

The potential use of otolith characters in identifying larval rockfish (*Sebastes* spp.)

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Rockfish of the genus *Sebastes* are commercially important in the northeast Pacific Ocean, where more than 60 valid species are recognized (Eschmeyer et al., 1983). Although morphological and chromatic characters are used routinely to identify adults of the genus, such traits are frequently ineffective for young of the year, especially larvae. Like most fish, early developmental stages of *Sebastes* spp. have less pigmentation, fewer hard structures (e.g. fin rays), and show less differentiation when compared with adults. However, an ability to discriminate among the larvae of the many rockfish species is critical to the advancement of early life history studies in this group.

A variety of methods have been used to identify larval and juvenile rockfish (Kendall, 1991). Although pigmentation is the most frequently used character (Moser et al., 1977; Laroche and Richardson, 1980; Moser et al., 1985; Kendall and Lenarz, 1987), size at extrusion (Moser et al., 1977), meristic counts (Moser et al., 1977), morphometrics (Morris, 1956), size at specific life history events (Stahl-Johnson, 1985), time of parturition in conjunction with geographic location (Moser et al., 1977), and electrophoretic patterns (Seeb and Kendall, 1991) have all been used to identify larval and juvenile rockfish.

A problem with many of these techniques, however, is that larval characters often undergo ontogenetic change. To overcome this problem, the larvae of many species have been reared in captivity and sequentially sacrificed. This is not only technically demanding, expensive, and time consuming, but differences in development between laboratory and wild fish may affect the number, size, and distribution of the attributes under investigation. Thus, permanent identifiable characters would be useful. A static trait, which retained its characteristics throughout early life, would increase our ability to positively identify rockfish larvae. Otoliths, being acellular aragonitic concretions, are good candidates to retain features produced during the larval stage. Likewise, otoliths have been shown to contain sufficient variation among species (Hecht and Appelbaum, 1982; Akkiran, 1985; Victor, 1987) and stocks (Messieh, 1972; Postuma, 1974; McKern et al., 1974; Neilson et al., 1985; Smith, 1992) to assign with accuracy group membership to individuals.

This study investigates the potential of using otolith microstructure to assist in the identification of larval rockfish. We assume that no change occurs to early larval otolith microstructure once it is deposited (Brothers, 1984; Steven-

son and Campana, 1992). Otolith characteristics (nuclear shading patterns, nuclear radius, and first increment width) produced during the early larval period were described and measured from late larval and pelagic juvenile stage specimens of eight species of rockfish: *Sebastes auriculatus*, *S. entomelas*, *S. flavidus*, *S. goodei*, *S. jordani*, *S. mystinus*, *S. paucispinis*, and *S. saxicola*. These species were the most numerous rockfish species collected off the central California coast during the study period.

Methods

Field collections

Samples of young-of-the-year pelagic juveniles and late larvae were collected with a midwater trawl (12 × 12 m) from the National Oceanic and Atmospheric Administration RV *David Starr Jordan*. From 1983 to 1989 nine cruises were conducted off central California (lat. 36°30'–38°10'N) during the months of April–June. Pelagic juvenile rockfish were frozen at sea and returned to the laboratory for final identification. Wyllie Echeverria et al. (1990) have described cruise sampling methodology in detail.

Laboratory procedures

Pelagic juveniles were identified to species from external characteristics, including pigmentation, fin-ray counts, and gill-raker counts (Laidig and Adams, 1991). The sagittal otoliths were removed and affixed whole to microscope slides and were prepared for viewing with the methods outlined in Laidig et al. (1991).

Otoliths were examined with a video image interfaced with a digitizer (Laidig et al., 1991). Distinct reoccurring shading patterns in the

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nucleus (i.e. the otolith core) were noted for each species. Increment counts were made beginning at the first clearly defined mark that completely encircled the primordium (see also Penney and Evans, 1985). This "extrusion" check forms at parturition (Ralston, unpubl. data) and defines the outer edge of the nucleus; the distance from the primordium to this mark is the nuclear radius (Fig. 1), and the increment immediately following the extrusion check is the first growth increment.

Data analysis

The mean radius of the nucleus and the mean width of the first growth increment were compared among species and years. An overall analysis of variance (ANOVA), incorporating separate pairwise *t*-tests, equivalent to Fisher's least-significant difference, was performed to detect differences in the size of the nuclear radius and the width of the first increment among species and years. We used a two-way factorial analysis and calculated least square means (Searle et al., 1980) to evaluate treatment effects among species, years, and the interaction of species and years. A parametric discriminant analysis, assuming a multivariate normal distribution with pooled covariance matrix, was used to determine the percentage of each species that was correctly classi-

fied by nuclear radius and first increment width, alone and in combination with each other.

To further evaluate species-specific differences in nuclear radius and first increment width, the data were pooled over years; however, we recognized the difficulty in isolating the effect of species alone. *Sebastes auriculatus* and *S. saxicola* were only sampled in one year; therefore they were not included in the annual variation analysis. For comparison, we treated these single-year studies as the pooled data for the other species.

A blind test was performed to determine the accuracy of the otolith characters in distinguishing between the eight rockfish species used in this study. One hundred otoliths representing all eight species (*S. auriculatus* [*n*=5]; *S. entomelas* [*n*=13]; *S. flavidus* [*n*=15]; *S. goodei* [*n*=14]; *S. jordani* [*n*=25]; *S. mystinus* [*n*=9]; *S. paucispinis* [*n*=11]; and *S. saxicola* [*n*=8]) were given to a reader. No other information (e.g. species, fish length, etc.) about the individual samples was provided. The reader then attempted to identify the correct species of rockfish, using both measured distances and shading patterns. The results of the tentative classification were compared with the actual species identities to determine percent agreement. The significance of this result was evaluated against a null multinomial distribution, by assuming assignments at random to species.

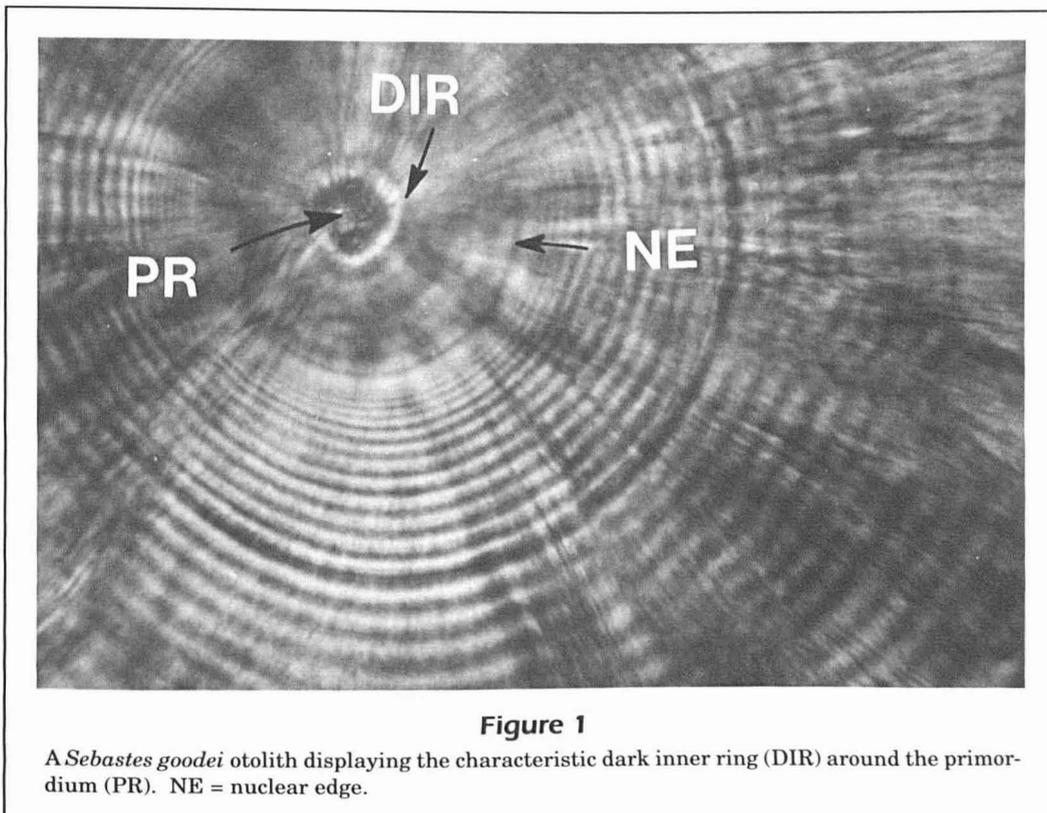


Figure 1

A *Sebastes goodei* otolith displaying the characteristic dark inner ring (DIR) around the primordium (PR). NE = nuclear edge.

Results

Seven specific nuclear shading characters were identified (Table 1): 1) the opacity of the primordium; 2) the opacity of the markings inside the nucleus; 3) the opacity of the increments directly outside the nucleus; 4) the opacity of the nuclear edge; 5) the existence of a dark inner band near the nuclear edge; 6) the existence of a light inner band near the nuclear edge; and 7) the existence of a dark inner ring encircling the primordium.

In some cases, combinations of the shading patterns were sufficient to characterize a species. For example, 84% of the otoliths of *S. goodei* were found to have a dark primordium, a dark nuclear edge, and a dark inner ring surrounding the primordium, while this combination was never found in the other species examined (Fig. 1). Likewise, in *S. jordani*, a light penumbra was regularly found (76% occurrence) adjacent to the inner edge of the nucleus, along with a dark primordium and many inner rings (faint microstructure occurring inside the nucleus). In *S. paucispinis* and *S. flavidus*, there was usually a dark inner band next to the edge of the nucleus (87% and 73% occurrence, respectively). No annual variation was observed for the nuclear shading patterns. However, in the remaining species, the shading patterns were too variable to establish consistent identifiable character states that would distinguish species.

Annual variations in nuclear radius and the width of the first increment were examined (Fig. 2). Annual variation in nuclear radius among the species was not significant; however, annual variability in the width of the first increment was significant ($P < 0.05$). In addition, there was a significant interaction ($P < 0.05$) between year and species. The mean width of the first increment of *S. paucispinis*, for

example, declined from 1984 to 1989, whereas that of *S. flavidus* increased (Fig. 2A).

Sebastes jordani was found to have the largest average nuclear radius (Table 2; Fig. 2B). The rank order for the remaining species, from largest to smallest average nuclear radii, was *S. goodei*, *S. auriculatus*, *S. paucispinis*, *S. flavidus*, *S. entomelas*, *S. saxicola*, and *S. mystinus*. Individually, the nuclear radii of *S. jordani*, *S. goodei*, *S. auriculatus*, and *S. mystinus* were significantly different ($P < 0.05$) from all other species and from each other.

Sebastes jordani was also found to have the largest average first increment of all species studied (Table 2; Fig. 2A). The rank order of the other species, from largest to smallest average widths of the first increment, was *S. paucispinis*, *S. goodei*, *S. auriculatus*, *S. saxicola*, *S. flavidus*, *S. entomelas*, and *S. mystinus*. *Sebastes jordani* and *S. paucispinis* had significantly larger average first increment widths ($P < 0.05$) than all other species studied (0.97 μm and 0.91 μm , respectively) (Table 2).

A discriminant analysis was performed on the classification of species by using nuclear radius and the width of the first increment as predictor variables (Table 3). *Sebastes goodei*, *S. jordani*, *S. mystinus*, and *S. paucispinis* were correctly identified from 57 to 83% of the time; *Sebastes auriculatus* and *S. flavidus* were classified correctly 33.9% and 41.8% of the time, respectively. Although these values are less than 50% correct, they represent the largest single classification for each species. Two species, *S. entomelas* and *S. saxicola*, were not often classified correctly (9.5% and 0%, respectively).

In a blind test, the reader correctly classified 70 of the 100 otoliths (70% correct; Table 4), demonstrating that otoliths provide useful information in species identification ($P < 0.001$). Greater than 90% of *Sebastes*

Table 1

Observed shading patterns of otolith nuclei for the eight species studied. An F means the attribute was faint, a D means it was dark, and a blank means that it was either variable or did not exist. For the last three attributes, an X means the character was present. aur = *S. auriculatus*, ent = *S. entomelas*, fla = *S. flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, pau = *S. paucispinis*, and sax = *S. saxicola*.

Attribute	Species							
	aur	ent	fla	goo	jor	mys	pau	sax
Primordium opacity	D	F	F	D	F	D	D	D
Inner ring opacity	F	F		D	F	F	F	
Outer increment opacity	F			D		F		
Nuclear edge opacity	F	F		D		D		
Dark inner band			X				X	
Light inner band			X		X			
Dark primordial ring				X				

goodei and *S. paucispinis* were correctly classified, whereas the remaining species showed lower accuracy varying from 56 to 68%.

Discussion

Otolith characteristics have been shown to vary among species and among stocks. Rybock et al. (1975) and Postuma (1974) used nuclear dimensions to identify different fish stocks. McKern et al. (1974) used otolith dimensions to separate seasonal stocks of steelhead trout, and Victor (1987) used otolith dimensions to separate different species of pomacentrids and labrids. Postuma (1974) related otolith opacity to nuclear size and compared this relationship between stocks. Messieh (1972) used otolith shape to distinguish among stocks of herring. Hecht and Appelbaum (1982) and Gago (1993) also used otolith shape to distinguish between species.

We have found that otolith characteristics can effectively distinguish certain species of *Sebastes*. Four of the species examined, *S. jordani*, *S. goodei*, *S. auri-*

culatus, and *S. mystinus*, had significantly different nuclear radii from each other and from the other species examined. *Sebastes jordani*, *S. goodei*, *S. paucispinis*, and *S. flavidus* each had unique shading patterns that may help in species identifications. *Sebastes jordani* and *S. paucispinis* were correctly classified over 90% of the time in the blind test, displaying the usefulness of otolith characteristics for identification.

The specificity of otolith characters gives researchers an opportunity to separate larvae using these characters alone. Without otolith data, the separation of *S. mystinus* larvae from other rockfish larvae is difficult. Although larval *S. jordani* are relatively easy to identify on the basis of pigmentation (Moser et al., 1977), identifications can be confirmed with a few additional otolith measurements.

We suggest from these findings that the difficult task of identifying rockfish larvae can be facilitated in some cases by employing otolith characters in combination with more traditional traits like pigmentation. Of the eight species examined, six species (*S. auriculatus*, *S. flavidus*, *S. goodei*, *S. jordani*, *S. mystinus*, and *S. paucispinis*) had distinctive otolith characters that allowed separation from other species. Many of the larval stages of the more than 60 species of rockfish found in the northeast Pacific Ocean are very similar, and pigmentation alone cannot always reliably separate species. Otolith character examination may be one further method that can aid researchers in accurately identifying species in this group.

Table 2

Mean nuclear radius and first increment width of otoliths (all years combined) of *Sebastes*. SD = standard deviation. n = sample size.

Species	n	Nuclear radius (μm)	SD	First increment (μm)	SD
<i>S. auriculatus</i>	56	14.07	1.48	0.82	0.20
<i>S. entomelas</i>	147	11.81	0.94	0.72	0.13
<i>S. flavidus</i>	122	12.09	0.69	0.72	0.16
<i>S. goodei</i>	230	15.15	0.89	0.85	0.17
<i>S. jordani</i>	541	16.96	0.99	0.97	0.23
<i>S. mystinus</i>	106	10.93	1.05	0.65	0.14
<i>S. paucispinis</i>	277	12.20	1.14	0.91	0.18
<i>S. saxicola</i>	12	11.58	0.90	0.73	0.23

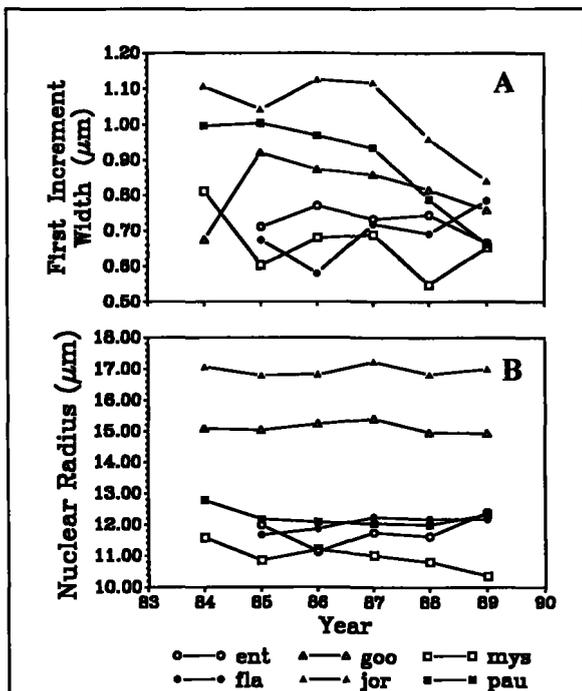


Figure 2

Annual variation in average nuclear radius and first increment width for the six species of *Sebastes* that had multiple year observations. ent = *S. entomelas*, fla = *S. flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, and pau = *S. paucispinis*.

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Table 3

Discriminant analysis for nuclear radius and first increment width of otoliths for all years combined showing percent classified as each species of *Sebastes*. aur = *S. auriculatus*, ent = *S. entomelas*, fla = *S. flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, pau = *S. paucispinis*, and sax = *S. saxicola*.

Species	Classification							
	aur	ent	fla	goo	jor	mys	pau	sax
aur	33.9	0.0	17.9	23.2	10.7	0.0	12.5	1.8
ent	1.4	9.5	38.8	1.4	0.0	22.5	12.9	13.6
fla	5.7	14.8	41.8	0.0	0.0	11.5	18.9	7.4
goo	23.5	0.0	0.9	59.6	14.4	0.0	1.7	0.0
jor	0.9	0.0	0.0	15.9	83.2	0.0	0.0	0.0
mys	0.9	4.7	6.6	0.0	0.9	72.6	6.6	7.6
pau	7.6	2.5	13.7	4.3	0.7	6.5	57.4	7.2
sax	0.0	8.3	25.0	0.0	0.0	33.3	33.3	0.0

Table 4

Results of blind test showing classification rates of *Sebastes* by species category. Bold numbers along the diagonal indicate correct classifications. aur = *S. auriculatus*, ent = *S. entomelas*, fla = *S. flavidus*, goo = *S. goodei*, jor = *S. jordani*, mys = *S. mystinus*, pau = *S. paucispinis*, and sax = *S. saxicola*.

Actual	Classified as:								% Correct
	AUR	ENT	FLA	GOO	JOR	MYS	PAU	SAX	
AUR	3	2							60%
ENT	2	8			1	2			61%
FLA		1	9			1	2	1	60%
GOO	1			13					92%
JOR	2			1	17	3	1	1	68%
MYS		1	1	1		5		1	56%
PAU							10	1	91%
SAX		2					1	5	63%

Literature cited

- Akkiran, N.**
1985. A systematic study on Carangidae (Pisces) employing the otolith characters in the eastern Mediterranean. Bilj. Notes Inst. Oceanogr. Ribar., Split. 63:1-9.
- Brothers, E. B.**
1984. Otolith Studies. In W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, and S. L. Richardson (eds.), Ontogeny and systematics of fishes, p. 50-57. Spec. Publ. 1, Amer. Soc. Ich. Herp. Allen Press, Inc., Lawrence, KS.
- Eschmeyer, W. N., E. S. Herald, and H. Hammann.**
1983. A field guide to Pacific coast fishes of North America. Houghton Mifflin Co., Boston, MA, 336 p.
- Gago, F. J.**
1993. Morphology of the saccular otoliths of six species of lanternfishes of the genus *Symbolophorus* (Pisces: Myctophidae). Bull. Mar. Sci. 52(3):949-960.
- Hecht, T., and S. Appelbaum.**
1982. Morphology and taxonomic significance of the otoliths of some bathypelagic Anguilloidei and Saccopharyngoidei from the Sargasso Sea. Helgolander Meeresunters 35:301-308.
- Kendall, A. W., Jr.**
1991. Systematics and identification of larvae and juveniles of the genus *Sebastes*. Environ. Biol. Fishes 30:173-190.
- Kendall, A. W., Jr., and W. H. Lenarz.**
1987. Status of early life history studies of northeast Pacific rockfishes. In Proceedings of the international rockfish symposium; October, 1986, Anchorage, Alaska, p. 99-128. Univ. Alaska, Alaska Sea Grant Rep. 87-2.
- Laidig, T. W., and P. B. Adams (eds.).**
1991. Methods used to identify pelagic juvenile rockfish (genus *Sebastes*) occurring along the coast of central California. U.S. Dep. Commer., NOAA Tech. Memo., NOAA-TM- NMFS-SWFSC-166, 180 p.

- Laidig, T. E., S. Ralston, and J. R. Bence.**
1991. Dynamics of growth in the early life history of shortbelly rockfish, *Sebastes jordani*. Fish. Bull. 89:611-621.
- Laroche, W. A., and S. L. Richardson.**
1980. Development and occurrence of larvae and juveniles of the rockfishes *Sebastes flavidus* and *Sebastes melanops* (Scorpaenidae) off Oregon. Fish. Bull. 77:901-923.
- McKern, J. L., H. F. Horton, and K. V. Koski.**
1974. Development of steelhead trout (*Salmo gairdneri*) otoliths and their use for age analysis and for separating summer from winter races and wild from hatchery stocks. J. Fish. Res. Board Can. 31:1420-1426.
- Messieh, S. N.**
1972. Use of otoliths in identifying herring stocks in the southern Gulf of St. Lawrence and adjacent waters. J. Fish. Res. Board Can. 29:1113-1118.
- Morris, R. W.**
1956. Early larvae of four species of rockfish, *Sebastes*. Calif. Fish Game 42:149-153.
- 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. U.S. Dep. Commer., NOAA Tech. Rep. NMFS Circ. 402, 71 p.
- Moser, H. G., E. M. Sandknop, and D. A. Ambrose.**
1985. Larvae and juveniles of aurora rockfish, *Sebastes aurora*, from off California and Baja California. In Descriptions of early life history stages of selected fishes: the 3rd international symposium on the early life history of fishes and 8th annual larval conference, p. 55-64. Can. Tech. Rep. Fish. Aquat. Sci. 1359, 82 p.
- Neilson, J. D., G. H. Geen, and B. Chan.**
1985. Variability in dimensions of salmonid otolith nuclei: implications for stock identification and microstructure interpretation. Fish. Bull. 83:81-89.
- Penney, R. W., and G. T. Evans.**
1985. Growth histories of larval redfish (*Sebastes* spp.) on an offshore Atlantic fishing bank determined by otolith increment analysis. Can. J. Fish. Aquatic Sci. 42:1452-1464.
- Postuma, K. H.**
1974. The nucleus of the herring otolith as a racial character. J. Cons. Int. Explor. Mer 35:121-129.
- Rybock, J. T., H. F. Horton, and J. L. Fessler.**
1975. Use of otoliths to separate juvenile steelhead trout from juvenile rainbow trout. Fish. Bull. 73:654-659.
- Searle, S. R., F. M. Speed, and G. A. Milliken.**
1980. Population marginal means in the linear model: an alternative to least squares means. Am. Statistician 34:216-221.
- Seeb, L. W., and A. W. Kendall Jr.**
1991. Allozyme polymorphism permit the identification of larval and juvenile rockfishes of the genus *Sebastes*. Environ. Biol. Fishes 30:191-201.
- Smith, M. K.**
1992. Regional differences in otolith morphology of the deep slope red snapper *Etelis carbunculus*. Can. J. Fish. Aquat. Sci. 49:795-804.
- Stahl-Johnson, K. L.**
1985. Descriptive characteristics of reared *Sebastes caurinus* and *S. auriculatus* larvae. In Descriptions of early life history stages of selected fishes: the 3rd international symposium on the early life history of fishes and 8th annual larval fish conference, p. 65-76. Can. Tech. Rep. Fish. Aquat. Sci. 1359.
- Stevenson, D. K., and S. E. Campana (eds.).**
1992. Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci. 117, 126 p.
- Victor, B. C.**
1987. Growth, dispersal, and identification of planktonic labrid and pomacentrid reef-fish larvae in the eastern Pacific Ocean. Mar. Biol. 95:145-152.
- Wyllie Echeverria, T., W. H. Lenarz, and C. Reilly.**
1990. Survey of the abundance and distribution of pelagic young-of-the-year rockfishes, *Sebastes*, off central California. U.S. Dep. Commer., NOAA NMFS Tech. Memo. NOAA-TM-NMFS-SWFC-147, 125 p.