Development of aquaculture technology for the flame angelfish, *Centropyge loriculus*

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Summary

The Marine Ornamental Aquaculture Project has allowed researchers at the Oceanic Institute to resolve a number of key challenges in developing captive production technology for the flame angelfish (*Centropyge loriculus*). Accomplishments of this project include daily year-round spawning, identification and culture of *Parvocalanus* copepod nauplii as first-feed for the small pelagic larvae, and early development of copepod-based hatchery technology leading to the first-ever rearing of flame angelfish. Current research is examining methods to increase survival through late hatchery and early juvenile stages, but overall, good progress has been made toward the development of captive production technologies as an alternative to wild-collection of the flame angelfish with potential application to a number of other high-value coral reef species.

Introduction

The marine aquarium trade exposes hundreds of millions of people around the world to the wonders of the undersea world, bringing a new appreciation of the beauty and complexity of the fragile coral reef ecosystem. However, the current reliance on wild-collection of specimens has created concern for long-term industry sustainability, leading many to advocate for the development of captive production technologies as a more sustainable approach to meeting industry needs while protecting our coral reef ecosystems. The development of aquaculture technology for coral reef species could also create new economic opportunities for many struggling island economies. Unfortunately, captive production of most of the coral reef species has proven challenging, mainly due to the small size of newly hatched larvae and lack of success in adapting available rotifer and *Artemia*-based hatchery technologies for rearing of these larvae (Ostrowski and Laidley, 2001). Therefore this project was undertaken to develop suitable broodstock, live feeds and larval rearing technologies for the flame angelfish as a model species for ornamental reef fish captive rearing. This manuscript reviews core technologies developed during the project and steps taken to successfully culture the flame angelfish for the first time in captivity.

***Acquisition and quarantine of broodstock*.** Flame angelfish broodstock were relatively easy to obtain through commercial trans-shippers. However, the transfer of fish through common holding systems exposes animals to a wide range of pathogens, including *Cryptocaryon irritans* and other ectoparasites. Therefore, fish were quarantined prior to stocking in broodstock holding systems. The quarantine process begins with a prophylactic freshwater dip (3-5 min.) prior to stocking in quarantine tanks and is followed by a rigorous protocol of hydrogen peroxide treatments (20min at 100ppm) three times/week for four weeks, with fish transfered to new quarantine tanks after each treatment. An alternative protocol that is also showing success includes a four week hyposalinity treatment (~12ppt), which is more effective in eliminating *Cryptocaryon*.

***Broodstock husbandry and holding system*.** After completing quarantine, flame angelfish stocks were paired, male and female, and stocked in relatively large (1,000L) broodstock holding tanks. Stocking in harems with multiple females provided little benefit. Tank size was shown to have significant effects on reproductive performance, with greater egg output with increasing tank size leading us to adopt relatively large tanks for such a small fish. Despite impressive egg production (>2,000 eggs/female/day) from such a small fish (Laidley et al. 2004), it was necessary to significantly scale-up broodstock holding capacity in order to generate sufficient numbers of eggs for marine ornamental hatchery operations. For example, it requires ~20 broodstock pairs to supply sufficient numbers of eggs to stock just one 1,000L larval rearing tank at typical stocking densities of 40 eggs/L. Therefore we designed and built a replicated broodstock holding system outfitted with individual surface egg collection systems (Fig. 1). The system includes both flow-through and recirculating water sources to maintain high-quality water sustaining long-term broodstock health and egg production.

Figure 1. Replicated (19 tank) marine ornamental broodstock holding system with surface egg collector system for gentle collection of small floating eggs from pelagic spawning reef species such as the flame angelfish.

After broodstock expansion we began experiencing egg quality issues which, after substantial trial-and-error effort, led us to identify water quality/source as a major factor affecting stock health, reproductive performance and egg quality (Callan and Laidley, in prep.). The ornamental reef species appear to loose condition when maintained on well water, with much improved egg output and improved egg quality when maintained on sterilized ocean water. The adoption of recirculation aquaculture system technologies not only allowed for greater control over water quality, but also provided for improved system biosecurity (less new water/less discharge) and tighter control environmental parameters including temperature and salinity.

Efforts were also focused on developing biosecure broodstock diets for maintaining broodstock health and viable egg supplies. Although work remains to fully identify the dietary requirements for the species; long-term stock health, reproduction, and egg quality were all shown to be impacted by broodstock diet. An initial “mixed diet” composed of high-quality *Spirulina*-based flake food combined with frozen ground shrimp, krill, fish eggs, *Artemia*, spinach, peas, broccoli and dried seaweed proved effective in supporting broodstock, while tested commercial diets proved inferior. Biochemical analysis of these diets and subsequent diet trials demonstrated a critical role for dietary lipid levels (Bou Mira, 2008.) and in particular the n-3 highly unsaturated fatty acids (Callan, 2007; Callan and Laidley, in prep.) on reproductive output and egg quality (Fig. 2).

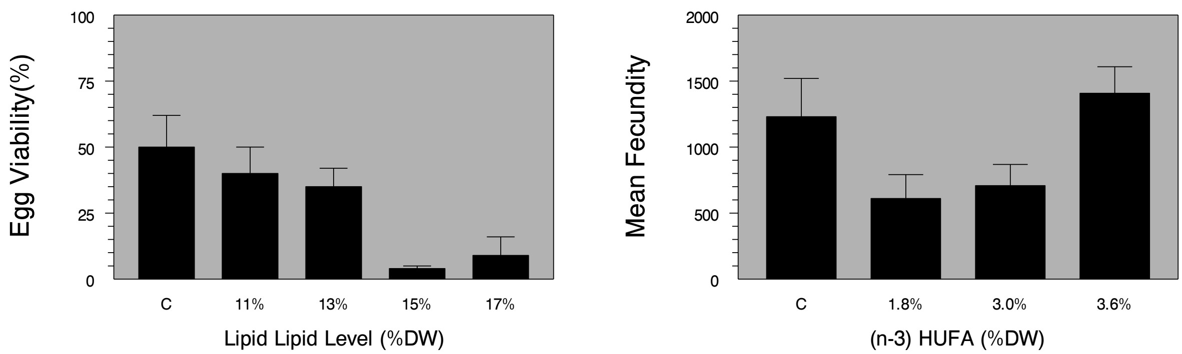
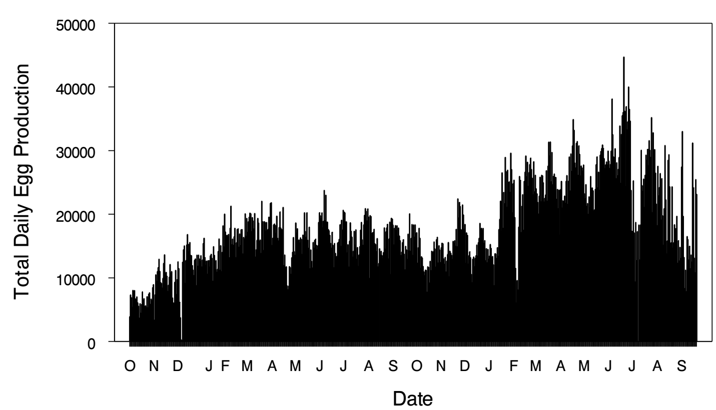


Figure 2. Effects of broodstock diet on reproductive performance and egg quality of flame angelfish broodstock. The left graph demonstrates negative effects of high lipid levels on egg viability while the right graph demonstrates improvements in fecundity with higher dietary n-3 HUFA levels.

The expansion of broodstock population to 18 mated flame angelfish pairs allowed us to generate baseline data for expected reproductive performance throughout the year for several years of operation. Based on this data, the expected egg output for this species is in the range of 1,000 to 2,000 eggs/day for a period of several years. Broodstock showed continued increases in egg output over time with egg production peaking at over 45,000 eggs/day (Fig. 3). Toward the end of the project we began seeing periodicity in egg production, with peak egg output observed during the dark phase of the lunar cycle.

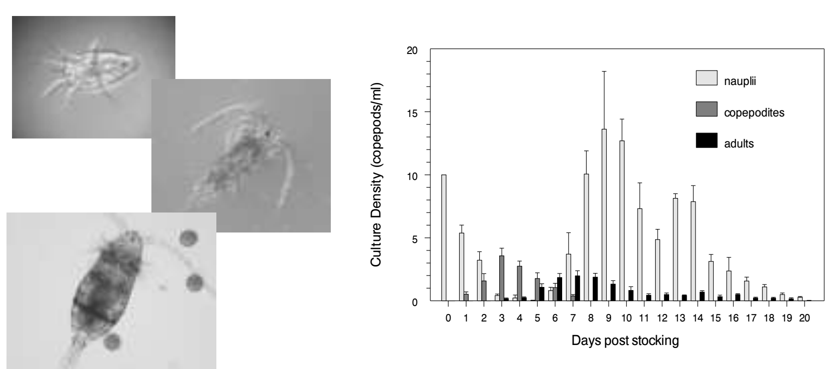
Figure 3. Daily total egg output from 18 pairs of flame angelfish broodstock over a period from October 2005 to September 2007.



Identification of suitable first-feed. Initial trials examining conventional rotifer- and *Artemia*-based feeding protocols confirmed previously reported (Holt and Riley, 2000) challenges in rearing the smaller ornamental reef species. Over the early stages of the project, we explored a range of potential diet sources for first-feeding larvae, including the use of pond water, sea urchin eggs, ciliates (*Euplotes*), oyster trochophores, and harpacticoid nauplii with little success. The larvae did consume oyster trochophores and harpacticoid nauplii but failed to gain condition or survive past yolk depletion. Limited success with pond water indicated that locally available marine plankton offered prospects, leading to isolation of a range of calanoid copepods from Kaneohe Bay waters. Using isolated copepod cultures, we quickly focused efforts on a single calanoid species identified as *Parvocalanus* sp. This species was shown to produce relatively small eggs (~62 µm) and early nauplii (~69µm), which were easily consumed by flame angelfish larvae, resulting in the first-ever rearing of flame angelfish in captivity (Shields and Laidley, 2003; Shields et al., 2005), which we presented at the November 2001 Marine Ornamentals meeting in Florida (Laidley et al., 2001). It should also be noted that at the same time a second Hawaii researcher successfully reared both Fisher’s and lemonpeel angelfish using wild zooplankton from Kaneohe Bay (Baensch, 2002).

Copepod culture. After identifying *Parvocalanus* as suitable feed item for rearing difficult-to-rear species such as flame angelfish, snapper and omilu, we worked to optimize culture conditions and develop a pilot-scale copepod production system to meet larval feed demands. Although copepods provide an excellent nutrient source for the early developing larvae, they are much more difficult to culture than rotifers. Rotifers can be maintained on paste and other commercially available feeds, have rapid life cycles, and are easy to culture at high densities (typically around 1,000/ml in batch systems, with densities greater than 20,000/ml achieved in high-density systems). Despite being more difficult to culture, *Parvocalanus* cultures were maintained relatively easily for periods of years on mixed diets of live *Isochrysis* and *Chaetoceros* algae. We were not successful in utilizing commercially available algae pastes for rearing copepods. Females mature and initiate egg production over a period of about one week and generate eggs for another two weeks. Although adults may be matured at high density (>100/ml), reproductive activity is inherently density-limited requiring the lowering of adult density at to ~1 adult/ml. Cultures usually peak at densities of 10-20 nauplii/ml (Fig. 4), although we have achieved densities greater than 70 nauplii/ml. It is important to note that actual daily nauplii output from continuously running production units currently range from 5-10 nauplii/ml/day. We are continuing to refine culture conditions having optimized feed density (~300,000 cells/ml), temperature (~25ºC), photoperiod (little effect), salinity (22ppt) and culture density (~1/ml)(Laidley and Bradley, in prep.).

Figure 4. Photomicrographs of *Parvocalanus* nauplius, copepodite and adult life stages (photos left), and typical copepod production cycle (right) with nauplii stocked at 10/ml on day 0 followed by maturation to the adult phase and subsequent nauplii production until culture senescence.



Rearing flame angelfish larvae. With the establishment of a reliable egg supply and a source of copepod nauplii, we began developing hatchery methods for rearing flame angelfish larvae. Early larval rearing attempts faced extremely low survival rates (<5%) during the pre-feeding period, explained by the very delicate nature of the newly hatchery larvae. These newly hatched larvae lack developed eyes and functional mouths and are significantly smaller (1.2mm) than any other species being reared today. Larval development through the first three days is extremely rapid, as the larvae exhaust yolk supplies and switch to exogenous feeding (Fig 5). Through a combination of improved water quality and reduced turbulence in the larval rearing system, we are now able to achieve day 3 survival nearing 70%. Starting on day 2 we maintain *Parvocalanus* copepod nauplii at densities of 5 to 10 nauplii/ml. By 5-7 days post-hatch, larvae begin to assume red coloration and inflate their swim-bladders.

Figure 5. Early development of flame angelfish larvae. Larvae hatch lacking mouth or functional eyes (left photo) and survive on yolk supplies until the initiation of exogenous feeding on day 3 (center photo). Successfully feeding larvae (right photo) assume red coloration with inflation of swim bladders by the end of the first week.

From day 7 onward, flame angelfish aggressively consume copepod nauplii resulting in rapid growth and major body transformations as larvae transition to a more laterally compressed body shape (Fig. 6). Unfortunately, around day 14 we begin seeing increasing mortalities, lasting until day 20. In part, we suspect that copepod nauplii may not be meeting larval nutritional requirements through the later larval period, as mortality rates remain high until the larvae are weaned onto newly hatched *Artemia* (day 18-20). Efforts to identify a transitional live prey remain, since we were not able to transition flame angelfish larvae onto rotifers, and it is challenging to maintain sufficient numbers of larger copepodites. During this phase, metamorphosing late stage larvae appear to have swim bladder hyperinflation problems (in our facilities) resulting in a loss buoyancy control, leading to erratic swimming behavior and eventual settlement on the surface, shortly before dying. On-going efforts are focused on upgrading both flow-through and recirculating water supplies with degassing systems to lower total gas pressure and reduce this source of mortality. Despite such challenges, flame angelfish larval rearing protocols have now been scaled to 1,000L larval rearing tank systems leading to the successful generation of large numbers (1,000's) of larvae through the early larval period out to day 14 (Fig. 6).

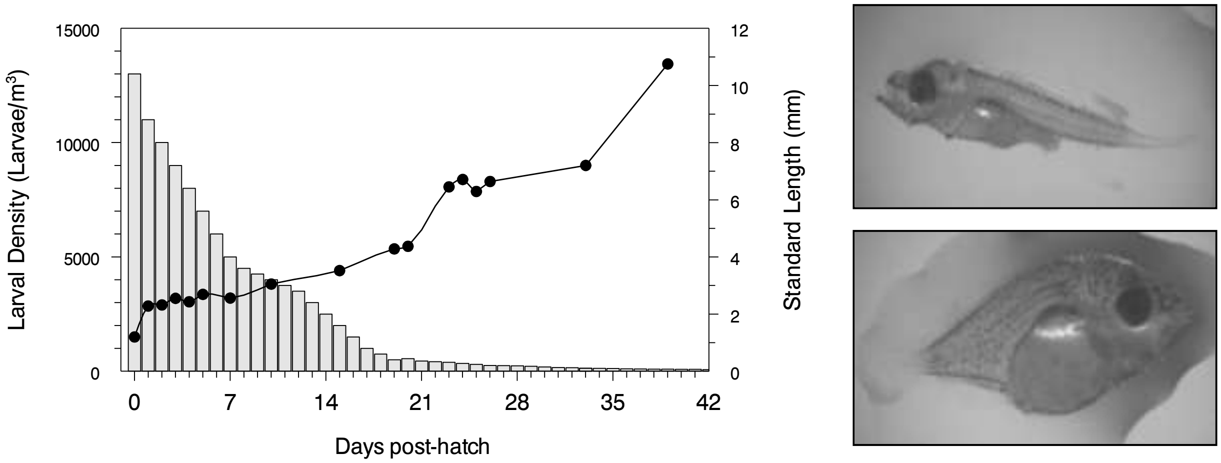
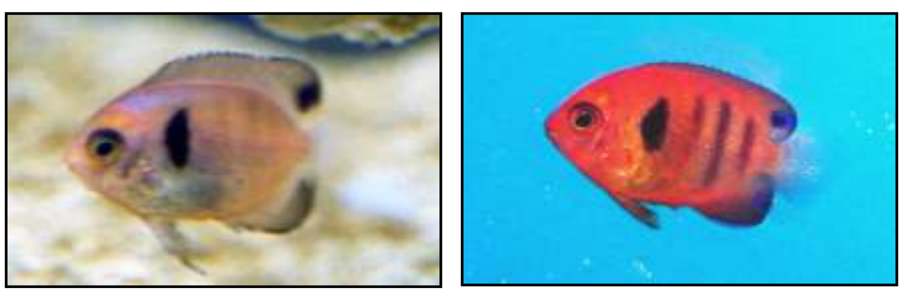


Figure 6. Flame angelfish larval survival and growth in 1,000L larval rearing system (graph on left). Larvae are fed *Parvocalanus* copepod nauplii at a density of 5 to 10 nauplii/ml from day 2 onward. Note the appearance of hyper-inflated swimbladders in day 15 and 23 larvae.

After weaning onto *Artemia*, mortality rates declined substantially as post-larvae transition through a rather prolonged terminal hatchery phase (~day 50) when juveniles can be transferred to nursery tanks. The relatively small numbers (dozens) of surviving fish appeared well adapted to handling and were integrated into a number of mixed ornamental tank systems with good results (Fig. 7). The captive reared flame angelfish appear physically normal, although their overall coloration is not as intense as wild-collected specimens. Recent trials are showing that the pale coloration and common occurrence of head-and-lateral line erosion disease in captive specimens may be caused by low light levels in research holding systems and thus relatively easy to address.

Figure 7. Pictures of day 90 (left) and day 145 (right) captive-reared flame angelfish juveniles reared at the Oceanic Institute.



Conclusions. This CTSA-funded Marine Ornamentals Project has successfully resolved a number of key challenges in the development of captive rearing technology for this never-before-reared group of high-value coral reef fishes. Our work with the flame angelfish led to the development of broodstock methods for reliably generating large numbers of high quality eggs on a daily-basis year-round, identified and developed culture technology for *Parvocalanus* copepod nauplii as the key first-feed item for small mouthed coral reef larvae, and developed rearing methods for getting large numbers of the fragile larvae through the critical early stages of larval development for the first-ever rearing of this key marine ornamental species. Further work (in progress) is needed to address challenges encountered late in larval development to generate commercial scale numbers of this species to market size. The captive generated fish appear relatively hardy and by 90 days can be easily handled and integrated into communal tanks system for hobbyists to enjoy.

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