Comparing the identification of southern California juvenile rockfishes (genus *Sebastes* spp.) by restriction site analysis of the mitochondrial ND3/ND4 region and by morphological characteristics

Zhuozhuo Li[1]
Mary M. Nishimoto[2]
Milton S. Love[2]
Anthony J. Gharrett[1]

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

[2] Marine Science Institute
University of California
Santa Barbara, California 93106-6150.
E-mail address (for A. J. Gharrett, contact author): **ffajg@uaf.edu**

Abstract—Rockfish (*Sebastes* spp.) juveniles are often difficult to identify by using morphological characters. This study independently applies morphological characters and a key based on mitochondrial restriction site variation to identify juvenile rockfishes collected in southern California during juvenile rockfish surveys. Twenty-four specimens of *Sebastes* were examined genetically without knowledge of the morphological assignment. Seventeen fish were identified genetically as *S. semicinctus*, *S. goodei*, *S. auriculatus*, *S. jordani*, *S. levis*, *S. rastrelliger*, and *S. saxicola*. Identities for the remaining fish were narrowed to two or three species: 1) three fish were either *S. carnatus* or *S. chrysomelas*; 2) one fish was either *S. chlorosticus*, *S. eos*, or *S. rosenblatti*; and 3) three fish could have been either *S. hopkinsi* or *S. ovalis*, the latter for which we now have distinguishing mitochondrial markers. The genetic and morphological assignments concurred except for the identity of one fish that could only be narrowed down to *S. hopkinsi* or *S. semicinctus* by using morphological characters. Genetics excluded more species from multispecies groupings than did the morphological approach, especially species within the subgenus *Sebastomus*. Species in the genetically unresolvable groups may be similar because of recent divergence or because of interspecies introgression.

Manuscript submitted 11 October 2004 to the Scientific Editor's Office.

Manuscript approved for publication 21 September 2005 by the Scientific Editor.

Fish. Bull. 104:376–382 (2006).

Sixty-five rockfish species (*Sebastes* spp.) inhabit the waters along the California coast (Moser, 1996). Within the genus, there is a high degree of similarity among many species in morphological characters. These similarities are in part due to recent divergence, but may also have resulted from convergence of congeners occupying similar habitats. Identification of *Sebastes* (and most species) is usually based on morphology; however, this approach may fail, especially for identifying sympatric species, which can be similar in coloration and overlap in morphological characters. Juvenile rockfishes are morphologically distinct from larvae and adults (Kendall, 2000), and juvenile stages of many species, especially the pelagic juvenile stage, have not yet been described; only a few species have complete ontogenetic descriptions (Matarese et al.,1989; Moser, 1996). The species of a few *Sebastes* larvae can be determined and adults can be misidentified.

Rockfishes are important ecologically and some species are economically valuable. *Sebastes* larvae are a large component of ichthyoplankton collections and rank third or fourth in abundance among all fish larvae taken during California Cooperative Fisheries Investigations (CalCOFI) surveys, which cover the entire length of the California and Baja California coast and now survey southern California. The ability to identify *Sebastes* accurately and efficiently at all developmental stages will, in turn, greatly increase our knowledge of their life histories, as well as our management and conservation efforts. An increased understanding of life history variation can improve the systematic descriptions of *Sebastes* species, which have mostly been based on the morphology of adults.

The analysis of restriction site variation of polymerase chain reaction (PCR)-amplified mtDNA is an effective and relatively simple method for species identification of adult specimens of fish species. The use of restriction enzymes to cleave the DNA can reveal variation in the mtDNA sequence at specific sites, and individuals or species can often be distinguished by the presence or absence of these sites. This method has advantages over sequencing in that it can be conducted relatively easily and used to survey large stretches of a DNA sequence, whereas sequencing,

because of its high cost, is usually limited to a much smaller span of DNA. Closely related species of several genera have been identified with this method; for example, snappers (Chow et al., 1993), eels (Aoyama et al., 2000; Lin et al., 2002), billfishes (Innes et al., 1998), and rockfishes (Gharrett et al., 2001). For identification of larvae and juveniles, restriction site patterns of adults can be used as references to ensure the reliability of the results. A limited database was used and a small number of samples were tested for a previous report on larval and juvenile identification of *Sebastes*, also based on restriction patterns of the mtDNA (Taylor, 1998).

The objective of this study was to compare the results of identifying pelagic juvenile rockfishes by the application of a key for delineating species based on mtDNA variation of adult rockfishes (Li et al., 2006) and by using only morphological characteristics. The questions we addressed were the following: Are the results for both genetic identifications and morphological identifications concordant? If not, how did these two methods differ? The targets of the comparison were samples of pelagic juvenile rockfishes collected from southern California. Both genetic and morphological analyses were conducted without knowledge of the results of the other.

Table 1

Sample identification numbers, sampling location, standard length (SL), weight, and method of tissue storage of juvenile specimens of *Sebastes* spp. Missing sample numbers are those for which the DNA could not be amplified: a = frozen on the boat, thawed on ice when genetic material was taken; b=put into ethanol on the boat.

Sample number	North latitude	West longitude	SL (mm)	Weight (g)	Preservation technique
1	34.20	120.24	41.3	1.200	a
2	"	"	42.2	1.282	a
3	"	"	35.8	0.756	a
4	"	"	36.1	0.777	a
5	"	"	31.0	0.478	a
8	"	"	29.5	0.360	a
9	"	"	24.1	0.206	a
15	34.26	120.15	28.9	0.265	b
16	"	"	37.6	0.565	b
17	"	"	34.0	0.654	b
18	"	"	23.2	0.150	b
19	"	"	26.0	0.326	b
20	"	"	35.1	0.591	b
21	"	"	24.8	0.273	b
22	"	"	22.6	0.190	b
23	"	"	21.0	0.140	b
25	"	"	25.0	0.273	b
26	34.23	119.93	27.6	0.472	b
27	"	"	26.8	0.235	b
31	"	"	28.0	0.345	b
44	"	"	37.9	0.799	b
45	"	"	35.6	0.590	b
48	"	"	24.2	0.189	b
49	"	"	24.3	0.168	b

Materials and methods

Collection of juveniles

Juvenile rockfishes were collected from the coast of Santa Barbara, California in June of 1998 and 2000 (Table 1). Fish were captured at night with a modified Cobb mid-water trawl with a nominal 12.2 m×12.2 m opening and 9-mm mesh net. Average trawling depth ranged from 18 to 29 m below the surface and above bottom depths ranged from 500 to 800 m, except for one specimen that was caught over a bottom depth of 88 m. Generally, specimens were sorted and then frozen on board the vessel. However, specimens from two hauls were sorted and immediately put in 95% ethanol aboard the vessel. Similar collection methods were used for both years. Details of the 1998 survey are provided in Nishimoto and Washburn (2002). The standard lengths (mm) of the specimens ranged from 16 to 42 mm.

Genetic identification

Following collection, each fish was identified in the laboratory by using morphological characters and meristics and pigmentation patterns. Specimens were chosen for genetic examination based on morphological differences. Fish representing different developmental stages (e.g., transition from larval to pelagic juvenile stage) of a species were included in the sample. We examined forty-nine specimens that included species that were easily identified based on pigmentation and morphological characters and individuals for which identification was less certain. All specimens were provided for genetic analysis without information on the morphological assignment.

For the genetic analysis, muscle tissue and skin from one side of the body was removed from each specimen in September 2000. Total genomic DNA was isolated by using a Purgene DNA™ isolation kit (Gentra Systems, Inc., Minneapolis, MN). Total DNA of each specimen was PCR-amplified and digested with specific restriction enzymes as directed in the key (Li et al., 2006). In some cases, digests with additional restriction enzymes were conducted to increase certainty of the identifications. Restriction fragments were separated electrophoretically on 1.5% agarose gels (one part agarose [Sigma-Aldrich, St. Louis, MO] and two parts Synergel™ [Diversified Biotech Inc., Boston, MA]) in 0.5×TBE buffer (TBE is 90 mM Tris-boric acid, and

Table 2

The most commonly occurring count (majority) and range of counts of dorsal-, anal-, and pectoral-fin rays among adults of *Sebastes* spp. identified in this study and for species that have morphologically similar pelagic juveniles or that are yet undescribed. (Compiled from Laidig and Adams, 1991; Moser, 1996; Love et al., 2002). Grouping of species is explained in the text.

	Dorsal-fin rays		Anal-fin rays		Pectoral-fin rays	
Species	Majority	Range	Majority	Range	Majority	Range
Species with distinct morphological characteristics						
S. levis	12–13	12–13	7	6–7	18	17–18
S. goodei	14	13–16	8	7–9	17	16–18
S. jordani	14–15	13–16	9–10	8–11	20–21	19–22
Group I						
S. saxicola	12	11–14	7	5–8	16	15–18
S. rastrelliger	13	12–14	6	6	19	18–20
S. semicinctus	13	12–14	7	6–8	17	16–18
S. auriculatus	13	12–15	7	5–8	18	15–19
S. wilsoni	13–14	13–15	6	5–7	17	16–18
S. hopkinsi	14–15	13–17	7	6–9	17	16–18
S. ovalis	14–16	13–16	8–9	7–9	18	17–19
Group II						
S. caurinus	12–13	11–14	6	5–7	17	16–18
S. carnatus	13	12–14	6	5–7	17	16–18
S. chrysomelas	13	12–14	6	5–7	17	17–18
S. atrovirens	14	12–15	7	6–8	17	16–18
Sebastomus group						
S. rosenblatti	12	11–13	6	5–6	17	16–18
S. lentiginosus	12	12–13	6	6–7	17	16–18
S. eos	12–13	11–13	6	5–7	17–18	16–18
S. umbrosus	12–13	11–14	6	5–7	16–17	15–18
S. rosaceus	12–13	11–14	6	5–7	17	16–18
S. chlorostictus	12–13	11–15	6	5–7	17	16–18
S. helvomaculatus	13	12–14	6	5–7	16–17	15–18
S. constellatus	13	12–14	6	5–7	17	16–18
S. ensifer	13	12–14	6	5–7	17	16–18
S. simulator	13	12–14	6	5–6	17	16–18

2 mm EDTA, pH 7.5). A 100 base-pair (bp) ladder provided molecular weight markers to estimate restriction fragment sizes. Gels were stained with ethidium bromide and photographed on an ultraviolet light transilluminator. Restriction fragments that could not be accurately measured from agarose gels were separated on 8% polyacrylamide gels and stained with SYBR Green I Nucleic Acid Stain™ (Molecular Probes, Eugene, OR) by using a 25-bp ladder for a molecular weight standard. Successful PCR amplification was obtained from 24 of the specimens. A key based on restriction site variation (Li et al., 2006) was used in the identification of the rockfish specimens.

Morphological identification

Some of the pelagic juveniles included in this study could be identified from body and fin pigment patterns and from gross morphological characteristics, whereas others had not yet developed species-specific characters. Meristics used to identify rockfish species included counts of dorsal, anal, and pectoral fin rays (Table 2). Key gross morphological characteristics have been described for *S. jordani* (Moser et al., 1977), *S. levis* (Moser et al., 1977), *S. goodei* (e.g., 35.6-mm-SL specimen illustrated in Sakuma and Laidig [1995]), *S. semicinctus* (Laidig and Adams, 1991), *S. saxicola* (e.g., 38.2-mm-SL specimen illustrated in Laidig et al. [1996]), and *S. auriculatus* (Moser, 1996). However, recently transformed pelagic juveniles of the latter three species do not yet possess distinguishing pigmentation patterns. Those species are included in group 1 in Table 2 and require both morphological and meristic examinations to distinguish them. Although the fin spines of transforming pelagic juveniles are not fully ossified, counts of dorsal-, anal-, and pectoral-fin rays can be made and are useful for separating similarly pigmented species. Body shape, color, and the presence or absence of certain head spines

Table 3

Restriction fragment patterns and consequent species assignments, and species assignments based on morphological features for *Sebastes* spp. examined. Haplotypes a_1 and a_2 are variants of *Mbo* I haplotype a, which was first observed in the present study.

Sample	*Bst*N I	*Bst*U I	*Cfo* I	*Dde* I	*Hin*d II	*Hinf* I	*Mbo* I	*Msp* I	*Rsa* I	Genetic assignment	Morphological assignment
1	X		D		A	C	p			*S. semicinctus*	*S. semicinctus*
2	Z		A		A	g	K			*S. hopkinsi* or *ovalis*	*S. hopkinsi*
3	Z		A				K			*S. hopkinsi* or *ovalis*	*S. hopkinsi*
4	Z		A			g	g			*S. hopkinsi*	*S. hopkinsi*
5	Z						g			*S. hopkinsi*	*S. hopkinsi*
8	X						p			*S. semicinctus*	*S. semicinctus*
9	Z									*S. hopkinsi*	*S. hopkinsi* or *semicinctus*
15	Y		D			O	a_1		E	*S. goodei*	*S. goodei*
16	Y		D			O	a_2		E	*S. goodei*	*S. goodei*
17	O	C	D			G	f		C	*S. saxicola*	*S. saxicola*
18	a						c			*S. jordani*	*S. jordani*
19	B					A	q			*S. levis*	*S. levis*
20	Z		A			g	K		C	*S. hopkinsi* or *ovalis*	*S. hopkinsi*
21	c		C	v		a	A		B	*S. rastrelliger*	*S. rastrelliger*
22	F		A	i	C	G	C	a	B	*S. carnatus* or *chrysomelas*	*S. carnatus* or *chrysomelas*
23	F		a	i	C	G	C	a	B	*S. carnatus* or *chrysomelas*	*S. carnatus* or *chrysomelas*
25	F		A	i	C		C	a	B	*S. carnatus* or *chrysomelas*	*S. carnatus* or *chrysomelas*
26	F		D			A	E		C	*S. chlorostictus* or *eos* or *rosenblatti*	*Sebastomus*
27	X		D			C	p			*S. semicinctus*	*S. semicinctus*
31	F		D	v	A	A	k		B	*S. auriculatus*	*S. auriculatus*
44	Z		A	n		g	K		C	*S. hopkinsi*	*S. hopkinsi*
45	Z		A			g	g		C	*S. hopkinsi*	*S. hopkinsi*
48	Z			n			K			*S. hopkinsi*	*S. hopkinsi*
49	X						p			*S. semicinctus*	*S. semicinctus*

(as in the case of *S. auriculatus*) can help to differentiate species with overlapping meristic ranges.

Species that could not be identified from their morphological features were assigned to a complex of species or a subgenus. Group 2 in Table 2 includes species that have similar pigmentation patterns during their pelagic juvenile stage. Meristics and head spination are used to identify *S. atrovirens*. However, the remaining three, *S. carnatus*, *S. caurinus*, and *S. chrysomelas*, are assigned as a species complex because of the uncertainty in identifying the species. The subgenus *Sebastomus* includes ten rockfish species that were found in the collection area and that cannot be distinguished by morphological characters and meristics or by pigmentation.

Results

The way in which the specimens were handled and preserved affected DNA quality and limited the number of genetic and morphological species assignments that could be compared. Only the DNA of seven of the 25 specimens frozen at sea and later thawed and processed was successfully amplified, whereas the DNA of 17 of the 24 specimens preserved in 95% ethanol at the time of capture was amplified. A total of 24 specimens were identified to species or species group based on restriction fragment patterns of the mtDNA using one or more restriction endonucleases (Table 3) and without knowledge of the morphological assignment. Table 4 sum-

Table 4

Developmental stages and counts of dorsal rays, anal rays, and pectoral rays used to assign species to *Sebastes* samples that were independently identified through **genetic analysis.** The number of specimens is given in parentheses. * indicates that the paired fins differed.

Morphological assignment	Genetic assignment	Number of samples	Developmental stage	Number of dorsal rays	Number of anal rays	Number of pectoral rays*
S. auriculatus	*S. auriculatus*	1	juvenile	13	7	18
S. carnatus, *chrysomelas*, or *caurinus*	*S. carnatus* or *chrysomelas*	3	transforming juvenile (3)	12(2), 13(1)	6(3)	17(3)
S. goodei	*S. goodei*	2	transforming juvenile (1), juvenile (1)	13(1), 14(1)	8(2)	17(2)
S. hopkinsi	*S. hopkinsi*	5	transforming juvenile (2), juvenile (3)	14(2), 15(2), 16(1)	7(5)	17(4), 17/18(1)
S. hopkinsi	*S. hopkinsi* or *ovalis*	3	juvenile (3)	14(1), 15(2)	7(3)	17(3)
S. jordani	*S. jordani*	1	transforming juvenile	14	9	20
S. levis	*S. levis*	1	transforming juvenile	13	6	18
S. rastrelliger	*S. rastrelliger*	1	juvenile	13	6	19
S. saxicola	*S. saxicola*	1	juvenile	12	6	16
subgenus *Sebastomus*	*S. chlorostictus*, *eos*, or *rosenblatti*	1	juvenile	12	6	18
S. semicinctus	*S. semicinctus*	4	transforming juvenile (2), juvenile (2)	13(4)	7(4)	17(2), 16/17(1), 18(1)
S. hopkinsi or *semicinctus*	*S. hopkinsi*	1	transforming juvenile	14	7	17

marizes the meristic data used to identify species morphologically. When a fish could not be assigned to a species, it was assigned to a species complex or subgenus.

The individuals genetically identified to species were six *S. hopkinsi*, four *S. semicinctus*, two *S. goodei*, one *S. auriculatus*, one *S. jordani*, one *S. levis*, one *S. rastrelliger*, and one *S. saxicola*. The restriction fragment patterns of these juveniles were identical to those of previously observed adult specimens, except for the two *S. goodei* juveniles, each of which differed by a single difference (presumably a site loss in one fish and a site gain in the other) from reference specimens for *Mbo* I (Table 3 and Li et al., 2006). The genetic species assignment of all but one of these specimens matched the identification based on morphological characters (Table 4). The discrepant fish was identified morphologically as either *S. semicinctus* or *S. hopkinsi*. The specimen had 14 dorsal rays, which is within the range of both species (Table 2) and was a transforming pelagic juvenile that had not yet developed the discernible body pigmentation pattern of older juveniles (Table 4).

At the beginning of the study, *S. ovalis* was not included in our database. When we added restriction site information for *S. ovalis* to the database, we found that the species is very similar to *S. hopkinsi* in its haplotype profile. Consequently, three individuals morphologically identified as *S. hopkinsi* were genetically assigned to the complex *S. hopkinsi-S. ovalis*. The restriction enzyme *Dde* I can delineate the two species; however, when we discovered the ambiguity, no samples remained for analysis. Based on meristics, the three specimens are more likely *S. hopkinsi* than *S. ovalis*. The range of counts for fin rays overlaps for the two species (Table 2). However, *S. ovalis* commonly has eight or nine anal rays, and the three specimens in question possessed seven rays that occur commonly in *S. hopkinsi* (Table 4).

Three specimens were assigned to the species complex *S. carnatus-S. chrysomelas-S. caurinus* according to morphological criteria. Haplotype information eliminated *S. caurinus*. Genetic analysis of the one specimen that was morphologically assigned to the subgenus *Sebastomus*, which includes 13 North Pacific species, reduced the possible species to *S. chlorosticus*, *S. eos*, or *S. rosenblatti*.

Discussion

Our results demonstrated that restriction site analysis of mtDNA is a simple and effective tool for identifying juveniles of *Sebastes*

species. The presumed intraspecific variation observed in *S. hopkinsi* and *S. goodei* did not interfere with the application of interspecific variation in species identification, and our other work (Gharrett et al., 2001; Li, 2004) indicates that intraspecific variation generally does not obscure delineation of species. The key we constructed for mtDNA restriction site variation can be especially helpful in learning about variation in the distinguishing morphological characters of the early life stages of *Sebastes*.

One factor that prevented identification of some juvenile specimens was poor DNA quality. Specimens must be properly preserved to provide high quality DNA for genetic analysis. Our success in amplifying DNA from specimens preserved in several volumes of 95% ethanol immediately after capture was substantially higher than that of specimens that had been frozen after capture and subsequently thawed for morphological studies.

Several small groups of species had similar restriction site patterns. Consequently, there was ambiguity in the genetic analysis of those groups. Specifically, we were unable to separate *S. carnatus* from *S. chrysomelas*, and we were unable to distinguish among *S. chlorostictus*, *S. eos*, and *S. rosenblatti*. However, morphological characteristics could not reveal a difference between *S. carnatus* and *S. chrysomelas*, nor could they distinguish any of the 13 *Sebastomus* species, including *S. chlorostictus*, *S. eos*, and *S. rosenblatti*.

Sebastes carnatus and *S. chrysomelas* are very closely related and, as adults, differ morphologically only in body coloration. *Sebastes carnatus* has flesh-colored blotches on an olive-brown background, and *S. chrysomelas* has yellow blotches on a black background (Love et al., 2002). They are found across similar geographic ranges, from northern California to Baja California, and are often sympatric, inhabiting rocky substrates, although they have different depth distributions (Larson, 1980), and their segregation is maintained by the social dominance of *S. chrysomelas* (Larson, 1980; Hoelzer, 1987). The two species may also differ slightly in gill-raker counts: *S. carnatus* typically has 28–30, and *S. chrysomelas* typically has 27–28 (Hallacher and Roberts, 1985). Their taxonomic status as distinct species is generally recognized from their different colorations, even though no fixed biochemical or genetic differences have been reported (Seeb, 1986; Seeb and Kendall, 1991; Hunter, 1994; Alesandrini, 1997). A recent study with the use of microsatellites in the nuclear DNA and mtDNA sequence variation has provided evidence that the two species *S. carnatus* and *S. chrysomelas* are genetically divergent (Narum, 2000).

Sebastes chlorostictus, *S. eos*, and *S. rosenblatti* are also morphologically similar, are present sympatrically, and are closely related (Chen, 1971; Love, 1996; Rocha-Olivares et al., 1999; Love et al., 2002). As adults, these species segregate by depth to some extent; *S. rosenblatti* occupies a somewhat narrower range than the other two (Love et al., 1990). Cladistic analyses indicate that *S. rosenblatti* is the most ancient of the three species (Rocha-Olivares et al., 1999). Estimates of divergence times of the subgenus *Sebastomus* may indicate that the three species are the result of the most recent speciation events within the subgenus, which may have begun less than 140,000 years ago (Rocha-Olivares et al., 1999).

The assignment of four specimens to multispecies groups in this study indicates the need for further effort to develop markers that will delineate the species in question. Approaches include screening additional regions of the mtDNA and application of additional restriction enzymes. If additional mtDNA regions and restriction enzymes do not provide species-specific information, other molecular techniques, such as the use of microsatellites, should be considered.

Acknowledgments

This work represents, in part, the master's thesis work of Z. Li at the University of Alaska Fairbanks. The project was supported by funding from the U.S. Geological Service (Biological Resources Division) Western Regional Office in Seattle, WA (R.W.O. 32) to AJG. Funding for MMN and MSL was provided by the Biological Resources Division under a cooperative agreement no. 1445-CA09-95-0836 with the Marine Science Institute, University of California, Santa Barbara.

Literature cited

Alesandrini, S.
1997. Genetic differentiation and population structure in the gopher rockfish (*Sebastes carnatus*) and the black-and-yellow rockfish (*S. chrysomelas*) along the California coast. M.S. thesis, 33 p. Univ. California. Santa Cruz, Santa Cruz, CA.

Aoyama, J., S. Watanabe, M. Nishida, and K. Tsukamoto.
2000. Discrimination of catadromous eels of genus *Anguilla* using polymerase chain reaction-restriction fragment length polymorphism analysis of the mitochondrial 16S ribosomal RNA domain. Trans. Am. Fish. Soc. 129:873–878.

Chen, L.-C.
1971. Systematics, variation, distribution, and biology of rockfishes of the subgenus *Sebastomus* (Pisces, Scorpaenidae, *Sebastes*). Bull. Scripps. Inst. Oceaogr. 18:1–115.

Chow, S., M. E. Clarke, and P. J. Walsh.
1993. PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjaninae): a simple method for species and stock identification. Fish. Bull. 91:619–627.

Gharrett, A. J., A. K. Gray, and J. Heifetz.
2001. Identification of rockfish (*Sebastes* spp.) from restriction site analysis of the mitochondrial ND-3/ND-4 and 12S/16S rRNA gene regions. Fish. Bull. 99:49–62.

Hallacher, L. E., and D. A. Roberts.
1985. Differential utilization of space and food by the inshore rockfishes (Scorpaenidae: *Sebastes*) of Carmel Bay, California. Environ. Biol. Fish. 12:91–110.

Hoelzer, G.
1987. The effect of early experience on aggression in two territorial scorpaenid fishes. Environ. Biol. Fish. 19:183–194.
Hunter, K.
1994. Incipient speciation in rockfish (*Sebastes carnatus* and *Sebastes chrysomelas*). M.S. thesis, 38 p. California State Univ. Northridge, Northridge, CA.
Innes, B. H., P. M. Grewe, and R. D. Ward.
1998. PCR-based genetic identification of marlin and other billfish. Mar. Freshw. Res. 49:383–388.
Kendall, A. W., Jr.
2000. An historic review of *Sebastes* taxonomy and systematics. Mar. Fish. Rev. 62:1–23.
Laidig, T. E., and P. B. Adams.
1991. Methods used to identify pelagic juvenile rockfish (genus *Sebastes*) occurring along the coast of central California. NOAA Tech. Memo. NMFS-SWFSC-166, 180 p.
Laidig, T. E., K. M. Sakuma, and M. M. Nishimoto.
1996. Description of pelagic larval and juvenile *Sebastes saxicola* (Family Scorpaenidae), with an examination of larval growth. Fish. Bull. 94:289–299.
Larson, R. J.
1980. Competition, habitat selection, and the bathymetric segregation of two rockfish (*Sebastes*) species. Ecol. Monogr. 50:221–239.
Li, Z.
2004. Phylogenetic relationship and identification of juveniles of the genus *Sebastes*. M.S. thesis, 146 p. Univ. Alaska Fairbanks, Fairbanks, AK.
Li, Z., A. K. Gray, M. S. Love, A. Goto, T. Asahida., and A. J. Gharrett.
2006. A key to selected rockfishes (*Sebastes* spp.) based on mitochondrial DNA restriction fragment analysis. Fish. Bull. 104:182–196.
Lin, Y., Y. Poh, S. Lin, and C. Tzeng.
2002. Molecular techniques to identify freshwater eels: RFLP analyses of PCR-amplified DNA fragments and allele-specific PCR from mitochondrial DNA. Zool. Studies 41:421–430.
Love, M. S.
1996. Probably more than you want to know about the fishes of the Pacific coast, 386 p. Really Big Press, Santa Barbara, CA.
Love, M. S., P. Morris, M. McCrae, and R. Collins.
1990. Life history aspects of 19 rockfish species (Scorpaenidae: *Sebastes*) from the southern California Bight. NOAA Tech. Rep. NMFS-87, 38 p.
Love, M. S., M. Yoklavich, and L. Thorsteinson (eds.).
2002. The rockfishes of the Northeast Pacific, 405 p. Univ. California Press, Berkeley, CA.
Matarese, A. C., A. W. Kendall Jr., D. M. Blood, and B. M. Vinter.
1989. Laboratory guide to early life history stages of northeast Pacific fishes. NOAA Tech. Rep. NMFS Circ. 80, 656 p.
Moser, H. G.
1996. Scorpaenidae: scorpionfishes and rockfishes. *In* The early stages of fishes in the California Current region (H. G. Moser, ed.), p. 793–795. CalCOFI Atlas no. 33.
Moser, H. G., E. H. Ahlstrom, and E. M. Sandknop.
1977. Guide to the identification of scorpionfish larvae (family Scorpaenidae) in the eastern Pacific with comparative notes on species of *Sebastes* and *Helicolenus* from other oceans. NOAA Tech. Rep. NMFS Circ. 402, 71 p.
Narum, S.
2000. Assortatative mating and genetic structure of gopher rockfish (*S. carnatus*) and black-and-yellow rockfish (*S. chrysomelas*): a case of incipient speciation. M.S. thesis, 90 p. Univ. California San Diego, San Diego, CA.
Nishimoto, M. M., and L. Washburn.
2002. Patterns of coastal eddy circulation and abundance of pelagic juvenile fish in the Santa Barbara Channel, California, USA. Mar. Ecol. Prog. Ser. 241:183–199.
Rocha-Olivares, A., R. H. Rosenblatt, and R. D. Vetter.
1999. Molecular evolution, systematics, and zoogeography of the rockfish subgenus *Sebastomus* (*Sebastes*, Scorpaenidae) based on mitochondrial cytochrome *b* and control region sequences. Mol. Phylogenet. Evol. 11:441–458.
Sakuma, K. M., and T. E. Laidig.
1995. Description of larval and pelagic juvenile chilipepper, *Sebastes goodei* (family Scorpaenidae), with an examination of larval growth. Fish. Bull. 93:721–731.
Seeb, L. W.
1986. Biochemical systematics and evolution of the Scorpaenidae genus *Sebastes*. Ph.D. diss., 176 p. Univ. Washington, Seattle, WA.
Seeb, L. W., and A. W. Kendall Jr.
1991. Allozyme polymorphisms permit the identification of larval and juvenile rockfishes of the genus *Sebastes*. Environ. Biol. Fish. 30:191–201.
Taylor, C. A.
1998. Phylogenetics and larval identification of rockfishes (genus *Sebastes*) using mitochondrial DNA techniques. M.S. thesis, 118 p. San Diego State Univ., San Diego, CA.