marks ventral end of anterior body bar; (**F**) 34.4-mm-SL pelagic juvenile. Note that not all head spines are included in the illustrations.

form in some specimens by 12.0 mm and was visible in most specimens by 14.0 mm (Fig. 1B). Snout pigment was represented by one or four melanophores. Anterior lower jaw pigment was heavy and confined to the tip of the jaw.

By 17.5 mm, the dorsal midline pigment had become much darker and denser (Fig. 1C, Table 4) and extended from the caudal fin to the head region, except for a gap where the nape pigment was beginning to form. All fins were unpigmented. Pigment along the ventral body midline began to form at 17.5 mm, with a few postanal melanophores. Lateral midline pigment formed in two locations. Melanophores near the peduncle increased anteriorly, and pigment began forming dorsal to the gut cavity and increased posteriorly toward the peduncle. A body bar began to form on the lateral surface above the pectoral fin between the spinous dorsal fin and the anterior lateral midline pigment. Opercular, eye, and head pigment all increased in density. Melanophores on the snout also became more prevalent between the tip of

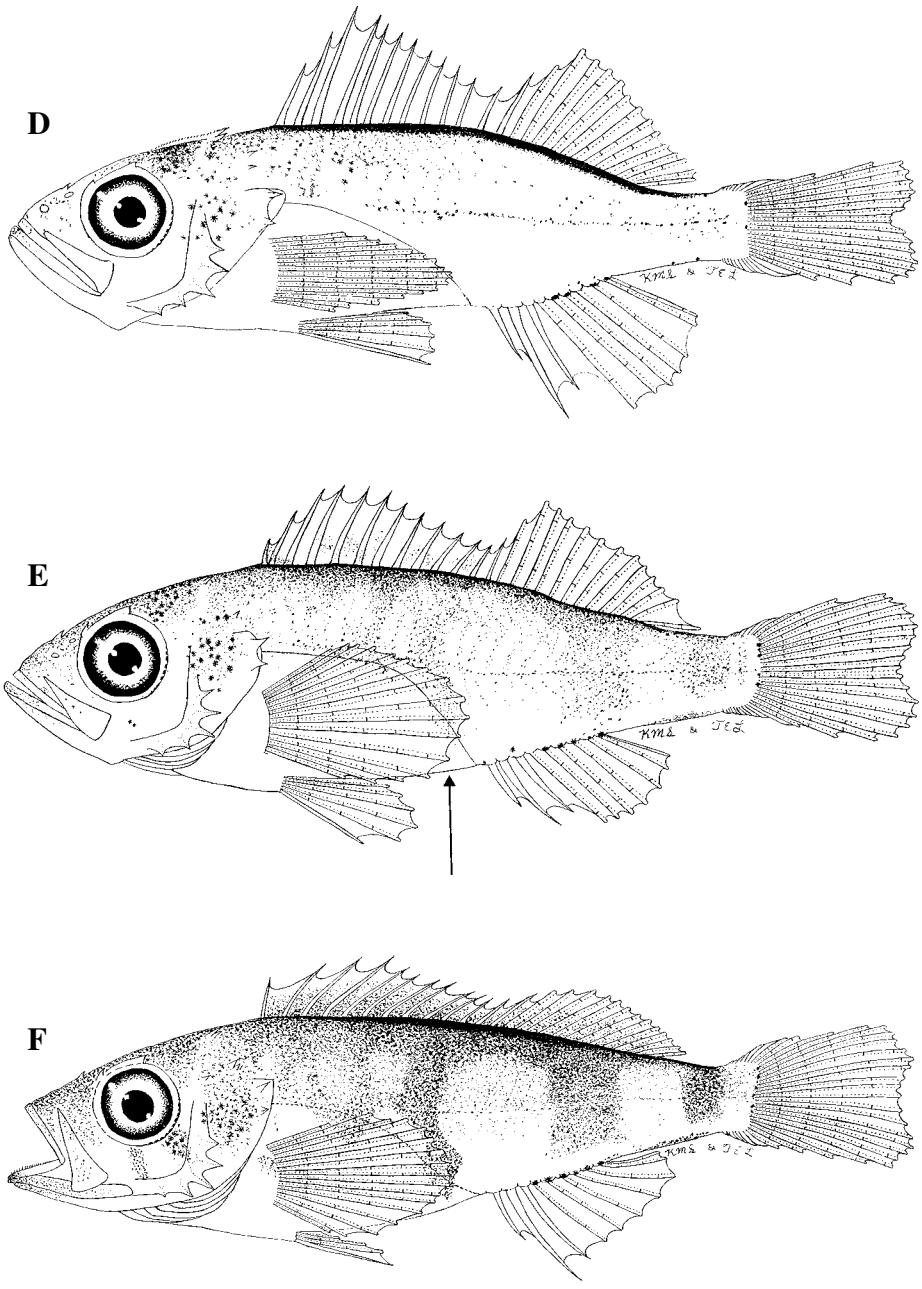

Figure 1 (continued)

the upper jaw and top of the head. Pigment on the tip of the lower jaw spread posteriorly and became denser than in smaller specimens.

When the pelagic juveniles reached 23.0 mm, the dorsal midline pigment was a dark strip extending from the caudal fin to the head (Fig. 1D, Table 4). Nape pigment almost merged with the head pigment, except for a small unpigmented area below the insertion of the parietal and nuchal spines. Hypural pigment was present distally in all individuals at this size and at larger sizes (n=30). Anterior and posterior lateral midline pigment merged to form a continuous line along the body. The number of melanophores increased on the ventral body surface, and a few melanophores were present at the anal-fin ray bases and along the ventral midbody posterior to the anal fin. The anterior body bar broadened and became more defined. A few melanophores on the flanks under the soft dorsal fin began to form a midbody bar. A third body bar began to form on the caudal peduncle. Pigment on the operculum, top of the

Table 4

Proportion of pygmy rockfish (*Sebastes wilsoni*) with melanophores present at various areas averaged over 1.0-mm size bins (range of ±1.0 mm). SL = standard length in mm. LJ = anterior tip of the lower jaw, EYE = posterior-ventral edge of the eye orbit, SNOUT = dorsal surface anterior to the eyes, OPER = operculum, CHK = radiating cheek bars, NAPE = nape pigment, DORS = dorsal body surface, VENT = ventral body surface, MID = along the lateral midline, HYP = hypural region, DFIN = spinous dorsal fin, PEC = blade of the pectoral fin, CAUD = caudal body bar, ANT = anterior body bar, MIDB = midbody bar, CANAL = clear area dorsal to anal fin, MAX = pigment on the side of the maxilla, UND = pigment under lower jaw.

SL	N	LJ	EYE	SNOUT	OPER	CHK	NAPE	DORS	VENT	MID	HYP	DFIN	PEC	CAUD	ANT	MIDB	CANAL	MAX	UND
8	2	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
9	3	1.0	0.0	1.0	1.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
10	6	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
11	10	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0
12	5	1.0	0.2	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
13	7	1.0	0.1	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
14	8	1.0	0.9	1.0	1.0	0.0	0.0	1.0	0.3	0.5	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
15	6	1.0	0.8	1.0	1.0	0.0	0.0	1.0	0.3	0.7	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
16	7	1.0	0.9	1.0	1.0	0.0	0.0	1.0	0.1	0.9	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
17	8	1.0	0.8	1.0	1.0	0.0	0.3	1.0	0.3	0.8	0.4	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
18	14	1.0	1.0	1.0	1.0	0.0	0.8	1.0	0.6	1.0	0.7	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
19	7	1.0	1.0	1.0	1.0	0.0	0.6	1.0	0.3	1.0	0.3	0.0	0.0	0.1	0.0	0.7	0.0	0.0	0.0
20	2	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.5	0.0	1.0	0.0	0.0	0.0
21	6	1.0	1.0	1.0	1.0	0.0	0.8	1.0	0.8	1.0	0.5	0.0	0.0	0.3	0.3	0.7	0.0	0.0	0.0
22	4	1.0	1.0	1.0	1.0	0.0	1.0	1.0	0.8	1.0	0.5	0.0	0.0	0.5	0.3	1.0	0.0	0.0	0.0
23	1	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
24	5	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	0.8	1.0	0.2	0.0	0.0
25	1	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
26	1	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0
27	2	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0
28	3	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0	0.7	0.7
29	6	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	0.3	1.0	1.0	1.0	1.0	0.7	1.0
30	3	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0
31	3	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	0.7	1.0
32	3	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
33	1	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
34	1	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

head, and snout also increased in density, and pigment formed posteriorly along the upper and lower jaws. Melanophores posteriorly around the eye socket increased in number. The fins remained unpigmented.

Pygmy rockfish 28.6 mm long had dorsal pigment that stretched continuously from the jaws to the caudal fin (Fig. 1E, Table 4). Pigmentation was heavy along the dorsal midline, head, and nape. Snout pigmentation also intensified. More melanophores were present on the hypural margin. Pigment along the ventral body surface darkened, especially in the area posterior to the anal fin. More melanophores were observed at the anal-fin ray articulations than on smaller specimens. The three body bars increased in width and length and were better defined than on smaller specimens. The bar on the caudal peduncle began to exhibit a rectangular shape that is characteristic of the juvenile stage. The midbody bar also took on a rectangular shape, although the dorsal half was indented. The midbody bar and the caudal peduncle bars did not reach the ventral midline. The anterior body bar extended from the spinous dorsal fin to the vent (see arrow Fig. 1E). Anteriorly, the bar formed a more or less rectangular pattern on the dorsal half of the body above the pectoral fin. In general, the lateral body surface became more heavily pigmented, especially on the dorsal half. The lateral midbody pigment line began to be incorporated into the body bars. Opercular pigment became denser and merged with the nape pigment. The area anterior to the nape and operculum was less pigmented than the surrounding areas. Pigment along the posteroventral portion of the orbit became denser than in smaller specimens. A cheek bar began to form ventral to the eye (as evidenced by the two melanophores in Fig. 1E). Melanophores formed along the ventral surface of the lower jaw and covered the lateral surface of the upper jaw. Pigment began to develop on the membranes of the spinous dorsal fin, typically with some unpigmented areas between the dorsal fin pigment and the dorsal body pigment.

The largest specimen, 34.4 mm, had the densest and most distinctive pigmentation (Fig. 1F, Table 4). Pigment was present on most of the body. Along the dorsal surface, the pigment formed a complete line from the tip of the upper jaw to the caudal fin. The number of melanophores increased along the hypural region, the postanal ventral midline, and at the anal-fin articulations. The mid- and caudal body bars were rectangular and still did not reach the ventral midline, leaving an unpigmented ventrolateral area. The anterior body bar comprised heavy pigment extending posteriorly between dorsal-fin spines VIII–XI and the vent, a lighter area just anterior to this, and another heavily pigmented area stretching from about dorsal-fin spines III–VI almost to the middle of the gut cavity. Anterior to this bar was an area of mottled pigmentation. Pigment was visible just anterior to the base of the pectoral fin. Pigment covered both the spinous and soft dorsal fins, except along the distal edge. All other fins remained unpigmented. Opercular pigment was dense and merged with the nape pigment, but these were separated from the head and eye pigment by an area of low pigment density. Two cheek bars radiated from the lower margin of the orbit. Pigment occurred along both jaws and covered the snout and ventral portion of the lower jaw.

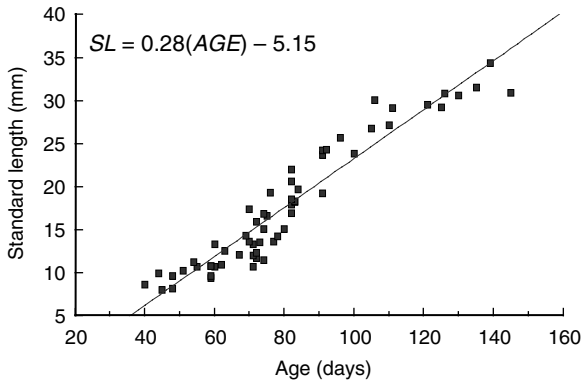

Figure 2

Standard length and age of pygmy rockfish (*Sebastes wilsoni*) (n=60). Solid line indicates predicted values from linear model.

Otolith examination

A linear relationship between standard length and age (as estimated from otolith increment counts) resulted in a good estimate of growth of pygmy rockfish (slope=0.28 mm/d; intercept=–5.15 mm; r^2=0.91; n=60; Fig. 2). The radius of the extrusion check ranged from 9.5 to 11.0 μm, averaging 10.5 μm (SD=0.29; n=60). Accessory primordia first appeared in a 19.8-mm specimen and were observed in otoliths from all larger specimens. Based on this character, transition from larval to pelagic juvenile stage occurs at around 20 mm SL.

Molecular confirmation

Interspecific levels of divergence, calculated among adult reference species, ranged from 0.13% (rougheye rockfish [*S. aleutianus*] vs. shortraker rockfish [*S. borealis*]) to 9.7% (black rockfish [*S. inermis*] vs. bocaccio) with an average of 4.1%. Two of the specimens (FT2 and FT3; Fig. 3A) were identical to one of the adult pygmy rockfish references (i.e. 0% sequence divergence) and differed from the other verified adult pygmy rockfish by a single nucleotide substitution (0.13% seq. div.). The remaining two specimens (FT1 and FT4; Fig. 3A) also were most similar to both adult pygmy rockfish references (0.13–0.40% seq. div.).

Although all four specimens were most similar to pygmy rockfish based on cytochrome *b* data, only a small number of nucleotide differences separated them from Puget Sound, redstripe, harlequin, and sharpchin rockfish (0.27–1.87%; Fig. 3A). A secondary data subset that included control region sequence (cyt-*b*+CR) yielded concordant results; all four larval specimens were

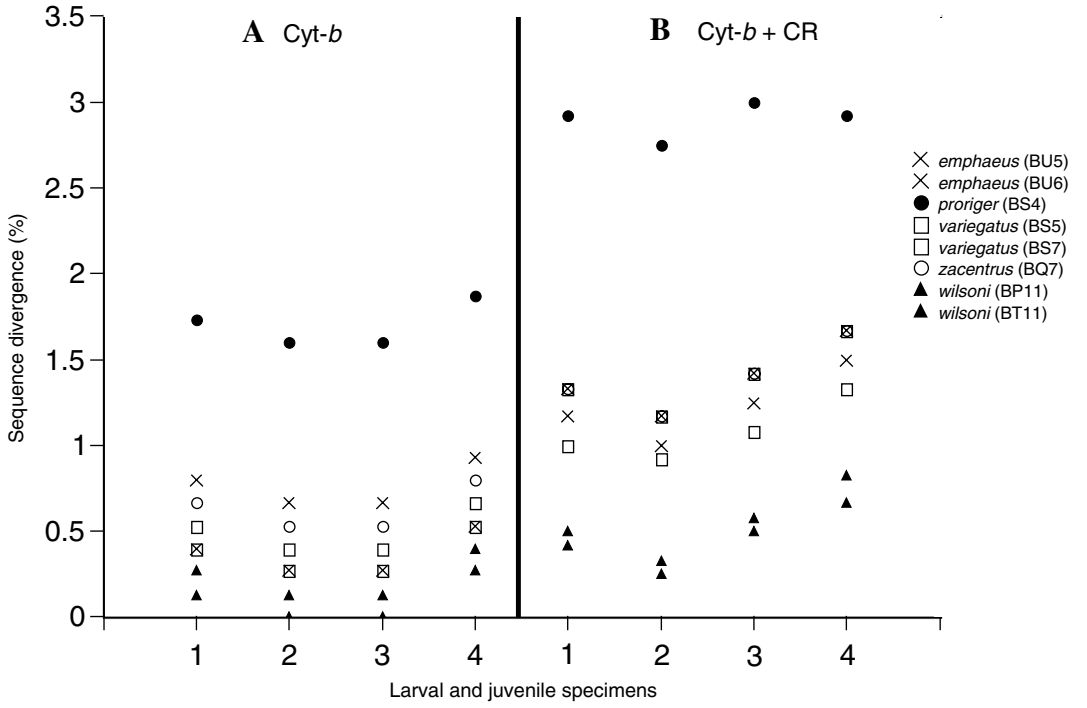

Figure 3

Percent sequence divergence based on the number of nucleotide differences in (**A**) cytochome *b* and (**B**) cytochrome *b* + control region between the four putative pygmy rockfish (*Sebastes wilsoni*) specimens (FT1–FT4) and five closely related reference species of *Sebastes*, including two adult *S. wilsoni*. Percent sequence divergence was calculated as the number of nucleotide differences over 750 base pairs (cyt-*b*) and 1200 base pairs (cyt-*b*+CR).

most similar to pygmy rockfish (0.25–0.83%; Fig. 3B). Increased levels of interspecific nucleotide variation, attributable to the faster evolving control region, resulted in more pronounced differences between the four specimens and the other species of *Sebastes* within the subset (range: 0.83–3.00%; Fig. 3B). Additionally, a distance-based analysis (UPGMA) of haplotypes (cyt-*b*+CR) clustered all four specimens with pygmy rockfish reference material.

Discussion

Postflexion larval pygmy rockfish can be identified through a combination of pigment and meristic characters. At approximately 8–10 mm, the larval pigment pattern is similar to only four of the 30 *Sebastes* species illustrated in the literature that occur within our geographic area (Matarese et al., 1989; Moser, 1996; Laroche[1]): yellowtail (*S. flavidus*), blue (*S. mystinus*), canary (*S. pinniger*), and sharpchin rockfish. Yellowtail and blue rockfish can be separated from pygmy rockfish because they exhibit ventral body and hypural pigment at this size—pigment that does not show up in pygmy rockfish until approximately 14 and 15 mm, respectively. In canary rockfish, the presence of ventral body pigment and dorsal midline pigment posterior to the soft dorsal fin (instead of at the base of the soft dorsal-fin rays as in pygmy rockfish) can help differentiate this species from pygmy rockfish. Pigmentation patterns of sharpchin rockfish are very similar to pygmy rockfish at 10 mm; however, sharpchin rockfish retain pigmented pelvic fins until 12.7 mm (Laroche and Richardson, 1981). Counts of anal-fin rays often can be used to differentiate these two species because pygmy rockfish have six rays and sharpchin have rockfish seven rays (Chen, 1986; Matarese et al., 1989; Moreland and Reilly, 1991; Laroche[1]). There is a small overlap in anal-fin ray counts (approximately 7%), and, because of this, 100% certainty of identification cannot be reached by anal-fin ray counts alone. Therefore, in order to increase confidence in identifications, a combination of pigmentation and fin-ray counts should be employed. After approximately 15 mm, a full complement of fin rays and gill rakers typically is present and can be used in combination with pigmentation patterns to differentiate pygmy rockfish from most other rockfish species. In these late-stage larvae, only three species (yellowtail, black (*S. melanops*), and blue rockfish) have a pigment pattern that could be confused with pygmy rockfish (Matarese et al., 1989; Moser, 1996; Laroche[1]),

but these patterns can be easily separated by using meristic characters.

Pelagic juvenile pygmy rockfish have a distinctive pigment pattern consisting of three body bars that can be used to discriminate this species from other *Sebastes* species. Yellowtail, halfbanded, and redstripe rockfish are the only species that have a similar three-barred pigment pattern (Matarese et al., 1989; Moser, 1996; Laroche[1]). Yellowtail rockfish can be distinguished by the lack of cheek bars and the presence of body bars that extend all the way to the ventral surface. Also, in yellowtail rockfish, the body bars form at a larger size than in pygmy rockfish. In halfbanded rockfish, the most anterior body bar is more densely pigmented than the other bars and typically forms a diamond shape. The caudal body bar is much wider and covers the entire peduncle. Redstripe rockfish are the most similar and are difficult to separate from pygmy rockfish by using pigmentation alone. However, these two species can be separated with greater than 90% certainty by using meristic counts. Pygmy rockfish have a mean anal-fin ray count of 6 (95% from the present study, and 93% from Laroche[1]), whereas redstripe rockfish have an average of 7 anal-fin rays (100% from Chen, 1986; 97% from Laroche[1]).

It should be noted that the only illustration of pygmy rockfish prior to our study was a 35.0-mm pelagic juvenile by Laroche,[1] which showed several pigment differences from our specimens of equivalent size. Laroche's illustrated specimen had only faint body barring, no cheek bars, and no ventral pigment, whereas all our specimens had prominent body barring, at least one cheek bar, and ventral pigment along the anal-fin articulations. At this time we cannot determine whether these differences were due to geographic variability in pigment patterns (Laroche's specimen probably was collected farther north than all of our specimens), or a misidentification of the original specimen illustrated by Laroche.[1]

The identification of larval and pelagic juvenile pygmy rockfish used in our study was confirmed by using DNA sequence analyses. Previous molecular identifications and subsequent descriptions of juvenile starry rockfish (*S. constellatus*) and swordspine rockfish (*S. ensifer*) also were based on mitochondrial cytochrome *b* data (Rocha-Olivares et al., 2000). In our study, orthologous cytochrome *b* sequence was sufficient for identification purposes, particularly for those specimens exhibiting exact haplotype matches to reference adult pygmy rockfish (e.g., FT2/FT3: 0.0% sequence divergence). Relatively low levels of interspecific genetic variation occurred between larval specimens and several reference species (pygmy, sharpchin, harlequin, and Puget Sound rockfish, and, to a lesser extent, redstripe rockfish). Rocha-Olivares et al. (1999a) used control region sequence, in addition to cytochrome *b*, to resolve phylogenetic relationships among recently diverged species of the *Sebastes* subgenus *Sebastomus*. In the present study, the control region sequence was used to increase divergence levels between species and to aid in insuring correct molecular identifications of specimens FT1 and FT4. Species assignment to pygmy rockfish was supported by the smallest divergence (based on cyt-*b* and cyt-*b*+CR) from reference pygmy rockfish compared with the other *Sebastes* species.

Larval and juvenile pygmy rockfish can also be separated from other *Sebastes* species by comparing the radius of the extrusion check on their otoliths. Of the fourteen other *Sebastes* species or species complexes with measured otolith extrusion check radii (Laidig and Ralston, 1995; Laidig et al., 1996; Laidig and Sakuma, 1998), only four species and two complexes have radii close to the average extrusion check radii for pygmy rockfish (10.5 μm, SD=0.3). Stripetail rockfish (*S. saxicola*) had an average extrusion check radius (11.6 μm, SD=0.5) that was larger than the largest radius for pygmy rockfish (11.0 μm). Quillback rockfish (*S. maliger*) had an average extrusion check radius of 9.1 μm (SD=0.1), which was smaller than the smallest radius for pygmy rockfish (9.5 μm). Species with extrusion check radii similar to pygmy rockfish were kelp rockfish (*S. atrovirens*) at 10.6 μm (SD=0.2), blue rockfish at 10.9 μm (SD=1.1), and the copper rockfish (*S. caurinus*, extrusion check radius=10.5 μm; SD=0.4) and gopher rockfish (*S. carnatus*, extrusion check radius=10.6 μm; SD=0.3) complexes (see Laidig et al., 1996, for complex definitions). Of these species, the only one that would be confused with pygmy rockfish, by pigmentation alone, would be blue rockfish at small sizes. However, pygmy rockfish and blue rockfish are easily separated by using meristic characters.

Growth rates of larval rockfish generally are slow during the first month of life and increase thereafter (Laidig et al., 1991; Sakuma and Laidig, 1995; Laidig et al., 1996). Because the youngest fish in our study was estimated to be 40 days old, our linear model can not be used to estimate early larval growth rates. For pygmy rockfish older than 40 days, the growth rate of 0.28 mm/day was somewhat slower than that observed for other *Sebastes*. Woodbury and Ralston (1991) found that, for fish older than 40 days, growth rates varied from 0.30 for widow rockfish (*S. entomelas*) to 0.97 mm/day for bocaccio. Other species exhibiting slightly faster growth rates after 40 days of age include stripetail rockfish (0.37 mm/day; Laidig et al., 1996), grass rockfish (*S. rastrelliger*; 0.36 mm/day; Laidig and Sakuma, 1998), and shortbelly rockfish (*S. jordani*; 0.53 mm/day; Laidig et al., 1991). Yellowtail rockfish had a more similar growth rate, ranging from 0.19 to 0.46 mm/day (Woodbury and Ralston, 1991). These differences in growth may reflect genetic variability or responses to environmental variables. Woodbury and Ralston (1991) suggested that annual variability in growth rates of juvenile rockfish was related to year-to-year changes in environmental conditions, especially temperature. Boehlert (1981) determined that temperature greatly affected growth rate of young splitnose rockfish (*S. diploproa*) in the laboratory. Boehlert and Yoklavich (1983) observed slower growth rates for black rockfish in colder temperatures. Lenarz et al. (1991) analyzed the vertical

distribution of late larval and pelagic juvenile rockfish and determined that pygmy rockfish were present on average in deeper, colder water than that favored by other rockfish species. This spatial separation of pelagic juvenile pygmy rockfish and other *Sebastes* spp. may explain the slower growth observed in pygmy rockfish.

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