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

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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 (*S. rastrelliger*), black-and-yellow (*S. chrysomelas*), gopher (*S. camatus*), copper (*S. caurinus*), quillback (*S. mali*ger; 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-fish 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.

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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 macrozoooplankton 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 flex-
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 myomeres 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 dorsal, 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.

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 species to the unknown larval haplotype was performed.

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. nattereri, 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. nattereri 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. Marlize (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 postflexion-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. 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.

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LITERATURE CITED


\(^\text{C. Taylor and R. Vetter, Gene flow of coastal and island populations of California kelp rockfish (Sebastes atrovirens) with implications for the design of marine protected areas (manuscript).}\)


