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# Growth and survival of porkfish (*Anisotremus virginicus*) larvae: comparing rotifers and copepod nauplii during first feeding

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**Abstract**. Eggs of porkfish (*Anisotremus virginicus*) were obtained from natural spawning events occuring at Seaworld Orlando and transported to the Tropical Aquaculture Laboratory. Eggs were stocked into 12 L experimental rearing tanks at 30/L to determine growth and survival of larvae fed rotifers (*Brachionus plicatilis* – Cayman strain) and copepod nauplii (*Acartia tonsa*) during first exogenous feeding through seven days post hatch (dph). Larvae fed rotifers during first feeding exhibited higher survival than those fed copepod nauplii (35.7 ± 4.0% and 23.8 ± 4.0% respectively). Conversely, larvae fed copepod nauplii exhibited greater body length and body depth than those fed rotifers. Results suggest that porkfish is an ideal candidate species for existing aquaculture technologies. This study highlights the Rising Tide Conservation Initiative, a collabortion of researchers and stakeholders invested in expanding marine ornamental aquaculture and research.
**Key Words**: *Anisotremus virginicus*, porkfish, larval rearing, copepods, rotifers.

**Introduction**. The marine ornamental fish trade relies primarily on wild-caught specimens to supply both private and public aquariums (Wabnitz et al 2003). Currently, aquaculture protocols are available for roughly 80 of the over 1,800 species of marine fishes traded in the international aquarium industry (Rhyne et al 2012; Wittenrich 2007). Aquaculture success with marine aquarium fish has typically been limited to demersal spawning species exhibiting parental care during incubation, low fecundity, and large larval size (Holt 2003; Olivotto et al 2011). Success with pelagic spawning species has been limited (Moe 1997, 2003; Ogawa & Brown 2001). As marine ornamental aquaculture moves progressively forward, developing production protocols for pelagic spawning species is becoming increasingly important (Sadovy et al 2001; Sadovy & Vincent 2002).

Bottlenecks to the commercial production of marine ornamental fishes have been generalized to include: initiating reproduction in captivity, development of eggs and larvae to the first feeding stage, and transition to first exogenous feeding (Olivotto et al 2006). Much research emphasis has been placed on the first feeding stage since successful feeding and subsequent growth of larvae determines the success or failure of aquaculture ventures (e.g. Moorhead & Zeng 2010; Wittenrich et al 2007; Olivotto et al 2006). Copepods have remained at the center of this research due their prevelance in the guts of wild collected larvae and high lipid content (Sampey et al 2007). The widespread use of copepods, however, has been limited due to captive production bottlenecks (Moorhead & Zeng 2010). Studies have demonstrated increased growth and survival of larvae fed copepod nauplii through the larval stages (Olivotto et al 2006, 2008), but it is still poorly known if limited or stage-specific feeding of copepod nauplii produce significant improvements to larval rearing.

The University of Florida's Tropical Aquaculture Laboratory (TAL) and SeaWorld Orlando (SWOR) are participants of the Rising Tide Conservation Initiative (RTCI).

Initiated by SeaWorld Parks and Entertainment in 2009, RTCI is a collection of research facilities, industry partners, and Association of Zoos and Aquariums (AZA) institutions committed to establishing viable aquaculture strategies for the commercial production of marine ornamental fish species (http://www.seaworld.org/rising-tide/index.htm). One of RTCI's initial research efforts utilize established populations of marine fish species spawning in public aquaria. Display aquariums are highly advantageous for research efforts focused on developing new species technology. Efforts of RTCI stakeholders take advantage of this valuable resource by collecting newly spawned eggs and/or larvae and shipping them to TAL for subsequent larval rearing studies; alleviating the necessity of on-site broodstock maintenance while providing screening of potential commercial species.

Recently, TAL and SWOR developed reliable techniques for harvesting and transporting the pelagic eggs of porkfish (*Anisotremus virginicus*) which lead to the establishment of larval rearing techniques for the species. Porkfish, a widespread Atlantic haemulid (Hoese & Moore 1998), are popular display animals in public aquariums due to their schooling behavior and bright colors. Similarly, juveniles of the species are captured for the aquarium trade where they display cleaning behavior (Brockmann & Hailman 1976). In the present study, pelagic eggs from SWOR were transported to TAL to determine the effects of first feeding larval diets on growth and survival. While porkfish have been reared in captivity prior to this study (Potthoff et al 1984) little information on the early life history and aquaculture potential is available. The main goal of this study was thus, to determine the relative growth and survival of larvae fed rotifers and copepod nauplii during the critical period of first feeding in hopes of contributing to an aquaculture protocol for the species and those closely related.

## Material and Method

**Spawning, collection, and egg incubation**. Marine fish eggs were collected from the floor aquarium located in the Jewels of the Sea exhibit building at SeaWorld in Orlando, FL. The octagonal shaped exhibit is 6 m wide and 2.1 m deep containing approximately 106,000 L of artificial saltwater. The life support system contains four 3-foot sand filters (Triton T-140), a protein skimmer (Emperor Aquatics), heat exchanger and an in-house constructed nitrogen-reduction chamber. Water flows from the exhibit through a bottom drain and side skimmer to a sump. Four circulation pumps (1.5 HP Jacuzzi pump) draw water from the sump manifold to the sand filters before returning it to the exhibit. Water is pumped through side loops (0.75HP Jacuzzi) that split to a protein skimmer and heat exchanger before returning to the sump. The exhibit contains 12 species of tropical marine fish. Fish are fed approximately 2,000 g of food (capelin, mackerel, silversides, krill, squid and shrimp) two times daily.

Eggs were collected from the side skimmer using aquarium nets (500 µm mesh) that were reshaped to fit inside the skimmer box. At 3 pm, the nets were set in place and remained overnight. At 7 am the following morning eggs were transferred to a 1 L plastic container where infertile or dead eggs were allowed to settle. Viable embryos were then transferred to an 18.9 L bucket and transported to TAL. The bucket was temperature acclimated for 1 hour before carefully decanting viable eggs into an acclimation vessel composed of equal parts transport water and larval rearing system water. After one hour the eggs were gently homogenized before determining the number of eggs. Species composition was determined by differences in egg diameter and pigmentation

**Larval rearing**. Larvae were reared in 12 L cylindrical, flat-bottom fiberglass tanks connected to a recirculating filtration system. Tank bottoms were painted white to facilitate behavioral observations and prey densities and tank walls were painted black. Natural filtered and sterilized seawater was circulated through mechanical filter (40 micron filter sock), biological filter (Kaldness beads), protein skimmer (Precision Marine) and 15 watt UV sterilizer (Aqua). Photoperiod was maintained at 24L:0D throughout the experiment with two 30 watt fluorescent lamps (6500K) suspended 20 cm above the surface of the water. Eggs were stocked at a density of 30 eggs/l yielding 360 eggs per

tank. Oxolinic acid was used at 1mg/L in the system water on days 1-10 post hatch. The initial water exchange rate was 400% total daily tank volume (TDTV) accompanied by gentle aeration. Beginning on 1 DPH, tanks were inoculated daily with Tahitian strain *Isochrysis galbana* (T-ISO). At 5 DPH, aeration was increased slightly and water exchange rate increased to 600% TDTV. At 10 DPH, aeration was again increased slightly and the water exchange rate increased to 800% TDTV for the remainder of the trial. Internal standpipes were fitted with 150 µm screen. Temperature was maintained at 28.3 ± 0.3°C, salinity 32.9 ± 0.8 g $L^{-1}$, pH 8.4 and dissolved oxygen 6.0 mg $L^{-1}$. Water quality via total ammonia-nitrogen was measured twice weekly with a Hach DR/4000U spectrophotometer. When TAN exceeded 0.015 ppm a 20% water change was performed on the total system volume via water exchange from the sump.

**Live feeds culture**. Rotifers, *Brachinous plicatilis*, Cayman strain (180µm lorica length), were cultured at 26°C and a salinity of 25ppt in four 110 L rectangular glass aquaria. Cultures were fed 1-4 L live *Nannochloropsis oculata* and Tahitian strain *Isochrysis galbana* (T-ISO) daily depending on rotifer density. Density of cultures was maintained below 500 $mL^{-1}$. *Acartia tonsa*, a calanoid copepod, were batch cultured at 28-30°C and a salinity of 22-25 ppt in four 150 L square polyethylene tanks and a 400 L cylindrical, conical-bottom tank with moderate aeration. Each 150 L culture was fed 0.5 L of T-ISO (15-20 million cells $mL^{-1}$) and the 400 L culture was fed 1.25 L. Nauplii were harvested twice daily from each tank via floating airlifts (Cassiano 2009). Harvested nauplii were then placed in a graduated beaker and quantified prior to feeding.

**Experimental design**. Two diet treatments were tested during first feeding (2-7 dph) to determine the relative growth and survival of larvae fed different diets during the transition to exogenous feeding. In group R, rotifers were maintained at a density of 2.0 $mL^{-1}$ on 2 dph, 5.0 $mL^{-1}$ from 3-5 dph, and 8.0 $mL^{-1}$ on 6 dph. Larvae in group AT received *A. tonsa* nauplii at a density of 1.0 $mL^{-1}$ on 2 dph, 2.5 $mL^{-1}$ on 3 dph, 3.0 $mL^{-1}$ on 4 dph, 4.5 $mL^{-1}$ on 5-6 dph.

Diet treatments were limited to the first feeding stage, defined as the period of reduced feeding performance, prior to development of the opercular-linkage (Wittenrich & Turingan 2011). On 7 dph both treatments were fed rotifers at 10.0 $mL^{-1}$ and increased to 15 $mL^{-1}$ from 8-12 dph. Similarly, both treatments were fed *Artemia* nauplii at 0.5 $mL^{-1}$ on 10 and 11 dph, 1.0 $mL^{-1}$ on 12 and 13 dph, and 2.0 $mL^{-1}$ on 14-15 dph. Live prey density was maintained by two daily feedings.

At 15 dph, all larvae were harvested and over dosed with MS-222 (tricaine methanosulfonate) prior to fixation in 10% buffered formalin. Specimens were transferred to 70% alcohol after two days. Transition to *Artemia*/dry feed greatly reduced mortality in previous trials at 15 dph and was used as the harvest date for the present study.

**Sample collection and morphometric analysis**. Morphometric analysis of fish larvae was adapted from Cassiano et al (2010). Twenty larvae from each experimental tank were haphazardly sub-sampled for morphometric analysis. Preserved larvae were placed on a sedgewick rafter cell, and photographed using a dissecting microscope at 40-X magnification with a digital camera (Sony Model DCRA-C171). SigmaScan Pro 5.0 image analysis software (SPSS Science) was used to measure: standard (SL) and body depth (BD). When measuring yolk-sac larvae, BD was considered the distance perpendicular to the longitudinal axis from the dorsal crest through the midpoint of the yolk-sac to the ventral most point of the body.

**Statistical analysis**. All statistical analyses were performed with SAS version 8.02 software (Cary, NC). Percentage data were arc-sine-square-root transformed prior to analysis. A Student's t test, following the TTEST procedure of SAS, was used to detect differences in treatment means for all dependent variables. All statistical tests were considered significant when $P \leq 0.05$.

## Results and Discussion

Two species of marine fish eggs were identified in the SWOR collection. Porkfish eggs were dominant at 70.4% and blue striped grunt, *Haemulon sciurus*, constituted 29.6% of the samples. Very few larvae of *H. sciurus* survived beyond 5 dph (23 total among 12 replicate tanks), presumably due to interspecific competition from porkfish larvae. Growth and survival was not analyzed due to low numbers and competitive interactions.

**Growth**. Newly hatched porkfish larvae (1 dph) measured 3.52 ± 0.21 mm SL (mean ± SD) and 1.16 ± 0.09 mm BD. At 15 dph, The SL of larvae fed the AT diet was significantly greater than larvae fed the R diet (7.07 ± 0.96 mm vs 6.33 ± 0.84 mm; T198 = 5.73; P < 0.0001) (Table 1). The BD of larvae fed the AT diet was significantly greater than larvae fed the R diet (2.14 ± 0.30 mm vs 1.95 ± 0.24 mm; T198 = 4.94; P < 0.0001).

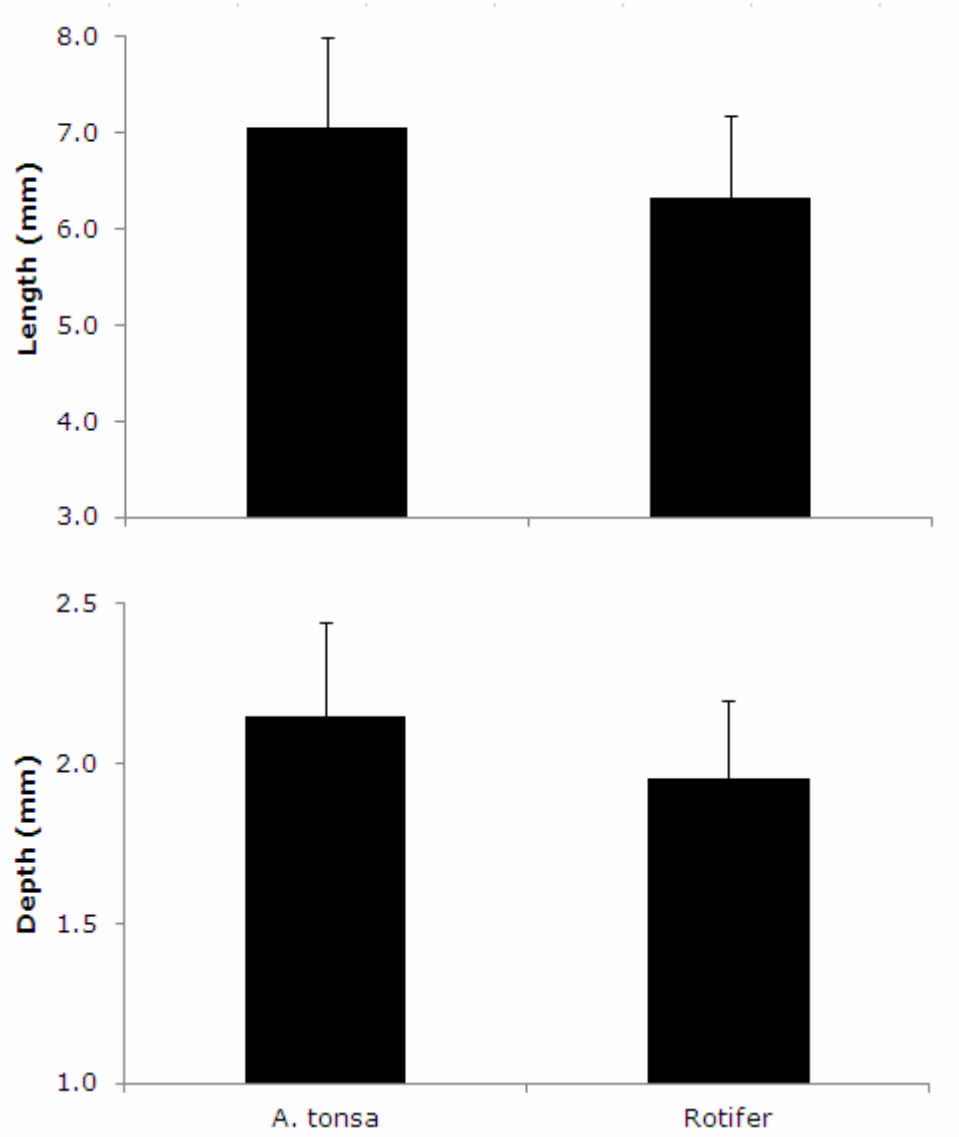


Figure 1. The standard length (top) and body depth (bottom) of 15 days post hatch porkfish larvae fed different dietary treatments during the experimental trial. Error bars represent standard deviation.

**Survival**. The survival of all larvae fed the R diet (35.7 ± 4.0%) was significantly greater than larvae fed the AT diet (23.8 ± 4.0%) (T8 = 4.64; P = 0.0017). The survival of

porkfish larvae fed the R diet (50.2 ± 5.4%) was significantly greater than porkfish larvae fed the AT diet (33.1 ± 4.9%) ($T8 = 5.20$; $P = 0.0008$) (Figure 2).

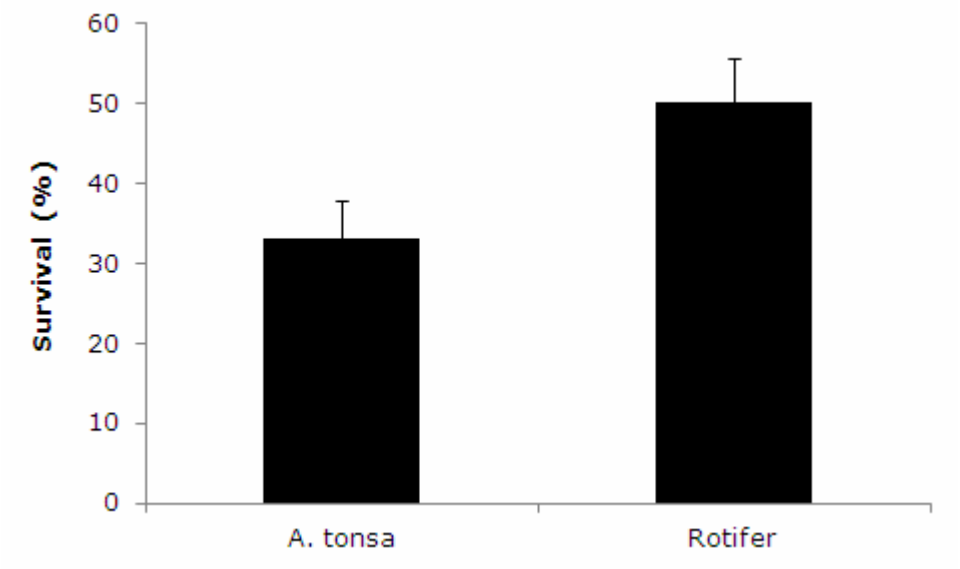


Figure 2. The percent survival of 15 days post hatch porkfish larvae fed different dietary treatments during the experimental trial. Error bars represent standard deviation.

Perhaps the most unique attribute of this examination was the utilization of marine fish eggs spawned in a public aquarium. As most production bottlenecks occur during the larviculture phase, continuous access to multiple species of marine fish eggs streamlines research efforts. RTCI has initiated a project using this valuable resource as a screening tool for the discovery of candidate species for marine fish aquaculture. With numerous species yet to be explored, examination of species readily spawning in public aquaria is viewed as a priority of research. This not only allows for multiple species to be examined simultaneously but reduces the need to acquire, acclimate, and condition broodstock. The porkfish is viewed as a successful model resulting from this approach with hopes that other species can follow suit. While the commercial value of porkfish within the aquarium hobby is limited, there is a sporadic, but significant demand for large numbers in public displays which may prove to be profitable for one or two producers.

Large-scale production of marine aquarium fishes is generally limited by the production of live feeds available during the transition to exogenous feeding. Many target species have not been successfully raised using traditional aquaculture prey items such as *Brachionus* spp. and *Artemia* spp. and there is continued concern that these prey types are nutritionally inferior to wild type zooplankters (Holt 2003). As a result, copepods have been heavily studied in recent years as a natural, nutritious first food choice for cultured marine fish larvae (Olivotto et al 2006, 2008). The success of using copepod nauplii for marine fish rearing is dependant on the species of fish in culture and the copepod chosen as feed. Tremendous diversity of mouth gape, prey capture performance, preference and nutritional requirements have been observed across marine fish families and it is likely that not all copepod species are suited for the culture of marine fishes. A few studies have noted that larval performance is greater in treatments co-fed with copepods and rotifers (Cassiano et al 2011; Stottrup & Norsker 1997). In species that recognize rotifers as prey items this technique can be advantageous. Unfortunately, many species don't identify rotifers as a first feeding organism and therefore the use of other prey items must be investigated.

Interspecific interactions were likely to have occurred in this study. Although unavoidable here, it is unlikely that growth and survival of porkfish larvae were greatly affected since these larvae appeared to be the dominant species and seemed to have a competitive advantage in size, mouth gape and behavior over *H. sciurus*. It is unlikely that this interaction improved the performance of porkfish larvae during this trial, but it

should be acknowledged. In subsequent larval rearing trials, with and without the presence of other species, porkfish have exhibited similar developmental and survival patterns as presented here (Moe personal communication).

In this study, porkfish larvae fed rotifers during first exogenous feeding exhibited higher survival compared to those fed copepod nauplii. The prey density of copepod nauplii was maintained lower than that of rotifers throughout the experiment. This is a common feeding strategy due to the higher nutritional content of copepod nauplii, however, the low prey density may have affected feeding performance of early larvae. The growth of larvae fed copepods, however, was greater than those fed rotifers. Improved growth in the copepod treatment could be explained by reduced larval density due to mortality, which then offered higher prey densities to surviving larvae. The acceptance of rotifers during first exogenous feeding, and the subsequent survival, suggests that copepods are not needed to implement large scale production of porkfish.

In Potthoff et al (1984), porkfish were reared, however, larvae were fed size sorted wild zooplankton with little details toward large scale culture. The present study shows that porkfish readily accept rotifers (*Brachionus plicatilis*) as a first food and are well suited to current commercial marine aquaculture technologies. Preliminary results at TAL encouraged a relationship between three industry partners and SWOR wherein eggs from SWOR were delivered to three commercial hatcheries for larviculture and growout. Two of the three facilities were successful in rearing large numbers of juveniles that were sold back to SWOR. This unique approach proved effective in obtaining viable numbers of eggs for examination. The model used here is viewed as a research priority for expanding marine ornamental species aquaculture.

**Conclusions**. The role of public aquariums to streamline marine fish aquaculture research is a great resource that should continue to be utilized. Based on the results of this study, porkfish have potential for commercial aquaculture as they fit the mold of current larviculture techniques (the primary bottleneck to production); specifically the live feed organism used. Futhermore, the practice of harvesting porkfish eggs from a public aquarium, growing them in a commercial facility, and selling them back to that public aquarium (and the retail market) has been demonstrated. This can be a model for all species in the quest for sustainability within public aquariums.

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