

EVALUATION OF MORPHOLOGICAL CHARACTERS TO IDENTIFY GROUPEL (SERRANIDAE: EPINEPHELINI) LARVAE IN THE GULF OF MEXICO USING GENETICALLY IDENTIFIED SPECIMENS

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ABSTRACT

The identities of early life stages of groupers worldwide are virtually unknown. Current identification strategies rely on characters not yet developed in small larvae (< 7 mm BL) and not always reliable in larger larvae. Genetically identified larval Epinephelini (280 specimens from 15 species) collected in the Straits of Florida, coupled with larvae collected during Southeast Area Monitoring and Assessment Program (SEAMAP) resource surveys in the Gulf of Mexico (500 specimens), were used to examine the utility of morphological characters in identifying epinephelin larvae to species. Through investigation of genetically identified larvae, we demonstrate that patterns of tail pigment and lower-jaw pigment are consistent within species and are often species-specific. These characters facilitate identification of grouper larvae when genetic analyses are not used. The coupling of molecular and morphological identification techniques proved to be a powerful and cost-effective tool in advancing our knowledge of larval groupers. Application of these combined techniques resulted in the first-time identification of the larvae of three species and the preflexion-stage larvae of 10 species of groupers. Six species, three species groups, and three morphological types could be identified in the SEAMAP samples. The synthesis of our findings with previously published descriptions of grouper larvae represents the most comprehensive treatment of larval groupers of the western North Atlantic to date.

Groupers are ecologically and economically important fishes found in tropical and subtropical waters worldwide (Parrish, 1987; Heemstra and Randall, 1993; Stallings, 2008). Grouper species vary in size and reproductive mode, with several being large apex predators, protogynous hermaphrodites, and/or aggregate spawners (Coleman et al., 1996). These characteristics make grouper species exceedingly vulnerable to overfishing (Huntsman and Schaaf, 1994; Coleman et al., 1999, 2000). Over the past decade, increasingly stringent management measures have been enacted to prevent or reduce overfishing on Gulf of Mexico grouper stocks (www.nmfs.noaa.gov). The first Marine Protected Areas (MPAs) in the Gulf of Mexico were designed specifically to protect *Mycteroperca microlepis* spawning aggregations (Koenig et al., 2000; Coleman et al., 2004). As with most marine fishes, groupers have a planktonic larval stage that lasts a few weeks and is the main source of connectivity between spawning and juvenile habitats, and between MPAs and fished areas (e.g., Sala et al., 2002; Gerber et al., 2005; Wielgus et al., 2007). Evaluation of the effectiveness of Gulf of Mexico MPAs for groupers has been problematic because actual larval transport pathways are largely unknown (Fitzhugh et al., 2005). A major obstacle to deciphering these transport dynamics is the lack of species-level temporal and spatial distribution data. Grouper larvae are difficult to identify to species because they are relatively rare in plankton samples and there are few complete developmental series

or diagnostic morphological traits. A further consequence of the failure to identify grouper larvae to species is that it prevents the use of larval abundance data, gathered during SEAMAP plankton surveys, in the stock assessments for grouper.

Conventional methods of larval fish identification are not adequate for species-level identification of larval Epinephelini at most sizes. Complete developmental series of grouper larvae are rarely available from field collections because too few larvae are collected and those larvae are often of similar size. Most grouper larvae routinely collected during ichthyoplankton surveys in the southeast United States and Gulf of Mexico are < 5.0 mm body length (BL; Houde, 1982; Lyczkowski-Shultz et al., 2003; Marancik et al., 2005). Moreover, the most comprehensive descriptions of grouper larvae (Johnson and Keener, 1984; Richards et al., 2005) are based on numbers of dorsal, anal, and pectoral-fin elements and the morphology of serrations on the dorsal and pelvic spines. Those morphological characters become useful only after their development (i.e., larvae > 5.0–7.0 mm standard length, SL). Even with these characters, species-level identifications have rarely been attempted because the larvae of so few species have been described within the relevant size ranges. Descriptions of laboratory-reared grouper larvae have provided information on larvae of smaller sizes and at earlier stages than descriptions based on field-caught specimens (*Hyporhodus niveatus*: Presley, 1970; *Epinephelus striatus*: Manday and Fernandez, 1966; Powell and Tucker, 1992; *Epinephelus morio*: Colin et al., 1996), but differences in morphology and pigmentation between laboratory-reared and wild-caught larvae and the lack of critical comparison to other species of groupers render those descriptions of limited use (Boglione et al., 2001; Strelcheck et al., 2003).

Coupling genetic techniques with traditional morphological analysis provides a way to limit the expense and effort of genetic analyses while still achieving reliable species-level identifications. Through molecular techniques such as DNA sequencing or barcoding (reviewed in Teletchea, 2009), eggs, larvae, post-settlement juveniles, and adult fish parts that could not be identified with conventional methods have been identified to species. Several genetic studies have also led to morphological descriptions and/or identification keys for fish eggs and larvae (Lindstrom, 1999; Welsford et al., 2004; Hyde et al., 2005; Luthy et al., 2005; Baldwin et al., 2009; Victor et al., 2009).

Unfortunately, genetic techniques cannot be used to identify all preserved larval fish specimens. Recent studies have reported low success rates (0%–20%) when attempting to extract and amplify DNA from formalin-fixed specimens after storage periods > 7–9 d (Bhadury et al., 2005; Chakraborty et al., 2006; Karaïskou et al., 2007; Skage and Schander, 2007). Most long-term plankton sampling regimes fix samples in formalin before storage, making molecular identification of larvae in these samples ineffective. Because of this, morphological means of identification are still highly valuable for identification of larval fishes. If diagnostic characters exist, they can be used to narrow larval grouper identification to species (ideal) or species groups of interest (for further genetic analysis). Limiting genetic analysis to a subgroup of specimens would considerably reduce cost and effort, even in programs using ethanol preservation. Therefore, our objectives were: (1) to use genetic techniques to establish a reference set of wild-caught larvae of known identity; (2) to describe patterns in pigmentation and spination of these larvae; and (3) to assess the diagnostic value of these patterns for identifying grouper larvae of the western North Atlantic Ocean. These new data taken from genetically identified larvae were subsequently used to

identify grouper larvae collected during Southeast Area Monitoring and Assessment Program (SEAMAP) resource surveys in the Gulf of Mexico to taxonomic levels and groupings that were previously unattainable.

MATERIALS AND METHODS

Currently, eight genera of western North Atlantic epinephelin groupers comprising 24 species are recognized (Table 1; Heemstra and Randall, 1993; Craig et al., 2001; Heemstra et al., 2002; Craig and Hastings, 2007; Eschmeyer and Fricke, 2009). Recent investigations into phylogenetic relationships within the family Serranidae has led to proposed revisions in grouper taxonomy (Craig and Hastings, 2007; Smith and Craig, 2007). However, due to its wide use among researchers, we have elected to follow the taxonomy found in Eschmeyer and Fricke (2009) which only recognizes the reassignment of four species of groupers, formerly in the genus *Epinephelus*, to the genus *Hyporthodus* following Craig and Hastings (2007).

COLLECTION OF GROUPEL LARVAE.—Larvae from two different sampling programs were used in this study. Grouper larvae used for genetic identification were collected from monthly sampling during 2003–2004 along a 17-station transect crossing the Straits of Florida at 25.5°N (Fig. 1; see Llopiz and Cowen, 2008 for sampling details). Specimens were captured with an asymmetrical MOCNESS (4-m² frame with 1000- μ m mesh nets and a 1-m² frame with 150- μ m mesh nets), which sampled from 0–100 m in 25-m increments (Guigand et al., 2005), and a combined neuston net (1 \times 2 m mouth with 1000- μ m mesh net and a 0.5 \times 1 m mouth with 150- μ m mesh net), which sampled the surface water. Samples were immediately preserved in 95% ethanol and then, after 2–5 d, were transferred to 70% ethanol for long-term storage. All larval fishes were removed from the samples and identified to the lowest taxonomic level possible. Epinephelin larvae were subsequently measured.

Additional grouper larvae used for morphological identification were collected seasonally during the Southeast Area Monitoring and Assessment Program (SEAMAP) resource surveys conducted in the United States Gulf of Mexico from 1982–2005 (Fig. 1). A detailed account of SEAMAP surveys, survey design, and plankton sampling methods can be found in Lyczkowski-Shultz and Hanisko (2007) and Rester et al. (2002). Most specimens of grouper larvae were captured with a bongo net (61-cm frame with 335- μ m mesh nets) which sampled obliquely from 2–5 m off the bottom or to a maximum depth of 200 m and a neuston net (1 \times 2 m mouth with 950- μ m mesh net) which sampled the surface water. Plankton samples were initially fixed in either 5%–10% unbuffered formalin (the majority of samples) or 95% ethanol. Formalin-fixed samples were transferred to 95% ethanol after 48 hrs, and samples initially fixed in ethanol were transferred to fresh 95% ethanol after 24–36 hrs. Fish larvae were removed from samples, identified to the lowest taxonomic level possible, and measured at the Sea Fisheries Institute, Plankton Sorting and Identification Center in Gdynia and Szczecin, Poland. Larval specimens were accessioned, curated, and made available to other researchers at the SEAMAP Archiving Center in St. Petersburg, Florida.

MOLECULAR IDENTIFICATION.—Voucher specimen DNA or sequences of cytochrome oxidase subunit I (COI) were obtained from two sources (Table 1): fin clips of adults collected from fisheries landings in the eastern Gulf of Mexico by the Florida Fish and Wildlife Research Institute; and/or the Barcode of Life Database (BOLD; Ratnasingham and Hebert, 2007). Sequences from voucher specimens obtained for this study were compared with published sequences from BOLD for the 15 species included in both data sets. In all instances, the voucher sequence had a > 99% match to the sequence of the same species in BOLD. No voucher specimens or sequences of COI were available in our collections, BOLD, or GenBank for three western North Atlantic epinephelin species (Table 1). Requests to private collections were not made.

The molecular identification of larvae followed the approach advocated by the Consortium for the Barcode of Life (Hebert et al., 2003) and the methodology described in Richardson et al. (2007). Briefly, DNA was isolated from an eyeball or a piece of tail (if no eyeball was

Table 1. List of the 24 grouper species that occur in the western North Atlantic. Included are the source of voucher specimen DNA or sequences for molecular identification and the number and size range (in mm) of genetically identified larval specimens by developmental stage examined in this study. EGOM = eastern Gulf of Mexico. BOLD = Barcode of Life Data System (Ratnasingham and Hebert, 2007). Parantheses in the DNA Source column contain the BOLD reference numbers. No COI Data = tissue was not collected and no sequences were available from BOLD.

Species name / Authority	Common name	DNA source	Genetically identified specimens					
			Preflexion		Flexion		Postflexion	
			Num.	Size rng	Num.	Size rng	Num.	Size rng
<i>Alphestes afer</i> (Bloch, 1793)	Mutton hamlet	BOLD	–	–	–	–	2	5.8–6.4
<i>Cephalopholis cruentata</i> (Lacépède, 1802)	Graysby	BOLD	6	3.3–4.1	20	3.4–5.7	16	4.9–7.7
<i>Cephalopholis fulva</i> (Linnaeus, 1758)	Coney	BOLD	8	3.4–4.9	4	4.5–6.0	2	6.9–7.7
<i>Dermatolepis inermis</i> (Valenciennes in Cuvier and Valenciennes, 1833)	Marbled grouper	BOLD	No specimens					
<i>Epinephelus adscensionis</i> (Osbeck, 1765)	Rock hind	Fin Clip: EGOM (EPIN041-09, EPIN040-09, EPIN039-09, EPIN038-09) and BOLD	No specimens					
<i>Epinephelus drummondhayi</i> Goode and Bean, 1878	Speckled hind	Fin Clip: EGOM (EPIN042-09, EPIN043-09, EPIN044-09) and BOLD	1	3.6	4	4.1–5.3	–	–
<i>Epinephelus guttatus</i> (Linnaeus, 1758)	Red hind	Fin Clip: EGOM (EPIN034-09, EPIN035-09, EPIN036-09, EPIN037-09) and BOLD	48	2.2–4.1	1	4.3	1	9.5
<i>Epinephelus itajara</i> (Lichtenstein, 1822)	Goliath grouper	BOLD	No specimens					
<i>Epinephelus morio</i> (Valenciennes in Cuvier and Valenciennes, 1828)	Red grouper	Fin Clip: EGOM (EPIN021-09, EPIN022-09) and BOLD	2	4.1–4.3	6	4.0–5.2	1	5.6
<i>Epinephelus striatus</i> (Bloch, 1792)	Nassau grouper	Fin Clip: Bahamas (EPIN054-09) and BOLD	6	3.2–4.4	2	4.5	–	–
<i>Hyporhamphus flavolimbatus</i> (Poey, 1865)	Yellowedge grouper	Fin Clip: EGOM (EPIN004-09, EPIN003-09, EPIN002-09, EPIN005-09, EPIN001-09) and BOLD	14	2.9–4.2	57	3.2–6.4	22	4.9–12.4
<i>Hyporhamphus mystacinus</i> (Poey, 1852)	Misty grouper	Fin Clip: EGOM (Epin006-09) and BOLD	–	–	2	5.3	2	5.1–6.7

Table 1. Continued.

Species name / Authority	Common name	DNA source	Genetically identified specimens						
			Preflexion		Flexion		Postflexion		
			Num.	Size mg	Num.	Size mg	Num.	Size mg	
<i>Hyporhodus nigrinus</i> (Holbrook, 1855)	Warsaw grouper	Fin Clip: EGOM (EPIN049-09, EPIN050-09, EPIN051-09) and BOLD	No specimens						
<i>Hyporhodus niveatus</i> (Valenciennes in Cuvier and Valenciennes, 1828)	Snowy grouper	Fin Clip: EGOM (EPIN013-09, EPIN012-09, EPIN014-09) and BOLD	8	2.2-4.6	3	4.0-5.3			
<i>Mycteroperca acuitirostris</i> (Valenciennes in Cuvier and Valenciennes, 1828)	Comb grouper	No COI Data	No specimens						
<i>Mycteroperca bonaci</i> (Poey, 1860)	Black grouper	Fin Clip: EGOM (EPIN023-09, EPIN024-09, EPIN025-09) and BOLD	2	3.3-3.6	2	5.1-5.3	1	8.4	
<i>Mycteroperca cidi</i> Cervigón, 1966	Venezuelan grouper	No COI Data	No specimens						
<i>Mycteroperca interstitialis</i> (Poey, 1860)	Yellowmouth grouper	Fin Clip: EGOM (EPIN019-09, EPIN018-09, EPIN017-09, EPIN020-09) and BOLD	2	3.6-3.8					
<i>Mycteroperca microlepis</i> (Goode and Bean, 1879)	Gag grouper	Fin Clip: EGOM (EPIN029-09, EPIN028-09, EPIN027-09, EPIN026-09) and BOLD	No specimens						
<i>Mycteroperca phenax</i> Jordan and Swain, 1884	Scamp	Fin Clip: EGOM (EPIN030-09, EPIN031-09, EPIN032-09, EPIN033-09, EPIN053-09) and BOLD	2	3.6-3.7	2	5.1-5.8			
<i>Mycteroperca tigris</i> (Valenciennes in Cuvier and Valenciennes, 1833)	Tiger grouper	BOLD	No specimens						
<i>Mycteroperca venenosa</i> (Linnaeus, 1758)	Yellowfin grouper	Fin Clip: EGOM (EPIN045-09, EPIN047-09, EPIN048-09) and BOLD	5	2.2-4.5	14	4.9-6.8			
<i>Paranthias furcifer</i> (Valenciennes in Cuvier and Valenciennes, 1828)	Creole fish	Fin Clip: EGOM (EPIN009-09, EPIN007-09, EPIN008-09, EPIN011-09, EPIN010-09) and BOLD	8	2.6-4.2	4	4.3-5.8			
<i>Gonioplectrus hispanus</i> (Cuvier in Cuvier and Valenciennes, 1828)	Spanish flag	No COI Data	No specimens						

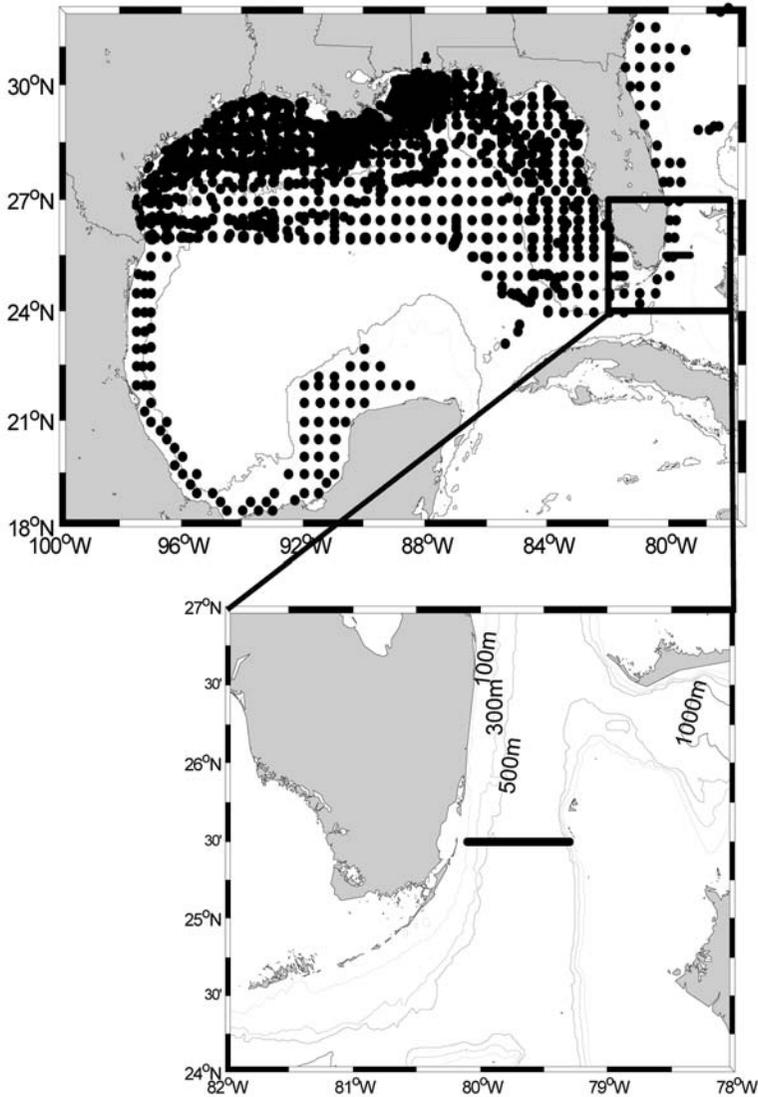


Figure 1. Map of Gulf of Mexico and the east coast of Florida showing the location of the sampling transect in the Straits of Florida and the ichthyoplankton sampling locations for the period between 1982 and 2005 during the Southeast Area Monitoring and Assessment Program surveys. Bathymetry for the Gulf of Mexico (100 m and 1000 m) and Straits of Florida (100 m, 300 m, 500 m, 1000 m) is indicated.

available), and an approximately 600 base-pair portion of the COI gene was amplified and sequenced. Samples showing signs of contamination (any sequence in the three negative controls on a 96-well plate) were discarded. Sequence analysis was primarily performed using the MATLAB script described in Richardson et al. (2007), comparing each larval sample sequence to the adult voucher sequences. If no strong match (> 98%) existed between the sequence from a larval fish and one of the voucher sequences, the larval fish sequence was queried against BOLD.

MORPHOLOGICAL CHARACTERS USED IN IDENTIFICATION OF GROUPER LARVAE.—Morphological characters were identified and evaluated for diagnostic value through examina-

tion of molecularly identified specimens and specimens that could be positively identified by their meristic characters and morphometrics. Developmental stage (preflexion, flexion, and postflexion following Moser, 1996), body length (either notochord or standard length), body depth through the cleithrum, and post-anal length were recorded. Spine length was not measured due to the high incidence of broken spines on net collected larvae. Spinelets on the elongate second dorsal and pelvic spines were characterized according to their relative length, shape, and whether the spinelet base was broad or narrow. In addition, the location, shape, and presence of serrations on the preopercular and supraorbital spines were examined. Melanistic pigment patterns on the lower jaw, head, nape, dorsal midline (dorsum), ventral midline (ventrum), gut, cleithral symphysis, and tail were recorded. Counts of dorsal, anal, and pectoral (sum of left and right) fin elements were recorded for large postflexion larvae following the methods described in Johnson and Keener (1984; Table 1). Numbers of dorsal spines was based on the number of pterygiophores, since the last two spines may be soft until about 20 mm SL. The distal radials of pterygiophores, examined using transmitted light, are flattened for spines and spherical for soft rays.

Examination of larvae was conducted using Nikon imaging software (NIS Elements BR 2.3) and a color digital camera (DXM1200C) mounted on a stereo microscope (SMZ1500) under both reflected and transmitted light. The images presented in Figures 7–22 reflect typical specimens at the different developmental stages examined for each species. Only diagnostic features of pigment are discussed in the text, but additional pigment may be present on the figured specimens.

RESULTS

Specimens from 15 of the 24 western North Atlantic grouper species, represented among the 280 genetically identified larval specimens, were examined to identify diagnostic morphological traits (Table 1).

MORPHOMETRICS.—Due to the variability within species and low sample sizes for most species, body measurements within developmental stage (ratios of body depth to body length, preanal length to body length, and body depth to preanal length) did not prove useful as diagnostic characters (Table 2). The exception was *Gonioplectrus hispanus* whose larvae are characteristically deep bodied with short postanal lengths relative to body length.

PIGMENTATION ON THE LOWER JAW.—Pigment on the lower jaw appears during the reflexion or flexion stage (Table 3; Figs. 4A, 5). Lower-jaw pigment was observed on only five species and is diagnostic for those species (Table 4).

PIGMENTATION AT THE CLEITHRAL SYMPHYSIS.—A melanophore at the symphysis of the cleithral bones was present on genetically identified specimens of four species (Table 3; Fig. 5): *Cephalopholis cruentata* (21 of 34 specimens), *Hyporthodus flavolimbatus* (1 of 93 specimens), *Epinephelus striatus* (1 of 8 specimens), and *Mycteroperca phenax* (2 of 4 specimens). Pigment at the cleithral symphysis may be linked to developmental stage as it was present in only 25% of reflexion *C. cruentata* but 86% of postflexion specimens, and in only the two largest *M. phenax* specimens. Due to its low incidence in small grouper larvae, pigment at the cleithral symphysis is only marginally useful as a distinguishing character until postflexion stage. In addition, pigment at the cleithral symphysis was absent on several SEAMAP-collected larvae with meristic characters indicative of *Mycteroperca* (10–13 anal-fin rays; 5.5–19.5 mm SL), suggesting that the presence of pigment at the cleithral symphysis is not diagnostic of the genus as previously stated (Johnson and Keener, 1984).

Table 2. Mean (range) in the ratios of three morphometric measurements for larvae of fifteen genetically identified species and one morphologically identified species of grouper. Specimens are grouped by developmental stage. If no range is given, all values were the same. Sample sizes are as listed in Table 1 except where labeled otherwise.

Species	Range in body depth: body length			Range in postanal length: body depth			Range in postanal length: body length		
	Preflexion	Flexion	Postflexion	Preflexion	Flexion	Postflexion	Preflexion	Flexion	Postflexion
<i>Alphistes afer</i>			0.34 (0.31–0.36)			1.20 (1.00–1.39)			0.40 (0.36–0.43)
<i>Cephalopholis cruentata</i>	0.34 (0.29–0.46)	^a 0.32 (0.24–0.40)	0.36 (0.28–0.42)	1.36 (0.75–1.75)	^a 1.33 (0.94–2.13)	1.06 (0.78–1.40)	0.45 (0.34–0.51)	^a 0.41 (0.31–0.50)	0.38 (0.32–0.43)
<i>Cephalopholis fulva</i>	0.30 (0.27–0.33)	0.28 (0.28–0.29)	0.34 (0.32–0.35)	1.48 (1.31–1.77)	1.61 (1.54–1.71)	1.12 (1.08–1.16)	0.44 (0.41–0.48)	0.46 (0.44–0.48)	0.38
<i>Epinephelus drummondhayi</i>	0.36	0.34 (0.30–0.37)		1.10	1.21 (1.00–1.44)		0.39	0.41 (0.37–0.43)	
<i>Epinephelus guttatus</i>	^a 0.28 (0.20–0.32)	0.30	0.34	^b 1.51 (1.00–2.43)	1.40	1.10	^b 0.41 (0.31–0.49)	0.42	0.37
<i>Epinephelus morio</i>	0.34 (0.30–0.37)	^a 0.35 (0.32–0.38)	0.34	1.40 (1.33–1.46)	^a 1.08 (0.87–1.24)	1.00	0.47 (0.44–0.49)	^a 0.37 (0.33–0.40)	0.34
<i>Epinephelus striatus</i>	0.29 (0.25–0.31)	0.29		1.46 (1.25–1.64)		1.30	0.42 (0.38–0.45)	0.38	
<i>Hyporhamphus flavolimbatus</i>	0.34 (0.29–0.41)	0.34 (0.23–0.40)	0.37 (0.31–0.43)	1.27 (1.00–1.46)	1.25 (0.94–2.09)	1.09 (0.85–1.37)	0.43 (0.34–0.49)	0.42 (0.35–0.50)	0.40 (0.34–0.48)
<i>Hyporhamphus mystacinus</i>		0.37 (0.36–0.38)	0.38 (0.37–0.39)		0.98 (0.95–1.00)	0.87 (0.84–0.90)		0.36 (0.34–0.38)	0.33 (0.31–0.35)
<i>Hyporhamphus niveatus</i>	0.33 (0.30–0.37)	0.37 (0.35–0.40)		1.34 (1.13–1.67)	1.05 (0.94–1.21)		0.43 (0.38–0.50)	0.39 (0.36–0.43)	
<i>Mycteroperca bonaci</i>	0.31 (0.30–0.31)	0.29 (0.28–2.9)	0.43	1.38 (1.36–1.40)	1.44 (1.27–1.60)	1.00	0.42	0.41 (0.37–0.45)	0.43
<i>Mycteroperca interstitialis</i>	0.29 (0.26–0.31)			1.63 (1.45–1.80)			0.46 (0.44–0.47)		
<i>Mycteroperca phenax</i>	0.29 (0.23–0.35)	0.30 (0.29–0.31)		1.86 (1.46–2.25)	1.58 (1.56–1.60)		0.51	0.48 (0.47–0.48)	
<i>Mycteroperca venenosa</i>	^a 0.33 (0.27–0.45)	0.29 (0.26–0.33)		^a 1.29 (0.90–1.50)	1.54 (1.28–1.79)		^a 0.40 (0.38–0.43)	0.44 (0.37–0.48)	
<i>Paranthias furcifer</i>	0.32 (0.29–0.35)	0.34 (0.33–0.37)		1.23 (1.00–1.50)	1.16 (1.05–1.21)		0.39 (0.33–0.44)	0.40 (0.37–0.43)	
<i>Gonioplectrus hispidus</i>		^a 0.41 (0.36–0.45)	^a 0.44 (0.43–0.44)		^a 0.89 (0.69–1.08)	^a 0.71 (0.63–0.78)		^a 0.35 (0.31–0.39)	^a 0.31 (0.27–0.35)

a: measurements from 18 of 20 specimens; b: 47 of 48 specimens; c: 5 of 6 specimens; d: 4 of 5 specimens; e: 2 specimens

TAIL PIGMENTATION.—Pigment on the tail (anus to notochord tip) of grouper larvae differed in the location, shape, and size of melanophores. Seven distinct and consistent patterns were observed among the genetically identified larvae making this one of the more useful characters for distinguishing grouper species or species groups (Table 3; Figs. 4B, 6). An additional pattern was observed only on specimens from SEAMAP collections (multiple melanophores tail pigment, Table 3; Fig. 4B8).

Patterns in tail pigmentation were generally consistent within species throughout development with a few exceptions (Fig. 6). By late postflexion stage, tail pigment on specimens of most of the species examined was the same. This pattern, called standard midlateral tail pigment, appears to result from the migration of melanophores from the ventrum to the lateral midline of the tail (Fig. 4B2). At this stage, the different tail pigment patterns described become indistinguishable, and dorsal, anal, and pectoral-fin elements in combination with spinelet morphologies and presence or absence of lower-jaw pigment are needed to identify specimens to species or species group (see Johnson and Keener, 1984; Table 4). Ontogenetic changes in tail pigmentation also were seen between preflexion and flexion stages of two species for which a species-specific pigment pattern was replaced by the standard tail pigment at larger sizes (Figs. 9, 20; Fig. 6). Although presence of tail pigment was the common condition, some specimens of *Cephalopholis cruentata* and *Cephalopholis fulva* lacked pigment on the tail (Fig. 6). No ontogenetic pattern was associated with this variability. Absence of tail pigment was only observed among specimens of three species: *C. cruentata* and *C. fulva* (described above), and *Hyporthodus mystacinus* (Fig. 6). Due to the rarity of this pattern, absence of tail pigment is diagnostic for these species.

DORSAL AND PELVIC-SPINE MORPHOLOGY.—Western North Atlantic grouper larvae have three serrate ridges running the length of the elongate second dorsal spine and four ridges along the elongate pelvic spines. The morphology of these serrations, or spinelets, on two of the dorsal-spine ridges (posterolateral wings pointing toward the posterior left and posterior right of larvae), and one of the four pelvic ridges (the primary or dorsomedial ridge usually pointing medially towards the body) is diagnostic for a number of species or species groups in conjunction with pigmentation and meristic characters (Johnson and Keener, 1984). Four types of spinelet morphologies were observed among genetically identified larvae (Table 3; Figs. 2–3). Spinelet morphology is similar on both dorsal and pelvic spines with the exception of *C. cruentata*.

Several patterns in spinelet development were observed. At least 12 of the 15 species examined develop simple, small, straight spinelets early in development (i.e., when these spines first become visible protruding from the larval finfold). For six species, spinelets remain small and straight throughout the size ranges examined (Fig. 3). But, for seven species, these small spinelets become long, broad-based, and curved during flexion at about 4.5–5.0 mm (Figs. 2C, 3). These long and curved spinelets are first evident near the tip of the spine, but by postflexion stage they extend over most of the length of the spine. Long, straight spinelets may form intermediately between the small and straight spinelet stage and the long and curved spinelet stage. This pattern was only observed in more than one specimen of *H. flavolimbatus* (Fig. 3). One species, *M. phenax*, develops long curved spinelets earlier in development than other species based on a 3.7 mm specimen with this type of spinelet (Fig. 3). Two spinelet morphologies are seen in specimens of *C. cruentata*. Dramatically long, curved, and narrow-based spinelets occur on the dorsal and pelvic spines (Figs. 2D, 3). In addi-

Table 3. Descriptions of diagnostic characters evaluated in this study. Only melanistic pigment is described.

Character Name	Code	Figure	Description
Pigmentation on the lower jaw	+	4A	This pigment is discernible on both rami of the lower jaw and ranges in intensity from fairly faint with two discrete melanophores (one on either side of the symphysis) to bold and connected (continuous across both rami).
Pigmentation at the cleithral symphysis	-	4A	Pigment absent on lower jaw.
	+		A surface melanophore lying just anterior to the ventral junction of the cleithral bones is present.
	-		Pigment absent at the cleithral symphysis.
Tail pigmentation	1	4B1	Standard tail pigment: a single large melanophore (2–3 myomeres wide) located along the ventrum approximately 3–4 myomeres from the end of the notochord or urostyle.
	2	4B2	Standard midlateral tail pigment: the standard tail pigment melanophore is displaced dorsally to a midlateral position and partially internal in larger postflexion specimens.
	3	4B3,2	Small melanophore tail pigment: resembles the standard tail pigment but the single melanophore is greatly reduced in size (< 1 myomere wide). This melanophore also migrates internally and dorsally to a midlateral position and becomes relatively larger in postflexion specimens.
	4	4B4	Ventrally along notochord tail pigment: 1–3 small (≤ 1 myomere wide), internal melanophores in an elevated position relative to the location of the pigment in the previous three patterns, and lying parallel to the notochord.
	5	4B5	Wispy caudal tail pigment: small melanophores located only in a more posterior position (than the previously described patterns) on the tail, just under the notochord with associated wispy streaks of pigment present ventrally on the caudal finfold. This pattern may co-occur with the standard pigment, but is only diagnostic in the absence of other tail pigment.
	6	4B6	No Pigment: absence of pigment anywhere on tail.
	7	4B7	Dorsal-ventral tail pigment: a melanophore on both the dorsum and ventrum of the tail, which occasionally extends laterally forming a band of pigment on the tail, approximately 4 myomeres from the notochord tip. This type of tail pigmentation was observed only on the smallest, 2.2–3.3 mm, preflexion larvae.
	8	4B8	Multiple melanophores tail pigment: This pattern consists of a series of small melanophores (≤ 1 myomere wide) on the ventrum, usually evenly spaced from the anus to the notochord tip or urostyle.
Dorsal and pelvic spine morphology	S	2A	simple, small, and straight.
	L	2B	long and straight.
	B	2C	simple, broad-based, long, and curved.
	N	2D	simple, narrow-based, long, and curved.
	A	2E	bifurcate or anvil shaped.
	C	2F	small and connected.

Table 4. Summary of characters useful in distinguishing species or species-groups of Gulf of Mexico grouper larvae. Presence (+) or absence (-) of pigment (length above which this pigment becomes consistently present) is noted. Spinelet types are abbreviated as N = narrow-based, long, and curved, A = anvil-shaped, S = simple, small, and straight, B = broad-based, long, and curved, and C = robust, small, and connected. ? = trait unknown. Included are meristics useful for identifying late stage larvae (from Smith, 1971 except where noted). Pectoral count is the sum of the pectoral rays from the left and right sides of the body. †Traits described in the literature for species which were not examined in this study. *published descriptions of reared *Epinephelus morio* note the presence of lower jaw tip pigment not observed among wild caught, genetically identified specimens.

Species	Lower-jaw pigment	Tail pigment	Nape pigment	Cleithral symphysys pigment	Spinelet morphology	Dorsal count	Anal count	Pectoral count
<i>Alphalex afer</i>	+/-	Standard	+/-	-	L (> 5.8 mm BL) S slight curve (> 60 mm BL) [†]	XI,17-18(16-19)	III, 9	34(33-36)
<i>Cephalopholis eruentata</i>	-	No pigment/Small, multiple melanophores?	+/-	+	N, A	IX,14(13-15)	III, 8(7-9)	32
<i>Cephalopholis fulva</i>	+/-	No pigment/Internal ventral noochord, standard (> 6 mm)	+/-	-	S	IX,15(14-16)	III, 9(8)	36(34-38)
<i>Dermatolepis thernis</i>	?	?	?	-†	L or slightly curved [†]	XI,19-20(17)	III, 9	36-38
<i>Epinephelus adscensionis</i>	?	Standard	+/-	-	S [†]	XI,16-17(18)	III, 8(9)	36-38(35-39)
<i>Epinephelus drummondhayi</i>	-	Standard	+/-	-	(S < 5.0 mm) B	XI,16(15)	III, 9	36(35)
<i>Epinephelus guttatus</i>	+ (> 3 mm)	Wispy caudal (< 4.0 mm); Standard (> 4.0 mm)	?	-	S slightly curved	XI(X),15-16(17)	III, 8-9	34(32-36)
<i>Epinephelus itajara</i>	?	?	?	+†	B (> 6.2 mm BL) [†]	XI,15-16	III, 8	37-38(36)
<i>Epinephelus morio</i>	-*	Standard	+/-	-	S	XI(X),16-17(15-18)	III, 9(8-10)	34(33-36)
<i>Epinephelus striatus</i>	+ (> 4 mm)	Standard	+/-	-	S	XI(X),16-17(18)	III, 8	36(35-38)
<i>Hyporhamphus flavolimbatus</i>	-	Standard	+/-	-	S	XI,14(15)	III, 9(8)	36(32)
<i>Hyporhamphus mystacinus</i>	-	No pigment	-	-	B (S < 5.0 mm) B	XI,14-15	III, 9	37-38(36)
<i>Hyporhamphus nigritus</i>	?	?	-	-†	B (> 5.3 mm BL)	X(XI),14-15	III, 9	36-38(39)
<i>Hyporhamphus niveatus</i>	?	Standard	+/-	-	B (> 9.1 mm BL) [†]	XI,14(15-16)	III, 9(8-10)	36(34-38)
<i>Mycteroperca acutirostris</i>	?	?	?	+†	(S < 4.0 mm) B	XI,15-17 [†]	III, 10-12 [†]	30-34 [†]
<i>Mycteroperca bonaci</i>	-	Standard	+	-	B (postflexion) [†]	XI,17(16-18)	III, 12(13)	34(35-36)
<i>Mycteroperca cidi</i>	?	?	+	-	(S < 5.0 mm) B	XI(XII),16(15-17)	III, 11(10-12)	32(30-36)
<i>Mycteroperca interstitialis</i>	-	Standard	+/-	-	B (postflexion) [†]	XI,17(15-18)	III, 11(12)	34(33)
<i>Mycteroperca microlepis</i>	-†	Standard [†]	+/-	+†	S (S < 3.8 mm unknown)	XI(X-XII),16-17(18)	III, 11(10-12)	34(32-35)
<i>Mycteroperca phenax</i>	-	Standard	+/-	+	B (postflexion) [†]	XI,17(16-18)	III, 11	32-33(31-34)
<i>Mycteroperca tigris</i>	-†	Dorsal-ventral (< 2.8 mm), standard (by flexion?) [†]	+/-	+/-	(S < 3.6 mm BL) B	XI,16-17	III, 11(10)	34(33-36)
<i>Mycteroperca venenosa</i>	+ (> 5.5 mm)	Dorsal-ventral (< 3.5 mm), standard (> 4.5 mm)	+/-	-	B (postflexion) [†]	XI,15-16(17)	III, 11(10-12)	34(32-35)
<i>Paranibichthys furcifer</i>	-	Standard	+/-	-	S	IX,18-19	III, 9(10)	40(39)
<i>Goniistius hispidus</i>	+/-	Standard	-	+	C	VIII,13 [‡]	III, 7 [‡]	32 [‡]

[†] Richards et al., 2005; [‡] Johnson and Keener, 1984; Kendall and Fahay, 1979

Table 5. Summary of the identification of grouper larvae collected during SEAMAP resource surveys in the Gulf of Mexico (1982–2005). Number and size range of specimens (all years pooled together) of each species, species group, or morphological type are segregated by developmental stage. Unknown stage columns include specimens too damaged to determine developmental stage. Spinelet types are abbreviated as N = narrow-based, long, and curved, A = anvil-shaped, S = simple, small, and straight, B = broad-based, long, and curved, NU = no or serrate spines, L = long and straight, and C = robust, small, and connected. * trait not observed among genetically identified specimens

Species or group	Preflexion			Flexion			Postflexion			Unknown			Pigment			Spinelet type		
	Num	Size range	Size range	Num	Size range	Size range	Num	Size range	Size range	Num	Size range	Size range	% Seamap groupers	Lower jaw	Cleithral symphysis		Nape	Tail
<i>Cephalopholis cruentata</i>	15	2.2–4.5	9	3.7–6.4	6	5.7–13.8	–	–	–	–	–	–	6%	–	+/-	+/-	No pigment / small	N, A
<i>Cephalopholis fulva</i>	–	–	2	4.2–4.8	–	–	–	–	–	–	–	–	0.40%	+/-	–	+/-	No pigment	S
<i>Gonioplectrus hispanus</i>	–	–	2	2.9–3.3	2	5.2–10.6	–	–	–	–	–	–	0.80%	+/-	+	–	Standard	C*
<i>Hyporhamphus mystacinus</i>	–	–	1	5.3	1	6.0	–	–	–	–	–	–	0.40%	–	–	–	No pigment	B
<i>Paranthias furcifer</i>	–	–	4	4.7–7.1	8	5.5–10.9	–	–	–	–	–	–	2.40%	–	–	+/-	Standard	S
<i>Epinephelus itajara</i>	–	–	–	–	1	24.3	–	–	–	–	–	–	0.20%	+	–	–	Standard	B
<i>Epinephelus striatus</i> / <i>Mycteroperca venenosa</i>	2	2.3–2.6	–	–	–	–	–	–	–	–	–	–	0.40%	+	–	+/-	Standard	S
Small epinephelini + standard pigment	143	1.2–4.3	–	–	–	–	–	–	–	–	–	–	29%	–	–	–	Standard	NU
Small spinelets species group	111	2.2–4.7	70	3.6–6.6	10	4.9–8.1	1	Damaged	–	–	–	–	38%	–	–	+/-	Standard	S
Long curved spinelets species group	4	3.0–4.8	23	3.6–6.8	17	5.0–9.2	2	4.2–4.6	–	–	–	–	16%	–	–	+/-	Standard	B
<i>Mycteroperca</i> spp.	–	–	3	5.5–6.5	15	6.1–19.5	–	–	–	–	–	–	–	–	+/-	+/-	Standard	B
<i>Epinephelus itajara</i> / <i>Mycteroperca</i>	4	2.7–5.2	7	3.9–5.8	3	5.3–10.1	1	5.0	–	–	–	–	–	–	+	+/-	Standard	(S < 5.0 mm) B
Multiple melanophores tail pigment	10	2.3–3.1	2	3.0–4.2	–	–	–	–	–	–	–	–	2.40%	–	+/-	+/-	Multiple melanophores*	N, A
Dorsal-ventral tail pigment	11	2.0–3.7	1	4.7	–	–	–	–	–	–	–	–	2.40%	–	–	+/-	Dorsal-ventral	NU or S
Long straight spinelets	1	3.3	2	4.3–4.4	–	–	–	–	–	–	–	–	0.60%	–	–	+/-	Standard	L*
Specimens with broken spines	4	2.6–4.1	2	4.6–5.0	–	–	–	–	–	–	–	–	1.20%	–	–	+/-	Standard	Unclassified

tion, one or more anvil-shaped spinelets occur on the pelvic spine of flexion and postflexion stage larvae (Figs. 2E, 3). Occasionally (eight of 44 specimens), bifurcation occurs on the proximal half of the dorsal spine, but in a less pronounced form than occurs on the pelvic spine. Anvil-shaped spinelets were never seen on any other species within the size ranges examined.

A fifth type of spinelet morphology was only seen on *G. hispanus* (Fig. 2F). This species develops short robust spines with small, straight, connected spinelets (furrowed appearance described by Johnson and Keener, 1984; Fig. 2F). Although we had no genetically identified specimens with which to compare, we feel confident in the identification of four *G. hispanus* specimens in our collections based on previously published descriptions (see species description below; Kendall and Fahay, 1979; Johnson and Keener, 1984).

IDENTIFICATION OF SEAMAP SURVEY GROUPER LARVAE.—In total, 500 grouper specimens, mostly preflexion larvae, were collected over 20 yrs of SEAMAP Gulf of Mexico surveys (Table 5). Most SEAMAP epinephelin specimens could be identified to one of six species (13% of specimens), three species groups (83% of specimens), or three morphological types (3% of specimens) using morphological characters (Table 5).

The majority of specimens collected were assigned to three large multi-species groups. The small spinelet species group (38% or 192 specimens ranging from 2.2 to 8.1 mm BL) and the long and curved spinelet species group (16% or 79 specimens ranging from 2.7 to 24.3 mm BL) are described below. Several specimens from these two species groups had dorsal and anal fins sufficiently developed to permit identification to a sub-group of species based on fin element counts. The third species group, represented by approximately 29% ($n = 143$ specimens) of the SEAMAP-collected specimens, were preflexion stage larvae (1.2–4.3 mm BL) with standard tail pigment, no lower-jaw pigment, and underdeveloped or no dorsal and pelvic spines (small Epinephelini + standard pigment species group; Table 5). The recently hatched larvae of 21 species of groupers may be represented within this morphological group of larvae. Specimens not assigned to one of the three groups were organized into types based on traits either not observed among the genetically identified specimens (e.g., multiple melanophores tail pigment) or not sufficiently linked by morphology to a species (e.g., dorsal-ventral tail pigment; Table 5).

A



Epinephelus morio 5.2 mm BL 30X with 1X lens

B



Hyporthodus flavolimbatus 5.0mm BL 40X with 1X lens

C



Hyporthodus flavolimbatus 5.6 mm BL 30X with 1X lens

D



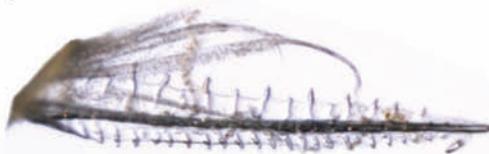
Cephalopholis cruentata 3.8 mm BL 40X with 1X lens

E



Cephalopholis cruentata 13.8 mm BL 20X with 1X lens

F



Gonioplectrus hispanus 3.3 mm BL 30X with 1X lens

Figure 2. Examples of the five spinelet types observed among genetically identified grouper larvae. (A) simple, small, and straight spinelets (S); (B) long and straight spinelets (L); (C) broad-based, long, and curved spinelets (B); (D) narrow-based, long, and curved spinelets (N); (E) anvil-shaped followed by long and curved spinelets (A), note the broader base of simple spinelets compared to panel D; (F) small and connected spinelets (C).

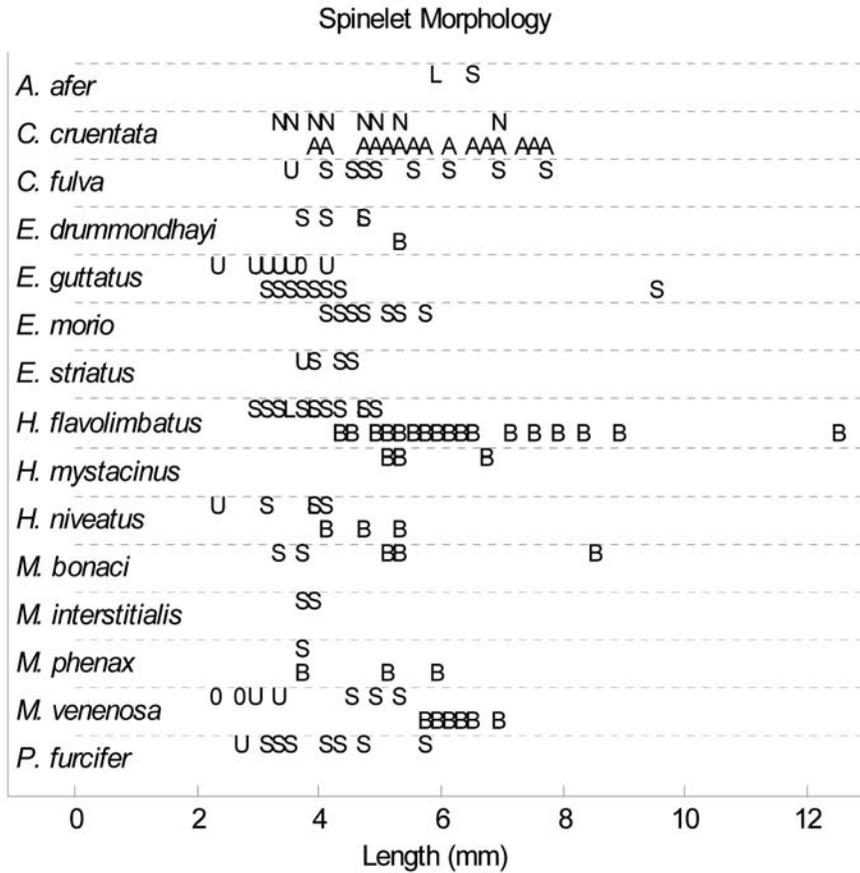


Figure 3. Dorsal and pelvic spinelet morphologies of genetically identified grouper larvae arrayed by specimen size and species. Only a subset of 20 specimens of flexion and postflexion *Hyporhamphus flavolimbatus* and preflexion *Epinephelus guttatus* are presented. The small connected spinelet pattern (Fig. 2F) found only on *Gonioplectrus hispanus* is not listed. 0 = no spines, U = unserrate spines, S = simple, small, straight spinelets, L = long and straight spinelets, B = broad-based, long, and curved spinelets, N = narrow-based, long, and curved spinelets, A = anvil-shaped spinelets.

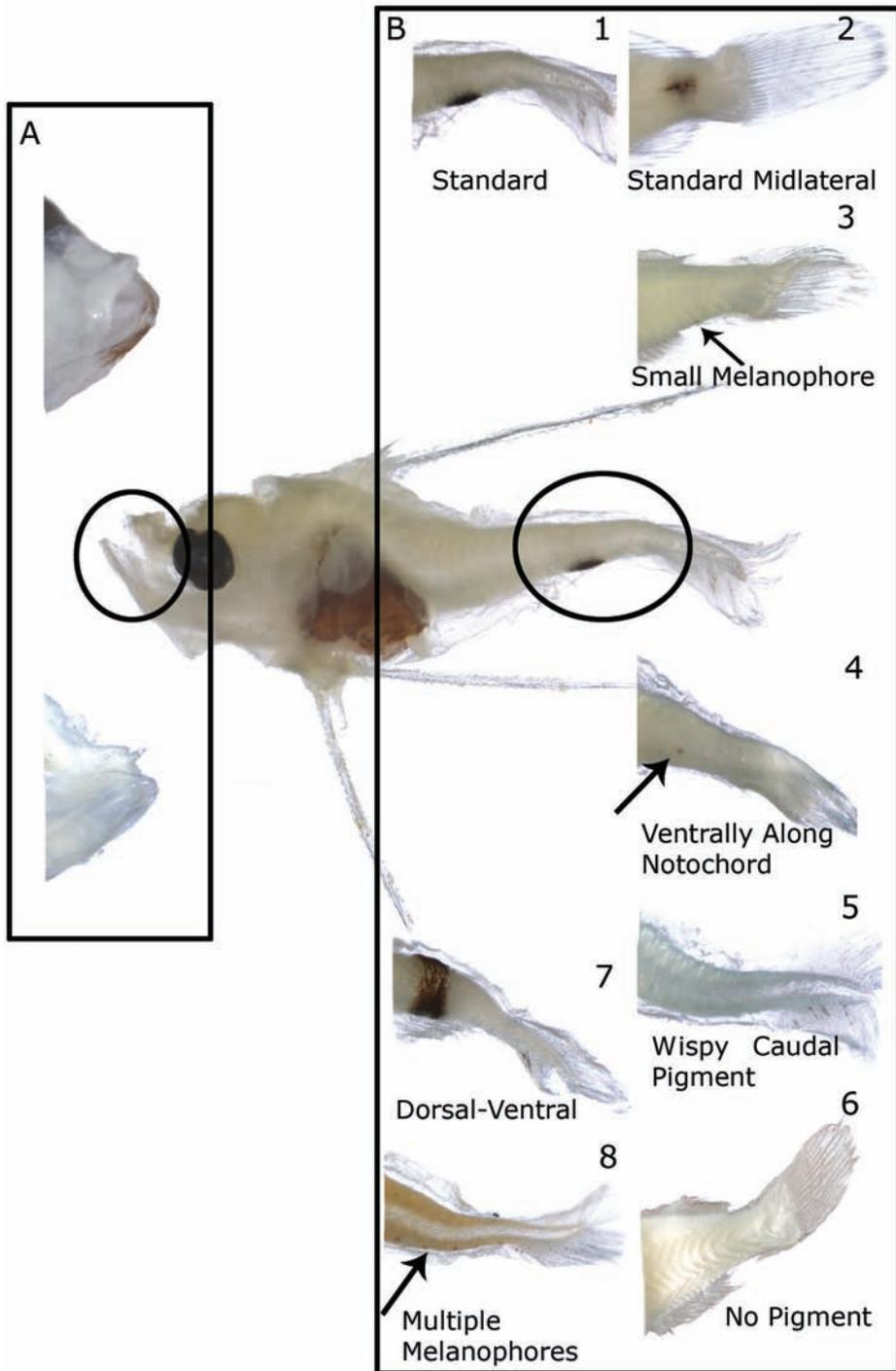


Figure 4. Diagnostic pigment patterns observed among genetically identified grouper larvae. Box A illustrates the two states of lower-jaw pigment. Box B depicts the eight patterns in tail pigment (see Table 3 for detailed description of these patterns).

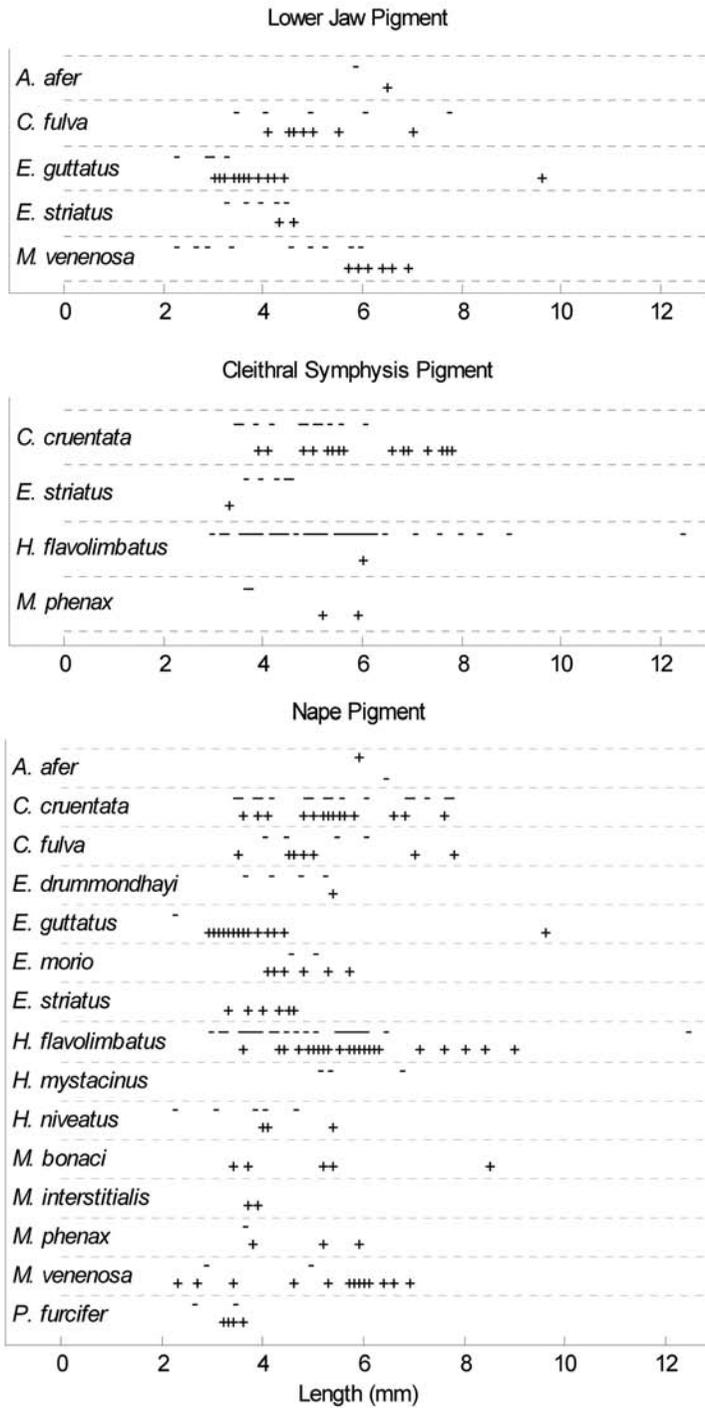


Figure 5. Presence (+) or absence (-) of pigment at the tip of the lower jaw, the cleithral symphysis, and the nape for the genetically identified specimens of each species arrayed by size. Species not listed did not display the pigment at any size.

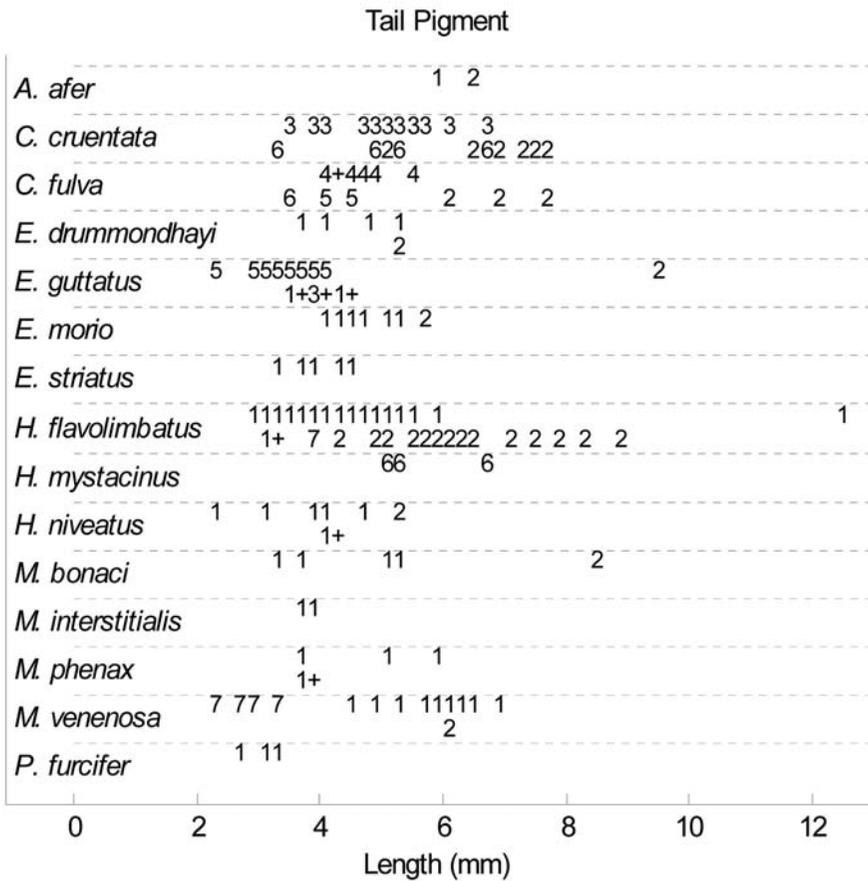


Figure 6. Tail pigment character states for the genetically identified specimens examined arrayed by specimen size and species. Tail pigmentation codes, 1–7, refer to panels in Figure 4B and are described in Table 3. (1) Standard tail pigment; (2) Midlateral; (3) Small; (4) Ventrally along Notochord; (5) Wispy Caudal; (6) No Pigment; (7) Dorsal-Ventral. Observations on a subset of 20 specimens of flexion and postflexion *Hyporthodus flavolimbatus* and preflexion *Epinephelus guttatus* are presented.

SPECIES DESCRIPTIONS

Alphestes afer

(Tables 1–3; Figs. 2–7)

Material Examined.—Two postflexion (5.8–6.4 mm BL) specimens of *A. afer* were collected (n = 2).

Description.—Lower jaw and nape pigment are occasionally present. The cleithral symphysis is not pigmented. The tail displays standard tail pigment, though tail pigment on the largest specimen is located in the midlateral position. Spinelets on the primary ridge of the pelvic spine are either long and straight (the smaller specimen)

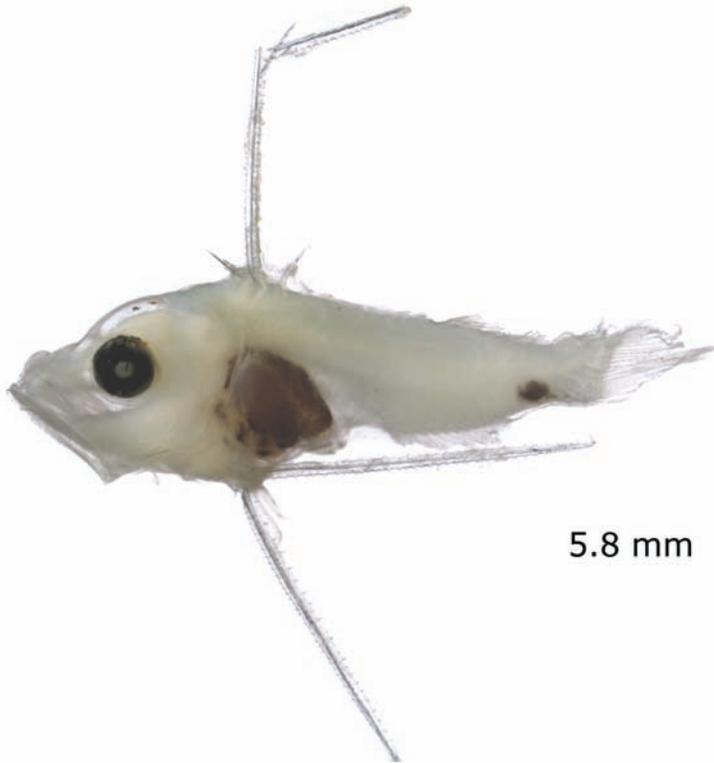


Figure 7. Genetically identified, 5.8 mm BL, postflexion *Alphestes afer* larva collected in the Straits of Florida (n = 2; 5.8–6.4 mm BL).

or widely spaced and slightly curved toward the tip of the spine (the larger specimen). Simple small spinelets were evident on all three ridges of the dorsal spine.

Remarks.—Johnson and Keener (1984) described 17 larval (10.5–19.5 mm BL), five transforming (25.2–29.1 mm BL), and three juvenile (33.2–62.0 mm BL) specimens identified based on fin-ray counts of D XI, 19–20; A III, 9; and P₁ 36–38. The spinelets of these specimens were small and straight on all dorsal spine ridges and on all but the fourth ridge of the pelvic spine. The proximal half of this ridge displayed slightly enlarged and straight spinelets angled toward the tip of the spine. This pattern may begin as long straight spinelets in early larvae (as observed on the smaller genetically identified specimen), that decrease in length with development. Johnson and Keener (1984) also observed cranial rugosity in large specimens (> 13.5 mm SL) of *A. afer* which appeared to be unique to the species. No cranial rugosity was observed on either specimen described here. Therefore, cranial rugosity does not reliably separate *A. afer* from other grouper species until late postflexion when the distinction between smooth and rugose becomes clear. *Alphestes afer* was indistinguishable from species whose larvae exhibit standard tail pigment and simple, small, and straight spinelets over most of their larval development (see “Small spinelet group description”).

Cephalopholis cruentata

(Tables 1–3; Figs. 2–6, 8)

Material Examined.—Six preflexion (3.3–4.1 mm BL), 20 flexion (3.4–5.7 mm BL), and 16 postflexion (4.9–7.7 mm BL) specimens of *C. cruentata* were collected (n = 44).

Description.—Pigment is absent on the lower jaw. Pigment at the cleithral symphysis is present on 25% of preflexion larvae and on 86% of postflexion specimens. Pigment at the nape is occasionally present irrespective of developmental stage. One of three pigment patterns occurs on the tail: no pigment (Fig. 4B6), small melanophore tail pigment (Fig. 4B3), or multiple melanophores tail pigment (Fig. 4B8). The spinelets on the posterolateral wings of the second dorsal spine and primary ridge of the pelvic spines are longer, narrower, and more curved than in the other western North Atlantic species examined (Fig. 2). These distinctive spinelets are already present in preflexion larvae < 3.3 mm BL. The dorsal spinelets of postflexion *C. cruentata* larvae begin to resemble the broad-based, long, and curved spinelets of other species, but by this stage, anvil-shaped spinelets on the base of the pelvic spines are apparent (Fig. 2). Anvil-shaped spinelets are also occasionally present on the wing margins of the dorsal spine in larger specimens.

Remarks.—Early larval stages of *C. cruentata* have not been described and these specimens represent the most extensive developmental series yet available. Johnson and Keener (1984) described the species-specific anvil-shaped spinelet morphology from 46 specimens of *C. cruentata* ranging in size from 5.2–20.5 mm identified by the meristic complement: D IX, 14; A III, 8; and P₁ 32. They observed that individual anvil-shaped spinelets occasionally occur in larvae of other grouper species but these are unlike the regular sequence of anvil-shaped spinelets present in large postflexion *C. cruentata* larvae.

Larvae of this species are readily identifiable due to the presence of three distinctive features (Table 4): (1) pigment at the cleithral symphysis (flexion and postflexion specimens), (2) narrow-based, long and curved spinelets on the dorsal and pelvic spines, and (3) anvil-shaped spinelets on the pelvic spine of postflexion larvae. Tail pigmentation further distinguishes *C. cruentata* larvae (Figs. 4B3, 6). If tail pigment is absent, *C. cruentata* and *H. mystacinus* (whose larvae also lack tail pigment) can be differentiated based on spinelet morphology.

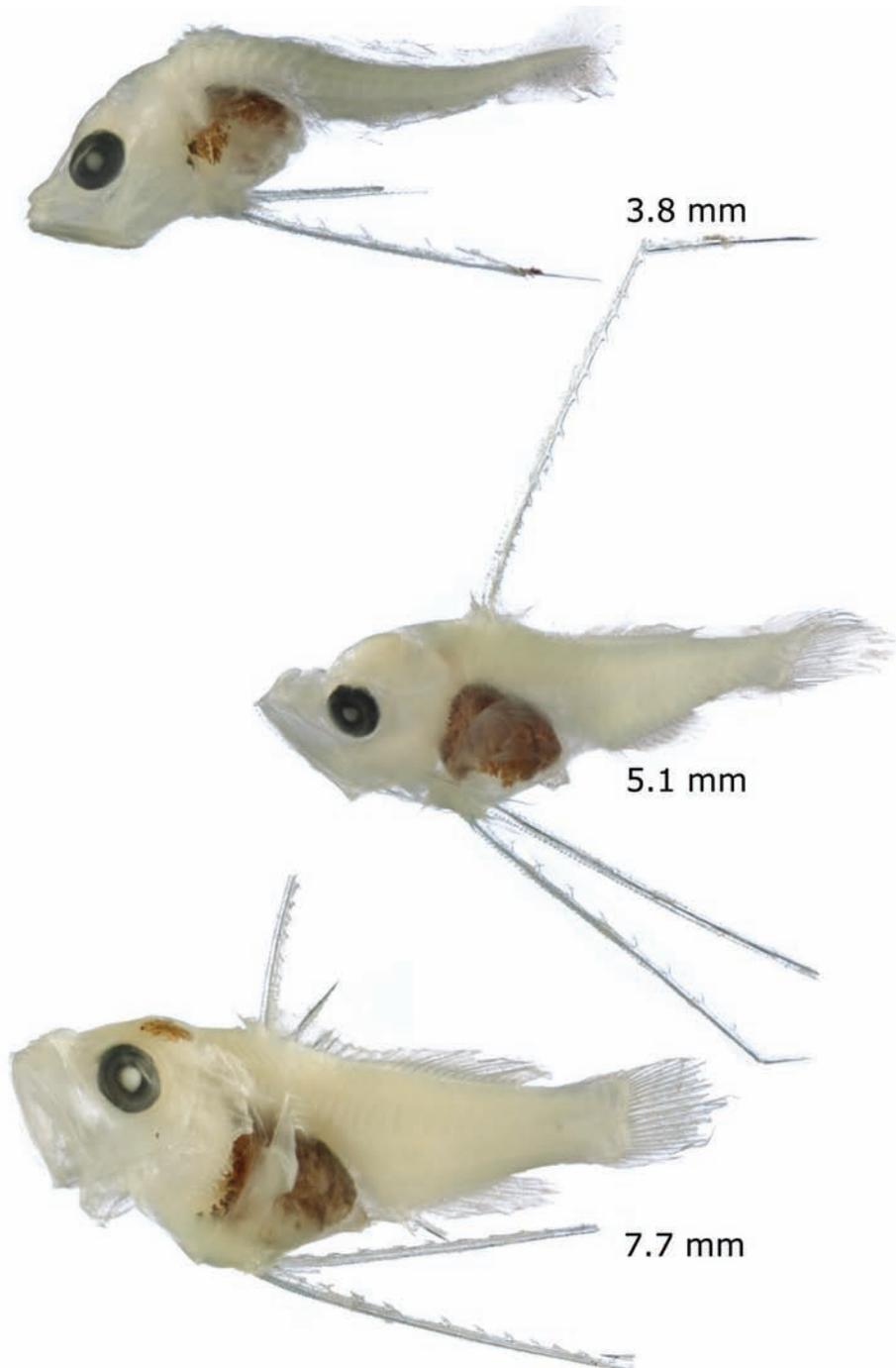


Figure 8. Genetically identified preflexion, flexion, and postflexion *Cephalopholis cruentata* larvae collected in the Straits of Florida (n = 44; 3.3–7.7 mm BL). Dorsal spine of the 3.8 mm specimen was broken off during capture.

Cephalopholis fulva

(Tables 1–3; Figs. 2–6, 9)

Material Examined.—Eight preflexion (3.4–4.9 mm BL), three flexion (4.5–6.0 mm BL), and two postflexion stage (6.9–7.7 mm BL) specimens of *C. fulva* were collected (n = 13).

Description.—Pigment on the lower jaw is present on many larval *C. fulva*. The cleithral symphysis is not pigmented, but the nape occasionally is. For preflexion and flexion staged larvae of the species, tail pigment is diagnostic and consists of the ventrally along notochord pattern (Fig. 4B4). By the postflexion stage, tail pigment is consolidated into the standard midlateral pattern. Dorsal and pelvic spinelets are small in preflexion and flexion larvae and remain small through metamorphosis (Johnson and Keener, 1984).

Remarks.—Two postflexion larvae, 5.7 and 9.4 mm SL, identified as *Epinephelus fulvus* are illustrated in Richards et al. (2005). Presumably, these specimens were identified by the unique dorsal (IX, 15) and anal (III, 9) counts which distinguish this species from all other western North Atlantic groupers. Johnson and Keener (1984) reported that the dorsal and pelvic spinelets in seven larval *C. fulva* ranging in length from 5.5 to 25.2 mm were simple, straight, and small. They also observed that most of the spinelets on the apex ridge (anterior surface) of the second dorsal spine were angled toward the spine tip.

Larvae of *C. fulva* can be distinguished from the other known grouper larvae by the combination of small spinelets on the dorsal and pelvic spines, tail pigment pattern, and/or the presence of lower-jaw pigment. In some cases, the difference between the tail pigment pattern on *C. fulva* and the standard tail pigment pattern was not obvious, in these cases *C. fulva* and *E. striatus* (which also exhibit lower-jaw pigment) were difficult to distinguish. Separation of these two species in this case can only be made if the tail pigment is clearly associated with the ventrum of the peduncle (*E. striatus*) or is in a ventrolateral position (*C. fulva*). The tail pigment of one specimen of *C. fulva* consisted of only one melanophore on the caudal finfold, similar to the wispy tail pigment of *Epinephelus guttatus* except that this melanophore did not spread along the caudal fin membrane.

Dermatolepis inermis

(Tables 1, 3)

Material Examined.—No specimens of this species were collected for genetic identification.

Remarks.—Johnson and Keener (1984) identified five specimens of *D. inermis*, 6.8–10.5 mm SL, based on the meristic complement: D XI, 19–20; A III, 9; P₁ 36–38. These specimens had deeply compressed bodies lacking strong cteni scales. The spinelet morphology of these specimens was unique in that the dorsal-spine ridges displayed long, straight, widely spaced spinelets over most of the length of the spine with slightly curved spinelets near the tip. The pelvic spines had spinelets that were large, narrow, and slightly curved toward the tip on the primary ridge and small, narrow, straight or slightly curved spinelets on the remaining pelvic ridges. Long



Figure 9. Genetically identified preflexion and flexion *Cephalopholis fulva* larvae collected in the Straits of Florida (n = 14; 3.4–7.7 mm BL).

straight spinelets also were observed among intermediately sized, genetically identified *H. flavolimbatus*. This condition may represent a transitional morphology between the small spinelets on preflexion specimens and the broad-based, long, and curved spinelets of postflexion specimens. Due to this similarity, care should be taken when attempting to identify flexion and early postflexion larvae with long straight spinelets.

Epinephelus adscensionis

(Tables 1 and 3)

Material Examined.—No specimens of this species were collected for genetic identification.

Remarks.—Johnson and Keener (1984) described late larvae and juveniles of this species based on approximately 200 specimens ranging in length from 10.5 to > 30.0 mm SL. *Epinephelus adscensionis* and *E. striatus* were indistinguishable based on meristic characters (D XI, 16–17; A III, 8; P₁ 36–38) and spinelet morphology, though the combination of both these characters separated these two species from all other western North Atlantic species. Both species displayed simple, small, and straight spinelets on all ridges of the second dorsal and pelvic spines. An illustration of a late postflexion (19.0 mm SL) specimen appears in Richards et al. (2005), likely identified based on the meristic complement, though neither the source of the illustration nor an explanation of how the specimen was distinguished from *E. striatus* was given. Lower-jaw pigment was not depicted in this illustration.

Epinephelus adscensionis are likely indistinguishable from the group of species whose larvae exhibit standard tail pigment and simple, small, and straight spinelets over the entire size range examined (see “Small spinelet group description”).

Epinephelus drummondhayi

(Tables 1–3; Figs. 2–6, 10)

Material Examined.—One preflexion (3.6 mm BL) and four flexion (4.1–5.3 mm BL) stage specimens of *E. drummondhayi* were collected (n = 5).

Description.—Pigmentation on the lower jaw or cleithral symphysis is not present on larval *E. drummondhayi*, and nape pigment is uncommon. Tail pigment consists of the standard single, large melanophore on the ventrum, though tail pigment on the largest specimens is located in the midlateral position. Dorsal and pelvic spinelet morphology transitions from simple, small, and straight to broad-based, long, and curved on specimens > 5 mm BL. This differed from published descriptions of the species (Johnson and Keener, 1984).

Remarks.—Johnson and Keener (1984) identified approximately 90 specimens of *E. drummondhayi*, *E. guttatus*, and *E. morio* ranging in size from 3.5 to 14.4 mm SL. These specimens were indistinguishable from each other based on spinelet morphology and the meristic complement: D XI, 15–17; A III, 9; P₁ 34, although a higher pectoral ray count of 36 should differentiate *E. drummondhayi* from the other two species. Unlike the large specimens of genetically identified *E. drummondhayi*, all three species, as described by Johnson and Keener (1984), had simple, small, and

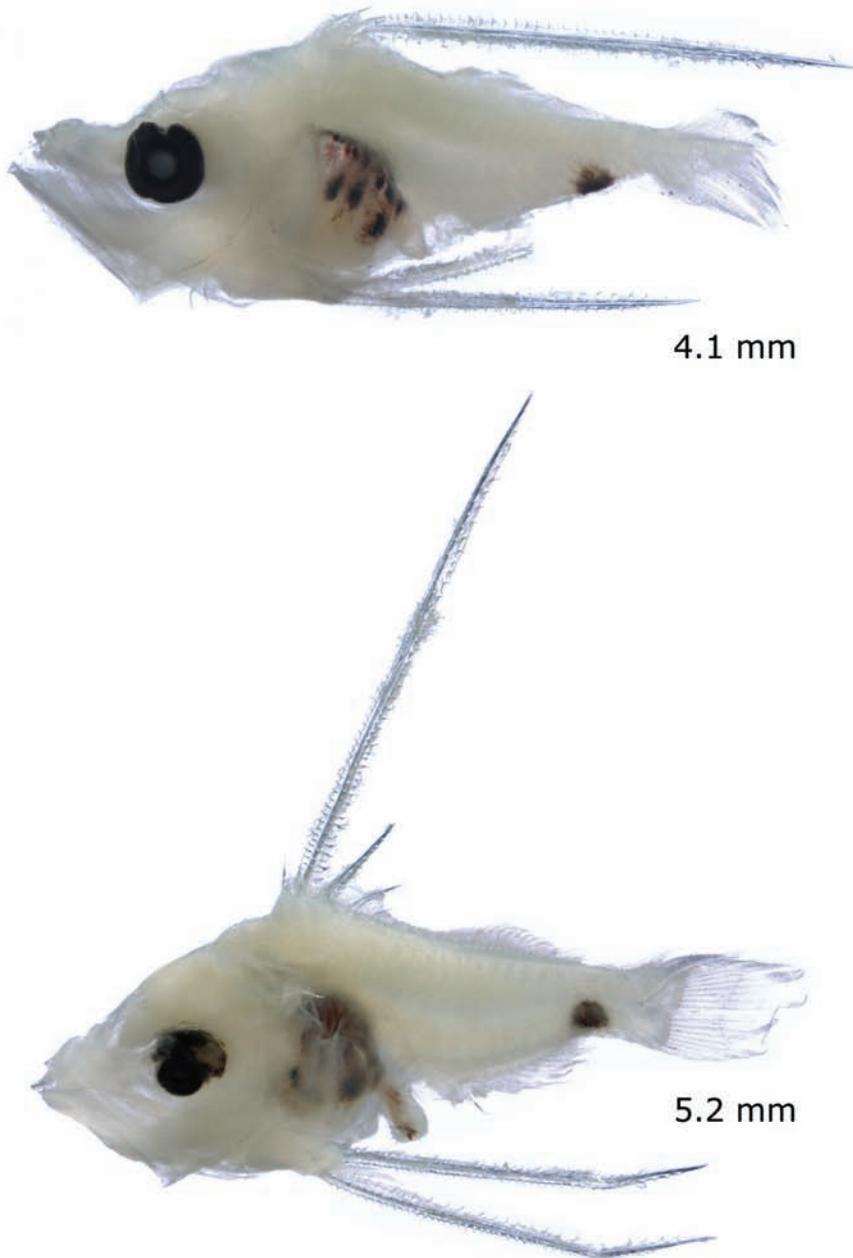


Figure 10. Genetically identified flexion and late flexion *Epinephelus drummondhayi* larvae collected in the Straits of Florida (n = 5; 3.6–5.3 mm BL).

straight spinelets on all ridges of the dorsal and pelvic spines. Several explanations for this discrepancy are addressed in the discussion. *Alphestes afer* also has similar meristic characters, however, the presence of cranial rugosity separates large *A. afer* from all other Gulf of Mexico species.

During preflexion and flexion stages, *E. drummondhayi* are indistinguishable from the group of species whose larvae exhibit standard tail pigment and simple, small, and straight spinelets (see "Small spinelet group description"). By approx. 5.2 mm BL, larvae of *E. drummondhayi* closely resemble a smaller group of species whose larvae bear standard pigment and broad-based, long, and curved spinelets (see "Long and curved spinelet group description").

Epinephelus guttatus

(Tables 1–3; Figs. 2–6, 11)

Material Examined.—Forty-eight preflexion (2.2–4.1 mm BL), one flexion (4.3 mm BL), and one postflexion stage (9.5 mm BL) specimens of *E. guttatus* were collected (n = 50).

Description.—Pigment on the lower jaw is frequently present. This pigment is often intense and forms a band from one side of the jaw tip to the other. Nape pigment is also common and usually more pronounced (not as deeply embedded) than in specimens of the other species examined. Pigment at the cleithral symphysis does not occur on larval *E. guttatus*. The wispy caudal tail pigment (Fig. 4B5) of preflexion *E. guttatus* is unique to the species. A very faint melanophore is occasionally present in the location of the standard tail pigment (three specimens). By flexion stage, the standard tail pigment is present, and moves midlaterally by postflexion. Spinelets on the dorsal and pelvic spines are simple, small, and straight by 3.0 mm BL and remain small throughout development.

Remarks.—Johnson and Keener (1984) found large *E. guttatus* to be inseparable from *E. morio* and *E. drummondhayi* based on meristic characters (D XI, 15–17 A III, 9 P₁ 34) and the simple, small, and straight spinelet morphology. These conclusions were based on approximately 90 specimens ranging from 3.5 to 14.4 mm BL. Richards et al. (2005) provide illustrations for two larval and one juvenile specimens of putative *E. guttatus* which also show lower-jaw pigment. The tail pigment pattern in these illustrations does not resemble the standard pigment of the genetically identified specimens.

Large *E. guttatus* larvae (the flexion and postflexion specimens) with standard tail pigment and lower-jaw pigment should be separable from all species except some *C. fulva* (which generally can be separated based on tail pigment patterns of either no pigment or one to three melanophores found ventrally along the notochord), *E. striatus* (which also has standard pigment and small spinelets), and *Mycteroperca venenosa* (which can be separated based on dorsal pigment during early preflexion stage and long curved spinelets later in development).

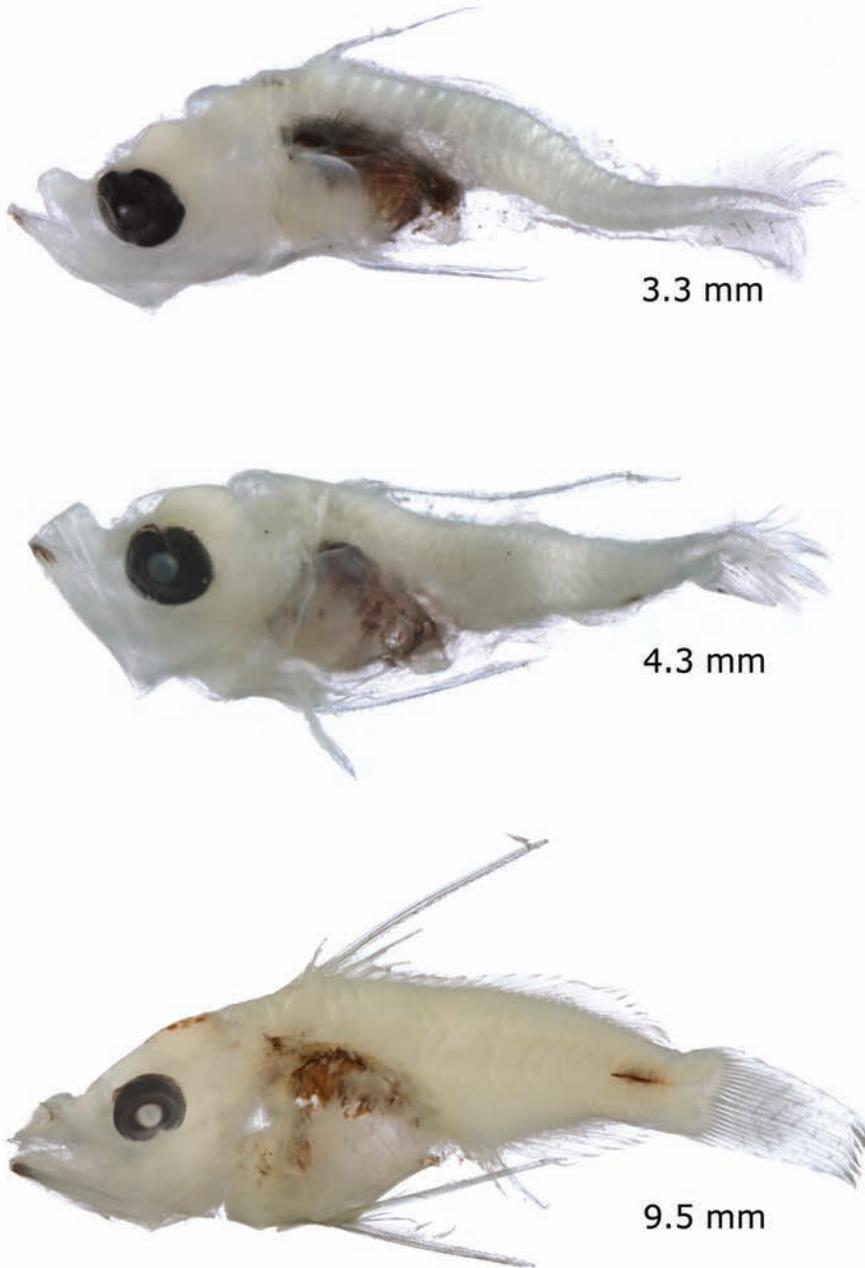


Figure 11. Genetically identified preflexion, flexion, and postflexion *Epinephelus guttatus* larvae collected in the Straits of Florida (n = 50; 2.2–9.5 mm BL).

Epinephelus itajara

(Tables 1 and 3)

Material Examined.—No specimens of this species were collected for genetic identification.

Remarks.—Johnson and Keener (1984) described five western North Atlantic specimens of late-stage larvae ranging from 6.2 to 17.4 mm BL. The meristic complement of D XI, 15–16, A III, 8, and P₁ 37–38 separate *E. itajara* from all species but *E. striatus* and *E. adscensionis* both of which have small dorsal and pelvic spinelets. The spinelet morphology of the *E. itajara* specimens consisted of large recurved spinelets along most of the length of the dorsal wing margins and on both the first and second pelvic-spine ridges with small spinelets at the base. Pigment at the cleithral symphysis was indicative of *E. itajara*, but Johnson and Keener (1984) did not mention the presence of pigment at the nape or on the lower jaw.

Epinephelus morio

(Tables 1–3; Figs. 2–6, 12)

Material Examined.—Two preflexion (4.1, 4.3 mm BL), six flexion (4.0–5.2 mm BL), and one postflexion stage (5.6 mm BL) specimens of *E. morio* were collected (n = 9).

Description.—Pigment on the lower jaw and cleithral symphysis are absent from larval *E. morio*. The tail displays the standard tail pigment, though tail pigment on postflexion specimens is located in the midlateral position. The elongate dorsal and pelvic spines possess simple, small, and straight spinelets throughout larval development.

Remarks.—This species also has been described from hatch to the juvenile stage of laboratory-reared larvae (Colin et al., 1996) and from late-stage wild-caught larvae (Johnson and Keener, 1984). Colin et al. (1996) described lower-jaw pigment on preflexion stage larvae which remained at least to the juvenile stage, but made no mention of pigment at the cleithral symphysis in the very detailed description of body pigment. The specimens described by Colin et al. (1996) lacked tail pigment until approximately 2.4 mm BL. By 2.6 mm BL, seven to nine melanophores were present, which condensed to a single large melanophore by 3.5 mm BL. This melanophore was joined by internal pigment at the midlateral position at 6 mm BL, and was no longer visible by 7.4 mm. The number of melanophores along the midlateral line increased with size, forming a line of pigment along the lateral midline. The small and straight spinelet morphology was also described by Johnson and Keener (1984) for approximately 90 larger specimens ranging from 3.5 to 14.4 mm BL. These specimens were identified based on the meristic complement D XI, 14–15 A III, 9 and P₁ 34, and were virtually indistinguishable from *E. guttatus* and some *E. drummondhayi*.

Epinephelus morio are indistinguishable from species whose larvae exhibit standard tail pigment and simple, small, and straight spinelets over the entire size range examined (see “Small spinelet group description”). The lower-jaw pigment described on the laboratory-reared specimens of Colin et al. (1996) may provide a character which limits identification to either *E. morio* or *E. striatus* for some specimens, but this pigment was not observed among the wild-caught genetically identified specimens.

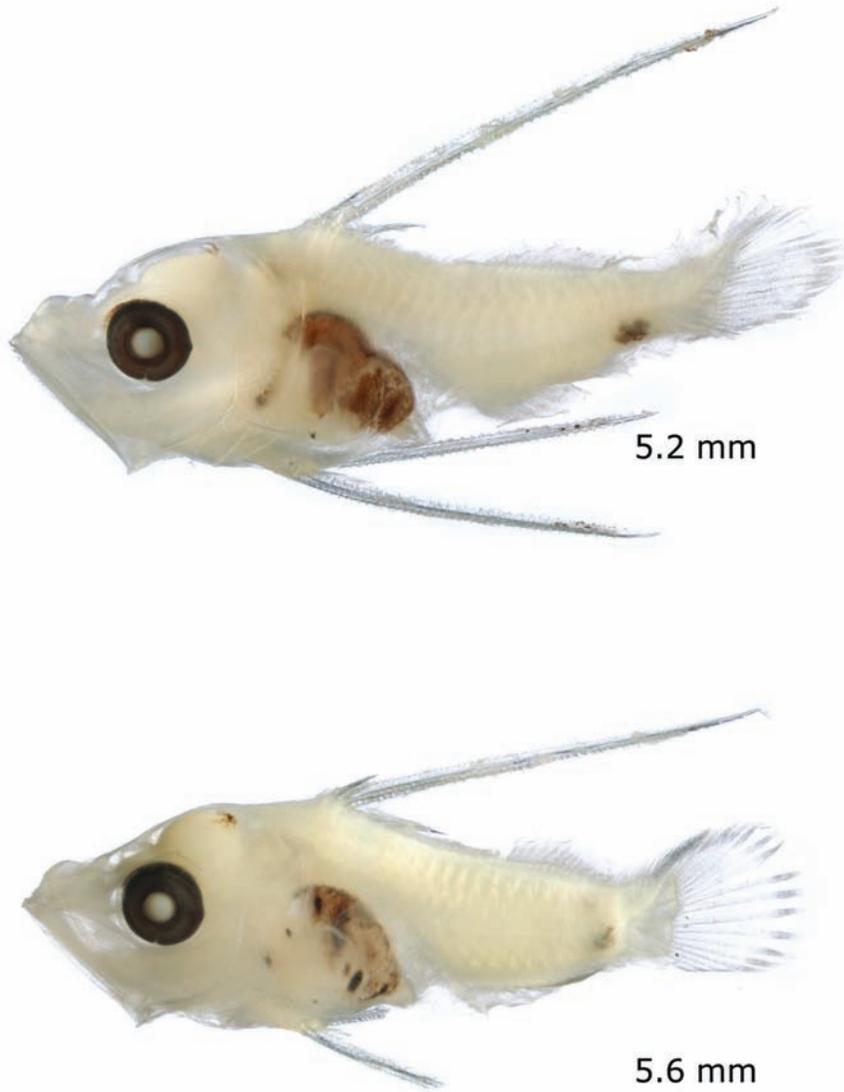


Figure 12. Genetically identified flexion and postflexion *Epinephelus morio* larvae collected in the Straits of Florida (n = 9; 4.1–5.6 mm BL).

Epinephelus striatus

(Tables 1–3; Figs. 2–6, 13)

Material Examined.—Six preflexion (3.2–4.4 mm BL) and two flexion (4.5 mm BL) stage specimens of *E. striatus* were collected (n = 8).

Description.—Pigment on the lower jaw occasionally occurs on larger larvae (> 4.0 mm BL). Pigment at the cleithral symphysis is rare, but pigment at the nape is common. The tail displays the standard tail pigment. Spinelets on the dorsal and pelvic spines form by 3.9 mm BL and are simple, small, and straight through transformation (Johnson and Keener, 1984).

Remarks.—This species is one of the most well-described grouper species in the literature. Manday and Fernandez (1966) described laboratory-reared larvae from the egg through hatching until the yolk was absorbed (2.6 mm BL). The only pigmentation mentioned in this description was pigment over the dorsal surface of the gut and a large single melanophore on the ventrum of the peduncle, which corresponds to standard tail pigment observed on the genetically identified specimens. Powell and Tucker (1992) give a very complete description of laboratory-reared larvae (1.8–13.5 mm BL) including the sequence of fin formation, size at flexion (5.0–6.5 mm BL), interdigitation patterns of the dorsal-fin pterygiophores (for larvae > 7.0 mm), gill raker counts (for larvae > 13.0 mm), and pigmentation. Among the areas of pigment described was an inverted saddle on the peduncle of yolk sac through early flexion stage specimens which is equivalent to the standard tail pigment described for the genetically identified larvae, nape pigment, and pigment at the tips of the upper and lower jaw of late flexion stage specimens. The tail pigment of late flexion and postflexion stage specimens had moved dorsally to the midlateral position (approximately 5.7 mm, notochord length, NL). No mention of pigment at the cleithral symphysis was made. Richards et al. (2005) contains illustrations of several putative *E. striatus*, at least one of which has not developed a full meristic complement of dorsal, anal, and pectoral elements. Lower-jaw pigment is indicated on the illustrations of the 5.8 mm SL and 9.1 mm SL specimens. The standard tail pigment is present on all, though juvenile pigment had begun forming on the largest specimen illustrated.

Once the lower-jaw pigment develops, *E. striatus* can be separated from all other species examined among the genetically identified specimens except *M. venenosa* (3.5–6.0 mm) based on the combination of standard tail pigment, lower-jaw pigment, and simple, small, and straight spinelets. In the absence of lower-jaw pigment (most specimens < 4 mm), preflexion and early flexion stage specimens of *E. striatus* are difficult to distinguish from several species that display the combination of no lower-jaw pigment, standard tail pigment, and simple, small, and straight spinelets (see “Small spinelet group description”). In a few cases, *E. striatus* can be confused with *C. fulva*, so care should be taken when determining the type of tail pigment present (see *C. fulva* description).



Figure 13. Genetically identified preflexion *Epinephelus striatus* larva collected in the Straits of Florida (n = 8; 3.2–4.5 mm BL).

Hyporthodus flavolimbatus

(Tables 1–3; Figs. 2–6, 14)

Material Examined.—Fourteen preflexion (2.9–4.2 mm BL), 57 flexion (3.2–6.4 mm BL), and 22 postflexion stage (4.9–12.4 mm BL) specimens of *H. flavolimbatus* were collected (n = 93).

Description.—Pigment on the lower jaw is not present on larval *H. flavolimbatus*, and pigment at the cleithral symphysis is very rare. The nape is frequently pigmented. The tail displays the standard tail pigment, though tail pigment on some flexion and most postflexion specimens is located in the midlateral position. Spinelets on the dorsal and pelvic spines are simple, small, and straight on all ridges till approximately 5.0 mm SL, at which time the posteriolateral ridges of the dorsal spine and primary ridge of the pelvic spines display broad-based, long, and curved spinelets.

Remarks.—Johnson and Keener (1984) gave a detailed description of the long and curved spinelet morphology of *H. flavolimbatus* and *H. niveatus* based on six larval specimens (4.0–19.0 mm BL) and six juvenile *H. flavolimbatus* specimens (29.1–33.0 mm BL). These identifications were made based on the meristic complement of D XI, 14 A III, 9 P₁ 36, which in combination with spinelet morphology may separate these two species from all other species occurring in the western North Atlantic. There is now some indication, based on the genetically identified specimens, that large *E. drummondhayi* share the same spinelet morphology as *H. flavolimbatus* and *H. niveatus* (see the *E. drummondhayi* species description).

During preflexion and flexion stages, *H. flavolimbatus* are indistinguishable from species whose larvae exhibit standard tail pigment and simple, small, and straight spinelets (see “Small spinelet group description”). By approx. 4.5 mm BL, larvae of *H. flavolimbatus* closely resemble a smaller group of species by the presence of standard pigment and broad-based, long, and curved spinelets (see “Long and curved spinelet group description”).

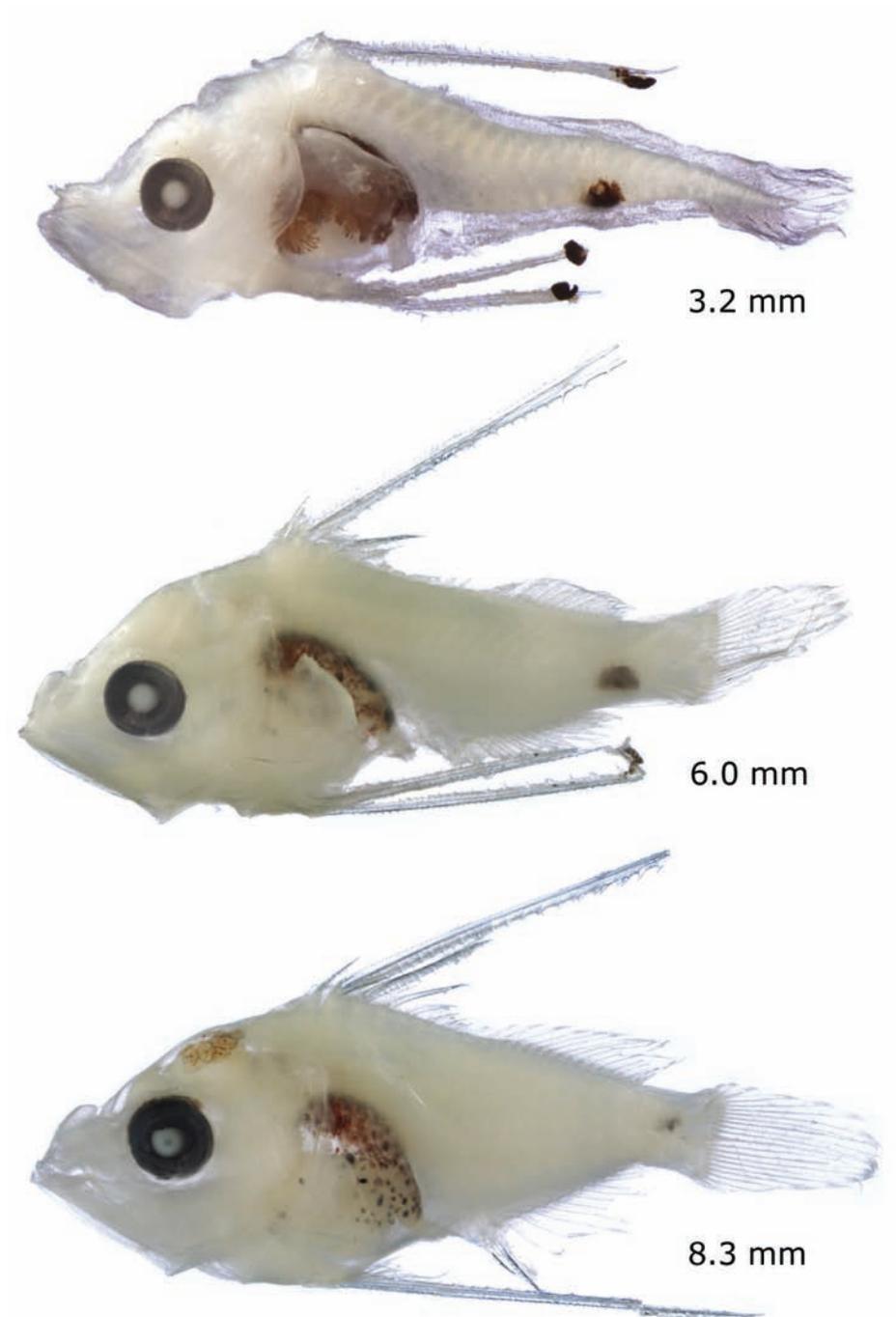


Figure 14. Genetically identified preflexion, flexion, and postflexion *Hyporthodus flavolimbatus* larvae collected in the Straits of Florida (n = 93; 2.9–12.4 mm BL).

Hyporthodus mystacinus

(Tables 1–3; Figs. 2–6, 15)

Material Examined.—Two flexion (5.3 mm BL), and two postflexion stage (5.1–6.7 mm BL) specimens of *H. mystacinus* were collected (n = 4).

Description.—Pigment is absent on the lower jaw, cleithral symphysis, nape, and tail of larval *H. mystacinus*. The spinelets on the dorsal and pelvic spines are broad-based, long, and curved on flexion and postflexion larvae. The spinelet morphology of preflexion larvae is unknown.

Remarks.—One 20.0 mm BL specimen was identified by Johnson and Keener (1984) based on the meristic complement of D XI, 14–15; A III, 9; and P₁ 37–38, which is similar to the meristic characters of *H. niveatus* and *H. flavolimbatus*. This specimen was identified as *H. mystacinus* based on the presence of long and curved spinelets on a second pelvic spine ridge. The genetically identified specimens had not formed an obvious second ridge of elongate spinelets. Three of the four specimens, however, had a second ridge with spinelets more widely spaced and slightly curved, which may be the precursory stage of the long and curved spinelets in this species.

The lack of tail pigment separates *H. mystacinus* from all species except some *C. cruentata* (for whom the dramatically long, narrow-based, and curved spinelets form early), *E. guttatus* (which have a unique tail pigment pattern of wispy pigment on the caudal finfold), and some *C. fulva* (which often have pigment at the tip of the lower jaw and do not appear to develop long and curved spinelets at any developmental stage).

Hyporthodus nigritus

(Tables 1 and 3)

Material Examined.—No specimens of this species were collected for genetic identification.

Remarks.—Johnson and Keener (1984) described one 9.1 mm BL specimen from the western North Atlantic. The identification was based on the unique meristic complement of D X, 14–15; A III, 9; and P₁ 36–38. The spinelet morphology of the dorsal and pelvic ridges was long, widely spaced, and curved. The specimen they described had broken pelvic spines, but evidence of a second ridge of elongate spinelets on the pelvic spine, similar to the pelvic spines of *H. mystacinus*, was observed.

Hyporthodus niveatus

(Tables 1–3; Figs. 2–6, 16)

Material Examined.—Eight preflexion (2.2–4.6 mm BL) and three flexion (4.0–5.3 mm BL) stage specimens of *H. niveatus* were collected (n = 11).

Description.—The lower jaw and cleithral symphysis are not pigmented, and the nape only occasionally bears pigment. The tail displays the standard tail pigment, though tail pigment on the largest specimens is located in the midlateral position. The dorsal and pelvic spines develop spinelets by 3.0 mm BL. These spinelets are



Figure 15. Genetically identified postflexion *Hyporthodus mystacinus* larva collected in the Straits of Florida (n = 4; 5.1–6.7 mm BL).

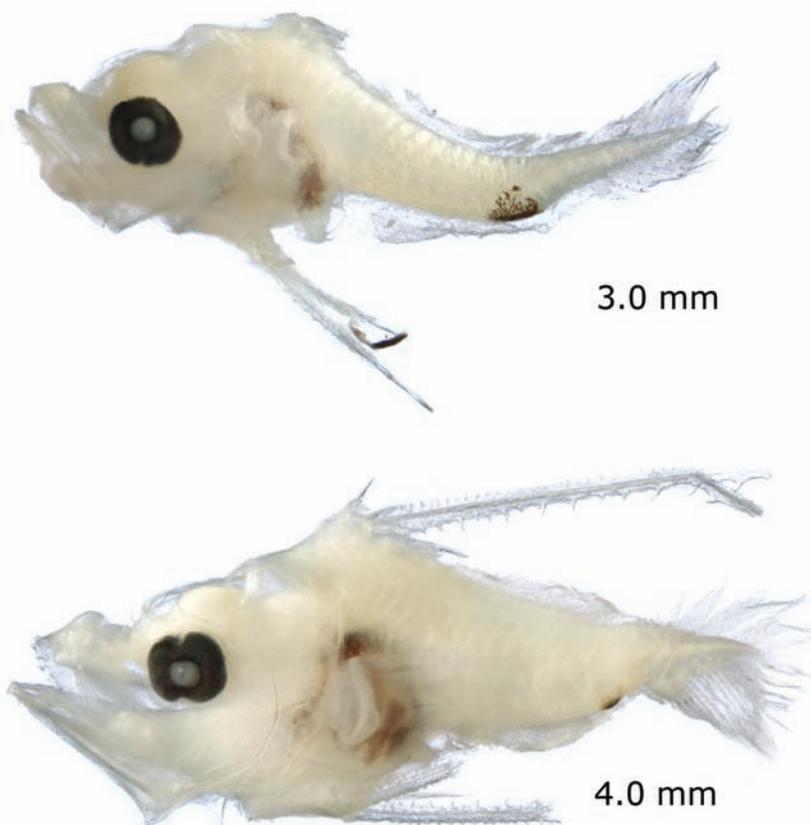


Figure 16. Genetically identified preflexion and flexion *Hyporthodus niveatus* larvae collected in the Straits of Florida (n = 11; 2.2–5.3 mm BL). Dorsal spine of the 3.0 mm specimen was broken off during capture.

simple, small, and straight on small larvae, but change to broad-based, long, and curved at approximately 4.0 mm BL.

Remarks.—Presley (1970) identified 16 specimens 5.5–10.3 mm BL based on meristic characters (D XI, 14; A III, 9; P₁ 36). The standard tail pigment was also present on these specimens, and no mention was made of pigment on the lower jaw or cleithral symphysis. Johnson and Keener (1984) also found long curved spinelets on the second dorsal and pelvic spines of six larval specimens (4.0–19.0 mm BL) and two transforming specimens (23.5–24.8 mm BL) that, based on meristic characters (D XI, 14 A III, 9 P₁ 36), were either *H. flavolimbatus* or *H. niveatus*.

During preflexion and flexion stages, *H. niveatus* are indistinguishable from species whose larvae exhibit standard tail pigment and simple, small, and straight spinelets (see “Small spinelet group description”). By 4.0 mm BL, larvae of *H. niveatus* closely resemble a smaller group of species by the presence of standard pigment and broad-based, long, and curved spinelets (see “Long and curved spinelet group description”).

Mycteroperca species

Remarks.—Kendall (1979) was the first to describe the larvae of the genus as a whole. Identifications were based on meristic characters, with *Mycteroperca* sharing a high anal-fin ray count (III, 10–13), which separates specimens of this genus from all other western North Atlantic genera. He also characterized the genus as having parallel lateral skull crests, general epinephelin shape with elongate second dorsal and pelvic spines, long and serrate preopercle angle spines, and pigment on the brain, the lateral surface of the gut, the tail, and on the elongate fin spines. Johnson and Keener (1984) were able to add detail from several hundred specimens ranging from 3.5 to 24.0 mm BL. These specimens also displayed pigment at the cleithral symphysis and the long and curved spinelet morphology similar to *H. niveatus* and *H. flavolimbatus*. Kendall (1984) and Richards et al. (2005) summarize *Mycteroperca* as having more anal rays than the other genera, species could not be distinguished from each other or several *Epinephelus* species, but suggest the presence of pigment at the cleithral symphysis as a way to separate *Mycteroperca* from most species of *Epinephelus* (Johnson and Keener, 1984). The genetically identified specimens of *Mycteroperca* species provided evidence that pigment at the cleithral symphysis may not be as ubiquitous among species or throughout development as previously believed. As such, pigment at the cleithral symphysis is not diagnostic at the genus level.

With the exception of large *M. venenosa*, *Mycteroperca* species cannot be distinguished from each other when pigment at the cleithral symphysis is present, and when the pigment is absent, species in this genus can not be separated from several other epinephelin species (see small spinelet group and long and curved spinelet group descriptions.) Pigment at the tail and cleithral symphysis may prove useful for further subdividing this group once more *Mycteroperca* specimens of known identity are available.

Mycteroperca acutirostris and *Mycteroperca cidi*

(Tables 1 and 3)

Material Examined.—No specimens of these species were collected for genetic identification.

Remarks.—To date, no species-specific larval descriptions of *M. acutirostris* or *M. cidi* have been published.

Mycteroperca bonaci

(Tables 1–3; Figs. 2–6, 17)

Material Examined.—Two preflexion (3.3–3.6 mm BL), two flexion (5.1–5.3 mm BL), and one postflexion stage (8.4 mm BL) specimens of *M. bonaci* were collected (n = 5).

Description.—The tip of the lower jaw and the cleithral symphysis are not pigmented on *M. bonaci*. Pigment at the nape is common. The tail has standard tail pigment, though tail pigment on postflexion specimens is located in the midlateral position. Spinelets on the dorsal and pelvic spines are simple, small, and straight on preflexion larvae, but the broad-based, long, and curved spinelets indicative of the genus appear by 5.0 mm SL.

Remarks.—To date, this is the only species level description of larval *M. bonaci*.

Mycteroperca interstitialis

(Tables 1–3; Figs. 2–6, 18)

Material Examined.—Two preflexion stage (3.6–3.8 mm BL) specimens of *M. interstitialis* were collected (n = 2).

Description.—Pigment on the lower jaw and cleithral symphysis are absent on larval *M. interstitialis*. Nape pigment is present and the tail displays the standard tail pigment. The dorsal and pelvic spines have simple, small, and straight spinelets on preflexion stage larvae.

Remarks.—To date, this is the only species level description of larval *M. interstitialis*. Only preflexion stage larvae were examined; the spinelets on the dorsal and pelvic spines may become broad-based, long, and curved, similar to other members of the genus at larger sizes.

Mycteroperca microlepis

(Tables 1 and 3)

Material Examined.—No specimens of this species were collected for genetic identification.

Remarks.—Kendall (1979) described several larval specimens identified as *M. microlepis* based on collection north of Cape Hatteras (smallest specimen was 4 mm). Several 12–35 mm specimens of late larvae and juveniles collected from Florida to

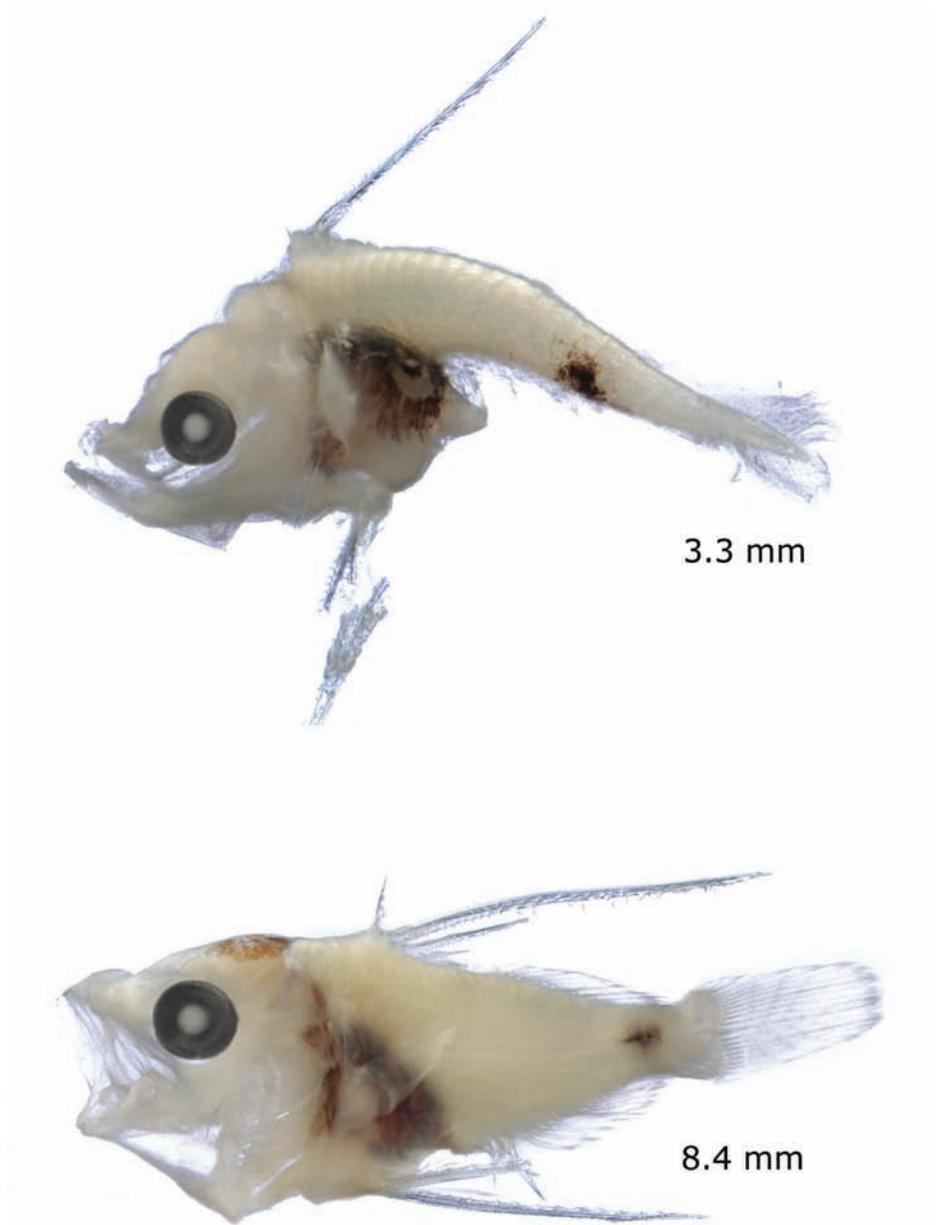


Figure 17. Genetically identified preflexion and postflexion *Mycteroperca bonaci* larvae collected in the Straits of Florida (n = 5; 3.3–8.4 mm BL).



Figure 18. Genetically identified preflexion *Mycteroperca interstitialis* larva collected in the Straits of Florida (n = 2; 3.6–3.8 mm BL).

New Jersey were also examined for developmental comparisons. Sequence of fin development and ossification were described. Morphometric relationships between body length and body depth (29%–35%), head length (31%–43%), preanal length (51%–66%), eye and snout length (9%–14%), second dorsal spine (40% at 4.6 mm, 60% at 5–10 mm, 11% at 35 mm), pelvic spine (68% at 8.3 mm, 12% at 35 mm), preopercular spine (0.5% at 5 mm, 1.1% at 8 mm, 0.2% at 35 mm) were also documented. Pigment changed little with size and was common on the brain case, internally on the nape, dorsolateral gut, cleithral symphysis in most specimens, ventrally on the tail in small larvae and midlaterally on the tail by 7.5 mm, and on the caudal fin base and rays. Opercle, supracleithrum, and posttemporal serrate spines were similar to other epinephelin species. Richards et al. (2005) includes illustrations of larvae and juveniles ranging from just after hatch to 40 mm SL. No pigment on the lower jaw or cleithral symphysis is indicated on these illustrations. Nape pigment is present on some, but not all. The standard tail pigment, shown on the illustrated specimens, moves dorsally to the midlateral position at sizes > 7.0 mm SL.

Mycteroperca phenax
(Tables 1–3; Figs. 2–6, 19)

Material Examined.—Two preflexion (3.6–3.7 mm BL) and two flexion (5.1–5.8 mm BL) stage specimens of *M. phenax* were collected (n = 4).

Description.—Lower-jaw pigment does not occur on larval *M. phenax*. The cleithral symphysis is pigmented in flexion and likely postflexion stage larvae, and nape pigment is common at all stages. The tail displays standard tail pigment. Small preflexion larvae have small and straight spinelets on the dorsal and pelvic spines, but broad-based, long, and curved spines are present on larger larvae. The transition in spinelet morphology occurs at smaller sizes than for most other species, with the broad-based, long, and curved spinelets present in larvae as small as 3.7 mm NL.

Remarks.—No published descriptions of this species' larval stages were found, but illustrations of the early larvae (1.7–3.1 mm BL; Koenig unpubl. data) appear in Richards et al. (2005). These illustrations do not show pigment on the lower jaw or cleithral symphysis, but do show the dorsal-ventral tail pigment pattern that we observed on the smallest genetically identified specimens of *M. venenosa*. This pigment pattern may be common among small larvae of the genus.

Mycteroperca tigris
(Tables 1 and 3)

Material Examined.—No specimens of this species were collected for genetic identification.

Remarks.—To date, no species-specific larval descriptions of *M. tigris* have been published. Illustrations of the early-stage larvae (1.7–2.8 mm BL; Koenig unpubl. data) appear in Richards et al. (2005). These illustrations do not show pigment on the lower jaw or cleithral symphysis, but appear to show the dorsal-ventral tail pigment, also observed on the smallest genetically identified specimens of *M. venenosa*. This pigment pattern may be common among the small larvae of the genus.

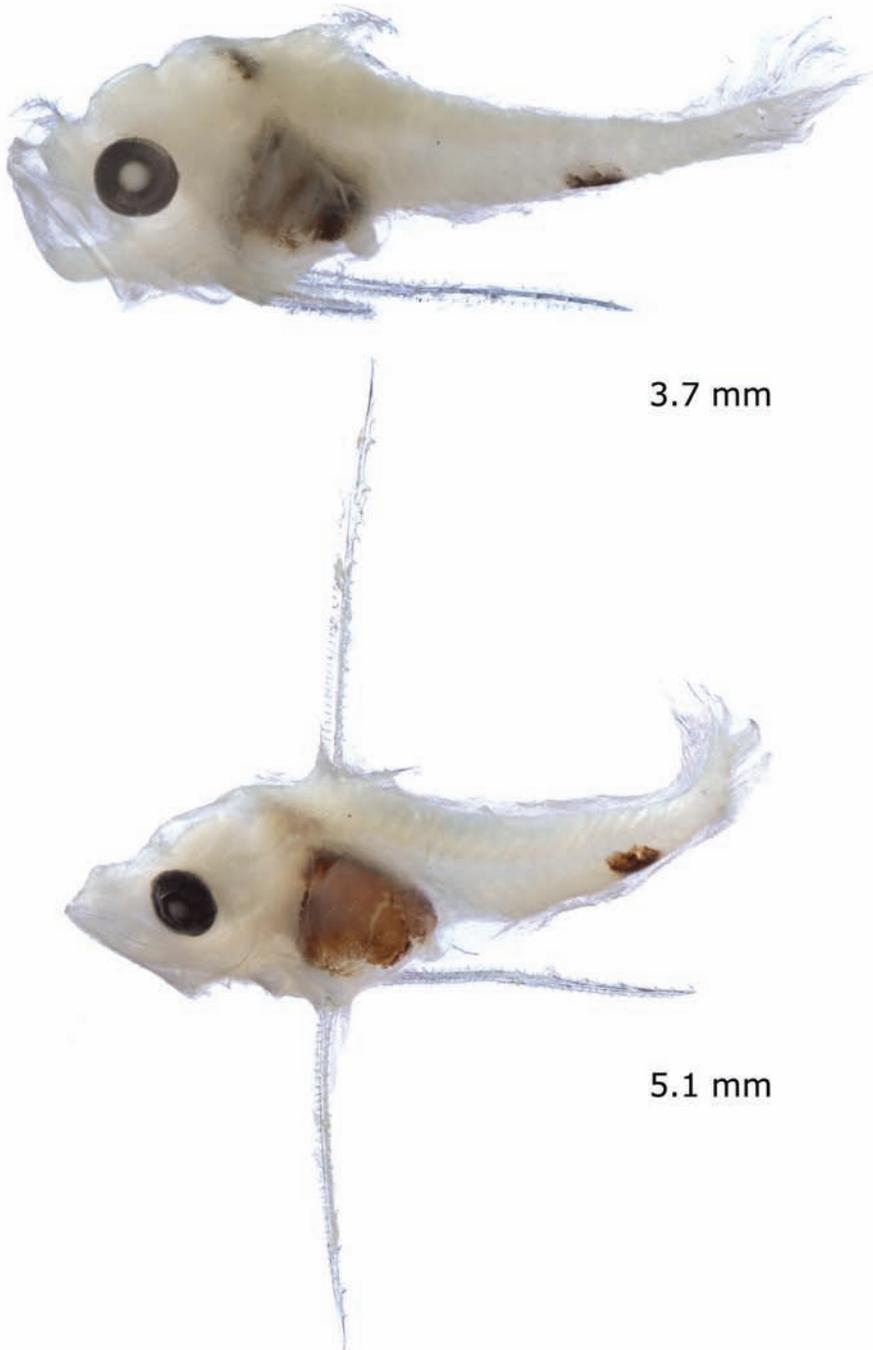


Figure 19. Genetically identified preflexion and flexion *Mycteroperca phenax* larvae collected in the Straits of Florida (n = 4; 3.6–5.8 mm BL). Dorsal spine of the 3.7 mm specimen was broken during capture.

Mycteroperca venenosa
(Tables 1–3; Figs. 2–6, 20)

Material Examined.—Five preflexion (2.2–4.5 mm BL) and 14 flexion (4.9–6.8 mm BL) stage specimens of *M. venenosa* were collected (n = 19).

Description.—Pigment at the tip of the lower jaw is diagnostic of this species and is consistently present on specimens ≥ 6.0 mm. Pigment at the cleithral symphysis is not present on larvae of *M. venenosa*; while pigment at the nape region is common (Fig. 5). Tail pigment changes with development. Small larvae (2.2–3.3 mm) display dorsal-ventral tail pigment (Fig. 4B7). This pattern was only observed among small *M. venenosa*, but may occur on several species (mostly the smallest *Mycteroperca* larvae). Larger larvae (> 4.5 mm NL) display the standard tail pigment (Fig. 4B1). By 4.5 mm NL, simple, small, and straight spinelets are present on all dorsal and pelvic-spine ridges, and by 5.6 mm, the posteriolateral dorsal ridges and primary pelvic ridge develop broad-based, long, and curved spinelets.

Remarks.—To date, this is the only species level description of larval *M. venenosa*. By late flexion and postflexion stages, *M. venenosa* can be separated from most other Gulf of Mexico grouper species by the combined presence of standard tail pigment, pigment at the tip of the lower jaw, and broad-based, long, and curved spinelets.

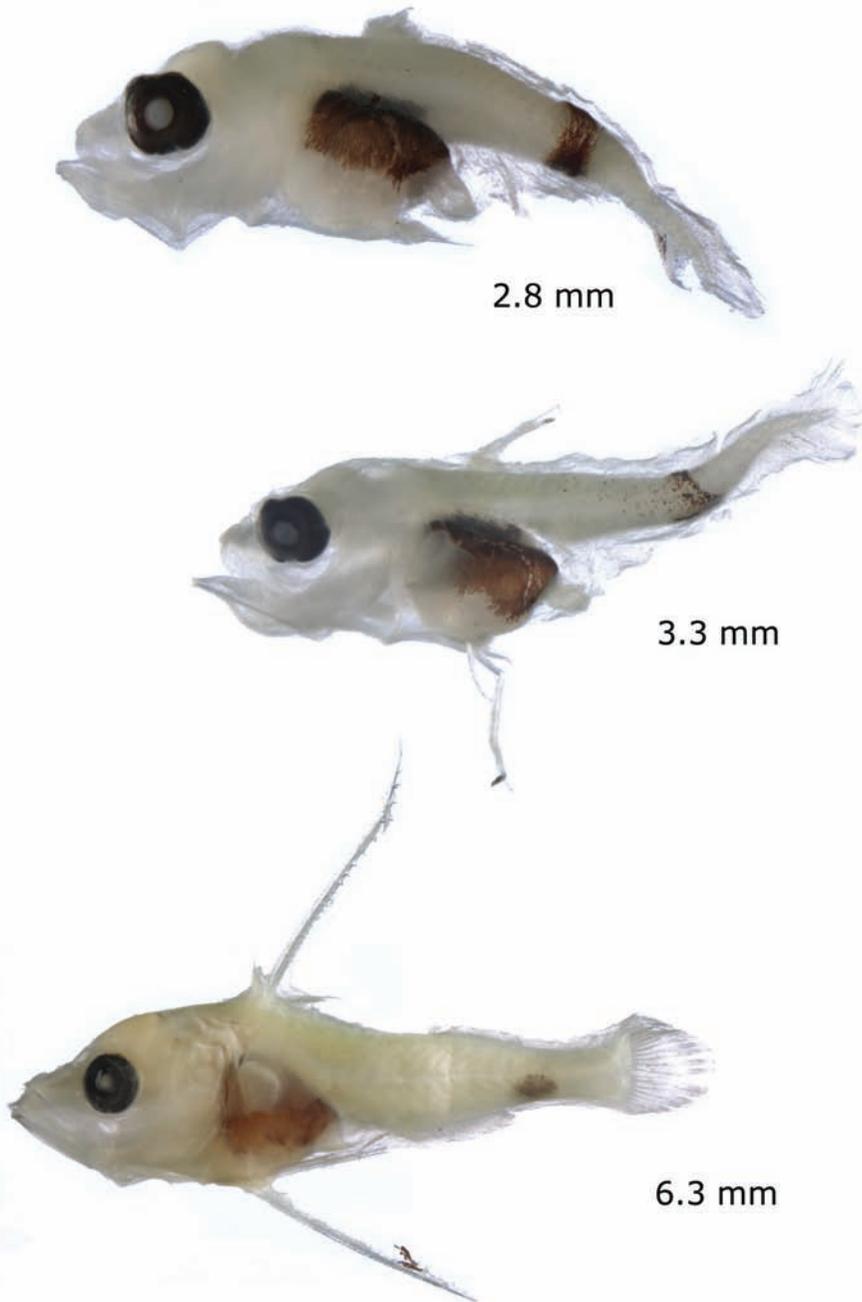


Figure 20. Genetically identified preflexion, late preflexion, and flexion stage *Mycteroperca venenosa* larvae collected in the Straits of Florida (n = 19; 2.2–6.8 mm BL). Dorsal spine of the 2.8 mm specimen has not erupted from the finfold.

Paranthias furcifer

(Tables 1–3; Figs. 2–6, 21)

Material Examined.—Eight preflexion (2.6–4.2 mm BL) and four flexion (4.3–5.8 mm BL) stage specimens of *P. furcifer* were collected (n = 12).

Description.—No pigment on the lower jaw or cleithral symphysis is present on specimens of *P. furcifer*. Nape pigment is frequently present, and the tail displays the standard tail pigment pattern. By 3.1 mm BL, simple, small, and straight spinelets are present on all ridges of the dorsal and pelvic spines. This spinelet morphology remains throughout development over the size range examined, and was observed among three late larval specimens (7.2–7.6 mm BL) from the western North Atlantic identified based on their unique meristic complement (D IX, 18–19; A III, 9, and P₁ 40; Johnson and Keener, 1984).

Remarks.—*Paranthias furcifer* was indistinguishable from species whose larvae exhibit standard tail pigment and simple, small, and straight spinelets over the entire size range examined (see “Small spinelet group description”).

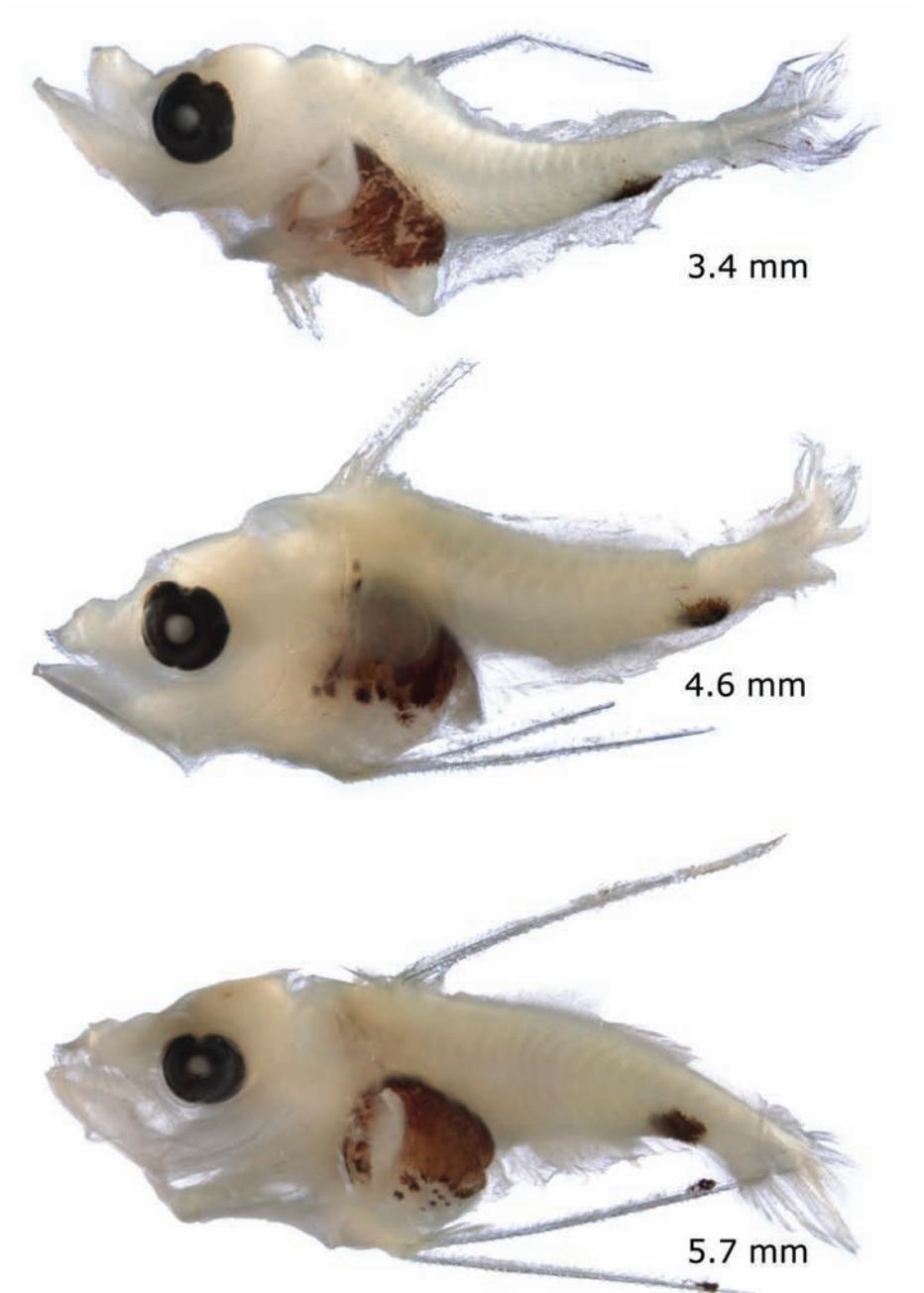


Figure 21. Genetically identified preflexion, flexion, and late flexion stage *Paranthias furcifer* larvae collected in the Straits of Florida (n = 12; 2.6–5.8 mm BL).

Gonioplectrus hispanus
(Tables 1 and 3; Figs. 2, 22)

Material Examined.—Two early flexion (2.9–3.3 mm BL) and two postflexion (5.2–10.6 mm BL) stage specimens of *G. hispanus* were collected in SEAMAP samples (n = 4).

Description.—*Gonioplectrus hispanus* are readily identifiable to species based on their deeply kite-shaped body. The average ratio of body depth to body length (approx. 0.41 for early flexion and 0.44 for postflexion stages) is high compared to the average of other similarly sized grouper larvae (< 0.37 for preflexion and flexion and < 0.43 for postflexion stages; Table 2). The preopercle spines of *G. hispanus* are notably long (0.5–0.7 mm), even among the smallest (early flexion) specimens, while the dorsal and pelvic spines are robust and notably shorter than the spines of other epinephelin species. The spinelets on these uniquely robust spines are small and connected (Fig. 2F). The tip of the lower jaw is frequently pigmented; pigment at the cleithral symphysis is common, and the tail displays the standard tail pigment (midlateral on postflexion specimens) through 10.6 mm BL.

Remarks.—These are the smallest larvae of the species described to date. Although these specimens were not identified genetically, their distinctive shape and spinelet morphology provide characters for species level identification. In addition, Kendall and Fahay (1979) described one 13.4 mm specimen, and Johnson and Keener (1984) described two, 13.4 mm and 14.0 mm BL, specimens identified using body shape and the unique meristic complement of D VIII, 13 and A III, 7. Kendall and Fahay (1979) observed a similarly high body depth to body length ratio (BD:BL of 0.5). The tail pigment of large postflexion specimens appears as a broad X shape (Kendall and Fahay, 1979; Johnson and Keener, 1984). Johnson and Keener (1984) described similar spinelet morphology and also observed that the second and third dorsal spines were approximately the same length. The relative length of these spines is also unique among grouper species.

Small Spinelet Species Group
(Table 5)

Remarks.—The combination of an absence of lower-jaw pigment, standard tail pigment, and simple, small, and straight spinelets was common to a number of species making them difficult to differentiate until dorsal, anal, and pectoral-fin elements could be reliably counted. This species group consisted of preflexion and some flexion stage specimens of *A. afer*, *E. drummondhayi* (< 5.2 mm BL), *E. morio*, *E. striatus*, *H. flavolimbatus* (< 4.5 mm BL), *H. niveatus*, *P. furcifer*, and unless pigment is present at the cleithral symphysis, *M. bonaci* (< 5.0 mm BL), *M. interstitialis*, *M. phenax* (< 3.7 mm BL), and intermediately sized (3.5–6.0 mm BL) *M. venenosa*. In addition, small specimens of the eight species not collected in this study may also fall into this group (*D. inermis*, *E. adscensionis*, *E. itajara*, *H. nigrinus*, *M. acutirostris*, *M. cidi*, *M. microlepis*, and *M. tigris*). Several species fall into this species group over their entire larval stage including: *E. morio*, *P. furcifer*, some *E. striatus*, and possibly *E. adscensionis* and *A. afer* (these last two based on published descriptions by Johnson and

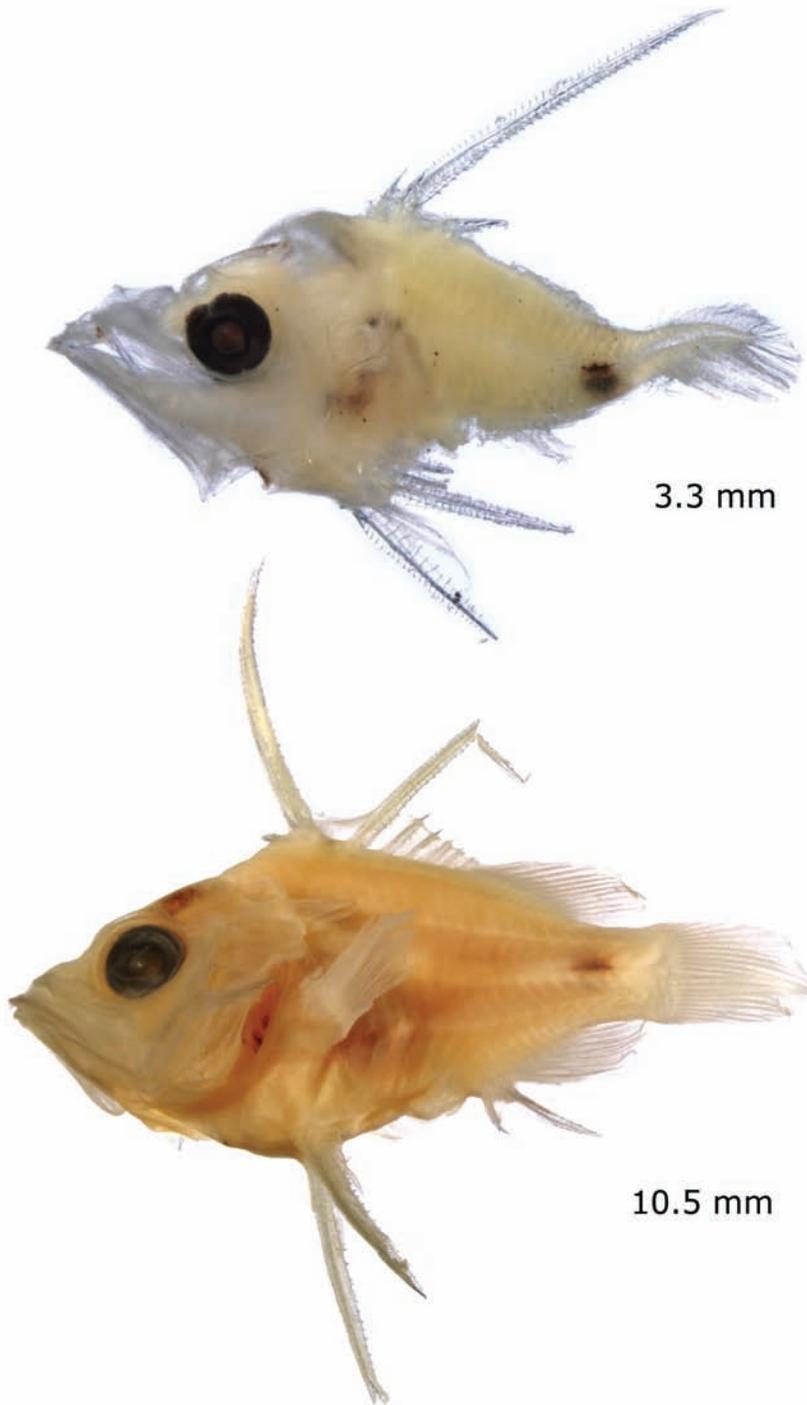


Figure 22. Flexion and postflexion larvae of *Gonioplectrus hispanus* (n = 4; 2.9–10.5 mm BL) collected during SEAMAP surveys in the Gulf of Mexico.

Keener, 1984). As more material, representing a more complete developmental series, becomes available, the number of species composing this group may be reduced.

Long and Curved Spinelet Species Group

(Table 5)

Remarks.—By the late flexion and postflexion stages (earlier for some species, e.g., *M. phenax*), when broad-based, long, and curved spinelets form, several species are still difficult to differentiate from each other including: *E. drummondhayi*, *H. flavo-limbatus*, *H. niveatus*, and unless pigment is present at the cleithral symphysis, *M. bonaci*, *M. interstitialis*, *M. phenax*, *M. venenosa*, and possibly *E. itajara*, *H. nigrinus*, *M. acutirostris*, *M. cidi*, *M. microlepis*, and *M. tigris* (these last five based on published descriptions by Johnson and Keener, 1984). As more material, representing a more complete developmental series, becomes available, the number of species composing this group may be reduced

DISCUSSION

Considerable progress in the identification of larval Gulf of Mexico groupers to the species level was made by examining morphological traits of genetically identified wild-caught larvae. In combination, the morphological characters considered in this study facilitate the identification of larvae from the preflexion stage until the spinelets begin to be reabsorbed late in the postflexion or early juvenile stages. Tail pigment patterns coupled with the presence or absence of lower-jaw pigment can be used to identify small preflexion larvae to species or a small group of species (Table 4). By early postflexion stage (4.5–5.0 mm or earlier for some species, e.g., *C. cruentata* and *M. phenax* due to early forming spinelets), presence of pigment at the cleithral symphysis and the morphology of spinelets on the second dorsal and pelvic spines provide additional aid in identification. By this stage, tail pigment for many species has changed or migrated to the lateral midline and is no longer useful for identification purposes. In contrast, lower-jaw pigment, once present, continues to be useful throughout the larval stage (Table 4 and Fig. 5). Pigment at the cleithral symphysis may be of use, but the lack of this pigment is not diagnostic. Dorsal, anal, and pectoral fin counts, pigment on the lower jaw, and pigment at the cleithral symphysis further help to refine identifications for larvae > 5–7 mm BL (Table 4).

Pigment patterns on the tail aid identification of species or species groups of Gulf of Mexico groupers. Leis (1986) also noticed that pigment patterns on the tail were useful in distinguishing small epinephelin larvae of Australia. In addition, the Australian larvae provided evidence that *Cephalopholis* species share a more plesiomorphic (primitive) tail pigment pattern (seen mostly in Indo-Pacific grouper larvae; Leis, 1986; Craig and Hastings, 2007) than other genera of groupers. This pattern consisted of no pigment or several small melanophores along the ventrum, rather than the single, large melanophore that characterizes the more apomorphic (derived) grouper species. The two species of the genus *Cephalopholis* collected in the Gulf of Mexico, *C. cruentata* and *C. fulva*, displayed similar plesiomorphic pigment patterns. Following this line of evidence and the observed spinelet morphologies, the SEAMAP specimens with the multiple melanophores tail pigment may be additional *C. cru-*

entata. Twelve SEAMAP specimens (2.4%) had opposing melanophores on both the dorsum and ventrum of the peduncle, no pigment on the lower jaw or cleithral symphysis, and were generally small with either no spines, or had not developed spinelets on the dorsal and pelvic spines. These specimens could be *Mycteroperca* species. Genetically identified specimens of *M. venenosa* exhibited this pigment pattern, and illustrations of *M. microlepis*, *M. phenax*, and *M. tigris* portray early larvae with this pigment (Richards et al., 2005).

Our data on spinelet morphology differed from that reported by Johnson and Keener (1984) in a few details. We described a lower spinelet diversity (five patterns rather than eight), which resulted from the composition of our sample, i.e., our samples represented fewer species and generally smaller specimens. The distinction between some of the spinelet patterns described in Johnson and Keener (1984) was unclear in the small larvae we examined.

Our descriptions of the spinelet morphology of two species differed from Johnson and Keener (1984) as well. *Cephalopholis cruentata* spinelets were not previously considered different from other long and curved spinelets; however, during preflexion through early postflexion, the spinelets of this species were distinctly different. This difference is likely related to size, with spinelets becoming broader based at larger sizes. The spinelet morphology of genetically identified *E. drummondhayi* differed more extensively from that reported by Johnson and Keener (1984). This species was believed to have simple, small, and straight spinelets on all ridges of the dorsal and pelvic spines throughout larval development. Two of the larger genetically identified *E. drummondhayi* had broad-based, long, and curved spinelets on the dorsal wing margins and primary pelvic ridges. There are several possible explanations for this discrepancy, one of which being that Johnson and Keener (1984) inadvertently confused large *E. drummondhayi* with *H. flavolimbatus* and *H. niveatus* specimens due to the similarity in their meristic characters (Table 4). Johnson and Keener (1984) based their identifications on modal meristic characters, which could have led to misidentification since the full ranges in number of dorsal soft rays observed by Smith (1971) did overlap slightly. Alternatively, the specimens with simple, small, and straight spinelets and 36 pectoral rays identified by Johnson and Keener (1984) could also have been *E. drummondhayi* that had not yet developed the broad-based, long, and curved spinelets of larger larvae. Unfortunately, lengths for these specimens were not reported. The genetic misidentification of these two specimens is a final possibility; however, it is considered unlikely given that the samples were run on separate plates, results from plates with any signs of contamination in the negative controls were discarded, and the sequence of *E. drummondhayi* and those of all other taxa are unlikely to be mistaken (difference > 7%).

SEAMAP data provide a real world example of the efficacy of using the traits described in this work to identify wild-caught larvae. Species-specific data for at least six species was collected in the Gulf of Mexico without further genetic analysis. The species we were able to identify were grouper of lesser economic importance, leaving the more economically important species, e.g., *M. microlepis*, *M. phenax*, and *E. morio*, identified as part of large species groups. For species with known spawning season and locations, further subdivision of the large species groups may be possible. Although species-level information is ideal, the morphological characters described in this study allow researchers to examine the larval ecology of species and groups

of economically important species. Where species-specific data are critical, targeted molecular analyses are possible.

The importance of our findings reaches beyond the immediate study area of the Gulf of Mexico and western Atlantic. By describing genetically identified larvae, we were able to demonstrate that variation in tail pigment and lower-jaw pigment is consistent within species and often species-specific. Larval groupers are notoriously difficult to identify in all their native habitats around the world. By documenting pigmentation on the tail and jaw, researchers may be able to enhance their ability to identify these larvae when genetic analyses are not used.

Coupling molecular and morphological identification techniques is a powerful and cost effective tool. At the start of this study, we sequenced approximately 40% of the grouper caught in the Straits of Florida samples. After subsequent morphological analysis of the entire catch, an additional 40% of the specimens was identified to species, leaving 20% identified to one of the large species groups. With the traits identified in this study, 56% of the Straits of Florida groupers could have been identified to species based solely on morphological characters, leaving only slightly more specimens for genetic identification than were originally sequenced. Had these traits been identified before we started, we could have focused genetic analysis on the specimens of the large species groups, resulting in all the Straits of Florida grouper identified to species without any appreciable increase in cost or effort. For the Gulf of Mexico SEAMAP groupers, the effort and expense required for genetic analysis could be greatly reduced by only sequencing the species groups containing species of interest. In this way, the cost effective combination of molecular and morphological methods will aid future work on grouper larvae as well as the larvae of other speciose and difficult to identify families.

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