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Abstract—Pelagic juvenile rockfish (*Sebastes* spp.) collected in surveys designed to assess juvenile salmonids and other species in the Gulf of Alaska in 1998 and 2000–2003 provide an opportunity to document the occurrence of the pelagic juveniles of several species of rockfish. Often, species identification of rockfish is difficult or impossible at this stage of development (~20 to 60 mm), and few species indigenous to Alaska waters have been described. Use of mitochondrial DNA markers for rockfish species allowed unequivocal identification of ten species (*S. aleutianus*, *S. alutus*, *S. borealis*, *S. entomelas*, *S. flavidus*, *S. melanops*, *S. pinniger*, *S. proriger*, *S. reedi*, and *S. ruberrimus*) in subsamples from the collections. Other specimens were genetically assignable to groups of two or three species. *Sebastes borealis*, *S. crameri*, and *S. reedi* were identified using morphological data. Combining genetic and morphological data allowed successful resolution of the other species as *S. emphaeus*, probably *S. ciliatus* (although *S. polyspinis* cannot be totally ruled out), and *S. polyspinis*. Many specimens were initially morphologically indistinguishable from *S. alutus*, and several morphological groups included fish genetically identified as *S. alutus*. This paper details the characteristics of these pelagic juveniles to facilitate morphological identification of these species in future collections.

Genetic and morphological identification of pelagic juvenile rockfish collected from the Gulf of Alaska

Arthur W. Kendall Jr.[1], Christine Kondzela[2], Zhuozhuo Li[3], David Clausen[2], and Anthony J. Gharrett[3]

[1] AJALA Enterprises
635 Wanapum Drive
LaConner, WA 98257
Email: Art.Kendall@noaa.gov

[2] Auke Bay Laboratories
Alaska Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
17109 Point Lena Loop Road
Juneau, AK 99801

[3] School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
11120 Glacier Highway
Juneau, AK 99801

Introduction

Rockfishes (*Sebastes* spp.) form an ecologically and economically important group of fishes in the Northeast Pacific (Love et al., 2002). They are quite diverse taxonomically with about 70 species occurring in the region and up to 30 in one location, and also ecologically diverse with, for example, some species occurring in tidepools and others at depths >1000 m. As adults, most species are demersal and associate with hard bottoms, but some are semi-pelagic. All species are viviparous, with individuals of some species yearly bearing up to 10^5 yolk-sac planktonic larvae. Some species are among the longest living fishes, reaching ages of over 100 years. Severe overfishing has reduced some species to less than 10% of their virgin biomass, and fisheries management efforts include restricting or closing fishing and creating marine reserves to rebuild populations. Assessing the size of the populations at various times in their life histories is required to evaluate the effectiveness of fisheries management efforts. Such assessments and other fisheries research on these fishes are hampered by the inability to identify their planktonic larval and pelagic juvenile stages in field collections. Adult morphological characters are not fully developed in pelagic juveniles; however, genetic characters, which persist throughout life history stages, and distinct pigment and other morphological features unique to the juvenile life history stage can be applied to species identification of juveniles.

After a larval stage of a few weeks, many rockfish species transform into pelagic juveniles, and some species remain in the water column for several months, while others settle to the bottom or to kelp within a few weeks (Moser and Boehlert, 1991). The pelagic juvenile stage, which may last for over six months (Boehlert, 1977), is ecologically and morphologically distinct from the generally demersal adult phase, and may be important in determining year-class strength.

Sampling for pelagic juvenile rockfish off central California provides an index of year-class strength for several species (Ralston and Howard, 1995; Ralston and Ianelli, 1998; Sakuma et al., 2006). Also, relationships between ocean conditions and the abundance and growth of juvenile rockfish have been documented through these surveys (Woodbury and Ralston, 1991). Such studies are predicated

on the ability to identify the rockfish juveniles that are collected in these surveys. Although morphological identification criteria for juveniles have been developed for many rockfish species that occur off California (Laidig and Adams, 1991), additional research is needed to identify species occurring off Alaska, due to latitudinal differences in species composition.

At present, little is known about the ecology of pelagic juvenile rockfish off Alaska. Most species have not even been described at this stage of development (see Kendall, 1991), and it is presently difficult or impossible to identify the larvae and juveniles of many species of rockfish from their morphology (Gray et al., 2006). Consequently, it is not possible to trace their early lives, to investigate their ecology, or to use them for population assessment.

Use of genetic information has proven valuable in taxonomic and systematic studies of adult rockfishes (see Seeb, 1986; Rocha-Olivares et al., 1999; Gharrett et al., 2001; Li et al., 2006a). Genetic techniques have also been applied successfully to identify field caught larval and juvenile stages of rockfishes from various areas in the Northeast Pacific (see Seeb and Kendall, 1991; Rocha-Olivares et al., 2000; LeClair and Buckley, 2001; Li et al., 2006b; Gray et al., 2006). In addition, Laidig et al. (2004) used genetic techniques to confirm the morphologically based identifications of *S. wilsoni* collected off California.

Recently, genetic methods have been developed to identify rockfish, including larvae and juveniles, using mitochondrial DNA (mtDNA) markers (Gharrett et al., 2001; Li et al., 2006c). With these markers most of the species found in the Gulf of Alaska (GOA) and along the North American Pacific coast can be identified. The few species that cannot be identified unequivocally form small groupings of two or three species.

It would be convenient to be able to identify juvenile rockfish visually in the field off Alaska. The challenge is that to use morphological characteristics to distinguish species, it is first necessary to know what the species are. The use of mtDNA markers can assist in evaluating morphological characteristics for species identification by determining the species independent of visual examination.

The primary objective of the present study was to use genetic analysis to establish the specific identity of field-collected pelagic juvenile rockfishes from the GOA, and then to document the morphological characteristics of these species. A secondary objective was to see if specimens could be sampled for genetic analysis without damaging important morphological characteristics before fixing them in formalin. Initial morphological identifications, based on adult meristic characters and published descriptions of pelagic juveniles, were confirmed or modified based on genetic analysis of the same specimens. Such analyses are needed to develop diagnostic morphological criteria to distinguish the species of field-collected pelagic juveniles of *Sebastes* spp.

Methods

Samples

The first batch of specimens (n = 23) to be examined was collected by the International Pacific Halibut Commission (and supplied to Alaska Department of Fish and Game) on 25 and 27 September 2002 in Shelikof Bay, off the coast of Southeast Alaska (Fig. 1; Table 1). The samples were collected during daylight in a small otter trawl (8 m headrope) towed on the bottom at 16.5–29.3 m water depth. It is assumed that the pelagic juvenile rockfish from these collections (except an 82 mm standard length (SL) specimen: see later) were caught as the net passed through the water column during setting and retrieval of the bottom trawl. The fish were photographed fresh, frozen, and shipped to the National Oceanic Atmospheric Administration (NOAA), National Marine Fisheries Service (NMFS), Alaska Fisheries Science Center (AFSC) in Seattle where they were thawed, photographed again, and a small fillet was removed for genetic analysis from the right side of the fish, taking care not to damage the head, left

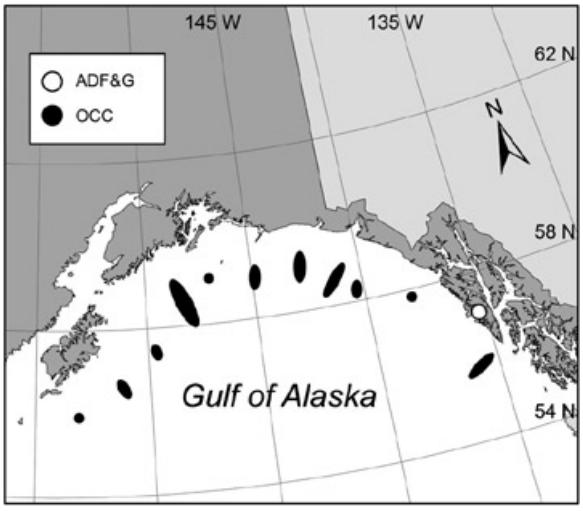

Figure 1

Sites in Alaska where pelagic juvenile *Sebastes* spp. analyzed in this paper were collected in 1998–2003. ADF&G: Alaska Department of Fish and Game, OCC: Ocean Carrying Capacity. Symbols indicate approximate geographic sites of collections.

Table 1

Numbers, length ranges, and sources of illustrations of pelagic juvenile *Sebastes* from the Gulf of Alaska.

		Sources of specimens*									
		Southeast Alaska		OCC-1		OCC-2		OCC-3			
Species	Common name	Number	Size range (mm SL)	Number	Size range (mm SL)	Number	Size range (mm SL)	Number	Size range (mm SL)	Totals	Sources of illustrations (standard length, mm: source)
S. aleutianus	Rougheye rockfish			1	29.5	4	16.0–27.4	4	19.3–29.2	9	19.3, 57.0: Matarese et al., 1989
S. alutus	Pacific ocean perch			40	27.0–49.5	86	13.8–54.7	77	19.7–48.8	203	57.0: Matarese et al., 1989
S. borealis	Shortraker rockfish			2	28.0–31.0	4	23.0–29.0	9	18.5–31.0	15	53.0: Laroche**
S. ciliatus	Dusky rockfish	3	32.0–35.4	2	29.5–29.6	2	22.6–27.8	17	21.6–35.4	24	44.0: Laroche**
S. crameri	Darkblotched rockfish			4	32.0–43.5	2	37.0–47.7	7	34.5–46.6	13	31.8: Richardson and Laroche, 1979, 39.0: Matarese et al., 1989
S. emphaeus	Puget Sound rockfish	14	43.0–54.5							14	16.4, 30.0: Matarese et al., 1989
S. entomelas	Widow rockfish			3	57.0–59.0	2	40.5–55.0	6	57.5–66.0	11	40.1: Laroche and Richardson, 1981, 48.0: Matarese et al., 1989
S. flavidus	Yellowtail rockfish							1	45.1	1	42.0: Matarese et al., 1989, 33.0, 46.8: Laroche and Richardson, 1980
S. melanops	Black rockfish							4	40.5–45.0	4	45.3: Laroche and Richardson, 1980
S. pinniger	Canary rockfish	6	25.8–31.4, 82.0***							6	20.0, 29.4: Richardson and Laroche, 1979, 40.0: Matarese et al., 1989
S. polyspinis	Northern rockfish					1	31.8			1	31.0: Matarese et al., 1989
S. proriger	Redstripe rockfish					1	28.7	1	24.7	2	16.8, 38.0: Matarese et al., 1989
S. reedi	Yellowmouth rockfish			3	38.6–40.4	10	31.1–43.6	13	23.1–40.8	26	49.0: Matarese et al., 1989
S. ruberrimus	Yelloweye rockfish							1	22.5	1	28.0: Matarese et al., 1989, 68.0: Laroche**
Totals		23		55		112		140		330	

* See Figure 1 for locations of collections, and text for details of collection methods.

** Text footnote 2.

*** Probably a demersal juvenile.

side, or fins. Fillets were placed in a DNA preservative solution (Seutin et al., 1991). The fish were then fixed in 10% formalin for about a week and then preserved in 70% ethanol.

The second (OCC-1, $n = 55$), third (OCC-2, $n = 112$), and fourth (OCC-3, $n = 140$) batches of juvenile rockfish were collected in the GOA in July and August by NOAA personnel during the 1998 and 2000–2003 Auke Bay Laboratory OCC/Global Ocean Ecosystem Dynamic (GLOBEC) salmonid research surveys onboard the contracted fishing vessel *Great Pacific*, a 38 m stern ramp trawler (Fig. 1; Table 1; see Farley et al.[1] for details). Samples were collected using a midwater rope trawl towed at or near the surface during daylight. At the time of collection, rockfish from each tow were frozen as a group at –70°C. The OCC-1 batch of fish was partially thawed to facilitate the removal of individuals from the masses of frozen fish. Several hauls were sampled from each year (1998 and 2000–2002). The fish were briefly examined to note obvious qualitative differences in morphology. As with the first batch of fish, a small fillet was removed and preserved from each specimen. The sampled fish were then refrozen, and later (at the AFSC in Seattle) were thawed, fixed in 10% formalin for a week, and then preserved in 70% ethanol for morphological examination. The OCC-2 and -3 batches were partially thawed, and as with the other batches of fish, a small fillet of tissue was removed from each specimen and preserved. Each specimen was then photographed, fixed in 10% formalin for about a week, and then preserved in 70% ethanol for morphological examination. A report on preliminary identifications of specimens from the OCC-1 batch has been generated (Kondzela et al., 2007).

Genetic analysis

Total cellular DNA was isolated using DNeasy® Tissue Kits (Qiagen, Valencia, CA). The target region, which included genes for the NADH dehydrogenase-3 and -4 subunits (ND3/ND4), was PCR-amplified from total genomic DNA using primers and methods described in Gharrett et al. (2001). Subsamples of the PCR-amplified mtDNA regions were subjected to restriction endonuclease digestion using conditions recommended by the manufacturers in order to detect species-specific restriction site fragments. Restriction fragments were separated by electrophoresis through 1.5% agarose (a mixture composed of one part Ultra Pure™ agarose [BRL Gibco, Grand, NY] and two parts Synergel™ [Diversified Biotech Inc., Boston, MA]) in 0.5×TBE buffer (TBE is 90mM Tris-boric acid, and 2mM EDTA, pH 8.3). The DNA in the gel was stained with ethidium bromide and digitally photographed on an ultraviolet light transilluminator. One kilobase and 100-base pair ladders were used as references to estimate restriction fragment sizes. Fragment sizes were estimated from digital images using ProRFLP 2.38 (DNA ProScan Inc., Nashville, TN). The restriction sites were positioned using the restriction site maps previously constructed for 71 *Sebastes* spp. (Gharrett et al., 2001; Li et al., 2006c). New haplotypes were analyzed by electrophoresis through 12% polyacrylamide (29:1 acrylamide:bisacrylamide) in 1×TBE and stained with SYBR Green 1 Nucleic Acid Stain™ (Molecular Probes, Eugene, OR) to accurately estimate the sizes of small (about 25 base pairs) restriction fragments. Species identifications were confirmed by including adult reference samples on gels; previous study has shown that intraspecific variation does not obscure species identification (Li et al., 2006c). Results of the genetic analysis of each batch of juvenile rockfish were not communicated until a thorough preliminary morphological analysis had been conducted.

Morphological descriptions

Morphology of the pelagic juveniles was examined to evaluate the utility of previously reported species-determining characters, to describe this life history stage of species for which the juvenile stage was previously unknown, and to look for useful identifying characters. For each batch of fish, initial morphological examination consisted of grouping the fish based on pigmentation and body shape. Later observations made on each specimen included standard length and body depth (measured at the deepest point, usually at the insertion of the pelvic fins); details of pigment patterns; head spine presence (head spine names are as in Matarese et al., 1989), appearance, and strength; and meristics of medial fins, pectoral fins, and vertebrae. Based on these observations and comparisons with published descriptions, a "best guess" was made for the identity of each specimen, without knowing the results of the genetic analysis. Subsequently, information from the genetic analysis was compared with the results from the morphological study, and identifications that did not concur with the genetic analysis were re-examined. The four batches of fish were examined sequentially and the results of each earlier batch informed the morphological analysis of subsequent batches.

The first batch of OCC fish was X-rayed at the University of Washington Fish Collection using a standard soft

[1] Farley, E. V., Jr., B. L. Wing, E. D. Cokelet, C. M. Kondzela, E. C. Martinson, N. Weemes, J. H. Moss, M. Auburn-Cook, and C. Fitch. 2001. Gulf of Alaska coastal research (July and August 2001) on juvenile salmon. North Pacific Anadromous Fish Commission Doc. 559. Auke Bay Laboratory, Alaska Fisheries Science Center, NMFS, NOAA, 11305 Glacier Highway, Juneau, AK 99801-8626. 19 p.

X-ray machine. It was not possible to count fin rays accurately on these X-rays, so the fish were X-rayed again using a digital X-ray machine at the AFSC in Seattle. The AFSC digital X-ray machine was used exclusively with the remaining batches of fish. Although vertebrae were countable, it was still difficult to count the fin rays on some of the X-rays, so representatives of each morphological group were lightly stained with Alizarin red to make the fin elements and head spines easier to see. Based on their appearance, it is possible that some of the fins had not reached their adult complements of rays in some of the smaller specimens (<20 mm SL) examined here.

Meristics for *Sebastes* spp. differ quantitatively, so proportional values of meristics (vertebrae, dorsal spines, and dorsal-, anal-, and pectoral-fin rays) of each *Sebastes* spp. occurring in the GOA (Orr et al., 2000) were generated based on adult data in Hubbs and Shultz (1933), Ishida (1984), and Chen (1986), and tabulated along with values observed in the juveniles identified by genetics and morphology in the present study (Table 2). To facilitate comparisons of the adults and juveniles, the maximum proportional value for each of the five meristic characters of the adults (from the literature) and juveniles (from the present study) of each species were summed (Table 2).

The sum of the maximal proportional values for adults of *Sebastes* spp. found in the GOA were compared with the maximal proportional values for the juveniles found in the present study (Table 3). For example, maximal proportional values for juvenile *S. alutus* found in this study were 13 dorsal spines (p = 0.98), 15 dorsal rays (p = 0.64), 8 anal rays (p = 0.84), 18 pectoral rays (p = 0.84), and 27 vertebrae (p = 0.95), for a total maximal proportional value of 4.25. The sum of the proportions of these meristic values for adult *S. aleutianus* is 2.98, while it is 4.31 for *S. alutus*, indicating that the meristics observed in the juveniles fit *S. alutus* more closely than *S. aleutianus*. This analysis of meristics helped establish the preliminary identity of juveniles based on morphology, before results of the genetic analysis had been communicated. Once the genetic results were made known, some changes in identification were possible.

Results

The removal of fillets from the right side of the fish for genetic analysis did not prevent thorough morphological analyses. Both spinous and soft fin rays could be counted in the dorsal and anal fins, and the soft fin rays were counted in the pectoral fins (Tables 2 and 3). Counts of abdominal and caudal vertebrae were made from X-rays, but much of the published comparative data includes only total vertebral counts (Table 2). At this stage of development, it is likely that the fin ray and vertebral numbers closely reflect the numbers observed in adults, but head spines may be obscured with further development in some species (Moser, 1996). In smaller specimens (<20 mm SL) the last dorsal and anal spines might not have yet transformed from soft rays. Meristics are of limited value in distinguishing the many *Sebastes* spp. in the northeast Pacific since they generally vary quantitatively and there is considerable overlap among species.

After this study was begun, two of the species of *Sebastes* occurring in the GOA were split into two additional species: *S. ciliatus* (dusky rockfish) into *S. ciliatus* (dark rockfish) and *S. variabilis* (dusky rockfish) based on morphological differences (Orr and Blackburn, 2004), and *S. aleutianus* (rougheye rockfish) into Type I and Type II based on genetic differences (Gharrett et al., 2005). The adult morphological characters used to separate *S. ciliatus* and *S. variabilis* were not useful with the juveniles in the present study. Thus, the name *S. ciliatus* is used in this paper realizing that specimens of *S. variabilis* may be included. *S. aleutianus* Type I and Type II were distinguished genetically, but not morphologically.

A total of 14 species of juvenile rockfish were identified using genetic and morphological analyses (Tables 1, 3, and 4): *S. aleutianus, S. alutus* (Pacific ocean perch), *S. borealis* (shortraker rockfish), *S. ciliatus, S. crameri* (dark-blotched rockfish), *S. emphaeus* (Puget Sound rockfish), *S. entomelas* (widow rockfish), *S. flavidus* (yellowtail rockfish), *S. melanops* (black rockfish), *S. pinniger* (canary rockfish), *S. polyspinis* (northern rockfish), *S. proriger* (redstripe rockfish), *S. reedi* (yellowmouth rockfish), and *S. ruberrimus* (yelloweye rockfish). *Sebastes alutus* was the predominant species in the GOA samples.

Comparison of the initial genetic and morphological analyses resulted in agreement of species identification on most specimens. Re-evaluation of the results led to a change in some morphological species determinations, removed some of the species identification ambiguity from the genetic results, and pointed out more variation in morphology of juvenile *S. alutus* than expected. Genetic analysis unequivocally detected 10 species (*S. aleutianus* [both Type I and Type II], *S. alutus, S. borealis, S. entomelas, S. flavidus, S. melanops, S. pinniger, S. proriger, S. reedi,* and *S. ruberrimus*) and reduced the remaining possibilities to one group that includes *S. crameri, S. ciliatus*, and *S. polyspinis*, and a second group that includes *S. emphaeus, S. variegatus,* and *S. wilsoni.* Morphologically, *S. aleutianus, S. ciliatus, S. entomelas, S. polyspinis,* and *S. proriger* were readily confused with *S. alutus,* which varied considerably, especially in the uniformity of body pigment. The mtDNA markers for *S. alutus* are quite distinctive; therefore, the variability in

Table 2

Comparisons of known meristics of *Sebastes* species from the Gulf of Alaska (based on data in Hubbs and Schultz, 1933; Ishida, 1984; and Chen, 1986) and pelagic juveniles in this study. All have three anal spines as adults.

	Fin rays																	
	Dorsal spines				Dorsal rays							Anal rays						
Species	12	13	14	15	11	12	13	14	15	16	17	5	6	7	8	9	10	
Pelagic juveniles found in this study																		
Sebastes aleutianus		9					2	2	2					6	2			
Sebastes alutus		179	4				2	26	112	30	2			10	151	17		
Sebastes borealis		15				2	9	2	1				3	8	2			
Sebastes ciliatus	1	22	1				1	5	18					4	15	4		
Sebastes crameri		13					2	6						13				
Sebastes emphaeus		14						12	1					14				
Sebastes entomelas		11						3	7	1				1	9	1		
Sebastes flavidus		1							1						1			
Sebastes melanops		4						1	3					1	3			
Sebastes pinniger		6						6						6				
Sebastes polyspinis			1					1							1			
Sebastes proriger		2						1	1					2				
Sebastes reedi		26					2	15	5					20	4			
Sebastes ruberrimus		1							1						1			
Species occurring in the Gulf of Alaska																		
Sebastes aleutianus		85	3			1	22	59	5					80	7			
Sebastes alutus		74	4				1	10	56	11				10	65	3		
Sebastes auriculatus		19				2	16	2					2	17				
Sebastes babcocki		29					4	20	5				3	24	2			
Sebastes borealis		33			1	14	105	49	5				9	140	26			
Sebastes brevispinis		9					2	5	3					10				
Sebastes caurinus		67			1	14	50	2				3	58	6				
Sebastes ciliatus		24						2	18	3			1		23	1		
Sebastes crameri	1	20					11	9	1					21				
Sebastes diploproa		62			1	31	28	2				1	11	50				
Sebastes elongatus		47				9	35	3				1	44	1				
Sebastes emphaeus		24					1	21	2				3	21				
Sebastes entomelas		19						1	16	2				1	18	1		
Sebastes flavidus	1	16						16	18					3	30			
Sebastes helvomaculatus	1	71	1			9	69	4					81	3				
Sebastes maliger		22				4	15	3					2	20				
Sebastes melanops		12	1					34	77	10				14	105	2		
Sebastes miniatus		17					4	12	1				2	15				
Sebastes mystinus		21							4	16	2				2	19	1	
Sebastes nebulosus		16				1	14	1					1	15				
Sebastes nigrocinctus	1	8				1	1	6	1				1	8				
Sebastes paucispinis		41	1	1			5	33	5						2	38	1	
Sebastes pinniger		17					1	14	3					18				
Sebastes polyspinis			18				5	20	33	1				12	25	22		
Sebastes proriger		22						9	13					22				
Sebastes rastrelliger		20				2	18						20					
Sebastes reedi							1	85	15					98	4			
Sebastes ruberrimus		7						1	5	1				7				
Sebastes saxicola		23			1	21	1						1	22				
Sebastes variegatus		9						5	4				1	18				
Sebastes wilsoni		25	1				6	17	1			1	23					
Sebastes zacentrus		25					2	22	1					23	2			

continued

Table 2 (Continued)

Comparisons of known meristics of *Sebastes* species from the Gulf of Alaska (based on data in Hubbs and Schultz, 1933; Ishida, 1984; and Chen, 1986) and pelagic juveniles in this study. All have three anal spines as adults.

	Fin rays							Vertebrae									
	Total Pectoral rays							Abdominal			Caudal			Total			
Species	14	15	16	17	18	19	20	10	11	12	15	16	17	25	26	27	28
Pelagic juveniles found in this study																	
Sebastes aleutianus				2	6				9			9				9	
Sebastes alutus		1		22	161	9			195	4	1	192	6		1	188	10
Sebastes borealis			1	3	7	4			15		1	9			1	11	
Sebastes ciliatus				5	17	1			17	5		19	4			13	11
Sebastes crameri				1	6	5		1	12		12	1			13		
Sebastes emphaeus			4	10				14				12	2			12	2
Sebastes entomelas					11				10	1	11				10	1	
Sebastes flavidus					1				1		1				1		
Sebastes melanops					2	2			4		4				4		
Sebastes pinniger			1	5				6			5	1			5	1	
Sebastes polyspinis					1					1		1					1
Sebastes proriger				1	1				2			2				2	
Sebastes reedi				1	6	19			26		26				26		
Sebastes ruberrimus						1			1		1				1		
Species occurring in the Gulf of Alaska																	
Sebastes aleutianus				8	95	5			24			24				28	
Sebastes alutus				7	76	8			28			27	1			40	1
Sebastes auriculatus			1	2	32	3									10	1	
Sebastes babcocki				2	8	47	1								10		
Sebastes borealis				3	34	88	3		7			7				75	1
Sebastes brevispinis				7	12										1		
Sebastes caurinus			1	113	24									1	7		
Sebastes ciliatus				2	44	4											5
Sebastes crameri					1	32	8								6		
Sebastes diploproa				22	103										49		
Sebastes elongatus			8	84	1										16		
Sebastes emphaeus			1	46	1											15	1
Sebastes entomelas					39	1									3	2	
Sebastes flavidus				3	62	2									6		
Sebastes helvomaculatus		4	135	29											5		
Sebastes maliger			1	33	10										1		
Sebastes melanops					20	148	2								4		
Sebastes miniatus				12	22										8		
Sebastes mystinus				1	39	2									17	1	
Sebastes nebulosus				2	30										7		
Sebastes nigrocinctus					3	15									5		
Sebastes paucispinis	4	76	5												5		
Sebastes pinniger			1	33	2										5		
Sebastes polyspinis				25	90	2											8
Sebastes proriger			2	42												10	
Sebastes rastrelliger					1	38	1								9		
Sebastes reedi					42	156	6								2		
Sebastes ruberrimus					1	13									1		
Sebastes saxicola			37	5	3										22		
Sebastes variegatus				4	70	1										4	
Sebastes wilsoni			1	44	3											17	1
Sebastes zacentrus			4	43	2											20	

Table 3

Comparisons of meristics (summed maximal proportions of five characters: dorsal spines, dorsal rays, anal rays, pectoral rays and total vertebrae) of adults of *Sebastes* spp. from the Gulf of Alaska (GOA) and of pelagic juveniles of species found in the present study.

		Juveniles of species found in the present study													
Species occurring in the GOA	Sum of maximal proportions for adults	*S. aleutianus*	*S. alutus*	*S. borealis*	*S. ciliatus*	*S. crameri*	*S. emphaeus*	*S. entomelas*	*S. flavidus*	*S. melanops*	*S. pinniger*	*S. polyspinis*	*S. proriger*	*S. reedi*	*S. ruberrimus*
S. aleutianus	4.44	3.82	2.98	4.02	2.98	3.44	3.64	1.98	1.98	1.15	2.64	1.67	3.02	2.61	1.15
S. alutus	4.31	3.61	4.31	2.90	4.31	2.04	2.26	3.34	3.34	2.59	1.28	1.85	2.85	1.29	2.59
S. auriculatus	4.45	2.83	1.93	3.63	1.93	3.75	2.14	2.75	2.75	1.99	2.96	0.94	2.04	2.98	1.99
S. babcocki	4.33	2.14	1.38	2.10	1.38	3.66	2.55	2.38	2.38	3.05	3.55	0.90	2.03	4.33	3.05
S. borealis	4.08	3.08	2.43	3.66	2.43	2.35	3.09	1.44	1.44	1.86	2.11	0.70	2.84	2.77	1.86
S. brevispinis	4.13	2.93	1.93	2.83	1.93	4.13	2.87	2.93	2.93	2.30	3.87	1.13	2.67	3.50	2.30
S. caurinus	4.31	1.26	1.17	2.01	1.17	2.17	1.94	2.05	2.05	1.88	2.81	0.20	1.91	1.99	1.88
S. ciliatus	4.58	2.66	3.58	1.88	3.58	1.97	1.13	3.58	3.58	2.78	1.13	2.89	1.82	1.17	2.78
S. crameri	4.26	2.02	1.02	2.50	1.02	3.41	2.38	2.02	2.02	2.78	3.38	0.45	2.00	4.16	2.78
S. diploproa	4.13	2.63	1.82	3.08	1.82	3.66	2.01	2.82	2.82	2.00	3.01	0.86	1.98	2.84	2.00
S. elongatus	4.60	1.03	1.01	1.78	1.01	2.10	1.99	2.01	2.01	2.00	2.99	0.07	1.92	2.09	2.00
S. emphaeus	4.65	2.92	2.04	2.88	2.04	2.77	4.65	1.10	1.10	1.08	3.71	0.96	3.85	2.75	1.08
S. entomelas	4.32	3.27	4.12	2.43	4.12	2.68	1.50	4.32	4.32	3.37	1.70	1.93	2.29	1.73	3.37
S. flavidus	4.31	2.49	3.31	1.96	3.31	3.43	1.55	4.31	4.31	3.41	2.55	2.31	1.61	2.53	3.41
S. helvomaculatus	4.58	1.01	0.97	1.85	0.97	2.06	1.23	1.97	1.97	1.97	2.23	0.06	1.18	2.06	1.97
S. maliger	4.34	2.14	1.23	2.82	1.23	3.27	2.80	2.23	2.23	2.00	3.80	0.36	2.66	3.05	2.00
S. melanops	4.30	1.79	2.54	1.16	2.54	2.44	1.32	3.54	3.54	4.30	2.32	1.34	1.68	3.19	4.30
S. miniatus	4.24	2.59	1.71	2.76	1.71	4.24	2.94	2.71	2.71	2.06	3.94	1.35	2.29	3.59	2.06
S. mystinus	4.46	2.17	2.26	1.98	2.26	2.87	1.08	3.15	3.15	2.26	1.97	1.02	1.26	1.99	2.26
S. nebulosus	4.75	2.88	1.94	3.75	1.94	3.94	2.06	2.94	2.94	2.00	3.06	1.00	2.00	3.00	2.00
S. nigrocinctus	4.28	2.06	1.17	2.06	1.17	3.61	2.44	2.17	2.17	2.83	3.44	0.83	1.89	4.28	2.83
S. paucispinis	4.54	1.07	1.12	1.07	1.12	2.72	1.72	2.12	2.12	2.12	2.72	0.84	1.07	2.72	2.12
S. pinniger	4.69	2.22	1.22	2.11	1.22	3.83	3.69	2.22	2.22	2.17	4.69	0.83	3.08	3.78	2.17
S. polyspinis	3.75	2.53	2.75	2.06	2.75	2.31	1.76	2.75	2.75	2.00	1.76	2.53	0.98	1.56	2.00
S. proriger	4.55	3.59	2.59	3.00	2.59	2.41	4.36	1.59	1.59	1.59	3.36	0.41	4.55	2.41	1.59
S. rastrelliger	4.85	1.03	1.03	1.93	1.03	2.03	1.00	2.03	2.03	2.95	2.00	0.03	1.00	2.95	2.95
S. reedi	4.57	2.32	1.39	2.18	1.39	4.01	2.80	2.39	2.39	2.95	3.80	1.09	2.11	4.57	2.95
S. ruberrimus	4.64	2.79	1.79	2.07	1.79	3.21	2.14	2.79	2.79	3.64	3.14	0.21	2.71	4.07	3.64
S. saxicola	4.69	2.02	1.07	2.07	1.07	3.02	2.07	2.07	2.07	2.00	3.07	0.07	2.07	2.96	2.00
S. variegatus	4.44	4.33	3.38	3.88	3.38	3.44	3.56	2.38	2.38	1.46	2.56	1.49	3.45	2.52	1.46
S. wilsoni	4.49	2.01	2.01	2.22	2.01	1.73	3.53	1.07	1.07	1.00	2.59	0.86	2.86	1.67	1.00
S. zacentrus	4.68	3.00	2.16	3.04	2.16	2.84	4.68	1.16	1.16	1.12	3.68	1.00	3.84	2.80	1.12

morphology is a feature that must be considered in determining diagnostic characteristics of juvenile *Sebastes*. Morphological analysis immediately identified *S. borealis*, *S. crameri*, and *S. reedi*. After reviewing the genetic identifications, pigment and meristic characters were used to identify *S. entomelas*, *S. ciliatus*, and *S. polyspinis*. Genetic results helped distinguish the morphologically similar *S. emphaeus* and *S. zacentrus*, and in turn, morphological characters were useful in distinguishing the genetically similar *S. emphaeus* from *S. variegatus* and *S. wilsoni*. Thus, in combination genetics and morphology identified all the specimens to species.

Genetic analysis

Ten species and two species groups were identified genetically: *S. aleutianus*, *S. alutus*, *S. borealis*, *S. entomelas*, *S. flavidus*, *S. melanops*, *S. pinniger*, *S. proriger*, *S. reedi*, *S. ruberrimus*, and *S. ciliatus* – *S. crameri* – *S. polyspinis* and *S. emphaeus* – *S. variegatus* – *S. wilsoni* (Table 4). Discrimination of species within each of the two groups could not be done with the markers currently used. In addition, *S. entomelas* could not be distinguished from *S. mystinus* haplotypes A and C (Li et al., 2006c) based on variation in the ND3/ND4

Table 4

Haplotype letter designations of juvenile *Sebastes* spp. using mtDNA regions digested with endonucleases for species identification. Haplotypes for regions ND3/ND4 and 12S/16S are based on Li et al. (2006c, Table 1), and haplotypes for region ND5/ND6 and alleles for microsatellite locus u*Sma6* are based on Gharrett et al. (2005).

		ND3/ND4								12S/16S	ND5/ND6	u*Sma6*
Species	n	*Mbo* I	*Bst*N I	*Dde* I	*Hinf* I	*Bst*U I	*Cfo* I	*Msp* I	*Rsa* I	*Msp* I	*Mbo* I	alleles
S. aleutianus Type I	1	K	F		E						a	*177/*177
	2	K	F	ForN	E						a	*177/*177
S. aleutianus Type II	4	K	F	ForN	E						b	*183/*183
	2	K	F	N	E						b	*183/*183
S. alutus	200	B	B									
	1	B_1[a]	B	J			A					
	2	B	A	J			A					
S. borealis	15	F	F	D								
S. ciliatus, crameri, polyspinis	9	F	A									
	16	F	A	M		D						
	1	F	A	M_1[b]								
	1	F	A_1[c]	M								
	2	F	A	M_2[d]								
S. emphaeus B, *variegatus, wilsoni* A	13	D	F	E					B			
S. entomelas	2	E	F	l						F		
	4	E	F	l_1[e]			J			F		
	5	E	F	l						F_1[f]		
S. flavidus	1	A	E	H								
S. melanops	4	E	D	F	B							
S. pinniger	6	C	F	s		C			B			
S. proriger	2	K	F	E	g		D	E	B			
S. reedi	3	r	A			D			F			
	23	r	A	M								
S. ruberrimus	1	I	C	B		C						

[a] B and B_1 differ at one site—site 758 lost.
[b] M and M_1 differ by one site—a site gained between sites 590 and 754 for fragment sizes 105 and 59 base pairs.
[c] A and A_1 differ at one site—site 829 lost.
[d] M and M_2 differ at one site—site 1601 lost.
[e] l and l_1 differ at one site—site 1601 lost.
[f] F and F_1 differ at one site—site 1259 lost.

region. These two species can be distinguished by a single site difference in the mitochondrial region, identifiable with the restriction endonuclease *Msp* I, that includes genes for 12S and 16S ribosomal RNA. Consequently, identification of all 11 fish in this group was resolved to *S. entomelas*.

The predominant species (203 of 330) was *S. alutus* (Table 1), which is easily identified because the mtDNA markers are distinct from all other rockfishes. A single specimen each of *S. flavidus*, *S. polyspinis*, and *S. ruberrimus* was observed. Six of the species or species groups had single haplotypes, that is, no intraspecific variation was detected in the *S. borealis*, *S. melanops*, *S. pinniger*, *S. proriger*, *S. reedi*, and *S. emphaeus – S. variegatus – S. wilsoni* samples. The *S. aleutianus* specimens were identified as a mix of Types I and II (Gharrett et al., 2005).

Three species or species groups exhibited some degree of intraspecific variation (Table 4). In the *S. alutus* samples, one common and two rare haplotypes were observed; one fish had a previously undescribed single restriction site loss. In the genetic species group *S. ciliatus – S. crameri – S. polyspinis*, all the fish morphologically identified as *S. crameri* and *S. polyspinis* in the OCC-2 and OCC-3 batches shared the same mtDNA haplotype. Most of the fish morphologically identified as *S. ciliatus* shared that same haplotype. New variation was observed in four *S. ciliatus* juveniles, with *Bst*N I or *Dde* I site losses in three fish and a *Dde* I site gain in one fish. Three

haplotypes were found in the *S. entomelas* samples, two of which are new and the result of single restriction site losses in *Dde* I and *Msp* I (region 12S/16S) digestions.

Morphological descriptions

Preliminary visual examination of pelagic juveniles grouped specimens by pigment pattern and body shape. The appearance of these groups was casually compared to published illustrations of pelagic juvenile *Sebastes* that occur in the GOA (e.g., Matarese et al., 1989). Comparisons of meristics of each specimen with those of adults known from the GOA helped in assigning specimens to groups and determining the specific identity of the groups. Results of the genetic analysis were then communicated and allowed confirmation of species assignments based on morphology or re-evaluation of them. In some individuals, the meristics more closely matched those of other species based on adult meristics, presumably because of the small sample sizes of the juveniles for some species and the fact that not all meristic elements may have been developed in the smaller specimens.

Results of the initial morphological (pigment, morphometrics, meristics) examination of the 23 juveniles from Southeast Alaska indicated that three morphological groups were present, which were found to be *S. ciliatus*, *S. emphaeus,* and *S. pinniger*, following genetic analysis (Table 1). The cursory visual examination of the OCC-1 batch of juveniles revealed five morphological categories, which closely aligned with genetic identifications (see Table 1). However, one species, *S. aleutianus*, and one species group, *S. ciliatus* and *S. polyspinis*, were indistinguishable from *S. alutus* in this cursory visual examination.

Among the deeper bodied specimens (body depth >25% SL) from the OCC surveys, the distinctively barred *S. crameri* and *S. reedi* juveniles were readily apparent, as were the juveniles of *S. borealis*, which have a saddle of pigment. Lightly pigmented deeper-bodied juveniles were separated and later found to be *S. aleutianus* and *S. ruberrimus*. The bulk of the juveniles were more slender bodied (body depth <25% SL) and more uniformly pigmented. These turned out to include several species (*S. alutus*, *S. ciliatus*, *S. polyspinis*, and *S. proriger*), predominated by *S. alutus*. It was not possible to separate these species based on preliminary morphological examination, and considerable variation in body pigmentation was observed in fish that were later identified genetically as *S. alutus*. Juveniles representing the subgenus *Sebastosomus* (here consisting of *S. entomelas*, *S. flavidus*, and *S. melanops*), which have a characteristic blotch of pigment in the membrane of their first dorsal fin, could not always be separated from the *S. alutus* group of specimens. Other than the dorsal blotch of pigment, which forms late in the juvenile period, these juveniles were morphologically similar to the *S. alutus* group (rather uniformly pigmented, slender body).

Accounts of individual species given below focus on distinguishing characteristics and pigmentation patterns, and comparisons of the present specimens with available literature. The few (6) smaller specimens (<25 mm SL) were late postflexion larvae and were not preserved well enough to allow detailed morphological descriptions. Descriptions of pigmentation refer to melanistic pigment unless otherwise noted. The photographs taken of the specimens when they were freshly thawed show additional pigment (e.g., red, yellow), which was not visible after the specimens were fixed in formalin. The drawings reproduced here are based on formalin-fixed specimens, and show only melanistic pigmentation. Because preopercular, opercular, and lower infraorbital spines are present in late larvae and pelagic juveniles of all specimens examined here, their presence is not noted in the following descriptions.

Sebastes aleutianus (Figs. 2A, 2B) In OCC-1 samples, there was one *S. aleutianus* (29.5 mm SL), in OCC-2 samples, there were four (16.0–27.4 mm SL); and in OCC-3 samples, there were four (19.3–29.2 mm SL). The 16.0 and 19.3 mm SL specimens are late postflexion larvae; the longer fish (23.9–29.5 mm SL) are pelagic juveniles. Among the commonly occurring species in the GOA, the meristics of these specimens fit *S. aleutianus* and *S. variegatus* (Table 3). Genetically, juveniles of both Type I and Type II *S. aleutianus* of Gharrett et al. (2005) were present in these collections, however it was not possible to distinguish between them morphologically. Description of juveniles of *S. aleutianus* is limited to an illustration of a 19.3 mm SL postflexion larva/pelagic juvenile, and a 57.0 mm SL early benthic stage juvenile (Matarese et al., 1989). The head on the OCC-1 specimen was slightly damaged, so accurate determination of head spines and dorsal fin spines was not possible. The other meristics of this specimen fit those of *S. aleutianus*. Its pigment pattern is similar to, but not as heavy as, that of the 19.3 mm postflexion larva illustrated in Matarese et al. (1989). All of the specimens have rather uniform body pigment, with the head and snout pigmented. However, the pigment is darker under the dorsal fins (there are scattered spots on the nape between the dorsal fin and the midlateral septum), and on the caudal peduncle. There are spots in the midlateral septum. On larger specimens there is more lateral body pigment, mainly as spots in the myosepta. The fins are unpigmented except for a line of spots at the base of the caudal-fin rays and a few spots on the bases of the dorsal-fin spines. In contrast, the 19.3 mm SL specimen illustrated in Matarese et. al. (1989) has small pigment spots scattered on all fins except the second dorsal and caudal. Body depth

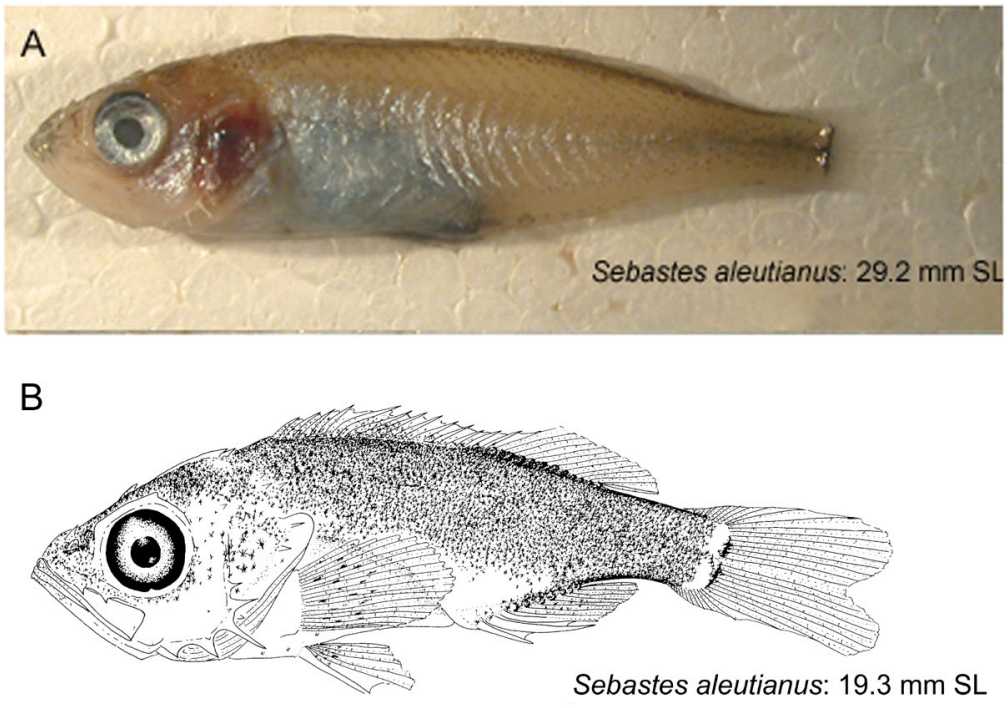

Figure 2

A) Photograph of *Sebastes aleutianus*, 29.2 mm SL. B) Illustration of *S. aleutianus*, 19.3 mm SL (Matarese et al., 1989).

is about 25% of standard length. Head spines consist of nasal, preocular, supraocular, postocular (serrate in the smaller two specimens), tympanic, parietal, and nuchal spines, and the parietal ridge is serrate.

Sebastes alutus (Figs. 3, 4, 5, 6A) Most of the specimens (203) from OCC-1 (40: 27.0–49.5 mm SL), OCC-2 (86: 13.8–54.7 mm SL), and OCC-3 (77: 19.7–48.8 mm SL) collections were identified genetically as *S. alutus*. Previous descriptions of juvenile *S. alutus* are limited to an illustration of a late pelagic juvenile (57.0 mm SL: see Matarese et al., 1989). There is considerable variation among the specimens examined here, mainly in the uniformity of body pigment, which is partially size-related. The photographs of *S. alutus* in Figures 3–5 are of representative specimens from OCC-3 arranged from uniform body pigment to increasingly barred body pigment. On the smaller fish (< 40 mm SL) there is no pattern of body pigment, whereas on some of the larger fish there is a blotch on the caudal peduncle and a blotch midlaterally near the insertion of the anal fin. Some have only the caudal peduncle blotch. Head and body pigment consists of fine spots, except on the ventral fourth of the body. Spots are concentrated in myosepta and along the lateral line. Other than spots at the bases of the anal-fin rays, there is no fin pigment until the fish are about 47 mm SL when spots are first seen in the dorsal fin membrane. A line of pigment on the hypurals extends slightly onto the caudal rays. There is a blotch of pigment on the opercle, and there is pigment ventrally on the caudal peduncle. On some of the larger fish, 42.6–47.8 mm SL, there is faint barring on the upper body under the spinous and rayed dorsal fin, and on the caudal peduncle, mainly dorsally. On the largest fish, 49.8–54.7 mm SL, the body is more uniformly pigmented, but there is a pattern of pigment in the dorsal fin with several bands in the membrane of the first dorsal fin, and a medial dark area in the second dorsal fin. They are moderately slender fish (body depth about 27% of standard length). The head has nasal, preocular, supraocular (present on specimens larger than about 30 mm SL), postocular, tympanic, parietal (serrate on specimens smaller than about 35 mm SL), and nuchal spines.

Sebastes borealis (Fig. 6B) Two *S. borealis* (28.0, 31.0 mm SL) were observed in the OCC-1 samples, four (23.0–29.0 mm SL) in the OCC-2 samples, and nine (18.5–31.0 mm SL) in the OCC-3 samples. Previous information on juveniles of *S. borealis* is limited to an illustration of a 53.0 mm SL early benthic juvenile

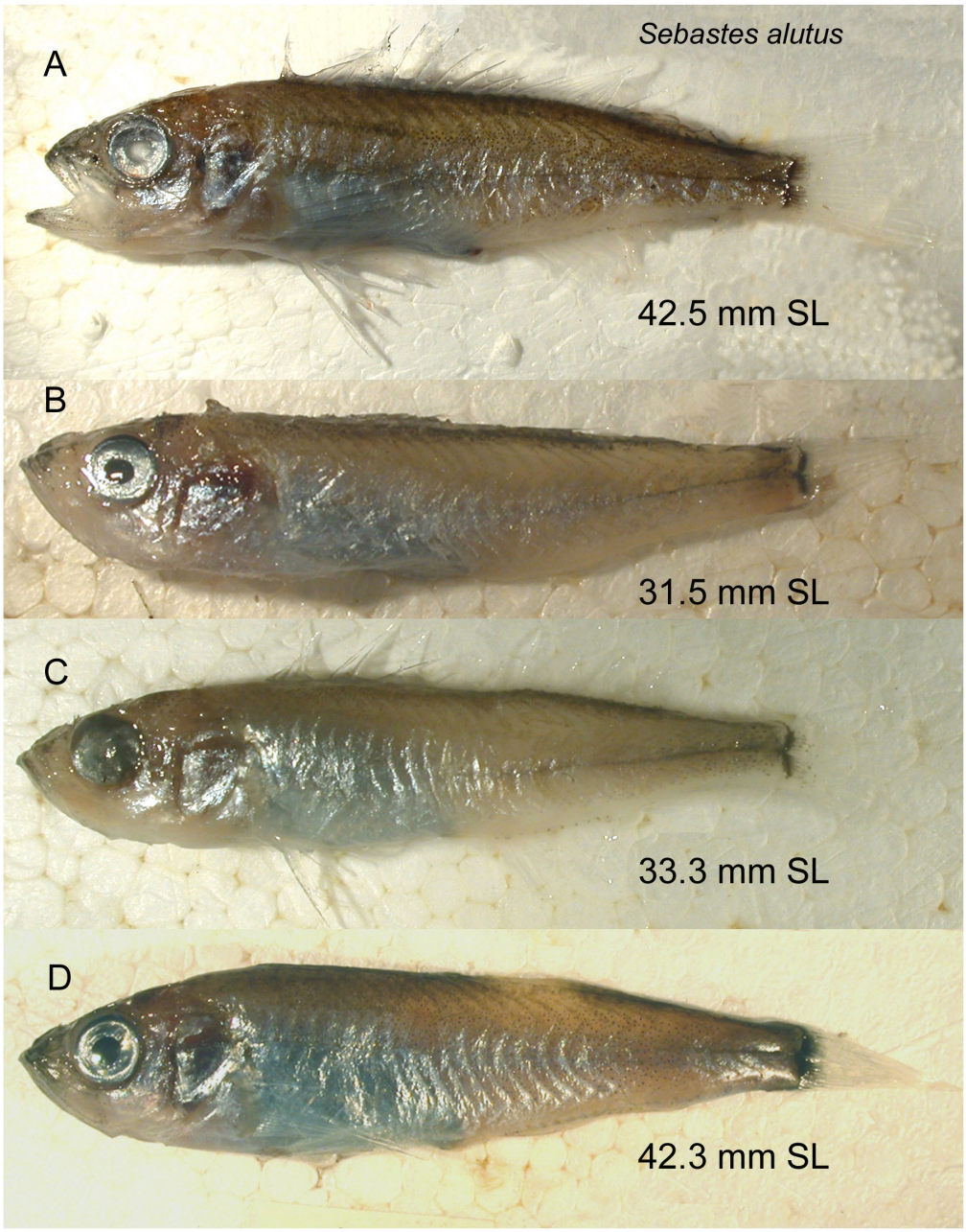

Figure 3

Photographs of pelagic juveniles of *Sebastes alutus* arranged from uniform to more patterned body pigment. A) 42.5 mm SL: uniform body pigment, heavier on caudal peduncle. B) 31.5 mm SL: uniform pigment, dark dorsally. C) 33.3 mm SL: fairly uniform, darker under second dorsal fin. D) 42.3 mm SL: light area under second dorsal fin, dark under first dorsal fin, caudal peduncle dark.

(Laroche[2]) which is considerably further developed than the specimens observed here. The pelagic juveniles found here are quite distinct from known juveniles of other *Sebastes* spp. Pigment consists of a saddle of fine spots on the spinous dorsal fin and onto the body at the paired fins. The paired fins are heavily pigmented and the head is pigmented. The jaws are pigmented in

[2] Laroche, W. A. 1987. Guide to larval and juvenile rockfishes (*Sebastes*) of North America. Unpublished manuscript. Stonefish Environ. and Taxon. Serv., Enosburg Falls, VT 05450.

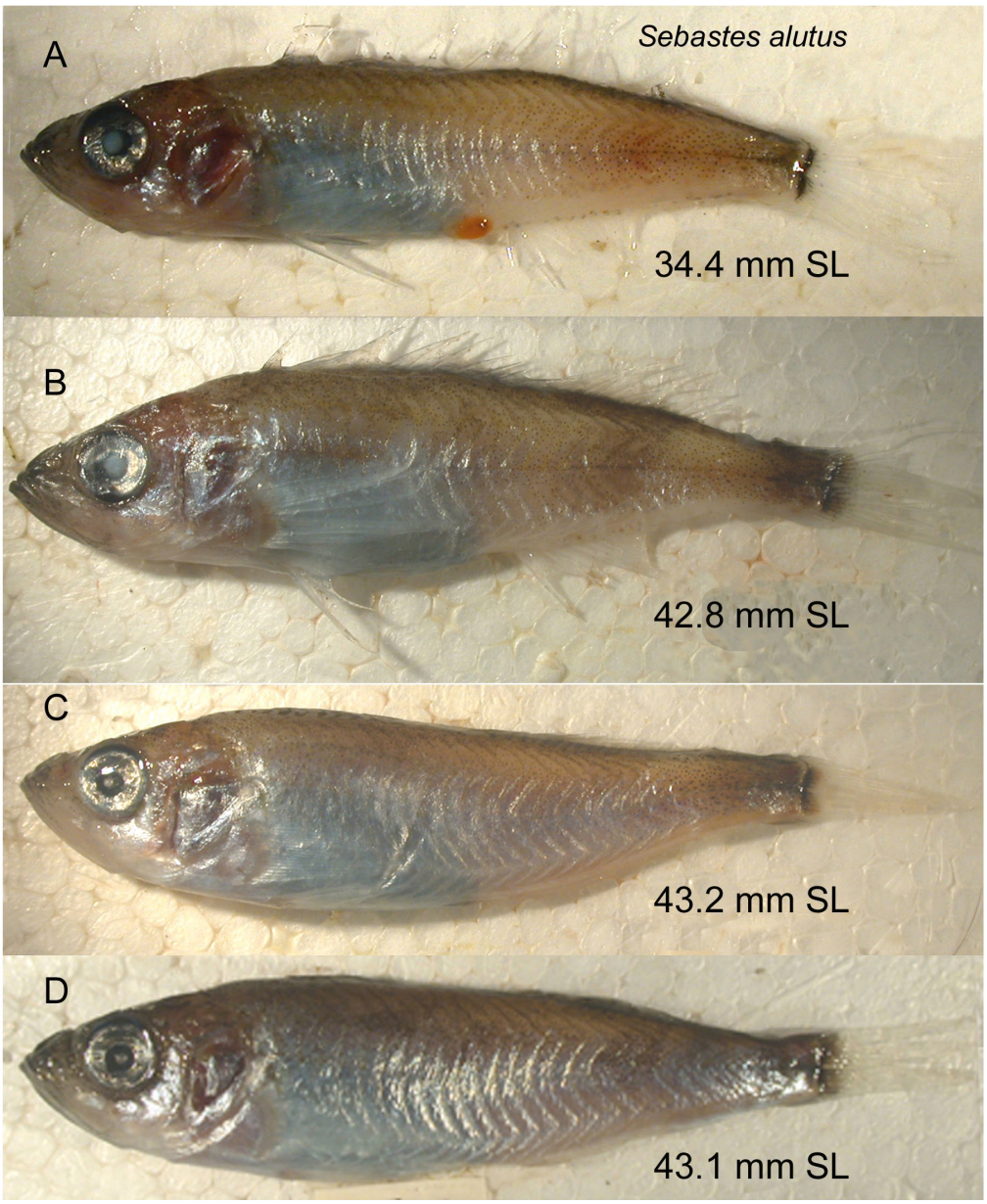

Figure 4

Photographs of pelagic juveniles of *Sebastes alutus* arranged from uniform to more patterned body pigment (continued). A) 34.4 mm SL: uniform, dark dorsally, under dorsal fins and on caudal peduncle, line at base of caudal fin. B) 42.8 mm SL: distinct caudal peduncle blotch, two blotches under dorsal fins. C) 43.2 mm SL: dark under dorsal fins and on caudal peduncle, faint blotch under second dorsal fin. D) 43.1 mm SL: rather uniform, faint barring, on caudal peduncle, at insertion of dorsal fin, pigment in dorsal fin membrane.

some specimens. There is pigment along the base of the dorsal fin. The posterior half of the midlateral septum is pigmented in the largest specimen examined. Most specimens have a midlateral spot on the caudal peduncle that may be embedded (the red blotch in this area shown in Fig. 6B does not persist in formalin-preserved specimens) and melanophores at the base of each caudal ray. They are relatively deep-bodied (body depth averaged 29% of standard length). Head spines are nasal, preocular, supraocular (on some: not related to fish size), postocular (serrate on smaller specimens), tympanic, coronal, parietal (usually serrate), and nuchal.

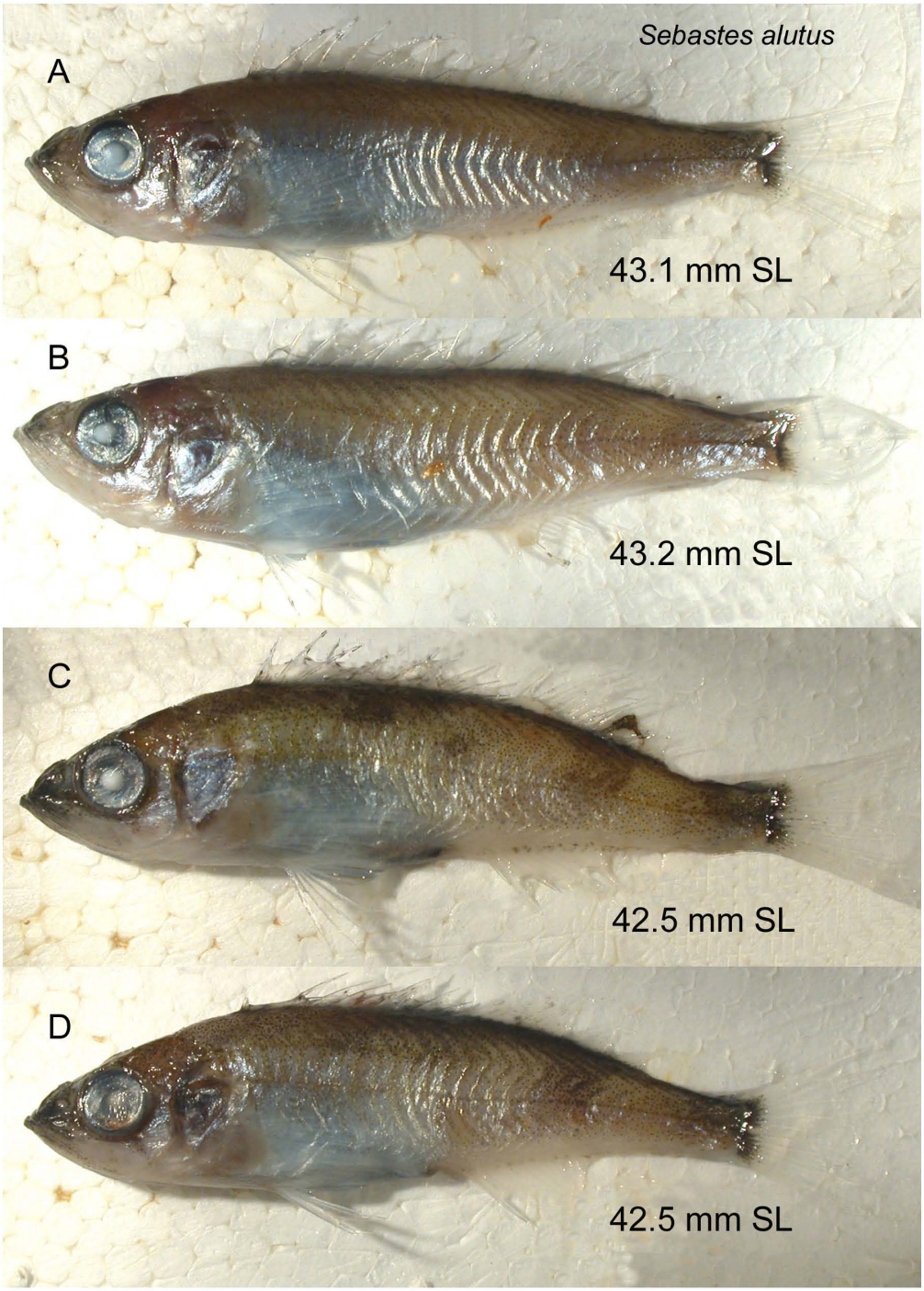

Figure 5

Photographs of pelagic juveniles of *Sebastes alutus* arranged from uniform to more patterned body pigment (continued). A) 43.1 mm SL: faint blotches, some pigment in dorsal fin. B) 43.2 mm SL: faint barring, under middle of first dorsal fin, at insertion of second dorsal fin, on caudal peduncle. C) 42.5 mm SL: faint blotches, some pigment in dorsal fin. D) 42.5 mm SL: faint blotches under first dorsal fin, posterior under second dorsal fin, on caudal peduncle; pigment in dorsal fin.

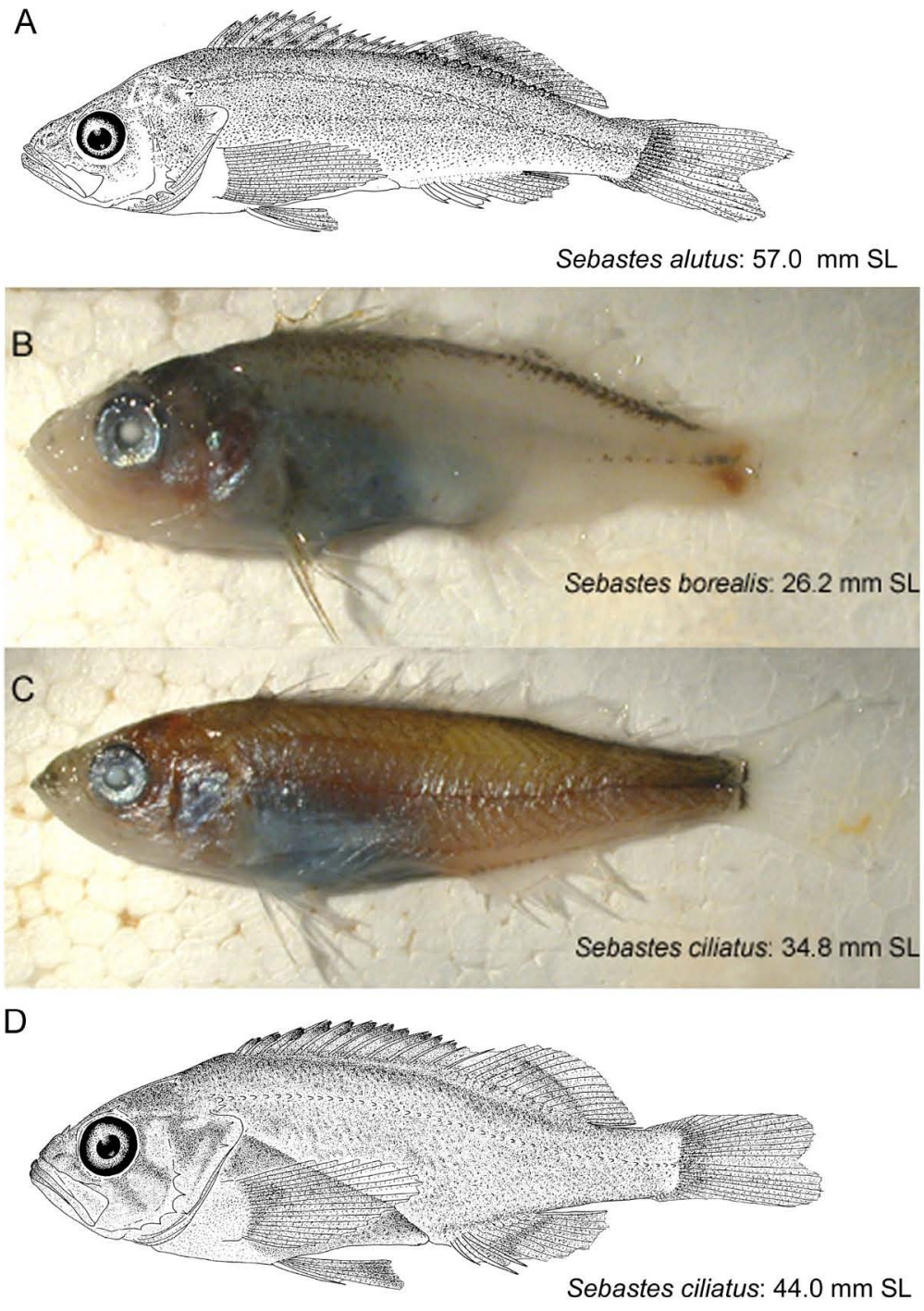

Figure 6

A) Illustration of *Sebastes alutus*, 57.0 mm SL (Matarese et al., 1989). B) Photograph of *S. borealis*, 26.2 mm SL. C) Photograph of *S. ciliatus*, 34.8 mm SL. D) Illustration of *S. ciliatus*, 44.0 mm SL (Laroche[2]).

Sebastes ciliatus (Figs. 6C, 6D) Based on the initial morphological examination, 24 specimens (3 from Southeast Alaska [32.0–35.4 mm SL], 2 from the OCC-1 collections [29.5, 29.6 mm SL], 2 from the OCC-2 collections [22.6, 27.8 mm SL], and 17 from the OCC-3 collections [21.6–35.4 mm SL]) were included in the category that was identified morphologically as *S. alutus*. However, genetic examination indicated that these were *S. crameri, S. ciliatus,* or *S. polyspinis*. Further morphological examination indicated that the most likely species is *S. ciliatus* (the juveniles of *S. crameri* are very distinctive in both morphology and pigmentation). Although *S. polyspinis* cannot be ruled out, none of these fish has the 14 dorsal spines characteristic of *S. polyspinis*. The last dorsal spine (or maybe the last two dorsal spines) in *S. polyspinis* probably develops as a soft ray and then transforms into a spine, as it does in other *Sebastes* spp. (see Laroche and Richardson, 1980), which may account for the lower count of dorsal spines in the smaller specimens examined here. However, the 31.8 mm SL *S. polyspinis* examined here has 14 dorsal spines (see later). The pigment and body shape of *S. ciliatus* are very similar to those of *S. alutus* and their meristics overlap, but *S. ciliatus* frequently (11/24) have 28 vertebrae, whereas *S. alutus* usually (193/205) have 27 (Table 2). Juveniles of *S. ciliatus* were previously unknown, except for a 44 mm demersal juvenile drawn by Laroche[2]. These specimens have rather uniform body pigment; however, on the larger specimens there are three faint bars on the body: one under the first dorsal fin, one decreasing in width from dorsal to ventral under the second dorsal fin, and a third more prominent bar across the caudal peduncle. The anterior two bars are most intense dorsally, not as wide ventrally, and may not extend to the ventral body margin. On the smaller specimens the dorsal aspect of the trunk is darkly pigmented along the bases of the dorsal fins and on the caudal peduncle. The midlateral septum (particularly the caudal portion) and the myosepta are pigmented. There are about five spots ventrally on the caudal peduncle. The paired fins are unpigmented, but there are spots at the base of each anal-fin ray, and in larger specimens there are spots at the bases of the dorsal spines. In larger juveniles, there is pigment in the membrane of the dorsal fin. These are slender fish (body depth is about 24% of standard length). Head spines consist of nasal, preocular, supraocular (occasional: on some specimens >32 mm SL), postocular, tympanic, parietal, and nuchal spines and the parietal ridge is serrate. Because adult *S. ciliatus* only consistently have nasal spines, the other spines seen on the pelagic juveniles would be resorbed or grown over (head spines are frequently lost during development of *Sebastes* spp.).

Sebastes crameri (Figs. 7A, 7B) Four pelagic juveniles from the OCC-1 collections (32.0–43.5 mm SL), two from the OCC-2 collections (37.0, 47.7 mm SL), and seven from the OCC-3 collections (34.5–46.6 mm SL) were identified morphologically as *S. crameri* and genetically as *S. crameri, S. ciliatus,* or *S. polyspinis*. The combination of numbers of dorsal rays, anal rays, and vertebrae resemble *S. crameri* more than the other two species (Table 2). Also the pigment pattern of these specimens is similar to that on illustrated pelagic juveniles (31.8 mm SL [Richardson and Laroche, 1979], and 39.0 mm SL [Matarese et al., 1989]) of *S. crameri*. They are heavily barred with a bar through the eye, a bar originating anteriorly at the spinous dorsal fin and extending onto the opercle, a double band at the middle and posterior portion of the spinous dorsal fin (roughly forming a "w" shape), a band from the rayed dorsal fin through the anal fin, and a caudal peduncle band. The bands medial to the dorsal and anal fins extend onto the associated fin membranes. The paired fins are heavily pigmented. These are deep-bodied fish (body depth is 34% of standard length). Head spines consist of nasal, preocular, supraocular, postocular, tympanic, parietal (infrequently serrate), and nuchal spines.

Sebastes emphaeus (Figs. 7C, 7D) Most of the specimens (14: 43.0–54.5 mm SL) from the Southeast Alaska collection are *S. emphaeus*. Morphological analysis could not rule out the possibility that these are *S. zacentrus;* the meristics of the two species are practically identical. Comparisons with published illustrations of both species are inconclusive. The development of *S. zacentrus* has been described by Laroche and Richardson (1981), but the early stages of *S. emphaeus* are only known from two illustrations of pelagic juveniles (Matarese et al., 1989: 16.4 and 30.0 mm SL). Based on genetic analysis, these fish were identified as *S. emphaeus* B, *S. variegatus*, or *S. wilsoni* A (see Li et al., 2006c). Genetic identification eliminated *S. zacentrus* and all specimens have seven anal rays, which reduced the likelihood that these fish were *S. wilsoni*, which typically has six anal rays. Furthermore, the juveniles examined here have 16 or 17 pectoral-fin rays, more like *S. emphaeus* than *S. variegatus*, which typically has 18 pectoral-fin rays. Pigment on the specimens examined here is concentrated dorsally, with an indication of more intense pigment in several areas where the dorsal fins meet the body. There is considerable yellow/orange pigment on the paired and anal fins on fresh specimens (Fig. 7C), which is lost in preservation. These are deep-bodied fish (body depth is about 31% of body length). Head spines consist of nasal, preocular, postocular, tympanic, and parietal spines.

Sebastes entomelas (Figs. 8A, 8B) A group of 11 specimens (40.5–66.0 mm SL) (3 from the OCC-1 collections, 2 from the OCC-2 collections and 6 from the OCC-3 collections) was identified genetically to

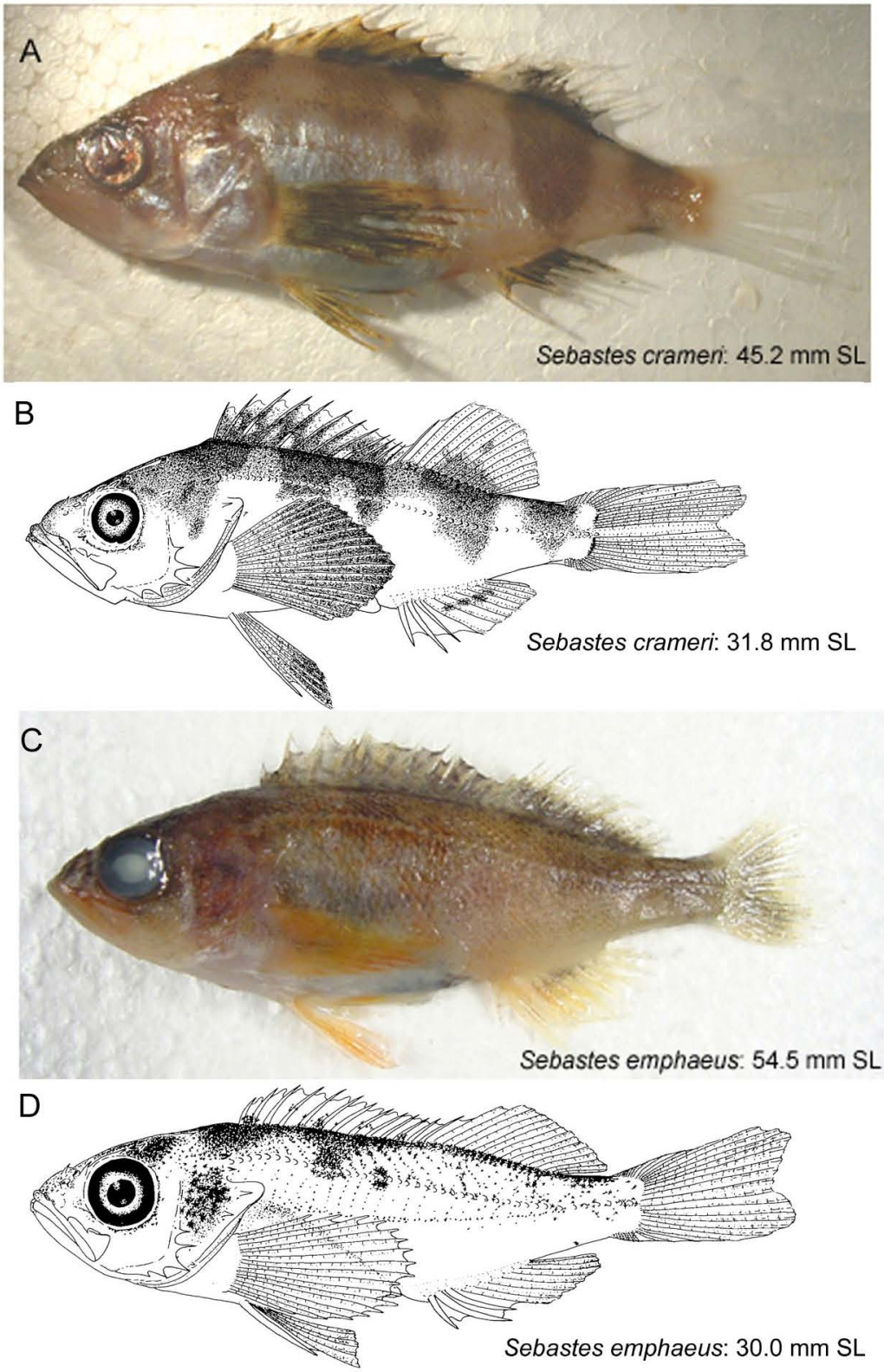

Figure 7

A) Photograph of *Sebastes crameri*, 45.2 mm SL. B) Illustration of *S. crameri*, 31.8 mm SL (Richardson and Laroche, 1979). C) Photograph of *S. emphaeus*, 54.5 mm SL. D) Illustration of *S. emphaeus*, 30.0 mm SL (Matarese et al., 1989).

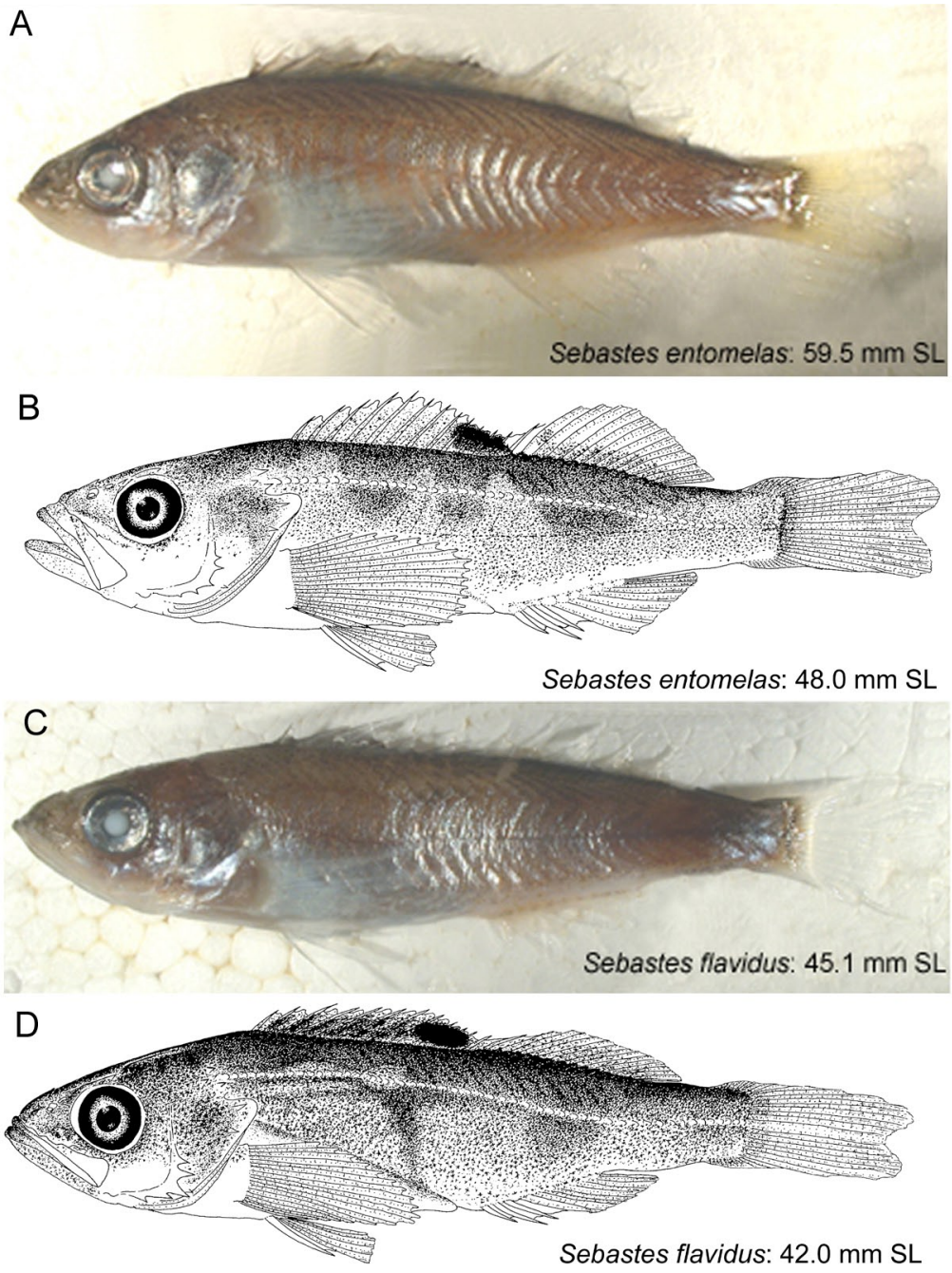

Figure 8

A) Photograph of *Sebastes entomelas*, 59.5 mm SL. B) Illustration of *S. entomelas*, 48.0 mm SL (Matarese et al., 1989). C) Photograph of *S. flavidus*, 45.1 mm SL. D) Illustration of *S. flavidus*, 42.0 mm SL (Matarese et al., 1989).

be either *S. entomelas* or *S. mystinus* using variation in the ND3/ND4 region. Additional genetic analysis confirmed the *S. entomelas* identification when the 12S/16S mtDNA region was amplified and digested with the *Msp* I endonuclease. The medial fin meristics, 15 dorsal soft rays, and 8 anal rays of these specimens are more consistent with *S. entomelas*; *S. mystinus* usually has 16 dorsal soft rays and 9 anal rays. These specimens fit published descriptions (Laroche and Richardson, 1981) and illustrations (Matarese et al., 1989) of *S. entomelas* pelagic

juveniles. However, Laroche and Richardson (1981) report transformation to benthic juveniles at about 44 mm SL. Based on the collections examined here, it appears that some specimens of *S. entomelas* continue to be pelagic in the GOA until at least 66 mm SL. Geographic distributions of the two species also supports identification of these specimens as *S. entomelas*: *S. mystinus* has a northern limit near the collection site, whereas *S. entomelas* is found northwest to Kodiak Island (Love et al., 2002). Except for the smallest specimen (40.5 mm SL), which was not initially distinguished from *S. alutus* based on morphology, these fish have the characteristic *Sebastosomus* blotch of pigment in the posterior part of the spinous dorsal fin. There is uniform superficial head and body pigment, with a darker line along the midlateral septum and more pigment in the myosepta. The dorsal aspect is dark. A line of pigment at the base of the caudal fin extends onto the fin forming a dark band. The paired, caudal, and anal fins are unpigmented, but some pigment occurs at the insertion of the dorsal spines and rays and in the dorsal fin membrane. These are rather slender fish (body depth is about 25% of standard length). They have nasal, preocular, supraocular, postocular, tympanic, parietal, and nuchal (overgrown or fused with the parietal spine in specimens longer than 55 mm SL) spines.

Sebastes flavidus (Figs. 8C, 8D) One specimen (45.1 mm SL) from the OCC-3 collection was determined to be *S. flavidus*. It was initially included in the specimens thought to be *S. alutus* on the basis of morphology. The development of *S. flavidus* has been described by Laroche and Richardson (1980) and a 42.0 mm specimen illustrated in Matarese et al. (1989) is similar to the specimen examined in this study. Differences between published descriptions and the specimen from the present study may be due to geographic differences in areas of collection (Oregon versus GOA). This specimen has more or less uniform body pigment, but is darker dorsally under the first dorsal fin and under the posterior part of the second dorsal fin. The caudal peduncle is dark and there is a line at the base of the caudal fin rays. There is ventral pigment on the caudal peduncle and a little pigment in the dorsal fin. It does not have the characteristic *Sebastosomus* blotch in the spinous dorsal fin; however, the dorsal-fin membrane is largely disintegrated. The other fins are unpigmented except there are melanophores at the bases of the anal-fin rays. This is a rather slender fish (body depth is 24% of standard length). This specimen has nasal, preocular, postocular, tympanic, parietal, and nuchal spines.

Sebastes melanops (Figs. 9A, 9B) In the OCC-3 collections, four pelagic juveniles of *S. melanops* (40.5–45.0 mm SL) were observed. The development of *S. melanops* has been described by Laroche and Richardson (1980); their illustration of a 45.3 mm pelagic juvenile closely resembles the specimens examined here. The body pigment is fairly uniform and dark, particularly dorsally under the dorsal fins and on the caudal peduncle. Laterally, pigment is concentrated in the myosepta (particularly on the upper half) and in the midlateral septum, and the caudal peduncle is dark. There is pigment at the bases of the anal-fin rays, ventrally on the caudal peduncle, and in a line at the base of the caudal-fin rays. The dorsal fin membrane has little pigment, which increases with growth. The 44.5-mm SL specimen has the characteristic *Sebastosomus* blotch of pigment in the membrane of the posterior part of the spinous dorsal fin. These are moderately slender fish (body depth is about 26% of standard length). Head spines are nasals, preoculars (bump), postoculars, tympanic, and parietals (serrate on the smallest specimen).

Sebastes pinniger (Figs. 9C, 9D) Six of the specimens (25.8–31.4 and 82.0 mm SL) from Southeast Alaska were identified as *S. pinniger*, based on genetics and morphology. The development of *S. pinniger* is fairly well known: 29.4 and 40.0 mm SL juveniles have been illustrated (Richardson and Laroche, 1979; Matarese et al., 1989), and an in situ photograph of a juvenile is included in Love et al. (2002: p. 235). The 82.0 mm SL specimen examined here may be a demersal juvenile, since the largest pelagic juvenile found by Richardson and Laroche (1979) was 42.4 mm SL and the smallest demersal juvenile was 59.4 mm SL. The characteristic black spot on the posterior part of the spinous dorsal fin, which extends onto the body in juvenile *S. pinniger,* was seen on the specimens examined here. The body of the smaller specimens (25.8–31.4 mm SL) is banded, but the body of the largest specimen (82.0 mm SL) is more uniformly pigmented. The smaller specimens have four bands as in the published illustrations of *S. pinniger*; however, the anterior band is indistinct and incomplete. It extends ventrally from the anterior spines of the dorsal fin onto the dorsal part of the body. The second saddle-like band is under the middle of the spinous dorsal fin and slants posteriorly. In some specimens pigment from the dorsal fin spot appears to join this band. The third, also saddle-like, band is under the second dorsal fin, and it decreases in width ventrally. The fourth band is across the caudal peduncle, and it also decreases in width ventrally. Besides these pigment characters, the meristics of the specimens examined here closely fit those of *S. pinniger*. These are deep-bodied fish (body depth is 30–34% of standard length). Head spines are nasals, preoculars, supraoculars, postoculars, tympanics, and parietals (serrate).

Sebastes polyspinis (Figs. 10A, 10B) A single specimen (31.8 mm SL) from the OCC-2 collection is consid-

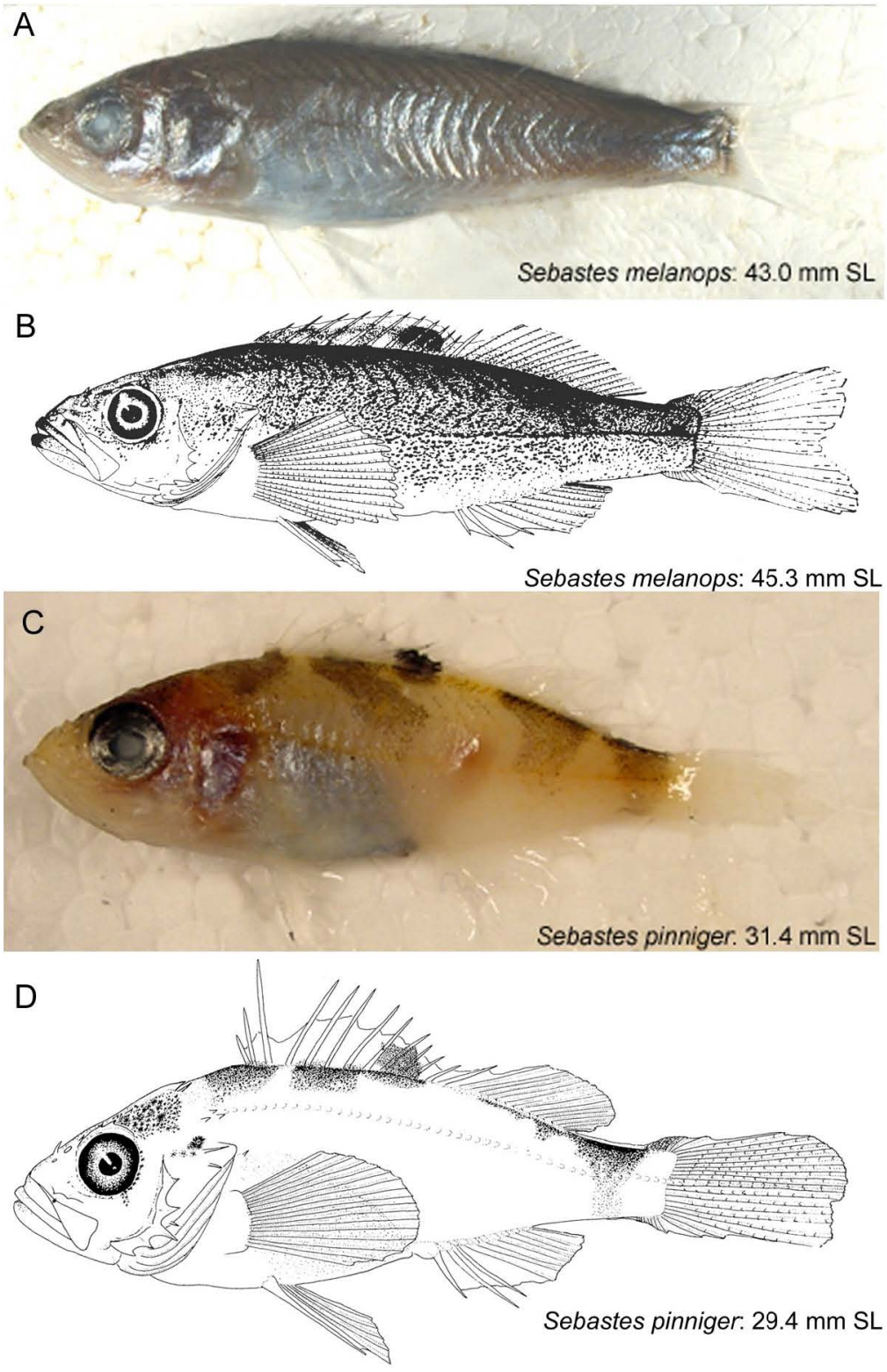

Figure 9

A) Photograph of *Sebastes melanops*, 43.0 mm SL. B) Illustration of *S. melanops*, 45.3 mm SL (Matarese et al., 1989). C) Photograph of *S. pinniger*, 31.4 mm SL. D) Illustration of *S. pinniger*, 29.4 mm SL (Richardson and Laroche, 1979).

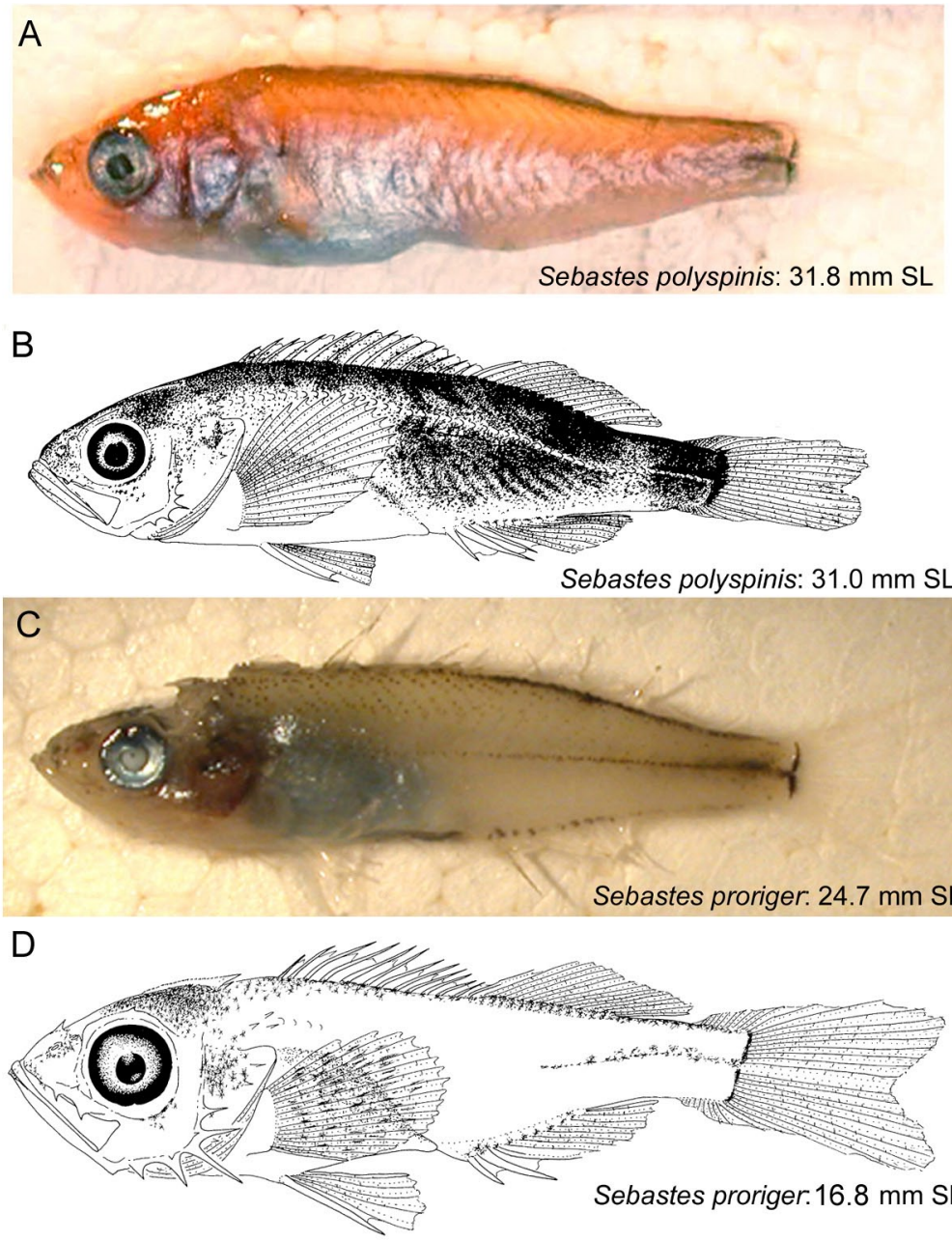

Figure 10

A) Photograph of *Sebastes polyspinis*, 31.8 mm SL. B) Illustration of *S. polyspinis*, 31.0 mm SL (Matarese et al., 1989). C) Photograph of *S. proriger*, 24.7 mm SL. D) Illustration of *S. proriger*, 16.8 mm SL (Matarese et al., 1989).

ered to be a pelagic juvenile of *S. polyspinis*. Genetically it was determined to be one of three species: *S. ciliatus*, *S. crameri*, or *S. polyspinis*. *Sebastes crameri* is distinctive morphologically and is dissimilar to the specimen examined here. The 14 dorsal spines of this specimen indicates that it is *S. polyspinis* since 13 dorsal spines is the common number for other *Sebastes* spp. from the GOA. The early life history stages of *S. polyspinis* are known only from illustrations of an extrusion larva (6.1 mm SL) and a pelagic juvenile (31.0 mm SL) (see Matarese et al., 1989). The specimen examined here is similar to the illustration of the 31 mm specimen, except it is not

as heavily pigmented, nor as developmentally advanced. The head and jaws are pigmented. The dorsal aspect of the body is darkly pigmented. There is pigment along the midlateral septum and in the myosepta. Laterally the body is fairly uniformly pigmented, with the heaviest pigment dorsally and on the caudal peduncle. There is an unpigmented area ventro-laterally along the base of the anal fin and along the caudal peduncle. There is a line of pigment at the base of the caudal fin. The fins are unpigmented except for the spinous dorsal fin where there is an indication of three bands of pigment in the fin membrane. This is a rather slender fish (body depth is about 25% of standard length). Head spines are nasal, preocular, supraocular (right only), postocular, tympanic, parietal (serrate), and nuchal.

Sebastes proriger (Figs. 10C, 10D) One pelagic juvenile (28.7 mm SL) from the OCC-2 collection and one (24.7 mm SL) from the OCC-3 collection were determined to be *S. proriger*. They were initially identified as *S. alutus* from morphological examination (the body shape and pigment pattern are very similar to *S. alutus* at this size); however, genetically they proved to be *S. proriger*, and their meristics fit *S. proriger* better than they do *S. alutus* (Table 3). There are published illustrations of 16.8 and 38.0 mm SL specimens of *S. proriger* (Matarese et al., 1989). The specimens examined here are similar to the published illustration of the 16.8 mm specimen. The head, including the lower jaw, is pigmented. The dorsal part of the opercle is fairly heavily pigmented. Dorsally there is heavy pigment along the base of the dorsal fin and along the dorsal aspect of the caudal peduncle. There are spots in the myosepta mainly dorsal to the midlateral septum. There is a line of spots in the midlateral septum which expands to become a blotch of pigment midlaterally on the caudal peduncle. The ventral midline of the caudal peduncle has two irregular parallel rows of melanophores. There are spots at the bases of the dorsal- and anal-fin rays and a line of pigment at the base of the caudal fin; otherwise the fins are unpigmented. These are relatively slender fish (body depth is 23% of standard length). Head spines are nasal, preocular, postocular, tympanic, parietal (serrate), and nuchal.

Sebastes reedi (Figs. 11A, 11B) Three pelagic juveniles from the OCC-1 collections (38.6–40.4 mm SL), 10 from the OCC-2 collections (31.1–43.6 mm SL), and 13 from the OCC-3 collections (23.1–40.8 mm SL) were identified as *S. reedi* from both genetic and morphological analysis. The meristics of these specimens fit *S. reedi* better than other *Sebastes* spp. that occur in the GOA (Table 3). Also, the pigmentation of these specimens is similar to that illustrated for a 49.0 mm SL *S. reedi* pelagic juvenile (Matarese et al., 1989). Body pigment consists of fine spots over the dorsal 3/4 of body and head. The myosepta and midlateral septum have spots. The distal halves of the paired fins are heavily pigmented. Bars are on the dorsal part of the body and onto the dorsal fins: anterior, medial, and posterior portions of the spinous dorsal fin; at the rayed dorsal fin; and on the caudal peduncle (extends below the lateral line). There is a line of pigment at the base of the caudal fin and a spot centered on the third anal spine. Body depth is moderate (body depth is 26% of standard length). Head spines are nasal, preocular, supraocular, postocular, tympanic, parietal (serrate), and nuchal.

Sebastes ruberrimus (Figs. 11C, 11D) One specimen (22.5 mm SL) from the OCC-3 collection was identified as *S. ruberrimus*. Knowledge of the development of *S. ruberrimus* is limited to illustrations of 28.0 and 68.0 mm SL juveniles (Matarese et al., 1989; Laroche[2]). The specimen examined here closely resembles the illustrated 28 mm specimen and looks somewhat like *S. borealis* at this size, but there are some significant differences. The body is generally lightly pigmented. There is pigment on top of the head, on the opercle, and dorso-laterally on the nape. There is a dark blotch of pigment on the caudal peduncle just posterior to the insertion of the second dorsal fin. There is no fin pigment except at the bases of the dorsal-fin spines associated with the nape pigment, and a line at the base of the dorsal caudal-fin rays. The orange pigment on the caudal peduncle (Fig. 11C) does not persist in preserved specimens. These are rather deep-bodied fish (body depth is 30% of standard length). Head spines are nasal, preocular, supraocular, postocular, tympanic, coronal, parietal (serrate ridge), and nuchal.

Discussion

In the present study, a combination of genetics and morphology was used to identify species of all the specimens under consideration. Other studies also demonstrate the utility of combining genetics and morphology to identify field-collected juvenile rockfishes. Electrophoretic analysis of allozymes was used to identify juvenile rockfishes collected near drifting algae and seagrass off Washington (LeClair and Buckley, 2001). The identity of the rather distinctive *S. diploproa* was confirmed, and other specimens were determined to be *S. melanops*, based on electrophoretic patterns and morphology. The remaining specimens, which all had similar morphologies, did not have allozyme patterns of any of the 11 reference species they used. Using sequence data from the mitochrondrial cytochrome *b* region, Rocha-Olivares et al. (2000) identified specimens of two closely related *Sebastes* spp. (*S. constellatus* and *S. ensifer*) collected off

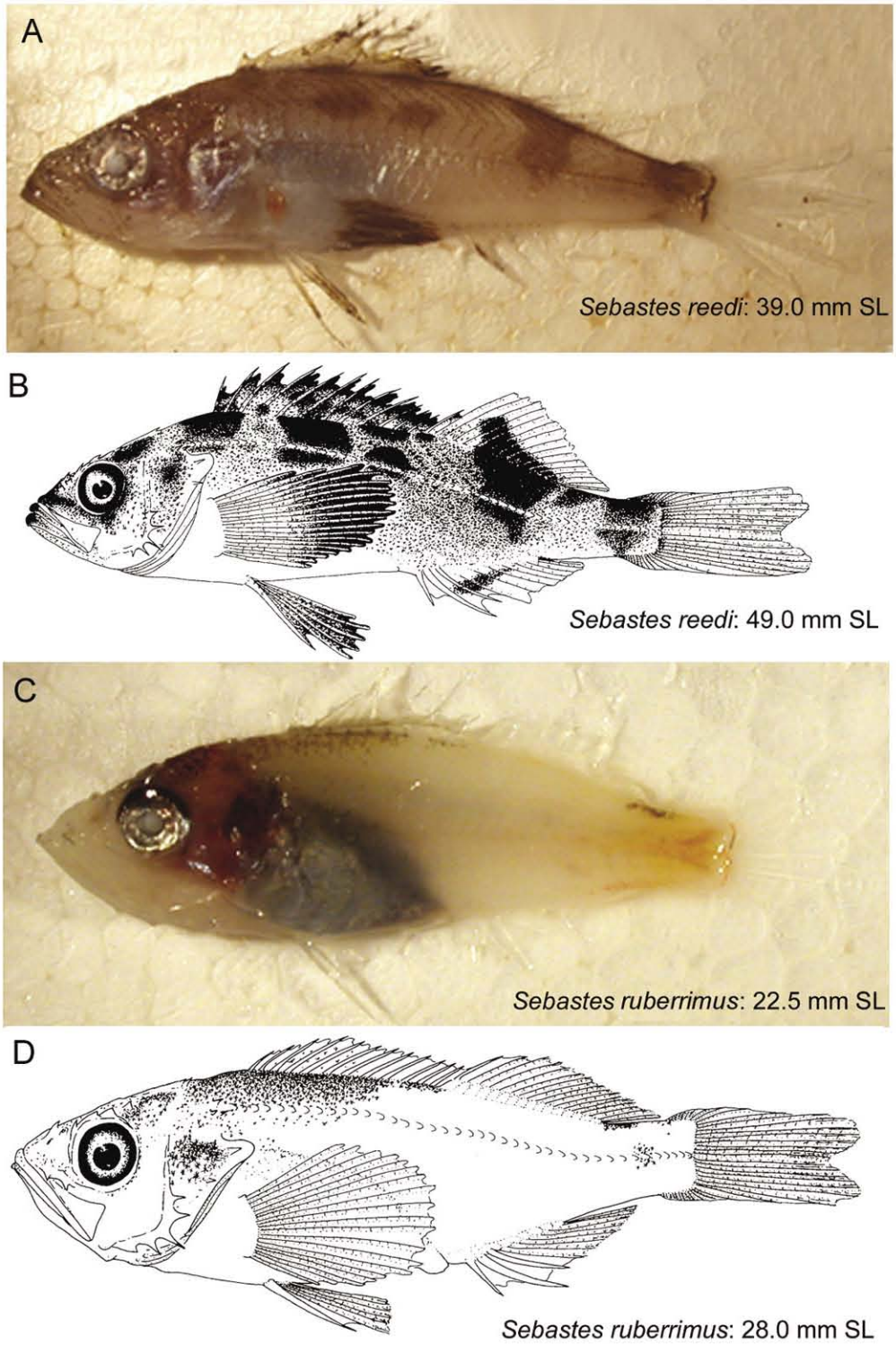

Figure 11

A) Photograph of *Sebastes reedi*, 39.0 mm SL. B) Illustration of *S. reedi*, 49.0 mm SL (Matarese et al., 1989). C) Photograph of *S. ruberrimus*, 22.5 mm SL. D) Illustration of *S. ruberrimus*, 28.0 mm SL (Matarese et al., 1989).

California. Once the genetic identities of the specimens were established, they found morphological characters that allowed the closely related species to be separated from each other and other species. Laidig et al. (2004) used mtDNA to confirm morphologically based identifications of field-collected *S. wilsoni*. Using restriction site analysis of the mitochondrial ND3/ND4 region, Li et al. (2006b) identified transforming larvae and pelagic juveniles of eight species of rockfishes collected off California, and reduced the possible identity of other specimens to groups of two or three species. Morphologically, most of these species could be distinguished; however genetic results were usually more restrictive. There is no overlap in species identified among these studies and those from the present study. Full descriptions of complete developmental series of more *Sebastes* spp. are needed to improve the ability to identify species based on morphology alone. Once more descriptions are available, it may be possible to develop and expand keys or other diagnostic tools to allow rapid visual identification of field-collected pelagic juveniles.

Three basic morphs occur among the pelagic juveniles found in the present study: a deep-bodied, heavily banded morph (*S. crameri*, *S. pinniger*, and *S. reedi*); a deep-bodied, lightly pigmented morph with a saddle of pigment on the nape (*S. borealis* and *S. ruberrimus*); and a slender morph with countershading pigment (*S. aleutianus*, *S. alutus*, *S. ciliatus*, *S. entomelas*, *S. flavidus*, *S. melanops, S. polyspinis*, and *S. proriger*). Some fish are lightly banded (some *S. alutus* and *S. emphaeus*). Larger specimens of some species have a dark blotch in the membrane of the dorsal fin in addition to other pigment (*S. entomelas*, *S. melanops*, and *S. pinniger*). As detailed in the following paragraphs, some of these morphological characteristics indicate systematic relationships while others may indicate convergence on characters that are adaptive for the environment occupied by the juveniles of these species.

The complex genus *Sebastes* has been subdivided into several subgenera; however, most had not been subjected to rigorous analysis (Kendall, 2000) until Hyde and Vetter (2007) used genetic analyses to investigate their systematics. Previously, two subgenera had been shown to be monophyletic in North American waters, and their limits established (*Sebastomus*: Rocha-Olivares et al. [1999] and *Pteropodus*: Li et al. [2006a]). Within each of these subgenera, the larval stage of species shows morphological similarities (see Moser, 1996; Watson and Robertson, 2004). Also, pelagic juveniles of species within *Sebastomus* are similar to each other (Rocha-Olivares et al., 2000). Within *Pteropodus,* the pelagic juveniles of too few species are known to evaluate similarities among them. Based on descriptions of pelagic juveniles within these two subgenera available to date, morphological characters alone may not permit species identifications; genetic analysis may be required.

The pelagic juvenile stage of a group of eight species (*S. brevispinis*, *S. entomelas*, *S. flavidus*, *S. melanops*, *S. miniatus*, *S. mystinus*, *S. pinniger,* and *S. serranoides*) has pigment in the membrane of the spinous dorsal fin (see Matarese et al., 1989; Laroche[2]). Five of these species (*S. entomelas*, *S. flavidus, S. melanops*, *S. mystinus,* and *S. serranoides*) have been placed in the subgenus *Sebastosomus* (Hyde and Vetter, 2007). The pigment blotch in the dorsal fin of *S. pinniger* and *S. miniatus* is somewhat different (round and contiguous with body pigment) from that seen in the *Sebastosomus* species, and they are sister species in the subgenus *Rosicola* (Hyde and Vetter, 2007). The spinous dorsal pigment blotch in *S. brevisipinis*, which has been placed in a separate clade (Hyde and Vetter, 2007), is not well defined and is continuous with adjacent body pigment (see Matarese et al., 1989). Thus, an oval blotch of pigment in the membrane of the spinous dorsal fin that is distinctly separate from pigment on the body might be a distinguishing character of pelagic juveniles of *Sebastosomus.*

The morphology of pelagic juveniles of *S. crameri* and *S. reedi* is similar: both species are rather deep-bodied and have banded body pigment extending onto the paired fins. *Sebastes crameri* and *S. reedi* are sister species and belong in a clade that includes *S. ciliatus*, *S. polyspinis,* and *S. variabilis* (Hyde and Vetter, 2007). Although the pelagic juveniles of these latter species could not be separated from each other in the present study, none of the specimens in this unresolved group has banded body pigment.

The different habitats occupied by juvenile *Sebastes* spp. are discussed by Moser and Boehlert (1991) and Love et al. (2002). Some species seemingly well adapted to an epipelagic existence have a slender streamlined body, terminal mouth, counter-shaded silvery pigment pattern, etc. Pelagic juveniles of other species are heavily banded and may reside with flotsam; however, Moser and Boehlert (1991) suggest that they occur deeper in the water column. Some species may become demersal soon after the larval phase so their juveniles would be rare in the pelagic habitat. Juveniles of some nearshore species (e.g., representatives of the subgenus *Pteropodus*: *S. maliger*) that were not found in the collections examined here may associate with kelp and would not be available to the near-surface water sampling used for the present collections. Convergence seems to be occurring in the genus *Sebastes* regarding morphology of the pelagic juveniles, which limits the usefulness of this stage in systematic studies.

The results of the present study document the occurrence of juvenile rockfish in the northern GOA during July and August. Because the subsamples of the collections used here were intended to determine which

species were present, they do not reflect abundances. In addition, not every collection was subsampled, so the species observed are not necessarily inclusive. Future analysis of these collections will provide information about spatial and temporal distribution in the GOA during summer months. In addition, this and future work based on larger numbers of specimens should help to better define morphological characters that can be used to identify the GOA rockfishes at this life stage.

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