UTILITY OF LARVAL PIGMENTATION TO IDENTIFY NEARSHORE ROCKFISHES OF THE *SEBASTES* SUBGENUS *PTEROPODUS* FROM SOUTHERN CALIFORNIA

CYNTHIA A. TAYLOR[1]
Scripps Institution of Oceanography
University of California, San Diego
9500 Gilman Drive
La Jolla, California 92093-0203
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, California 92037-1508
ctaylor@illumina.com

WILLIAM WATSON
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, California 92037-1508

ABSTRACT

Unidentified larval rockfishes, *Sebastes* spp., were sorted from ichthyoplankton collected during the April 1999 CalCOFI cruise and categorized according to their similarity to the pigmentation typical of larval *Pteropodus*, a subgenus of nearshore rockfishes heavily targeted in the California live-fish fishery. The 62 larvae, ranging in size from 3.5 to 11.2 mm, were identified by molecular genetic methods to test the hypothesis that this pigment-based classification technique can reliably distinguish *Pteropodus* from all other *Sebastes* larvae. All larval *Pteropodus* were correctly classified, and only 5% of the non-*Pteropodus* were misclassified as *Pteropodus*, but there were so few *Pteropodus* in the CalCOFI plankton samples that the number of misclassified larvae was larger than the number of *Pteropodus* larvae. Larval *Pteropodus* may remain close to the shallow-water adult habitat shoreward and closer to islands than most CalCOFI stations. Ichthyoplankton sampling near shore (within the 200 m depth contour) would be required to examine this hypothesis, to provide the samples required for a better test of the pigment-based classification method, and, perhaps ultimately, to provide fishery-independent data on population trends in *Pteropodus.*

INTRODUCTION

A primary source of fishery-independent data used in current fisheries assessments is the ichthyoplankton collected during California Cooperative Oceanic Fisheries Investigations (CalCOFI) biological/oceanographic surveys off southern California. Larval rockfishes of the genus *Sebastes* are common and abundant in CalCOFI plankton collections (Moser et al. 2000). These larvae may represent more than 50 species in the Southern California Bight (SCB), most of which are targeted in commercial and sport fisheries (Miller and Lea 1972; Eschmeyer et al. 1983). However, only seven *Sebastes* species are unambiguously identifiable from CalCOFI samples by means of pigmentation and morphological criteria (Moser 1996; Moser et al 2000). Ichthyoplankton data for three of these—bocaccio (*Sebastes paucispinis*), cowcod (*S. levis*), and shortbelly rockfish (*S. jordani*)—have been used in recent assessments (Butler et al. 1999, 2003; MacCall et al. 1999; Ralston et al. 2003).

Attempts have been made to classify larval *Sebastes* into subgeneric or other categories based on larval pigmentation (e.g., Kendall and Lenarz 1987; Kendall 1991; Kendall and Gray 2001), but we have not found these classifications useful for routine analysis of ichthyoplankton samples. The CalCOFI collections potentially provide a rich source of data for constructing temporal abundance trends for the unidentified *Sebastes* species, but when larvae cannot be identified to species, their abundance trends are not available to use for indices of adult biomass.

We focus on nearshore rockfishes generally considered to belong to the *Sebastes* subgenus *Pteropodus*: grass (*Sebastes rastrelliger*), black-and-yellow (*S. chrysomelas*), gopher (*S. carnatus*), copper (*S. caurinus*), quillback (*S. maliger*; rare in southern California), China (*S. nebulosus*; rare in southern California), calico (*S. dallii*), brown (*S. auriculatus*), and kelp (*S. atrovirens*) rockfishes (Taylor 1998; Kendall 2000). This nearshore group is targeted by the live-finfish fishery that developed off California in the mid-1980s (Walters 2001). We chose this complex because reliable abundance estimates are lacking and because the larvae share pigmentation features that might be unique to the group. This could provide a means to reliably select these larvae for later molecular identification to species for use in fishery-independent abundance estimates. Alternatively, an abundance estimate may be developed for the subgenus as a whole.

We sorted possible *Pteropodus* larvae from other *Sebastes* species in CalCOFI collections on the basis of their pigmentation characters, and used molecular genetic methods to test the hypothesis that a pigment-based sorting technique can reliably distinguish *Pteropodus* from all other *Sebastes* larvae. Molecular genetic data, a constant at all life-history stages, provides a method by which we may assign species identifications to larvae by comparisons with known adult reference data.

[Manuscript received 2 February 2004.]

[1]Current address: 9885 Towne Center Drive, San Diego, CA 92121-1975

MATERIALS AND METHODS

Oblique bongo net tows through the upper 212 m of the water column (from 15 m above the bottom in shallower water) are routinely collected according to standard protocols (Kramer et al. 1972; Ohman and Smith 1995) during quarterly CalCOFI cruises. The sample from one side of each bongo cast is fixed in 5% neutral buffered formalin and, beginning in 1997, the sample from the other net has been fixed and preserved in 5% tris-buffered ethanol, which is changed within 24 hours after fixation. For this study, we used samples collected during the April 1999 CalCOFI cruise (fig. 1), a time when larval *Sebastes* were abundant (Ambrose et al. 2001).

All fish larvae were sorted from the macrozooplankton in the laboratory and subsequently identified to the lowest possible taxon. For the ethanol-preserved samples, *Pteropodus*-like larvae were sorted from other unidentified *Sebastes* according to pigmentation characters. Other characters that may be helpful in conjunction with pigmentation include the relatively slender body and relatively large size at parturition (~ 4.5 to 5.5 mm) of the *Pteropodus* larvae (e.g., Watson and Robertson, 2004). In addition to the pigmentation of the head and gut through most or all of larval development that is common to most *Sebastes* species, larval *Pteropodus* share a common pigment pattern (fig. 2), the main elements of which are (1) a long melanophore row along the dorsal margin of the tail, commonly of about 5–30 melanophores between about myomeres 9–23, often extending forward onto the trunk; (2) a long melanophore row along the ventral margin of the tail, commonly of about 30–60 melanophores, usually originating at the last preanal or first postanal myomere and extending to myomere 23–25; and (3) little or no pigment on the pectoral fins (Watson and Robertson, 2004). For purposes of larval classification, this pattern is most useful for preflexion and flexion stages; pigmentation of postflexion-stage larvae converges progressively on a pattern shared with many other species (e.g., Watson and Robertson, 2004). Nevertheless, because ~ 90% of the *Sebastes* larvae collected in standard plankton tows off California are preflexion stage (Moser 1996), the method is potentially valuable.

In total, 298 unidentified *Sebastes* larvae were sorted from the samples; 121 of these were identified as possible *Pteropodus*. Details of pigment were noted for 62 of the 121 larvae that were in good condition, and they were placed into the following categories: (1) *Pteropodus*; (2) probable *Pteropodus*; (3) possible *Pteropodus*; and (4) probable non-*Pteropodus*. Larvae classified as *Pteropodus* displayed the exact *Pteropodus* pigment pattern; the other categories deviated progressively from that pattern. The 62 selected larvae ranged from 3.5 to 11.2 mm: 71% were preflexion stage (3.5 to 7.1 mm), 23% were flexion stage (6.8 to 8.3 mm), and 6% were postflexion stage (9.5 to 11.2 mm). The remaining 59 possible *Pteropodus* larvae (98% preflexion stage) generally were in poorer condition; they were not classified further, and details of their individual pigment patterns were not recorded. We focus here on the 62 larvae examined in detail.

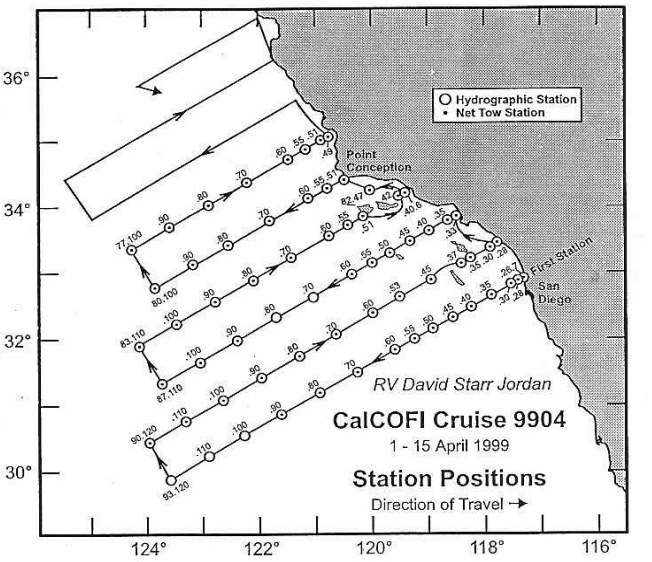

Figure 1. Cruise track and stations occupied during CalCOFI cruise 9904, 1–15 April 1999. Circles indicate standard CalCOFI stations; dots indicate net tow stations on cruise 9904 (from Ambrose et al. 2001).

Genomic DNA was extracted from tail or muscle tissue of larvae by means of a chelex extraction protocol (Walsh et al. 1991). We used PCR to amplify mtDNA in a 1× buffer containing 20 mM Tris HCl, 50 mM KCl, and 1.5 mM $MgCl_2$ with 0.3 μM of each primer. Primers included published (GluRF and CB3RF, Rocha-Olivares et al. 1999) and internal custom primers (CB306F 5'-TTACTACGGCTCVTACCT-3, Cb521R 5'-GTTG CATTGTCTACTGAG-3', and CB364F, 5'-CTAGT TATAATAACTGCTTT-3'). We used Qiaquick kits (Qiagen, Inc.) to clean PCR products and cycle-sequenced them according to manufacturer protocols with an ABI 3100 automated sequencer. Chromatogram data for sequenced DNA were aligned by means of the biosequence analysis and editor program Sequencher (ver. 4.1.1, Gene Codes, Inc.).

Larval sequences were compared to DNA reference sequence data of identified adult *Sebastes* found in the Southern California Bight by means of an iterative approach within the software program Phylogenetic Analysis Using Parsimony (PAUP* 4b10; Swofford 2000), with the optimality criterion set to distance. Adult sequences in the PAUP file included only species known to be limited to the northeastern Pacific. We used nonparametric bootstrapping (100 replications, MAXTREES set to 1000) to cluster the unknown larval haplotype within a database of consensus haplotypes (consensus =

TABLE 1
Molecular Identification of the 62 *Sebastes* Larvae Classified as Potential *Pteropodus* Candidates

	Classification			
Identification	*Pteropodus*	Probable *Pteropodus*	Possible *Pteropodus*	Probable non-*Pteropodus*
S. carnatus	1			
S. caurinus	1			
S. hopkinsi	2	16	25	8
S. ovalis		1		2
S. rufus			1	
S. saxicola	1		3	
S. semicinctus		1		
Total	5	18	29	10

most common of intraspecific haplotypes available) from known adults for putative identification for 384 haplotypes representing 65 species of *Sebastes* found in the northeast Pacific (voucher specimens maintained at NOAA Fisheries, La Jolla Laboratory). If a larva clustered with a single reference haplotype with a bootstrap value ≥ 90%, this was accepted as the identification of the larva. If a larva clustered with a single haplotype with a bootstrap value < 90%, this was accepted as a first-pass identification, and a secondary analysis that included all available haplotypes of the three nearest (in uncorrected "p") species to the unknown larval haplotype was performed.

RESULTS

Among the 62 *Sebastes* larvae, only five (8%) displayed the full *Pteropodus* pigment pattern (tab. 1). Another 18 larvae (29%) classified as "probable *Pteropodus*" displayed pigmentation very much like the *Pteropodus* pattern, except that in all cases the melanophore series on the ventral margin of the tail originated at the second to fourth postanal myomere (commonly the second or third) rather than the first. The 29 "possible *Pteropodus*" (47%) all had the moderately long to long *Pteropodus*-like row of melanophores on the dorsal margin, but the ventral series on the tail originated at postanal myomere 3-7 (commonly 4-6). The ten (16%) "probable non-*Pteropodus*" had ventral pigmentation like the "possible *Pteropodus*," and either a shorter dorsal melanophore series or a predominantly double row of melanophores on the dorsum, in contrast to the long, predominantly single row typical of *Pteropodus* larvae.

Molecular identification revealed that among the 62 larvae, only two were *Pteropodus*: one each of *S. carnatus* and *S. caurinus*, both correctly classified (tab. 1). Two of the remaining three larvae classified as "*Pteropodus*" were *S. hopkinsi*, and the third was *S. saxicola*. Among the "probable *Pteropodus*," 16 (88%) were *S. hopkinsi*, and there was one each of *S. ovalis*, and *S. semicinctus*.

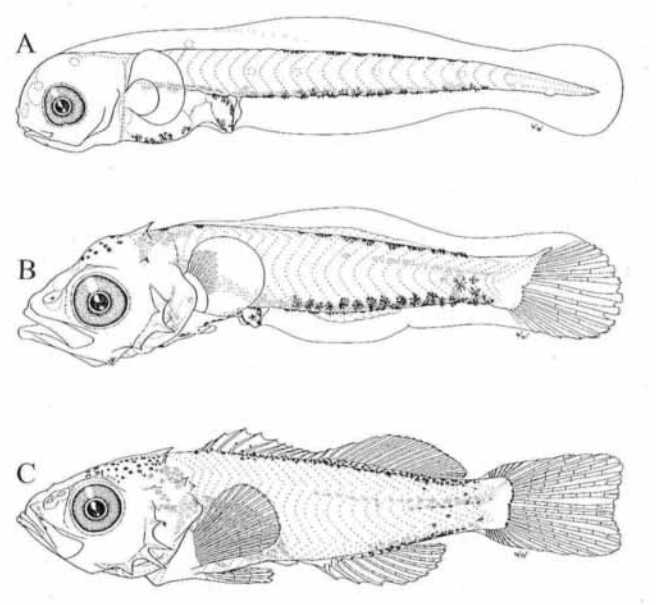

Figure 2. Larval *Sebastes atrovirens* showing an example of the typical *Pteropodus* larval pigment pattern. *A*, preflexion stage, 4.6 mm; *B*, flexion stage, 8.0 mm; *C*, postflexion stage, 14.6 mm (from Watson and Robertson, 2004).

The "possible *Pteropodus*" also were predominantly *S. hopkinsi* (25 larvae: 86%); three larvae (10%) were *S. saxicola* and one (3%) was *S. rufus*. The "probable non-*Pteropodus*" likewise were mostly *S. hopkinsi* (80%); two larvae (20%) were *S. ovalis*. Thus, among the 62 *Pteropodus* candidates, two (3%) actually were *Pteropodus* larvae, 51 (82%) were *S. hopkinsi*, four (6%) were *S. saxicola*, three (5%) were *S. ovalis*, and one each (2%) were *S. rufus* and *S. semicinctus*.

DISCUSSION

This experiment demonstrates the value of molecular identification for validating identifications based on visual methods. The experiment also demonstrates that the characteristic *Pteropodus* pigmentation pattern should be strictly adhered to in attempting to classify larvae, because some other species (e.g., *S. hopkinsi*) have pigmentation patterns much like the *Pteropodus* pattern. These other species differ from the *Pteropodus* pattern primarily in lacking ventral pigment on the first 1–7 postanal myomeres, whereas larvae of most *Pteropodus* species typically have ventral pigment on those myomeres through at least flexion stage. For example, in laboratory-reared *S. atrovirens*, *S. caurinus*, *S. chrysomelas*, and *S. rastrelliger*, all larvae had ventral pigment at the first postanal myomere through flexion stage, although in laboratory-reared *S. auriculatus* only 26% had pigment on that myomere (ventral pigment originated at the second postanal myomere in the remaining 74%; Watson and Robertson, 2004). Thus strict adherence to the typical *Pteropodus* pattern in order to minimize misidentification of non-

Pteropodus larvae may result in some underestimation of the number of *Pteropodus* larvae.

Owing to the very small number of *Pteropodus* collected during the April 1999 CalCOFI cruise (the 59 potential *Pteropodus* not examined in detail also included two *Pteropodus*, both *S. atrovirens*; there were none among the 177 larvae not considered possible *Pteropodus*), it is difficult to evaluate the pigment-based classification. Nevertheless, one might argue that the classification was successful in that both *Pteropodus* larvae among the group of 62 were correctly identified, and only 5% of the non-*Pteropodus* were misclassified as *Pteropodus*. However, because so few *Pteropodus* were collected, the small number of misclassified larvae actually represents 60% of the total classified as *Pteropodus*. If this result is typical, the method clearly would not yield data useful for management purposes based on CalCOFI sampling. Molecular identification of additional *Pteropodus* larvae, or laboratory rearing of multiple broods, is needed to define the range in pigment variation for each species.

Parturition of larval *Pteropodus* is primarily in late winter-spring (e.g., Wyllie Echeverria 1987). If the *Sebastes* species composition in the April 1999 CalCOFI plankton samples is typical for the time of year in the SCB (the La Niña event at that time should not have adversely affected parturition), then larval *Pteropodus* must be rare off southern California as far seaward from adult habitat as the nearest CalCOFI stations. Observations on laboratory-reared *S. atrovirens*, *S. auriculatus*, *S. caurinus*, *S. chrysomelas*, and *S. rastrelliger* suggest that larval *Pteropodus* may prefer to remain near the edges of structures (in this case, primarily the edges of 500-1,700 L holding tanks and 5-19 L rearing containers), and most apparently are capable of doing so from birth even in modest currents (in contrast, laboratory-reared larvae of deepwater species such as *S. constellatus* show no affinity for the edges of the rearing containers) (pers. obs., W.W., July 1999). If this behavior also occurs in the field, most larval *Pteropodus* may not range far from adult habitat. Marliave (1986) showed that the larvae of several rocky intertidal fish species are able to maintain position inshore, near rocky habitat, from hatching through settlement. One of the two larval *Pteropodus* among the group of 62, a post-flexion-stage *S. caurinus*, was collected inshore (CalCOFI station 93.28) near adult habitat; the other, a flexion-stage *S. carnatus*, was collected in deep water off Catalina Island (CalCOFI station 90.45). Both *S. atrovirens* in the group of 59 possible *Pteropodus* larvae were collected off Santa Rosa Island (CalCOFI station 83.55).

Morphological and more recent population genetic studies of *S. atrovirens*, a kelp forest associated species within *Pteropodus* suggest a clinal (Love and Larson 1978) or stepping-stone pattern of gene flow and an average dispersal distance of less than 20 km along the coast and at the northern Channel Islands.[2] Ichthyoplankton sampling near shore (within the 200 m depth contour), where the principal adult habitat is located, would be required to determine the absolute extent of pelagic dispersal for these species. If they are limited to the nearshore zone, nearshore sampling would be required to provide samples to adequately test the pigment-based classification method and perhaps, ultimately, to provide fishery-independent data on population trends in *Pteropodus*.

ACKNOWLEDGMENTS

Samples were obtained through CalCOFI in cooperation with the California Department of Fish and Game, the Scripps Institution of Oceanography, colleagues in NOAA Fisheries, and scientists and crew on the NOAA RV *David Starr Jordan*. We are especially indebted to R. Vetter and J. Hunter for their support of this work; to A. Hays, D. Griffith, and R. Charter, for their assistance in obtaining samples and data to support this work; and to J. Hyde for his valuable feedback in the laboratory. We acknowledge D. Ambrose, S. Charter, and H. G. Moser for their time and thoughtful comments on earlier drafts of this manuscript. We also wish to thank the Oceanids Foundation and the Frieda Daum Urey scholarship committee for their generous endowment to CAT. The genetic data collection was funded through NOAA Fisheries base funds and support was provided through the Biological Oceanography Fellowship at Scripps Institution of Oceanography.

LITERATURE CITED

Ambrose, D. A., R. L. Charter, and H. G. Moser. 2001. Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations survey cruises in 1999. NOAA Tech. Memo. NOAA-TM-NMFS-SWFSC-311, 69 pp.

Butler, J. L., L. D. Jacobson, J. T. Barnes, and H. G. Moser. 1999. Stock assessment of cowcod. *In* Appendix to the status of the Pacific coast groundfish fishery through 1999 and recommended acceptable biological catches for 2000. Pacific Fishery Management Council, 2130 SW Fifth Avenue, Suite 224, Portland, Ore. 97201.

———. 2003. Biology and population dynamics of cowcod rockfish (*Sebastes levis*) in the Southern California Bight. Fish. Bull. 101:260–280.

Eschmeyer, W. N., E. S. Herald, and H. Hammann. 1983. A field guide to Pacific Coast fishes of North America. Houghton Mifflin, Boston, 336 pp.

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 A. K. Gray. 2000. An historical review of *Sebastes* taxonomy and systematics. Mar. Fish. Rev. 62 (2):1–15.

———. 2001. A comparison of genetic identifications and pigment patterns of *Sebastes* larvae caught on NOAA ship *John N. Cobb* cruise 9809. U. S. Dep. Commer., Nat. Mar. Fish. Serv., Alaska Fish. Sci. Center, AFSC Processed Rep. 2001–2002, 31 pp.

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. Univ. of Alaska Sea Grant Prog., pp. 99–128.

[2] C. Taylor and R. Vetter, Gene flow of coastal and island populations of California kelp rockfish (*Sebastes atrovirens*) with implications for the design of marine protected areas (manuscript).

Kramer, D., M. Kalin, E. G. Stevens, J. R. Thrailkill, and J. R. Zweifel. 1972. Collecting and processing data on fish eggs and larvae in the California Current region. NOAA Tech. Rep. NMFS Circ. 370, 38 pp.

Love, M., and R. Larson. 1978. Geographic variation in the occurrence of tympanic spines and possible genetic differentiation in the kelp rockfish (*Sebastes atrovirens*). Copeia 1978:53-59.

MacCall, A. D., S. Ralston, D. Pearson, and E. Williams. 1999. Status of the bocaccio off California in 1999 and outlook for the next millennium. *In* Appendix to the status of the Pacific Coast groundfish fishery through 1999 and recommended acceptable biological catches for 2000. Pacific Fisheries Management Council, 2130 SW Fifth Avenue, Suite 224, Portland, Ore. 97201.

Marliave, J. B. 1986. Lack of planktonic dispersal of rocky intertidal fish larvae. Trans. Am. Fish. Soc. 115:149–154.

Miller, D. J., and R. N. Lea. 1972. Guide to the coastal marine fishes of California. Calif. Dep. Fish. Game Fish. Bull. 157, 235 pp.

Moser, H. G. 1996. Scorpaenidae: scorpionfishes and rockfishes. *In* The early stages of fishes in the California Current region, H. G. Moser, ed. Calif. Coop. Oceanic Fish. Invest. Atlas 33:733–795.

Moser, H. G., R. L. Charter, W. Watson, D. A. Ambrose, J. L. Butler, S. R. Charter, and E. M. Sandknop. 2000. Abundance and distribution of rockfish (*Sebastes*) larvae in the Southern California Bight in relation to environmental conditions and fishery exploitation. Calif. Coop. Oceanic Fish. Invest. Rep. 41:132–147.

Ohman, M. D., and P. E. Smith. 1995. A comparison of zooplankton sampling methods in the CalCOFI time series. Calif. Coop. Oceanic Fish. Invest. Rep. 36:153–158.

Ralston, S. J., R. Bence, M. B. Eldridge, and W. Lenarz. 2003. An approach to estimating rockfish biomass based on larval production, with an application to *Sebastes jordani*. Fish. Bull. 101:129–146.

Rocha-Olivares, A., R. 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. Phylog. Evol. 11 (3):441–458.

Swofford, D. L. 2000. PAUP*, Phylogenetic Analysis Using Parsimony (* and other methods), Version 4, Sinauer Associates, Sunderland, Mass.

Taylor, C. A. 1998. Phylogenetics and identification of rockfishes (genus *Sebastes*) using mitochondrial DNA techniques. Master's thesis, Biological Sciences, San Diego State University, 113 pp.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513.

Walters, K. 2001. Live fishes. *In* Review of some California fisheries for 1999: market squid, Dungeness crab, sea urchin, prawn, abalone, Pacific sardine, Pacific herring, Pacific mackerel, reduction, white seabass, and recreational, L. Rogers-Bennett, ed. Calif. Coop. Oceanic Fish. Invest. Rep. 41:22–24.

Watson, W., and R. L. Robertson. 2004. Development of kelp rockfish, *Sebastes atrovirens* (Jordan and Gilbert 1880), and brown rockfish, *S. auriculatus* (Girard 1854), from birth to pelagic juvenile stage, with notes on early larval development of black-and-yellow rockfish, *S. chrysomelas* (Jordan and Gilbert 1880), reared in the laboratory (Pisces: Sebastidae). NOAA Prof. Pap. NMFS 3, 30 pp.

Wyllie Echeverria, T. 1987. Thirty-four species of California rockfishes: maturity and seasonality of reproduction. Fish. Bull. 85(2):229–250.