

Rearing tank size effects feeding performance, growth, and survival of sergeant major, *Abudefduf saxatilis*, larvae

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Abstract. This study examined the interplay between food and environment during larval rearing of the sergeant major (*Abudefduf saxatilis*) and potential constraints to aquaculture production. To address this objective, larvae were reared in two different size rearing tanks (60 and 120 L) and fed a diet of rotifers and size-sorted copepod dominated zooplankton. Prey-capture success was significantly lower in 60 L rearing tanks and larvae consumed significantly lower numbers of prey when compared to larger rearing tanks. Growth rates were significantly higher in 120 L rearing tanks. No larvae survived beyond 10 DPH in 60 L rearing tanks. The pattern of mortality observed in the 60 L tanks was attributed to poor prey-capture performance generated by inappropriate rearing tank conditions.

Key Words: *Abudefduf saxatilis*, larval rearing, survival, growth, marine ornamental.

Introduction. Marine ornamental fish aquaculture has become a well established industry over the last several decades, however, the number of species that have been economically produced on commercial farms is limited to fewer than 80 of the more than 1,800 species of coral reef fishes traded globally (Rhyne et al 2012; Green 2003; Holt 2003; Arvedlund et al 2000). In the face of increasing anthropogenic stressors such as habitat destruction, increased coastal development (Hughes et al 2005), and the ambiguity of sustainable yields for coral reef species (Sadovy et al 2001) the need to develop reliable culture protocols for marine ornamental species has become increasingly important. Several groups of popular marine aquarium fishes have been targeted by scientific and commercial facilities although culture bottlenecks have limited their production in most cases.

Early larval stages of coral reef fishes have proven difficult to rear using traditional methods. Crucial bottlenecks limiting the expansion of the industry are typically attributed to the first feeding stage of larvae and rearing tank design (Holt 2003). The first feeding stage, when larvae switch from relying on endogenous yolk reserves to feeding exogenously, is an important critical period during early ontogeny (sensu Hjort 1914). Many factors are known to influence the feeding performance of marine fish larvae such as the development of the visual system and other sense organs (Job et al 1997), feeding mechanism (Wittenrich et al 2007; Turingan et al 2005) and search behavior (MacKenzie & Kiorboe 1995). The size (Krebs & Turingan 2003), color (Checkley 1982), density (Lasker 1975), and swimming behavior (Beck & Turingan 2007) of zooplankton prey organisms also influence feeding success and prey selectivity; however, to exploit these traits under captive culture conditions more diverse and nutritionally acceptable live prey organisms need to be obtained or cultured. Rotifers (*Brachionus* spp.) and *Artemia* spp. remain the most commonly used live feed organism in the culture of marine fishes; however, these prey organisms have proven inadequate for rearing many species of marine fishes, independent of enrichment (Wittenrich et al 2007; Holt 2003; Danilowicz & Brown 1992). The benefits of naupliar stages of copepods as a first

food for marine fish larvae have been realized for over a century (Støttrup & Norsker 1997) although their widespread use has not been realized due to difficulty in collecting or maintaining long term cultures (Holt 2003).

Rearing tank volume (Başaran et al 2004), shape (Büke et al 2005), and flow fields (Sakakura et al 2007) are known to influence the success rate of rearing marine fish species, with larger tanks generally accounting for more success. Although the effect of tank size in rearing marine food fish has been demonstrated (Duray et al 1997), the role that tank size may have on successful rearing of marine ornamental fish species is largely unknown. The ability of fish larvae to capture prey may be constrained by the physical design of the rearing environment. Tank design and size likely influence the circulation pattern within the tank, which in turn affect the distribution of prey and fish larvae. The interaction between tank design/size and prey, as well as fish distribution within the tank may influence the ability of fish larvae to select and capture prey. Prey selectivity has implications for the development of feeding protocols that guarantee feeding success in marine fish larvae.

Our goal in this study is to examine how the interplay between food and environment affects feeding performance and survivorship in marine fish larvae using the sergeant major damselfish, *Abudefduf saxatilis* (Linnaeus, 1758), as a model (Figure 1). Despite their nearly ubiquitous distribution in tropical marine environments and their high prevalence in the aquarium trade, the majority of pomacentrids, with the exception of the clownfishes (Amphiprionae), have proven to be difficult to rear through the early larval stages. A handful of reports describe rearing methods for damselfishes, though most attempts have yielded low survival (Murphy et al 2007; Olivotto et al 2003; Tanaka & Yamada 2001; Alshuth et al 1998; Danilowicz & Brown 1992). This study addresses the following questions: 1) Does rearing tank size affect survival and feeding performance of *A. saxatilis* larvae? and 2) Does growth rate vary between rearing tank sizes?



Figure 1. Juvenile *A. saxatilis* at 75 days post hatch, reared as part of this study.

Materials and Methods

Collection of embryos, incubation and hatching. Embryos of *A. saxatilis* were collected from established nests in shallow water (< 2m) along the North rock jetty at Sebastian Inlet, Sebastian, FL in May 2007 (Figure 2). To limit parental effect during the study 250–500 embryos were collected from six brooding males. Embryos were collected by scraping the base of the embryos with a plastic card in a forward motion at a roughly 45° angle to the smooth surface in which they were attached. Nests were selected based on the developmental stage of the embryos and only embryos just prior to eye pigmentation stage were selected (Figure 3). During this developmental stage embryos

are least prone to mortality during transport (Wittenrich, unpublished data). Embryos were mixed together and transported to the Fish Ecophysiology lab at Florida Institute of Technology within one hour of collection.

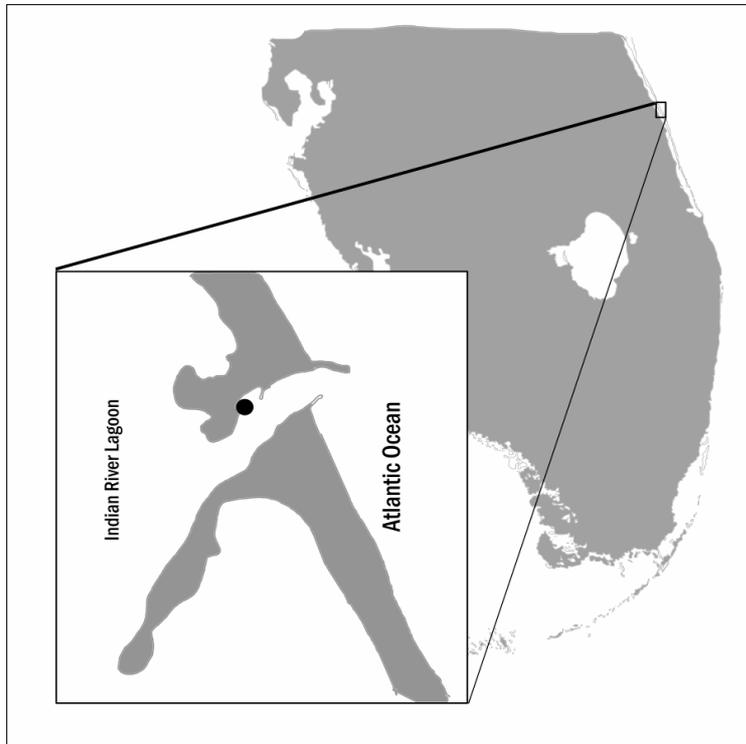


Figure 2. Location of embryo collection (●) in Sebastian Inlet, Florida.

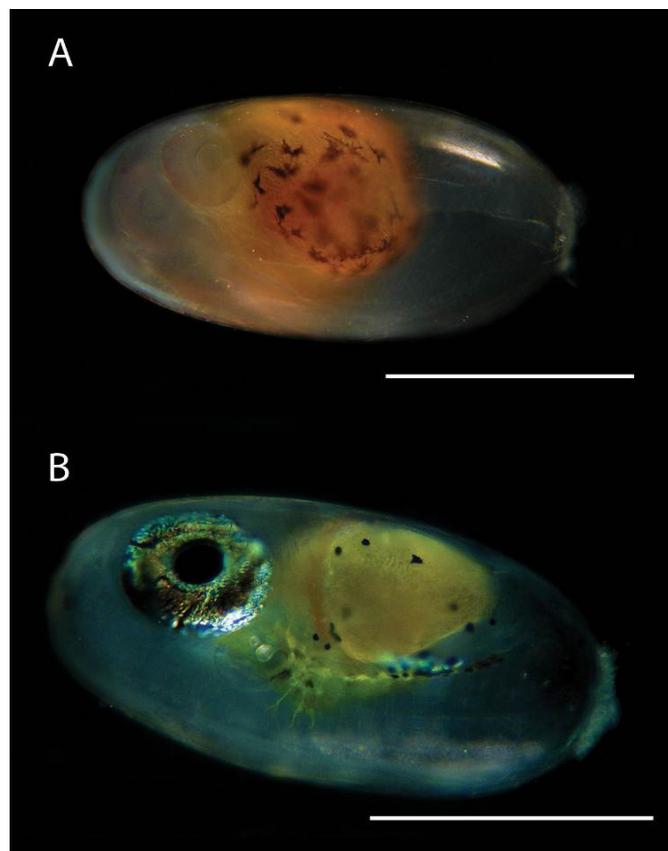


Figure 3. Embryo at collection showing the scraped adhesion point; estimated at 5 days post fertilization (A). Embryo prior to hatching 3 days post collection (B) (scale bar = 1 mm).

Upon arrival, embryos were divided into treatment tanks and placed in incubators within each tank. Incubators consisted of cut lengths of 10 cm ID (inside diameter) PVC pipe with 100 μm screen attached to the bottom. Incubators were suspended 1 cm below the surface and supplied with a steady supply of seawater (800-1000 mL/min) from recirculating systems through a 6mm ID flexible tube directed at a 45° angle to the wall. Incubators kept the embryos in constant motion until hatching. Hatched larvae swam from the top of the incubator into the rearing tank.

Effects of rearing tank size on larval survival. Two different size rearing tanks were used to determine larval survivorship (60 L and 120 L). Sixty liter black conical tanks measured 40 cm ID x 35 cm H (height) to the top of the cone. One hundred and twenty liter black conical tanks measured 80 cm ID x 50 cm H to the top of the cone. Water quality was maintained by a recirculation system connected to a biological filter tower, ultraviolet sterilizer, and protein skimmer with an exchange rate of 100 mL/min provided to the 60 L tanks and 200 mL/min provided to the 120 L tanks. Water temperature was maintained at 28°C, salinity 35 ppt, pH 8.2, NO_2 and $\text{NH}_3 < 0.02$ ppm, and $\text{NO}_3 < 10$ ppm. Photoperiod was maintained at 24L:0D during all trials with two 40W fluorescent bulbs (6500 K) mounted 10 cm above the tanks. Greenwater was maintained in the rearing tanks during all trials until 15 dph by adding 0.5 mL of diluted *Nannochloropsis oculata* algae paste (Reed Mariculture) to the 60 L tanks and 1 mL to the 120 L tanks each morning.

One-day old larvae were stocked at a density of 8 L⁻¹ in three replicate rearing tanks of each size. Larvae were fed *Brachionus plicatilis* at 2 mL⁻¹, 35-90 μm size-sorted zooplankton (2 mL⁻¹) and 90–250 μm size-sorted zooplankton (2 mL⁻¹). Mortality was quantified 2x daily (9:00 am and 3:00 pm) by turning off all air and water flow to the rearing tank and scanning the surface and floor of the rearing tank for dead larvae. A 3 mm ID rigid tube was used to siphon bottom debris each morning to search for dead larvae and aid in visual inspection of the bottom.

Effects of rearing tank size on feeding performance and growth. Three thousand embryos from 8 wild nests were divided into two 120 L and two 60 L rearing tanks and used to determine feeding performance and prey selectivity of larvae reared in each tank size. One-day old larvae were stocked at a density of 8 L⁻¹. Larvae were pulse fed each morning (9:00 am) with *B. plicatilis*, 35-90 μm size-sorted zooplankton, 90–250 μm size-sorted zooplankton, 12 hr *Artemia* sp., and 48 hr *Artemia* sp. each stocked to 2 mL⁻¹. This diet treatment was designed to examine the functional constraints of the larval feeding apparatus (Wittenrich 2007). Larvae were allowed to feed for 2 hours, after which 20 larvae were haphazardly sampled, anesthetized with MS-222 (Tricaine Methanesulfonate, Western Chemical) to prevent regurgitation of ingested prey and fixed in 10% formalin for 24 hours before being transferred to 70% ethanol for later analysis. To test the hypothesis that feeding performance varied among treatments the entire digestive tract of all larvae from the feeding trials were excised and examined.

Data analysis. To test the hypothesis that larval survival was affected by rearing tank size during subsequent days a one-way repeated measures ANOVA was conducted with tank size as the main effect, days as the repeated factor and survival as the dependant variable. To test if feeding performance, expressed as the mean number of prey consumed by larvae during the 2 hour feeding trial, was affected by rearing tank size a one-way repeated measures ANOVA was conducted with tank size as the main effect, days as the repeated factor and average number of prey consumed during feeding trials as the dependant variable. Prey-capture success, defined as the number of larvae that consumed at least one prey organism during the two hour feeding trial, was compared between rearing tank sizes using a two-way ANOVA with age and tank as factors and feeding performance as the response variable. To determine if growth rate was affected by rearing tank size a factorial ANOVA was performed using time as a covariate (Field 2005). Statistical analysis was performed using SPSS v. 16 with a probability value of 0.05.

Results

Effects of rearing tank size on larval survival. The highest larval survival rate (6.6%) was observed in the 120 L rearing tank. Larvae in the 60 L tanks exhibited complete mortality by 10 DPH. Initial mortality was similar in both treatments until 7 DPH when larvae in the 60 L tank exhibited significantly higher mortality ($F = 20.79$, $df = 1$, $p < 0.001$, Figure 4).

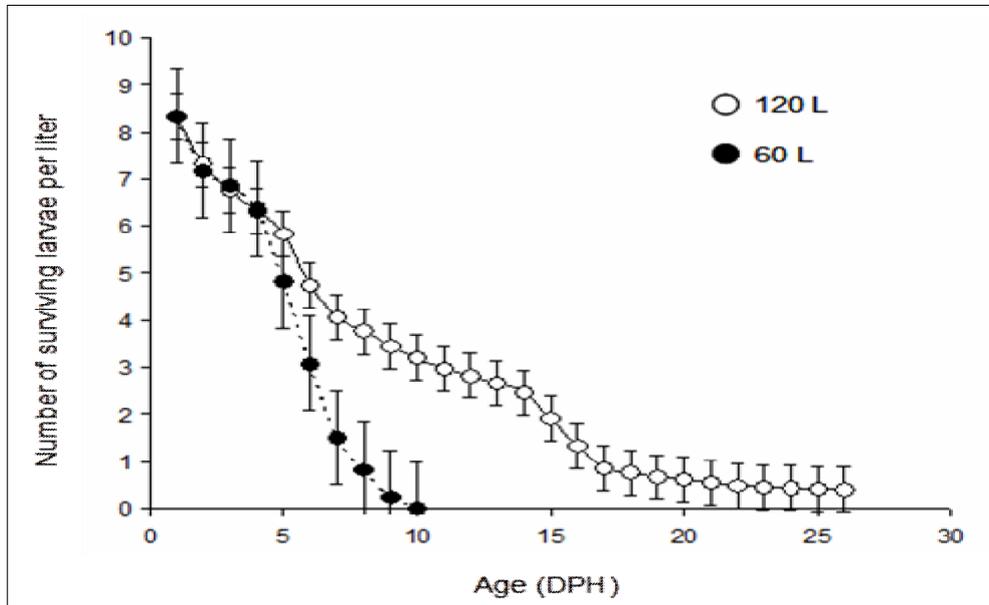


Figure 4. Effect of rearing tank and diet on daily rates of mortality through the larval cycle of *A. saxatilis*.

Effects of rearing tank size on feeding performance and growth. There was a significant difference in prey-capture success between tanks ($F = 231.47$, $df = 1$, $p < 0.001$) and age of larvae ($F = 135.58$, $df = 4$, $p < 0.001$). Only 24.67% of first feeding larvae in the 60 L tanks were successful in capturing at least one prey organism during the 2 hour feeding trial compared to 61.67% prey capture success of first feeding larvae in the 120 L tanks ($p < 0.05$). By 3 DPH larvae in the 120 L tanks exhibited a prey capture success rate of 74.33%, whereas larvae in the 60L tank performed significantly lower at 38% ($p < 0.05$). At 5 DPH 99.33% of larvae in the 120 L tanks exhibited successful prey-capture compared to 62.5% in 60 L tanks ($p < 0.05$). Prey capture success at 9 DPH was similar in both tanks ($p > 0.05$).

First feeding larvae in the 120 L rearing tanks consumed mainly small, non-elusive prey such as tintinnids, dinoflagellates, and copepod nauplii. The average number of 35–90 μm zooplankton ingested increased from 2–10 organisms in the first 10 days followed by an abrupt transition from small, non-elusive zooplankton (35–90 μm) to larger, more elusive zooplankton (90–250 μm) such as copepod adults and metanuplii around 12 DPH.

Larvae in the 60 L rearing tanks exhibited no clear pattern of prey selectivity and consumed significantly less prey during the 2 hour feeding trials than larvae in the 120 L tanks ($F = 14.01$, $df = 1$, $p = 0.020$, Figure 5).

Growth rates of larvae were significantly different between the 120 L and 60 L treatments with larvae in the 120 L treatment exhibiting a faster growth rate ($F = 4.9$, $df = 4$, $p = 0.002$, Figure 6).

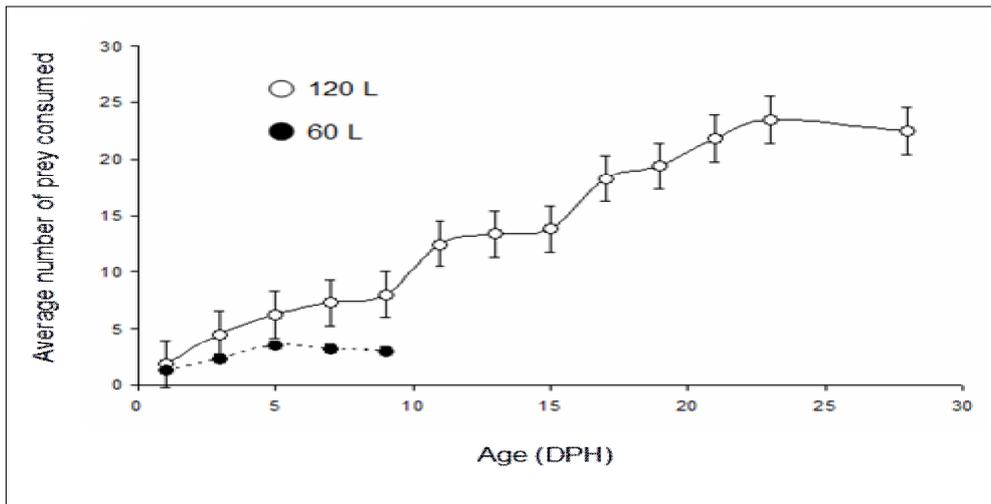


Figure 5. Feeding performance of *A. saxatilis* expressed as the average number of each of five prey types consumed through early ontogeny in 120 L tanks and 60 L tanks.

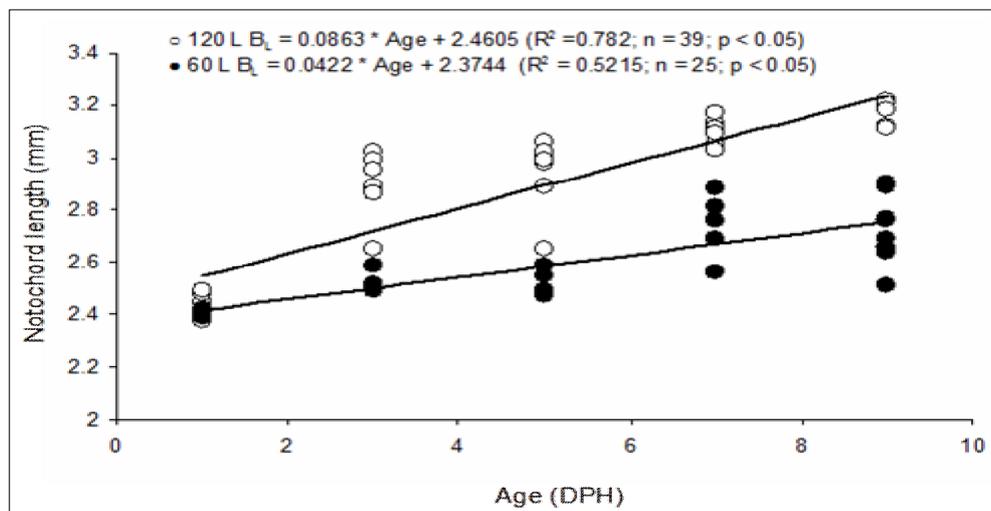


Figure 6. Notochord length at age of *A. saxatilis* larvae reared in: ○ 120 L tanks and ● 60 L tanks. Regression of length on age demonstrate that larvae in the 120 L tanks grow faster and attain significantly larger lengths by 3 dph.

Discussion. Here, we demonstrate that the rearing tank can have profound effects on larval survival through suppression of feeding performance. Larval development is illustrated in Figure 7. Though appropriate food organisms were present to promote growth and survival, as attested by the survival in the 120 L tanks, complete mortality occurred in the 60 L tanks. Finding suitable prey will only promote survival under ideal rearing conditions and it is probable that a multitude of factors will become important to identify conditions that optimize feeding performance.

Damselfish are among the most popular marine aquarium fishes and consequently those most heavily collected for the trade. Over 112 species of pomacentrids are collected worldwide accounting for 42% of all fishes traded internationally (Green 2003). Ironically, little progress has been made in developing culture technologies for this important group of reef fish. Danilowicz & Brown (1992) reported good success with two species of *Dascyllus* using a combination of large rearing tanks, size-sorted zooplankton, and flow through seawater systems; however, this remains the only successful report for the genus within the last 16 years. Members of the genus *Chrysiptera* are among the most popular members of the group (Green 2003) with reports of three species being successfully reared (Olivotto et al 2003; Tanaka & Yamada 2001). Several more species of pomacentrids have been reared in limited numbers for scientific research (Gardner 2008; Murphy et al 2007; Tanaka et al 2007; Job et al 1997; Moe 1992) though there seems to be no common methodology toward larval rearing or repeated successes. The

sergeant major (*A. saxatilis*) is a common species available throughout the aquarium trade with all specimens captured from the wild. Alshuth et al (1998) described the eggs and larval development of this species and contributed the first successful rearing report of the species.

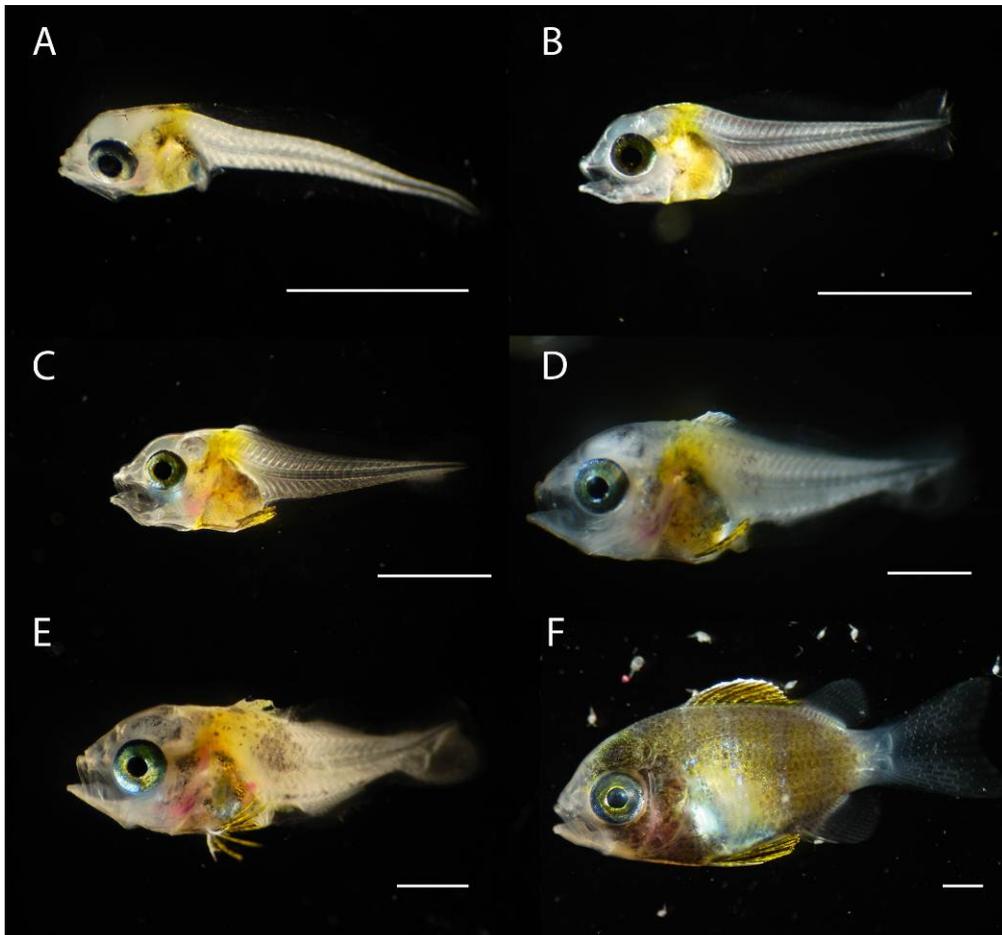


Figure 7. Larval development of laboratory-reared *A. saxatilis* at: A) 1 dph, B) 5 dph, C) 9 dph, D) 11 dph, E) 14 dph, and F) 22 dph (scale bar = 1 mm).

The future availability of many pomacentrids may depend on the development of successful rearing protocols. The reproductive biology of many common species is highly advantageous for their captive culture; being small, site-attached, and reliable and prolific demersal spawners (Thresher 1984). Commercial rearing bottlenecks revolving around rearing tank and feeding management strategies remain the major obstacles in the culture of marine fishes (Holt 2003). Many researchers have targeted larval critical periods from vastly different angles and it is now apparent that broodstock genetics (Green & McCormick 2005), nutrition (Ostrowsky & Laidley 2004), age (Berkeley et al 2004), and environmental induced stress (Schreck et al 2001) can effect larval quality before captive spawning ever takes place. Photoperiod (Olivotto et al 2003; Arvedlund et al 2000), prey type (Olivotto et al 2005; Gardner 2003), temperature and salinity (Hart et al 1996), development of appropriate digestive enzymes (Kolkovski et al 1993), live feed enrichment (Olivotto et al 2005), development of the feeding mechanism (Wittenrich et al 2007), rearing tank design (Büke et al 2005), and flow field (Sakakura et al 2007) have all been shown to effect larval survival in marine fishes. In developing rearing protocols it is important to consider the combined effects and interplay between intrinsic and extrinsic factors known to effect survival.

Conclusions. In developing rearing protocols it is important to consider the combined effects and interplay between intrinsic and extrinsic factors known to affect larval feeding

performance and survival. Larvae of *A. saxatilis* exhibit a high degree of prey selectivity and seem to have a narrow window of environmental conditions that elicit a feeding response. Based on the results of this study, rearing tanks should be designed to maximize prey encounter rates through optimization of horizontal flow fields. Though we have shown that survival of *A. saxatilis* larvae was only attained through utilization of a larger rearing tank, we can only speculate why. The environmental conditions and prey base that elicit a feeding response in larvae changes dramatically with ontogeny. As marine ornamental aquaculture continues to expand, employing rearing tanks that provide environmental gradients of light and current will likely aid in advancing the field.

Acknowledgements. Special thanks to A. Didoha, JR. Kerfoot and Z. Jud for assistance with this project. Funding was supplied by a Florida Sea Grant to RGT.

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Received: 10 August 2012. Accepted: 12 August 2012. Published online: 06 November 2012.

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How to cite this article:

Wittenrich M. L., Turingan R. G., Cassiano E. J., 2012 Rearing tank size effects feeding performance, growth, and survival of sergeant major, *Abudefduf saxatilis*, larvae. *AAFL Bioflux* 5(5): 393-402.