

**Abstract**—Although the Florida pompano (*Trachinotus carolinus*) is a prime candidate for aquaculture, the problematic production of juveniles remains a major impediment to commercial culture of this species. In order to improve the understanding of larval development and to refine hatchery production techniques, this study was conducted to characterize development and growth of Florida pompano from hatching through metamorphosis by using digital photography and image analysis. Newly hatched larvae were transparent and had a large, elongate yolk sac and single oil globule. The lower and upper jaws as well as the digestive tract were not fully developed at hatching. Rotifers were observed in the stomach of larvae at three days after hatching (DAH), and *Artemia* spp. were observed in the stomach of larvae at 14 DAH. Growth rates calculated from total length measurements were  $0.22 \pm 0.04$ ,  $0.23 \pm 0.12$ , and  $0.35 \pm 0.09$  mm/d for each of the larval rearing trials. The mouth gape of larvae was  $0.266 \pm 0.075$  mm at first feeding and increased with a growth rate of  $0.13 \pm 0.04$  mm/d. Predicted values for optimal prey sizes ranged from 80 to 130  $\mu$ m at 3 DAH, 160 to 267  $\mu$ m at 5 DAH, and 454 to 757  $\mu$ m at 10 DAH. Based on the findings of this study, a refined feeding regime was developed to provide stage- and size-specific guidelines for feeding Florida pompano larvae reared under hatchery conditions.

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## Development and growth of hatchery-reared larval Florida pompano (*Trachinotus carolinus*)

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Jacks and pompanos of the family Carangidae are represented by 33 genera and approximately 140 species found in tropical and subtropical oceans around the world (Helfman et al., 2003). Because carangids are highly regarded as food and game fishes, many species are exploited worldwide. Common to the southeastern Atlantic and Gulf of Mexico coasts of the United States, the Florida pompano (*Trachinotus carolinus*) is a prime candidate for aquaculture and stock enhancement programs because of its high value and limited availability from commercial harvests (Watanabe, 1995; Craig, 2000).

The larvae and early juveniles of 28 species of carangids of the South Atlantic and Gulf coasts have been described with some detail (Goode, 1882; Starks, 1911; Ginsburg, 1952; Aprieto, 1974; Laroche et al., 1984; Fahay, 2007). Fields (1962) described wild-caught Florida pompano ranging in size from 4.0 to 42.1 mm total length (TL), and more recently Fahay (2007) described fish ranging in size from 3.1 to 14.8 mm TL. Although these reports provide essential information on the early life history of jacks and pompanos for fishery biologists, additional knowledge on the morphological development, growth, and trophic requirements is essential to evaluate new species for culture

and to develop effective hatchery rearing techniques and feeding regimes.

Significant interest in the culture of Florida pompano developed in the United States during the 1960s and 1970s and, as a result, efforts were initiated to develop spawning and rearing techniques for this species (Moe et al., 1968; Hoff et al., 1972, 1978a; McMaster, 1988). Florida pompano exhibit a number of desirable characteristics for aquaculture (Weirich et al., 2006). The species exhibits a high rate of growth, readily accepts commercially prepared diets, adapts to low salinity environments, and has been successfully cultured in tanks, ponds, cages, and offshore netpens. Unfortunately, the production of a reliable supply of juveniles to stock grow-out operations has been a limiting factor with respect to commercial culture of this species. Hoff et al. (1972) reported producing an average of only 300 juveniles per spawning event, and until recently, researchers and commercial culturists alike were largely unsuccessful at producing the quantities of eggs and larvae needed for commercial production or stock enhancement programs. Weirich and Riley (2007) reported that in a series of nine spawning trials conducted over a two-year period, 3.2 million fertilized eggs were produced by 40 adult Florida pompano (1:1 sex ratio). These

values approximate the necessary quantities needed for mass production. The advent of new broodstock management techniques for domestication and controlled reproduction in captivity offer great promise for the culture of Florida pompano. However, there is a need to develop and refine hatchery technologies for this species because larvae undergo major functional and morphological changes throughout their early life history.

Florida pompano eggs are typical of marine fishes with pelagic eggs. In a single spawning event, one female can produce 200,000 to 400,000 small, buoyant eggs that range in size from 0.87 to 1.00 mm in diameter (Hoff et al., 1978a). Florida pompano eggs normally have a single oil globule, although eggs from some broodfish reportedly have several small oil globules. The size and number of oil globules within eggs can serve as an indicator of egg quality and correlate with the amount of energy available for developing larvae (Barbaro et al., 1991). The yolk that is deposited during vitellogenesis must provide nutrition for the developing embryo and larvae. Newly hatched Florida pompano larvae are approximately 2.0 mm TL and are not well developed (Hoff et al., 1978b). Depending on water temperature and developmental rates, larvae use yolk reserves for two to three days after hatching (DAH), which coincides with pigmentation of the eyes, mouth formation, and first feeding. Florida pompano larvae have been cultured by using a variety of live zooplankton, including copepods, rotifers, and *Artemia* spp. Florida pompano undergo metamorphosis at 24 DAH at 15 mm TL and can easily transition to dry feeds (McMaster, 1988).

Previously published descriptions of Florida pompano larvae provide limited details on development and growth under hatchery conditions. To improve the understanding of larval development of this species, the present study was conducted to measure the growth of larvae from hatching through metamorphosis by using digital photography and image analysis. The specific objectives were 1) to compare morphological variation among larvae from three different spawning trials; 2) to document time of occurrence for critical periods including first feeding, yolk and oil globule exhaustion, gas bladder inflation, transition in diet, and onset of metamorphosis; and 3) to develop a model feeding regime for Florida pompano larvae.

## Materials and methods

### Spawning and egg incubation

This study presents data regarding developmental characteristics and growth of larvae obtained from captive reproduction of Florida pompano broodstock held at the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) Center for Reproduction and Larviculture in Fort Pierce, Florida (Weirich and Riley, 2007). Broodstock (sex ratio, 1:1) were held in recirculating tank systems under controlled photothermal condi-

tions and were sampled periodically to assess health and reproductive condition. To initiate spawning, ripe females (mean oocyte diameter  $\geq 500 \mu\text{m}$ ) and males were implanted with a 75- $\mu\text{g}$  slow-release pellet of gonadotropin-releasing hormone analogue (Syndel International, Inc., Vancouver, BC). Fish spawned volitionally approximately 36 hours after hormone implantation, and eggs were collected and stocked into aerated 100-L incubation tanks (24–26°C). Hatching occurred approximately 30–36 hours after fertilization.

### Larval culture

In three independent larval rearing trials (initiated 15 June 2004, 17 June 2004, 17 August 2005), approximately 50,000 larvae (0 DAH) were stocked into a 1.0-m<sup>3</sup> round fiberglass tank. The tank was filled with 800 L of natural seawater that had been subjected to biological and mechanical filtration, in addition to ultraviolet sterilization, before use. Water quality was monitored daily with a multiparameter dissolved oxygen probe (YSI Incorporated, Model 85, Yellow Springs, OH). Water was not exchanged from zero DAH through five DAH. After five DAH, water quality was maintained by daily water changes ranging from 50% to 200% through 20 DAH.

Although similar production methods were used during each year of the study, trials were conducted within a greenhouse during the first year of the study and within an insulated, climate-controlled hatchery during the second year. After stocking, tanks were gently aerated and surface light levels were maintained at 300 lux (model LI-189, LI-COR, Lincoln, NE) for 16 hours daily. At two DAH, the aeration level was increased, tanks were inoculated with cultured microalgae (*Nannochloropsis oculata*) (Instant Algae, Reed Mariculture, Campbell, CA) to maintain green water culture conditions, and surface light levels were increased to 2000 or 3000 lux. Larvae were fed enriched rotifers (*Brachionus plicatilis*; 53–225  $\mu\text{m}$ ) from two DAH through 15 DAH. Rotifer strains and size distributions differed among years (Fig. 1). Larvae were fed *Artemia* spp. nauplii (~480  $\mu\text{m}$ ) from 12 DAH through 20 DAH (Embryon, INVE, Salt Lake City, UT). Live feed organisms were fed three times daily and were maintained at densities of one to three individuals per mL. Artificial feed (400–800  $\mu\text{m}$  diameter particles) was offered to larvae beginning at 10 DAH (INVE NRD Micro Pellet, Salt Lake City, UT).

### Sample collection

Samples of 10 larvae were randomly collected daily from hatching through completion of metamorphosis at 20 DAH. Larvae were euthanized by brief immersion in cold seawater (4°C), placed on glass slides, and photographed by using a dissecting microscope at 4 $\times$  magnification. A compound microscope at 100 $\times$  magnification was used to photograph the head and mouth of each larva from 0 DAH through 5 DAH; thereafter, the head and mouth of each larva was photographed by using the dissect-

ing microscope. All larvae were photographed on their left sides in the sagittal plane, and a fine-point needle was used to position larvae as necessary. Microscopes were equipped with high-resolution digital cameras (Sony DSC-FS17, San Diego, CA), and photographs were recorded as uncompressed files in tagged image file format (TIFF) at 6 megapixels. For calibration, a 0.01-mm micrometer scale bar was photographed for each larval series and for both microscopes.

### Image analysis

Larvae and selected anatomical features were measured and analyzed by using SigmaScan Pro 5.0 image analysis software (SPSS Science, Chicago, IL). All morphometric measurements listed below were recorded to the nearest 0.001 mm and calibration errors were maintained at less than 1  $\mu\text{m}$  ( $\leq 0.1\%$  of 1 mm). The total length (TL) and standard length (SL) of larvae was measured along lines parallel to the longitudinal axis of the fish (Snyder 1983).

**Body depth**—The distance, perpendicular to the longitudinal axis of the body from the insertion of the first dorsal spine to the ventralmost point of the body. For yolk-sac larvae, the distance perpendicular to the longitudinal axis of the body from the dorsal crest through

the midpoint of the yolk to the ventralmost point of the body.

**Head length**—The distance, parallel to the longitudinal axis of the head, from tip of snout to the edge of the operculum.

**Eye diameter**—The distance, parallel to the longitudinal axis of the head, from anteriormost to posteriormost points of bony orbit.

**Yolksac length**—The distance, parallel to longitudinal axis of the body, from anteriormost to posteriormost points of the yolk sac.

**Yolksac depth**—The distance, perpendicular to the longitudinal axis of the body, from ventralmost to dorsalmost points of yolk sac.

**Oil globule diameter**—The distance, parallel to longitudinal axis of the body, from anteriormost to posteriormost points of oil globule.

**Length of upper jaw**—The length of the premaxillae and maxillae to the point of articulation with the dorsal process of the dentary.

**Length of lower jaw**—The length of the dentary to the point of articulation with the angular and maxillae.

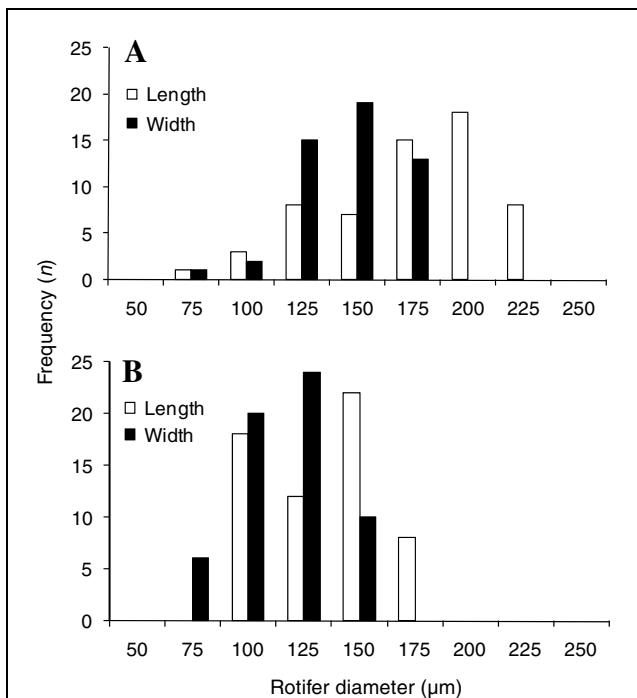
At the time of measurement, observations of larval development state were recorded to identify the chronological sequence of events. The following critical periods were noted when first observed: 1) hatching; 2) mouth formation; 3) body pigmentation; 4) eye formation and pigmentation; 5) stomach and digestive tract formation; 6) first feeding; 7) yolk exhaustion; 8) oil globule exhaustion; 9) diet transition from rotifers to *Artemia* spp.; and 10) metamorphosis. Completion of the larval stage and metamorphosis was defined as the point when the axial skeleton is ossified and fish acquire the anatomical and morphological characteristics of juveniles.

The relationship between TL and age; SL and age; and mouth gape and age were plotted separately for each larval rearing trial. Total and standard length data of larvae were fitted to a simple curvilinear equation (i.e.,  $y = a + b \times x^{0.5}$ ). The comparison between these plots allowed assessment of somatic growth pattern through time. Repeated-measures analysis of variance was then used to statistically compare growth rates among rearing trials. To normalize observations and stabilize the variance, data were logarithmically transformed before statistical analysis. Tukey's test was used to determine if significant differences existed among treatment means. Differences were considered significant at  $P \leq 0.05$ . The general linear model function in SigmaStat 3.0 (SPSS Science, Chicago, IL) was used for all analyses.

Regression equations were calculated for total and standard length, yolk volume, and oil globule volume for larvae cultured from each spawn. Yolk volume was determined by using the equation for a prolate spheroid:

$$\text{Yolk volume} = \frac{4}{3} \pi [\text{yolk-sac length}] [\text{yolk-sac depth}]^2. \quad (1)$$

Oil globule volume was determined by using the equation for a sphere:



**Figure 1**

Size-frequency histograms of rotifer cultures used to feed larval Florida pompano (*Trachinotus carolinus*). Stock cultures of rotifers (*Brachionus plicatilis*) were obtained from (A) Aquaculture Center of the Florida Keys (ACFLK, Marathon, FL) in 2004 and (B) Oceans Reefs and Aquariums (ORA, Fort Pierce, FL) in 2005.

$$\text{Oil globule volume} = \frac{4}{3} \pi [\text{oil globule diameter}/2]^3. \quad (2)$$

Length and volume data were then plotted against age. The curvilinear equation,  $y = a + b \times x^{-1}$ , was fitted to the yolk and oil globule volume data and plotted with 95% confidence limits.

The mouth gape was determined by using length measurements of the upper and lower jaws and the Law of Cosines equation for a triangle with two known sides and an angle between them:

$$a^2 = b^2 + c^2 - 2bc \cos \alpha, \quad (3)$$

where  $a$  = mouth gape;

$b$  = upper jaw;

$c$  = lower jaw; and

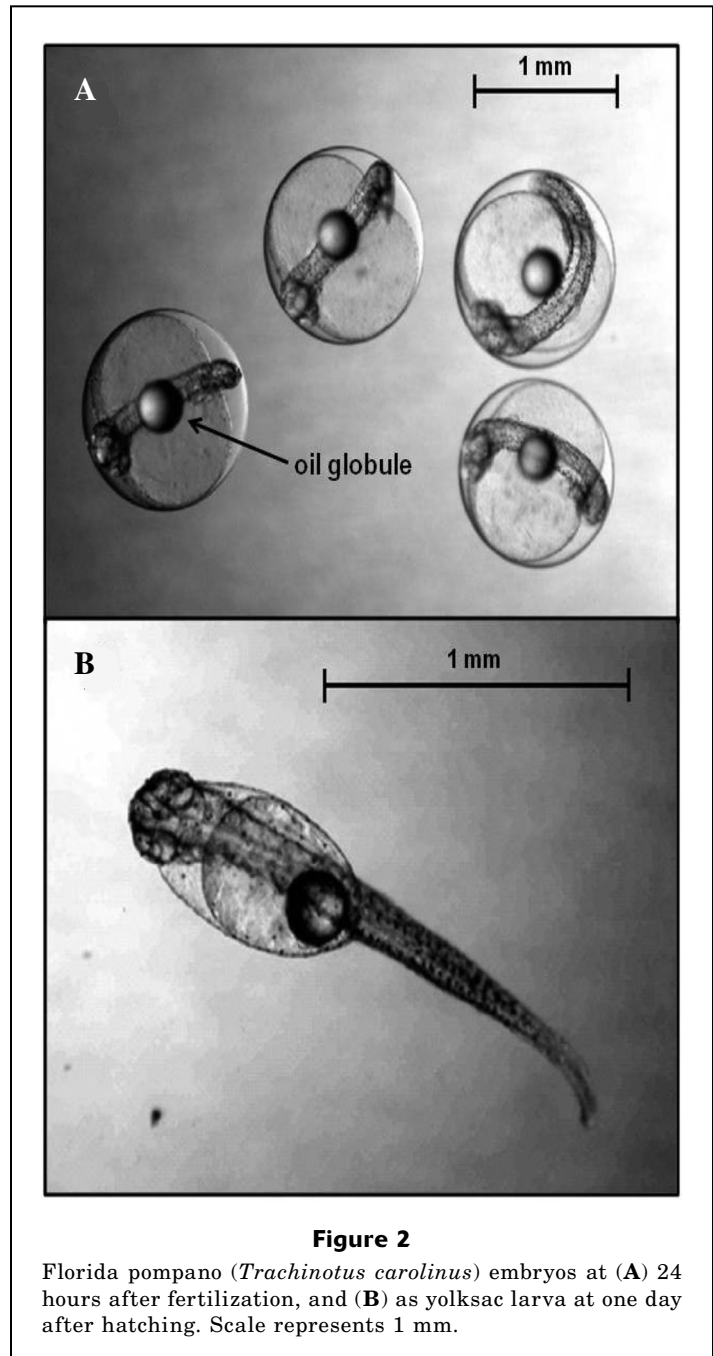
$\alpha$  = angle that forms the degree of mouth opening.

Calculations were based on the assumption that during active feeding the mouth of larvae opens to an angle ranging from  $90^\circ$  to  $120^\circ$  to capture prey (Shirota, 1970). Optimal prey sizes were estimated at 30% and 50% of mouth gape for larvae (Yasuda, 1960; Shirota, 1970; Hunter and Lasker, 1981; Cunha and Planas, 1999).

## Results

During each 20-day trial, production of postmetamorphic juvenile Florida pompano ranged from 1.5 to 5.0 fish/liter. Although water temperature is an important factor governing growth, there was no significant difference among any of the water quality parameters measured among rearing trials ( $P=0.67$ ). In the first rearing trial, dissolved oxygen was  $5.7 \pm 0.2$  mg/L (mean  $\pm$  SE), temperature was  $25.5 \pm 0.2^\circ\text{C}$ , and salinity was  $34.9 \pm 0.6$  g/L. In the second rearing trial, dissolved oxygen was  $5.6 \pm 0.2$  mg/L, temperature was  $25.5 \pm 0.2^\circ\text{C}$ , and salinity was  $34.8 \pm 0.5$  g/L. In the third rearing trial, dissolved oxygen was  $5.7 \pm 0.1$  mg/L, temperature was  $25.0 \pm 0.6^\circ\text{C}$ , and salinity was  $34.5 \pm 0.6$  g/L.

Eggs collected from each spawning event were uniform in shape and appearance. Fertilized eggs were  $0.99 \pm 0.04$  mm in diameter and contained a single oil globule (Fig. 2A). Newly hatched larvae were transparent, small (TL= $2.6 \pm 0.4$  mm), and not well developed (Fig. 2B). As typical of carangids, larvae hatched with large, elongate yolk sacs extending beyond the head and along the ventral region of the head and gut. A single oil globule was situated at the posterior end of the yolk sac. At hatching, the lower and upper jaws, as well as the digestive tract, were not fully developed. Pigmented eyes and functional mouth parts had formed by the end of two DAH when the larval swimming pattern became stronger and feed-



**Figure 2**

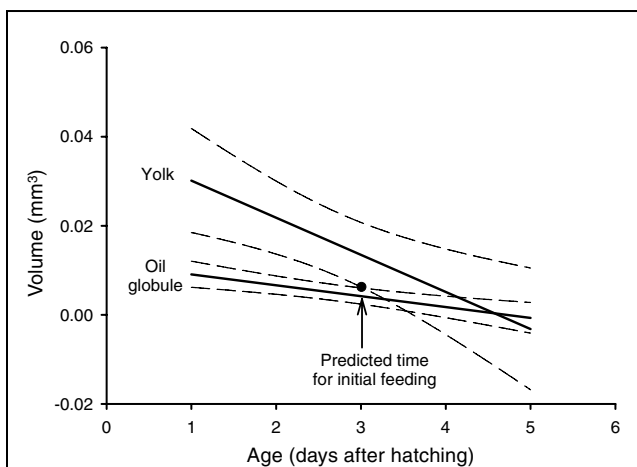
Florida pompano (*Trachinotus carolinus*) embryos at (A) 24 hours after fertilization, and (B) as yolk sac larva at one day after hatching. Scale represents 1 mm.

ing behavior was first observed. Melanophores were observed forming along the head and dorsal surface of the body at two DAH. The stomach and a primitive intestine were observed forming at two DAH, and the intestine had connected with the anus at three DAH. Rotifers and algae were first observed in the stomachs of larvae at three DAH. Larvae had exhausted yolk reserves at three to four DAH and were completely transitioned to exogenous feeding at five DAH (Fig. 3; Fig. 4A). Larvae at seven DAH exhibited a fully formed

and well developed digestive system (Fig. 4B). *Artemia* spp., which were first offered 12 DAH, were observed in the stomach of larvae along with rotifers at 14 DAH. Artificial feeds, which were offered beginning 10 DAH, could not be detected through observation with a dissecting microscope. Although Florida pompano lack a gas bladder, approximately 10% of larvae consumed small air bubbles (129–235  $\mu\text{m}$ ) that were trapped in the peritoneal cavity from 5 to 10 DAH.

Swimming ability improved and larvae were noticeably stronger with pectoral fin development at four to five DAH. The dorsal, pelvic, and anal fins began developing at nine DAH and the formation of the caudal fin and fin rays was observed at 12 DAH. Morphological and osteological development at six and seven DAH was noticeably more advanced in the third rearing trial than in specimens collected in the previous trials. As evidenced by the formation of hypural elements, notochord flexion was first observed at 10 to 12 DAH in the first two rearing trials. In the third rearing trial, notochord flexion was observed 8 to 11 DAH. Larvae completed flexion at 12 to 16 DAH in the first two rearing trials, and at 11 to 12 DAH in the third rearing trial. Postflexion and the onset of transformation were apparent at 15 to 18 DAH in the first two rearing trials (Fig. 5A), and at 13 to 14 DAH in the third rearing trial. Larvae in the first two rearing trials completed transformation and had a full complement of fins and scales by 20 DAH (Fig. 5B), whereas fish in the third rearing trial completed transformation by 17 DAH.

Differences in growth were observed among trials. Mean growth rates calculated from TL measurements were  $0.22 \pm 0.04$ ,  $0.23 \pm 0.12$ , and  $0.35 \pm 0.09$  mm/d for each of the larval rearing trials. Larvae in the third trial grew faster than fish in the first and second trial.

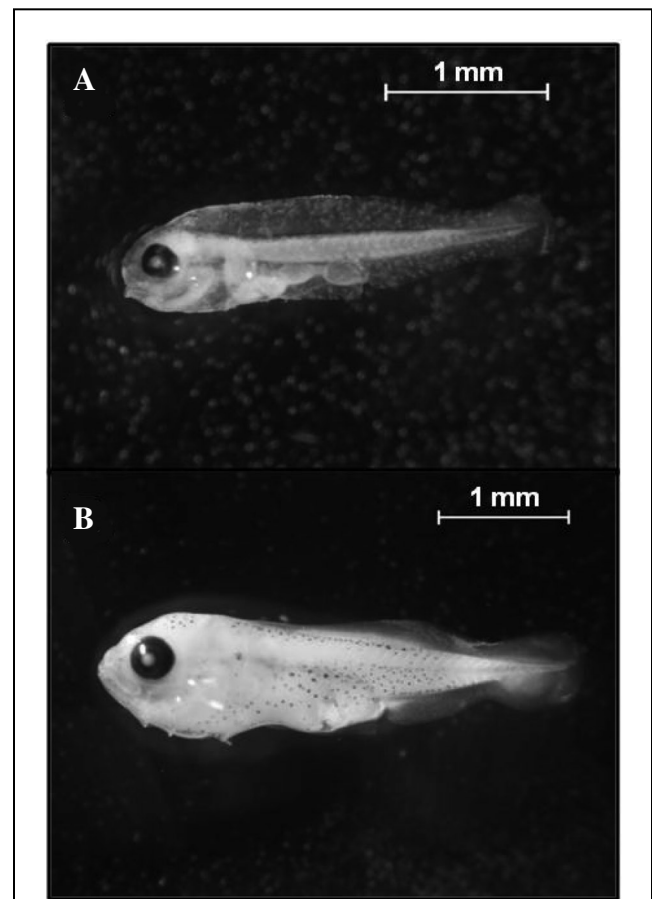


**Figure 3**

Regression (solid line) with 95% confidence limits (dashed line) of yolk volume on oil globule volume for Florida pompano (*Trachinotus carolinus*). Data represent combined measurements of three larval production trials.

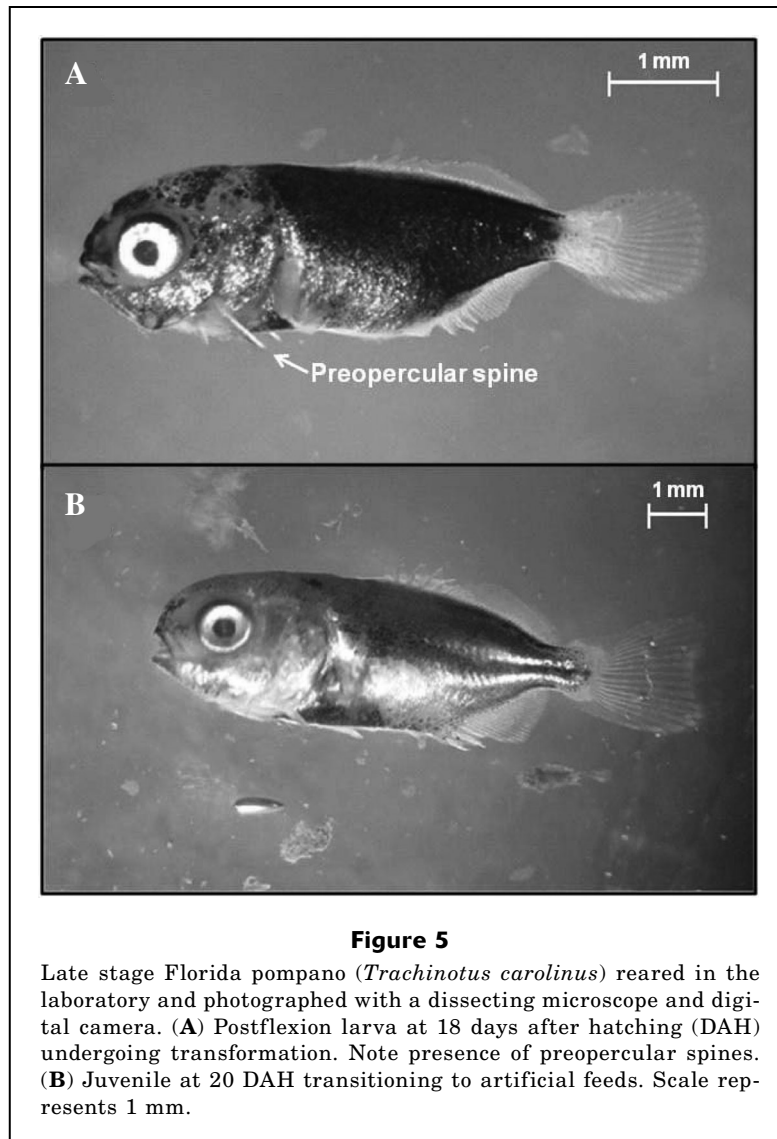
Measured growth parameters of Florida pompano larvae reared from one through 20 DAH are summarized in Table 1. Statistical analysis evaluating TL and age (Fig. 6) revealed no significant differences among growth rates from hatching through six DAH ( $P=0.84$ ); however, a significant difference in growth rate was detected for larvae reared in the third trial ( $P=0.007$ ) after seven DAH (Table 2). No significant differences were detected between the first two rearing trials ( $P=0.12$ ).

Similar results were observed with a statistical analysis of larvae, where SL and age (Fig. 7) were used to determine growth rates. Mean growth rates were  $0.18 \pm 0.03$ ,  $0.18 \pm 0.10$ , and  $0.31 \pm 0.08$  mm/d for each of the larval rearing trials. No significant differences were observed among growth rates from hatch through eight DAH ( $P=0.75$ ); however, a significant difference in growth rate was detected for larvae reared in the third



**Figure 4**

Early stage Florida pompano (*Trachinotus carolinus*) reared in the laboratory and photographed with a dissecting microscope and digital camera. (A) Larva at three days after hatching (DAH) near initiation of exogenous feeding and with eye pigmentation. (B) Larva at 7 DAH with fully formed digestive system, distinctive pigmentation, and initial development of head spines. Scale represents 1 mm.



trial ( $P < 0.001$ ) after nine DAH. Larvae in the third trial grew significantly faster than fish in the first and second trial ( $P < 0.001$ ). No significant differences were detected among the first two rearing trials ( $P = 0.18$ ).

No significant differences were observed in larval mouth size among rearing trials ( $P = 0.16$ ). The mean mouth gape of larvae was  $0.266 \pm 0.075$  mm at first feeding and increased with a growth rate of  $0.13 \pm 0.04$  mm/d (Table 3). The length of the upper and lower jaws and mouth gape increased linearly with age (Table 4). Predicted values for optimal prey sizes ranged from 80 to 130  $\mu\text{m}$  at three DAH, 160 to 267  $\mu\text{m}$  at five DAH, and 454 to 757  $\mu\text{m}$  at 10 DAH. These values correspond closely with the sizes of rotifers and *Artemia* spp. nauplii fed to larvae during each of the rearing trials.

Larvae that exhibited aggressive behavior progressed through metamorphosis earlier than their cohorts. Cannibalism, defined by Smith and Reay (1991) as the act of killing and consuming the whole, or major part, of

an individual belonging to the same species regardless of its age or stage of development, was not observed in the rearing trials. The dominant, aggressive behavior observed was that of inflicting injury that resulted in mortality in small premetamorphic individuals.

## Discussion

As a direct result of aquaculture research, the larval development of a number of valuable marine fish species has been described and characterized. The early life stages of species such as striped bass (*Morone saxatilis*; Brown et al., 1998), red drum (*Sciaenops ocellatus*; Lee et al., 1984), and red snapper (*Lutjanus campechanus*; Drass et al., 2000) were studied to support efforts for food production and stock enhancement in the United States, and global research on the early life history of a number of valuable species has led to the develop-

**Table 1**

Morphometrics of Florida pompano (*Trachinotus carolinus*) from one day after hatching (DAH) through 20 DAH cultured at 25°C. Metamorphosis was completed at 17–19 DAH. The relationship of head length to standard length ranged from 33% to 49%. Values are means  $\pm$  standard error (SE) for larvae sampled from three rearing trials ( $n=600$ ).

Days after hatching	Total length (mm)	Standard length (mm)	Body depth (mm)	Head length (mm)	Eye diameter (mm)
1	2.27 $\pm$ 0.50	2.14 $\pm$ 0.58	0.61 $\pm$ 0.06	—	0.19 $\pm$ 0.02
2	2.62 $\pm$ 0.31	2.52 $\pm$ 0.50	0.67 $\pm$ 0.10	—	0.25 $\pm$ 0.00
3	2.71 $\pm$ 0.27	2.69 $\pm$ 0.54	0.65 $\pm$ 0.04	—	0.28 $\pm$ 0.01
4	2.84 $\pm$ 0.76	2.76 $\pm$ 0.25	0.68 $\pm$ 0.06	—	0.28 $\pm$ 0.03
5	3.05 $\pm$ 1.01	2.80 $\pm$ 0.25	0.66 $\pm$ 0.07	—	0.29 $\pm$ 0.02
6	3.25 $\pm$ 1.26	2.96 $\pm$ 0.24	0.60 $\pm$ 0.07	1.08 $\pm$ 0.19	0.34 $\pm$ 0.17
7	3.55 $\pm$ 1.52	3.06 $\pm$ 0.30	0.74 $\pm$ 0.07	1.17 $\pm$ 0.17	0.31 $\pm$ 0.08
8	3.67 $\pm$ 1.50	3.31 $\pm$ 0.51	0.74 $\pm$ 0.03	1.25 $\pm$ 0.27	0.35 $\pm$ 0.12
9	3.95 $\pm$ 1.36	3.67 $\pm$ 0.47	0.87 $\pm$ 0.07	1.42 $\pm$ 0.24	0.39 $\pm$ 0.17
10	4.20 $\pm$ 1.64	3.80 $\pm$ 0.53	0.91 $\pm$ 0.04	1.42 $\pm$ 0.27	0.42 $\pm$ 0.26
11	4.37 $\pm$ 1.78	4.10 $\pm$ 0.88	0.95 $\pm$ 0.01	1.53 $\pm$ 0.45	0.45 $\pm$ 0.26
12	4.49 $\pm$ 1.67	4.46 $\pm$ 1.24	0.95 $\pm$ 0.02	1.68 $\pm$ 0.59	0.47 $\pm$ 0.37
13	4.87 $\pm$ 1.96	4.79 $\pm$ 1.39	0.98 $\pm$ 0.09	1.77 $\pm$ 0.68	0.47 $\pm$ 0.32
14	4.95 $\pm$ 1.35	4.95 $\pm$ 2.06	1.20 $\pm$ 0.23	1.89 $\pm$ 0.62	0.53 $\pm$ 0.39
15	5.18 $\pm$ 1.48	5.15 $\pm$ 2.20	1.15 $\pm$ 0.03	1.90 $\pm$ 0.74	0.52 $\pm$ 0.43
16	5.51 $\pm$ 1.44	5.54 $\pm$ 2.13	1.31 $\pm$ 0.32	1.99 $\pm$ 0.78	0.55 $\pm$ 0.61
17	5.71 $\pm$ 1.46	5.42 $\pm$ 0.20	1.39 $\pm$ 0.22	2.15 $\pm$ 0.69	0.61 $\pm$ 0.49
18	5.99 $\pm$ 1.46	5.48 $\pm$ 0.17	1.51 $\pm$ 0.05	2.22 $\pm$ 0.74	0.59 $\pm$ 0.40
19	6.72 $\pm$ 1.44	5.92 $\pm$ 0.11	1.61 $\pm$ 0.10	2.23 $\pm$ 0.94	0.60 $\pm$ 0.47
20	8.55 $\pm$ 1.80	6.92 $\pm$ 1.88	2.53 $\pm$ 0.85	2.59 $\pm$ 0.86	0.79 $\pm$ 0.50

ment of standardized hatchery practices to support the growth of commercial aquaculture. Examples of such species include barramundi (*Lates calcarifer*; Kohno et al., 1986), milkfish (*Chanos chanos*; Kohno et al., 1996), mangrove red snapper (*L. argentimaculatus*; Doi et al., 1997), gilthead seabream (*Sparus auratus*; Polo et al.,

1992) and European sea bass (*Dicentrarchus labrax*; Kuzir et al., 2004).

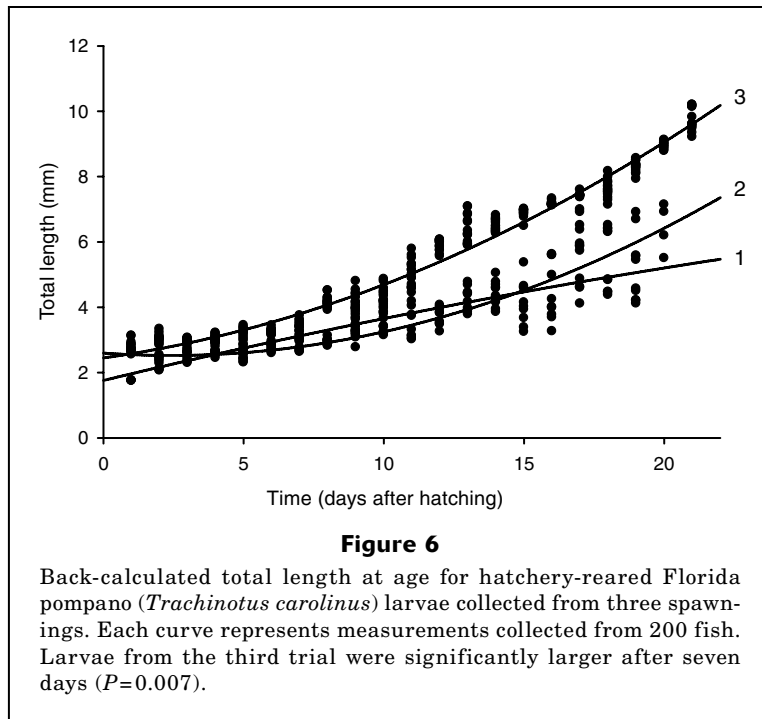
In this study, image analysis proved to be a useful tool (with a high degree of accuracy) for documenting the early development of larvae and for obtaining measurements of larval characters. The process of digitally photographing larvae and measuring growth was relatively quick and uncomplicated. Measurements were accomplished in real-time during rearing trials and therefore offered a broad spectrum of possible applications for research or commercial hatcheries. With the exception of overall growth measured as TL and SL, no substantial amount of morphological variation was expressed among cohorts of larvae from an individual spawning event or multiple spawnings. Developmental characteristics of cultured larvae were not significantly different from previous descriptions of wild-caught Florida pompano larvae from 7.2 to 11.0 mm SL (Fields, 1962); however, it was determined that fish changed morphologically from larvae to juveniles with full fin-ray counts at 17 to 19 DAH, instead of at a previously observed time in which metamorphosis of cultured fish occurred at 24 DAH (McMaster, 1988).

Similar to the challenges in rearing any marine fish species with small eggs and larvae, the culture of Florida pompano larvae is difficult and time consuming. It is unfortunate that larvae will not readily consume

**Table 2**

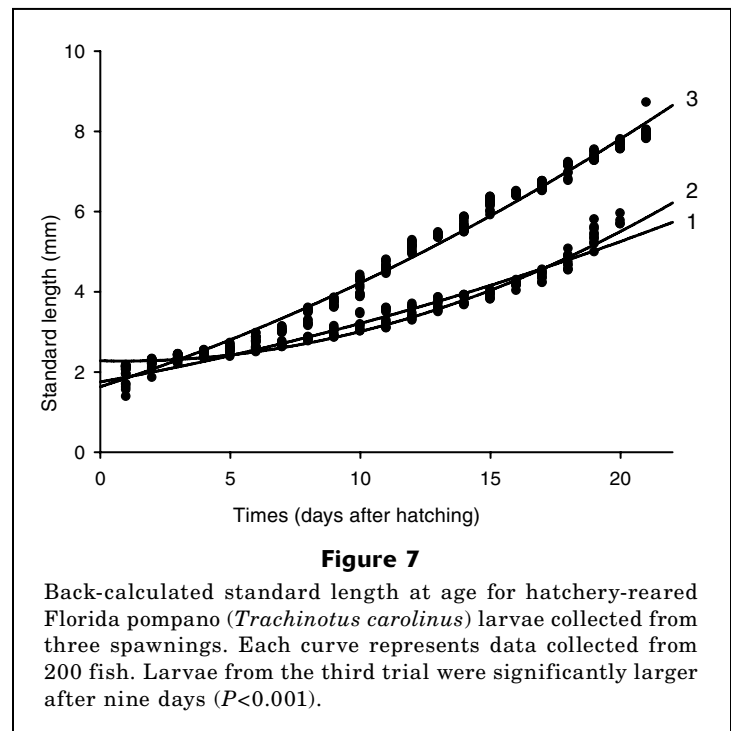
Growth equations based on total length (TL) and standard length (SL) for Florida pompano (*Trachinotus carolinus*) reared at 25°C. Larval lengths were measured in mm and age was measured as number of days after hatching. Separate growth equations were developed for each rearing trial because significant differences in growth were observed ( $P < 0.001$ ).

Trial	Equation	$n$	$r^2$
1	$TL = 2.058e^{0.0533 \text{ Age}}$	200	0.96
	$SL = 1.967e^{0.0479 \text{ Age}}$	200	0.98
2	$TL = 2.066e^{0.9438 \text{ Age}}$	200	0.94
	$SL = 1.893e^{0.0508 \text{ Age}}$	200	0.93
3	$TL = 2.381e^{0.0672 \text{ Age}}$	200	0.98
	$SL = 2.859e^{0.0697 \text{ Age}}$	200	0.96



artificial feed or *Artemia* spp. at first feeding. In nature, larval Florida pompano prey upon a wide variety of different types and sizes of zooplankton. Larvae have small mouths with limited yolk reserves and undeveloped digestive systems at first feeding. As a consequence, larvae require small, slow-moving prey that are recognizable as potential food items. Given that the optimal prey size for marine fish larvae is 25% of mouth gape at first feeding and increases to 50% within a few days (Hunter and Lasker, 1981), the production of appropriate size live feeds must be considered an essential component of larviculture protocols.

The marine rotifer, *B. plicatilis* (so-called large or small morphotypes), is the most commonly cultured and mass produced species of zooplankton worldwide (Yoshimura et al., 1996; Lubzens et al., 2001). Recent studies have shown that the strain and morphotypes of rotifer stocks within a hatchery can vary greatly, and rotifer stocks from commercial hatcheries often represent a mixture of species, strains, and morphotypes (Papkostas, 2006). Commercial hatcheries frequently buy or trade rotifer cultures with other hatcheries to meet production quotas, which can exceed one billion rotifers per day (Lubzens, 2001). In this study, we used image analysis to document the size distribution of rotifers obtained from local hatcheries. Image analysis coupled with routine sampling allowed us to monitor growth, reproduction, and size-frequency distribution of rotifer stocks. Determination of rotifer size-frequency distributions was



useful for ensuring that a sufficient number of small individuals were available for first feeding larvae. Although rotifer strains and size distributions differed among years, there is little evidence that larval growth and survival was affected. Although production methods for marine rotifers and *Artemia* spp. are currently



**Table 3**

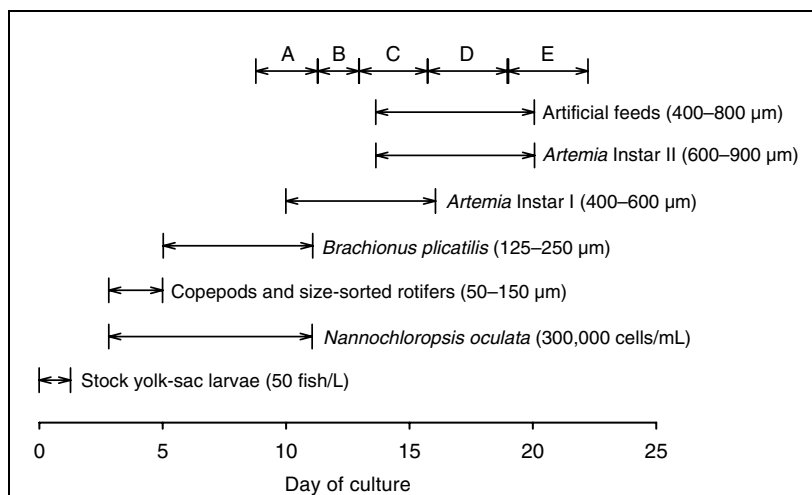
Mouth size of Florida pompano (*Trachinotus carolinus*) at the onset of opening (three days after hatching, DAH) through 20 DAH. Values represent measurements (means  $\pm$  standard error [SE]) for larvae sampled from three rearing trials. Mouth gape estimates were based upon calculations assuming the mouth opens 90° (min) to 120° (max) during feeding and prey capture.

Days after hatching	Upper jaw length (mm)	Lower jaw length (mm)	Min mouthgape (mm)	Max mouthgape (mm)
3	0.28 $\pm$ 0.02	0.24 $\pm$ 0.00	0.213	0.319
4	0.29 $\pm$ 0.02	0.27 $\pm$ 0.05	0.250	0.372
5	0.30 $\pm$ 0.01	0.28 $\pm$ 0.05	0.427	0.640
6	0.36 $\pm$ 0.09	0.38 $\pm$ 0.09	0.525	0.784
7	0.37 $\pm$ 0.12	0.39 $\pm$ 0.19	0.618	0.924
8	0.39 $\pm$ 0.04	0.43 $\pm$ 0.12	0.763	1.132
9	0.47 $\pm$ 0.11	0.52 $\pm$ 0.16	1.065	1.550
10	0.53 $\pm$ 0.17	0.59 $\pm$ 0.26	1.221	1.805
11	0.55 $\pm$ 0.02	0.62 $\pm$ 0.22	1.912	2.838
12	0.61 $\pm$ 0.03	0.68 $\pm$ 0.29	2.265	3.392
13	0.70 $\pm$ 0.03	0.69 $\pm$ 0.35	3.250	4.863
14	0.77 $\pm$ 0.09	0.73 $\pm$ 0.45	3.472	5.197
15	0.79 $\pm$ 0.03	0.75 $\pm$ 0.39	4.002	5.979
16	0.88 $\pm$ 0.13	0.85 $\pm$ 0.39	4.383	6.575
17	0.89 $\pm$ 0.07	0.86 $\pm$ 0.50	5.216	7.757
18	0.94 $\pm$ 0.06	0.89 $\pm$ 0.50	5.306	7.958
19	1.01 $\pm$ 0.18	0.89 $\pm$ 0.62	5.501	8.252
20	1.06 $\pm$ 0.19	1.03 $\pm$ 0.55	5.878	8.802

the standard for commercial hatcheries, a variety of small copepods, protozoans, cladocerans, and molluscan larvae offer great promise as feed, provided they offer adequate nutrition.

As a result of the findings of this study a refined feeding regime for Florida pompano was developed (Fig. 8). The regime directly addresses the importance of feeding small prey items (80–130  $\mu$ m) to larvae at

first feeding (three DAH), and it provides a stage- and size-specific guideline for feeding larvae reared under laboratory conditions at 25°C. Future work should address the bioenergetics and nutritional requirements specific for Florida pompano larvae and determine the effect of increased temperature on larval growth and development. Although research conducted in this study was performed within a small-scale marine hatchery, the techniques could be used to formulate commercial hatchery production protocols for other subtropical and tropical marine species with similar early life-history patterns.

**Figure 8**

Recommended hatchery feeding regimen for rearing Florida pompano (*Trachinotus carolinus*) from hatching through transformation. Chronological stages of development are noted by the upper bar: A—preflexion; B—flexion; C—postflexion; D—metamorphosis, and E—juvenile.

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**Table 4**

Growth equations for upper jaw, lower jaw, and mouth gape of Florida pompano (*Trachinotus carolinus*) reared from hatching through metamorphosis. Jaw length and mouth gape were measured in mm and age was measured as days after hatching.

Equation	<i>n</i>	<i>r</i> <sup>2</sup>
Upper jaw = 0.0492 Age + 0.0562	510	0.98
Lower jaw = 0.0447 Age + 0.1452	510	0.96
Mouth gape = 0.0367 Age - 2.0308	510	0.92

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