DESCRIPTION OF REARED PREFLEXION GRAY TRIGGERFISH, BALISTES CAPRISCUS, LARVAE FROM THE NORTHERN GULF OF MEXICO

Carrie M. Simmons and Stephen T. Szedlmayer

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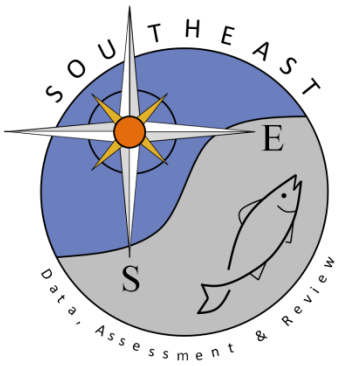

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NOTE

DESCRIPTION OF REARED PREFLEXION GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, LARVAE FROM THE NORTHERN GULF OF MEXICO

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ABSTRACT

Here we describe and illustrate reared gray triggerfish, *Balistes capriscus* (Gmelin, 1789), larvae from the northern Gulf of Mexico less than 3 mm notochord length (NL). Eggs were collected by scuba divers from active nests with a guarding female. Gray triggerfish eggs had a mean diameter of 0.62 (SD 0.03) mm. Eggs hatched from 24 to 48 hrs after fertilization based on samples brought back to the laboratory. Post-hatch larvae were distributed throughout the water column of the rearing tanks. Larvae were maintained in laboratory rearing tanks but none survived beyond 6 d post-hatch. Morphological development of live gray triggerfish larvae (n = 10) for each day is described and a representative specimen is illustrated. Larvae hatched at a mean size of 2.2 mm NL and 20 myomeres, without pigmented eyes or a functional mouth. This study provides descriptive information on smaller and less developed larvae than has previously been described. This new material will improve researchers' ability to identify gray triggerfish from field collections.

Gray triggerfish, *Balistes capriscus* (Gmelin, 1789), is a widely distributed species found throughout the Gulf of Mexico and the eastern and western Atlantic Ocean (Briggs 1958, Moore 1967). Homogeneity of mitochondrial DNA sequences between gray triggerfish collected in the Gulf of Mexico and western Atlantic Ocean suggests that there is one stock (Antoni et al. 2011). This widely distributed species can be found in a variety of habitats including artificial reef structures (Frazer and Lindberg 1994, Vose and Nelson 1994, Kurz 1995, Wilson et al. 1995, Simmons and Szedlmayer 2011, 2012) and natural reefs (Johnson and Saloman 1984, Vose and Nelson 1994). Recent stock assessments indicate that the gray triggerfish is overfished in the Gulf of Mexico (SEDAR-9 2006, SEDAR-9 Update 2011).

Fishery-independent ichthyoplankton surveys in the Gulf of Mexico, when available, play an important role in the stock assessment process as indices of adult abundance (Lyczkowski-Shultz and Hanisko 2007). The National Marine Fisheries Service in cooperation with the gulf states has been conducting ichthyoplankton surveys since 1982 under the Southeast Area Monitoring and Assessment Program (SEAMAP). For these ichthyoplankton surveys to be used as an index of adult abundance, larval identification keys are needed. Postflexion gray triggerfish larvae from the Gulf of Mexico and southern Brazil have been described and illustrated (Matsuura and Katsuragawa 1981, 1985, Lyczkowski-Shultz and Ingram 2005). However, the present study provides new descriptive information of reared preflexion gray triggerfish larvae, which facilitates identification of the earliest stages of gray triggerfish.

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Methods

Eggs were collected from active gray triggerfish nests with a guarding female by scuba divers around artificial habitats that were located 26–50 km south to southeast of Dauphin Island, Alabama, in the northern Gulf of Mexico. Eggs were collected from 2004 to 2006 in June–July during the peak spawning season while reproductive behavior of gray triggerfish was recorded (Simmons and Szedlmayer 2012). Eggs were removed from active nests and placed in 3.8-L plastic bags. At the surface, eggs were placed into aerated coolers at 20 to 21 °C, similar to the bottom water temperature, and transported to the laboratory. Larval rearing was attempted six times from 2004 to 2006 during June and July of each year. Several different methods of rearing gray triggerfish larvae were attempted, but only methods with survival up to 6 d are reported. All larval rearing attempts were completed in a closed system, with gentle aeration. Larvae from three different gray triggerfish egg masses were reared in circular polyvinyl chloride (PVC) 200-L containers with the temperature maintained from 21 to 22.5 °C and salinity constant at 33. Stocking density ranged from 10 to 15 larvae L^{-1}. Live wild zooplankton were collected with two 63-μm plankton nets every 2 d. Larvae were fed all zooplankton within the 63–150-μm size fraction daily.

Photographs were taken of live eggs and larvae each day. Digital photographs of larvae and a micrometer were taken with an Olympus BH-2 compound microscope with 4× and 10× objective lenses, a Sony AVC-D7 video camera, and a FlashPoint 128-4M digitizing board (Integral Technologies, Inc., Indianapolis, Indiana). Digitized egg and larval images were measured (nearest 0.01 mm) using Image Pro v4.5 software. Measurements of 10 gray triggerfish larvae were taken each day and terminology follows: (1) total length (TL) = tip of snout to end of tail; (2) snout length = tip of snout to anterior margin of the eye; (3) notochord length (NL) = tip of the snout to end of the notochord; (4) oil globule in the yolk sac = largest horizontal measure from the anterior to the posterior margin of the oil globule; (5) yolk sac = largest horizontal measure from the anterior to the posterior margin of the yolk sac; (6) eye diameter = largest horizontal measure from the anterior to the posterior margin of the eye; (7) lower jaw length = tip of the lower jaw to posterior margin of the jaw (Matsuura and Katsuragawa 1981, Kimmel 1993, Moser 1996). Illustrations of the larvae were made each day using the digitized images of live larvae. For pigment location, larvae preserved in NOTOXhisto fixative (Scientific Device Laboratory, Glenview, Illinois) were examined using a Leica MZ6 (Leica Microscopy Systems, Ltd., Heerbrugg, Switzerland) dissecting scope and compared with several (3–5) digitized images of live larvae without fixative.

Results

Mean egg diameter was 0.62 (SD 0.03) mm (n = 90) based on samples from nine different nests (Fig. 1A). Gray triggerfish eggs hatched from 24 to 48 hrs after fertilization based on samples brought back to the laboratory. Rearing attempts resulted in larvae living through 6 days post hatch (dph). Post-hatch larvae were distributed throughout the water column of the rearing tanks. The rearing tank height ranged from 0.61 to 1.52 m. Mean morphometric characters of larvae from hatching through 6 dph are shown in Table 1. After rearing experiments were completed and larvae preserved, we examined preserved larval gray triggerfish for the presence of a tuft of spinules on the cheek, a characteristic feature of Balistidae larvae. These spinules were detected in preserved larvae, but due to deterioration the exact timing of formation was undetermined. Spinules were not visible in photographs or documented in the illustrations of live larvae.

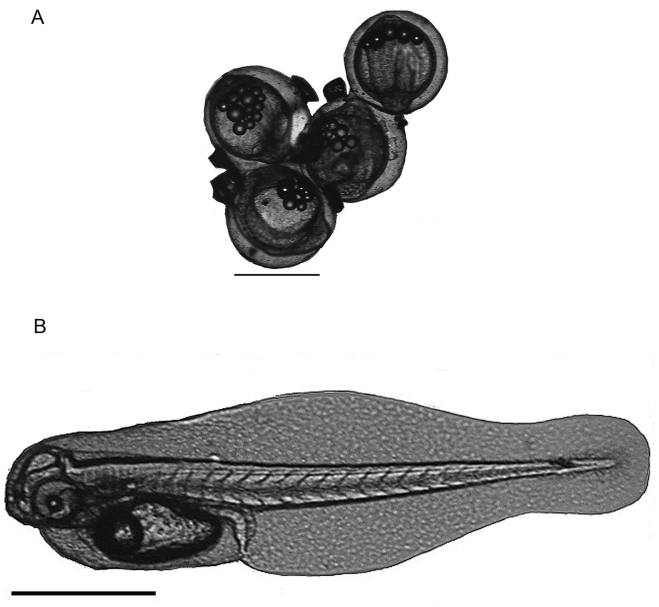

Figure 1. Photographs: (A) gray triggerfish eggs. Mean egg diameter = 0.62 (SD 0.03) mm; (B) gray triggerfish larvae at 0 d. Larvae hatched without pigmented eyes or functional mouth. Scale bars = 0.5 mm.

Day 0.—Mean TL of gray triggerfish larvae at hatching was 2.27 (SD 0.04) mm (Fig. 1B, Table 1). Larvae hatched without pigmented eyes or a functional mouth. At 0 dph, there were 20 myomeres, with four to five pre-anus, 15 to 16 post-anus, and the otoliths were visible. The number of myomeres did not change from 0 dph throughout the study. Nine to 12 melanophores were visible on the anterior portion of the oil globule and yolk sac, with four to five melanophores on the dorsal margin of the peritoneal cavity. One to two melanophores were visible on the dorsal portion of the yolk sac. The forebrain, midbrain, and hindbrain were visible, with one melanophore on the forebrain and one to two melanophores on the midbrain.

Day 1.—At 1 dph, mean TL was 2.32 (SD 0.02) mm (Fig. 2A). Eyes were partially pigmented, with melanophores spreading from the anterior and dorsal portion of eye towards the ventral margin of the eye. Many overlapping melanophores were visible on the anterior margin of the yolk sac and oil globule. The gut was

Table 1. Mean (SD) morphometric measures of live larval gray triggerfish by day (n = 10).

Day	Total length	Snout length	Notochord length	Eye diameter	Oil globule	Yolk sac	Jaw length
0	2.27 (0.04)	0.10 (0.01)	2.16 (0.04)	0.16 (0.01)	0.18 (0.01)	0.46 (0.02)	–
1	2.32 (0.02)	0.11 (0.02)	2.19 (0.05)	0.18 (0.02)	0.18 (0.01)	0.35 (0.02)	–
2	2.34 (0.08)	0.12 (0.02)	2.20 (0.06)	0.22 (0.01)	0.15 (0.01)	0.22 (0.02)	–
3	2.34 (0.02)	0.13 (0.01)	2.20 (0.03)	0.20 (0.02)	0.11 (0.01)	0.20 (0.02)	0.17 (0.03)
4	2.27 (0.06)	0.10 (0.01)	2.12 (0.05)	0.20 (0.01)	0.08 (0.01)	0.12 (0.03)	0.22 (0.03)
5	2.29 (0.05)	0.11 (0.01)	2.13 (0.04)	0.20 (0.01)	–	–	0.21 (0.03)
6	2.22 (0.05)	0.06 (0.01)	2.08 (0.05)	0.20 (0.01)	–	–	0.25 (0.02)

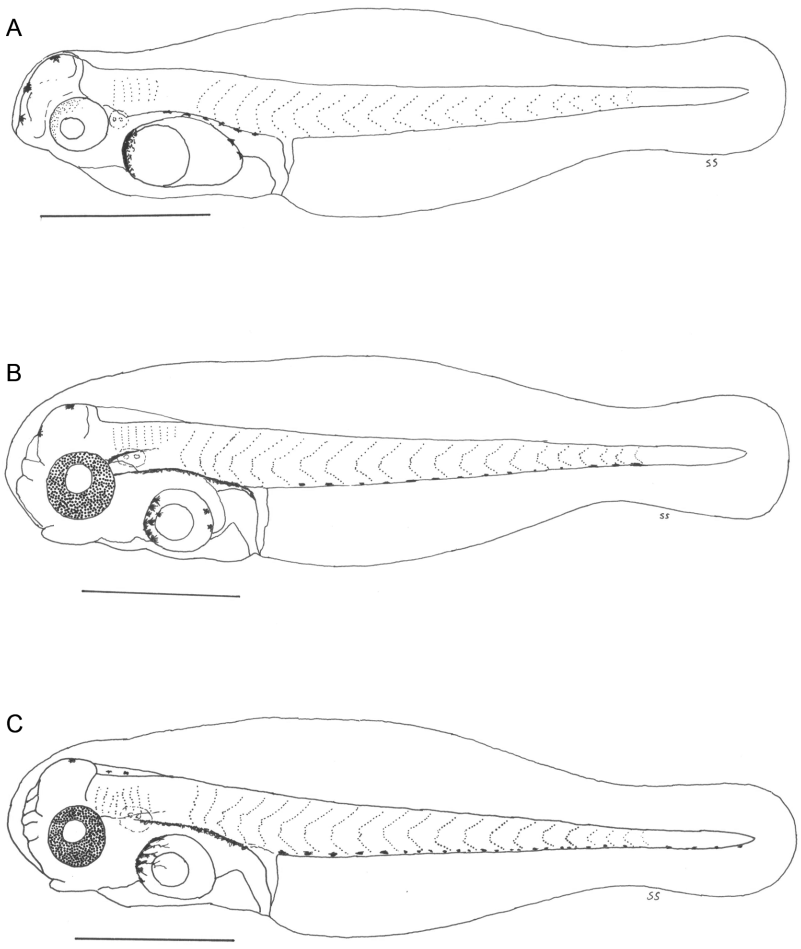

Figure 2. Illustration of gray triggerfish larvae over the first six days post hatch (dph). (A) At 1 dph, eyes were partially pigmented and many overlapping melanophores were visible on the anterior margin of the yolk sac. (B) At 2 dph, eyes were completely pigmented, lower jaw was visible, and the gut was coiled. (C) At 3 dph, melanophores were visible on the ventral portion of the myomeres posterior to the anus. (D) *(Opposite page)* At 4 dph, melanophores are so numerous on the ventral portion of the myomeres they form a continuous line to the end of the notochord. (E) At 5 dph, the yolk sac, oil globule, and otoliths were no longer visible. (F) At 6 dph, the gut was more tightly coiled and the lower jaw that extended anteriorly beyond the adjacent margin of the head. For all images, scale bar = 0.5 mm.

more visible as the yolk sac decreased in size. Two melanophores were present on the ventral margin of the gut, adjacent to the yolk sac. Two large melanophores were visible on the forebrain and the midbrain had one large melanophore. The pectoral fins, otoliths, heart, and gills were visible, but only changes by day are described after 1 dph. Otoliths were included in illustrations up to 5 dph, when they were no longer visible.

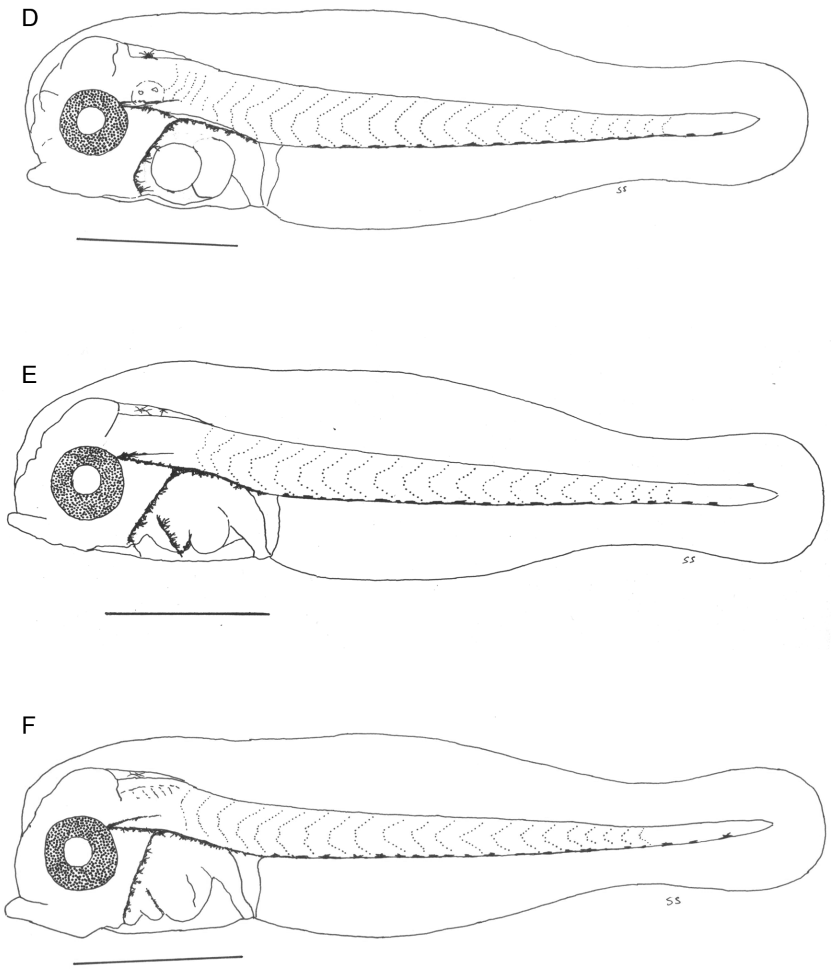

Figure 2. Continued.

Day 2.—At 2 dph, mean TL was 2.34 (SD 0.08) mm (Fig. 2B). Eyes were completely pigmented. The gut was coiled. A series of melanophores was present on the ventral margin of the tail starting at the first or second post anal myomere. The dorsal margin of the peritoneal cavity, just ventral to the myomeres, had numerous melanophores that formed a continuous line. Posterior to the eye, across the otoliths, and into the hindbrain were numerous melanophores that formed a short line. The lower jaw was now visible for the first time. Pectoral fins were longer. Two distinct melanophores were present on the midbrain. Larger and more numerous melanophores were present on the anterior margin of the yolk sac and oil globule. Two or three melanophores were present on the posterior margin of the yolk sac, anterior to the coiled gut.

Day 3.—At 3 dph, mean TL was 2.34 (SD 0.02) mm (Fig. 2C). Eye pigment did not change from 3 dph through 6 dph. A series of melanophores were visible on the ventral margin of the tail beginning on the first myomere posterior to the anus. Large single melanophores were present on the five myomeres posterior to the anus, followed by two melanophores on each of the next seven myomeres, followed by single melanophore on the next three myomeres. Three or four melanophores were also located ventrally near the tip of the notochord. The forebrain lobes became more distinct and larger just anterior to the eye. There was one melanophore on the midbrain and two distinct melanophores on the hindbrain. The yolk sac decreased in size and melanophores on the anterior portion of the yolk sac were numerous and formed a solid patch of pigment (i.e., individual melanophores could not be distinguished). The pectoral fins were longer and the gut larger with melanophores forming a solid patch of pigment, dorsally over the peritoneal cavity. The lower jaw became more prominent and projected anteriorly.

Day 4.—At 4 dph, mean TL was 2.27 (SD 0.06) mm (Fig. 2D). The melanophores on the ventral margin of the myomeres posterior to the anus were larger on each myomere so individual melanophores were not distinguishable, until the tip of notochord. The hindbrain was more developed. The yolk sac was smaller and more difficult to distinguish from the oil globule. Therefore the yolk sac was not included in the illustration. Melanophores formed a continuous line on the anterior and dorsal margins of the peritoneal cavity, and also posterior to the eye across the otoliths into the hindbrain. The lower jaw was pronounced and extended anteriorly beyond the adjacent margin of the head. Two large melanophores were located on the indented portion of the hindbrain, but only one was visible from a lateral view.

Day 5.—At 5 dph, mean TL was 2.29 (SD 0.05) mm (Fig. 2E). Otoliths were no longer visible in 5 and 6 dph. Melanophores were more numerous and formed a continuous line from the posterior margin of the eye along the ventral margin of the myomeres to the tip of the notochord for 5 and 6 dph. One large melanophore was visible on the dorsal side of the notochord tip. The lower jaw was more prominent and extended anteriorly beyond the adjacent margin of the head. The yolk sac and oil globule were no longer visible. Melanophores were also numerous on the anterior and dorsal margin of the peritoneal cavity as well as ventral margin of the gut forming a continuous line for 5 and 6 dph. Individual melanophores were no longer distinguishable, but formed a solid line of pigment from the posterior margin of the eye to the hindbrain for 5 and 6 dph. The forebrain and hindbrain continued to show more distinctive lobes. Four large melanophores were located on the indented portion of the hindbrain, but only two were visible from the lateral view.

Day 6.—At 6 dph, mean TL was 2.22 (SD 0.05) mm (Fig. 2F). Larvae looked similar to 5-dph larvae, except the melanophore present on the dorsal portion of the notochord tip on 5 dph was no longer visible. The lower jaw and peritoneal cavity was similar to 5 dph, except the gut had larger more numerous coils. The coiled gut had increased in size and the line of melanophores on the anterior margin of the gut was thinner and less visible as it appeared more tightly coiled. Four melanophores on the hindbrain were not visible as separate melanophores as they

were at 5 dph; instead they formed one large area of pigmentation on 67% of the photographs and preserved specimens. Dorsal spine formation was not observed in any of the reared larvae up to 6 dph.

Discussion

Gray triggerfish larvae hatched between 24 and 48 hrs based on observations of active nests, collection of eggs, and transport back to the laboratory for rearing. The exact time of fertilization was unknown as scuba divers located active nests by searching for guarding females post-spawning before collecting eggs (Simmons and Szedlmayer 2012). However, we did make observations of pre-spawning behavior just prior to fertilization, and on follow up scuba diver searches located guarding females with newly deposited egg masses (Simmons and Szedlmayer 2012). Our study estimates a shorter incubation period than the 50–55 hrs estimated by Lyczkowki-Shultz and Ingram (2005). We also documented gray triggerfish hatching at a mean size of 2.16 mm NL, larger than the size (1.7 mm NL) suggested by Lyczkowski-Shultz and Ingram (2005). Another difference documented here was that gray triggerfish had 20 myomeres from hatching until at least 6 dph. However, Lyczkowski-Shultz and Ingram (2005) documented gray triggerfish at 1.7 mm NL with 18 vertebrae. One explanation for these differences in myomere counts could be attributed to the two myomeres at the end of the notochord that become the caudal complex and fusion of the third and fourth hypurals at approximately 4.7 mm NL (Matsuura and Katsuragawa 1985).

Comparisons of developmental milestones by size (e.g., formation of coiled gut, lower jaw development, and dorsal spines) between our study and previously documented literature is not possible due to live larvae used here and documented shrinkage of preserved specimens (Theilacker 1980, Matsuura and Katsuragawa 1981, 1985, Lyczkowski-Shultz and Ingram 2005). However, we note that in the present study, the lower jaw was present at 3 dph and continued to grow in length through 6 dph. The yolk sac and oil globule were not visible after 4 dph, when fish apparently feed exogenously. Food was visible in the gut of some larvae at this time, but difficult to distinguish in many larvae because of the coiled gut. Eye diameter was largest at 2 dph, and remained consistent in size through 6 dph. The reared gray triggerfish in our study showed no development of dorsal spines during the first 6 dph, therefore dorsal spines must develop after this time period. At 6 dph, our description of melanophores was similar to the melanophores documented for the 3-mm NL gray triggerfish described by Matsuura and Katsuragawa (1981); specifically, the melanophores were similar on the ventral margin of the myomeres post-anus to the tip of the notochord. A distinguishing characteristic for Balistidae documented in post-flexion larvae is a tuft of spinules on the cheek (Leis and Rennis 2000, Lyczkowski-Shultz and Ingram 2005). In the present study, these spinules were overlooked in the live larvae. Although we observed check spinules in the preserved larvae, their precise timing of formation could not be determined due to deterioration of preserved larvae. This overlooked but distinct property requires additional characterization in pre-flexion gray triggerfish larvae.

Benthic habitat use by larval gray triggerfish has been suggested in other studies because of their absence in plankton tows (Matsuura and Katsuragawa 1981,

Lyczkowski-Shultz and Ingram 2005). However, in our study, larvae were observed throughout the water column of all rearing tanks, which coincides with post-flexion larvae being found in pelagic ichthyoplankton tows (Lyczkowski-Shultz and Ingram 2005) and their use of pelagic habitat and close association with *Sargassum* spp. (Dooley 1972, Fahay 1975, Bortone et al. 1977, Wells and Rooker 2004). Our description of larval gray triggerfish is also consistent with the description of pre-flexion larvae of the orange-lined triggerfish, *Balistapus undulates* (Park, 1797), described as free swimming (Lobel and Johannes 1980). It is possible that the absence of small gray triggerfish larvae (<3 mm NL) in the plankton may be due to the difficulty of distinguishing gray triggerfish larvae from other larval fishes found in the plankton.

There are several possibilities why larval gray triggerfish reached the maximum mean TL and NL at 3 dph as well as why larval gray triggerfish in this study did not survive past 6 dph. The decrease in larval length after 3 dph could be an artifact of rearing conditions such as incorrect food type or concentration. Some larval rearing studies suggests that shrinkage occurs during metamorphosis, likely due to starvation, after which some larval fish supplied with the correct food type and concentration will resume normal growth (Farris 1959, Rogers and Westin 1981,Tsukamoto and Okiyama 1993). The present study used wild-caught plankton, and prey concentrations may have been too low or the plankton provided may not have been the correct food type. For example, red snapper larval rearing studies found higher rates of survival in captivity when a ciliate, *Fabrea salina* (Henneguy, 1890), was provided as prey in addition to copepod naupli (Rhodes and Phelps 2008). Field studies also suggest that ciliates play an important role in early feeding of larval fish; however, most ciliates do not have hard parts so they are difficult to detect in the gut of larvae (Stoecker and Govoni 1984, Fukami et al. 1999).

While morphometric description in the present study can be used to distinguish the smallest larval gray triggerfish (<3 mm NL) from other similar species from the same plankton tows, there presently are few descriptions of such larvae <3 mm NL (Lyczkowski-Shultz and Ingram 2005; e.g., such as Monacanthidae, Zapfe and Lyczkowski-Shultz 2005; Tricathodidae, Lyczkowski-Shultz 2005) and continued work on the description of the earliest stages of marine fish larvae is needed.

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Addresses: (CMS) *Gulf of Mexico Fishery Management Council, 2203 North Lois Avenue, Suite 1100, Tampa, Florida 33607.* (STS) *Department of Fisheries and Allied Aquacultures, Auburn University, 8300 State Highway 104, Fairhope, Alabama 36532*. Corresponding Author: (CMS) *Email: <carrie.simmons@gulfcouncil.org>.*