Aquaculture of Marine Ornamental Species, Year 3

General Information

Reporting Period June 1, 2002–September 30, 2004

Funding Level

Year	Amount
1	$128,735
2	$104,135
3	**$102,325**
TOTAL	$335,195

Participants

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Objectives

Oceanic Institute (OI)

1. Maintain and expand centralized broodstock populations of yellow tang (*Zebrasoma flavescens*) and flame angelfish (*Centropyge loriculus*).
2. Evaluate the effects of harem size on reproductive activity and spawning performance of angelfish broodstock.
3. Determine appropriate stocking densities and sex ratio for yellow tang broodstock.
4. Test pilot-scale rearing system for mass production of angelfish fry.

Waikiki Aquarium (WAQ)

5. Determine uptake rate, survival, and growth of angelfish and yellow tang larvae, and/or other available species fed wild zooplankton collected from inshore waters in South Oahu.

Anticipated Benefits

Successful completion of this project will assist the aquarium industry by providing hatchery techniques to culture several species of marine ornamentals, thereby offering a more environmentally sustainable alternative to wild collection practices. Consistency in production would ensure a solid base for development of an industry and transfer of reliable technologies. Techniques to mature and spawn the species chosen could be transferred to other highly desired ornamental fish, allowing for the rapid development of new aquacultured species. As expressed in the June 1997 newsletter of the American Marine Life Dealers Association, several benefits, apart from cost savings, will accrue to the industry from the financial investment in research and development of captive propagation, including new economic development, job creation, and an increased emphasis on the importance of maintaining coastal resources. Additional economic benefits will flow throughout the industry, strengthening aquarium and pet retail stores and benefiting consumers with healthier fish.

Work Progress and Principal Accomplishments

Years 1 and 2

During Years 1 and 2 of this project, broodstock populations of two highly popular marine ornamental species, flame angelfish and yellow tang, were established at OI for seedstock production. Experiments were carried out to determine optimal tank configuration for adult flame angelfish, in terms of system volume and to induce spawning behavior. Systems and husbandry protocols were developed based on 750-L holding tanks, mimicking essential parameters in the fishes' natural reef environment, in combination with a natural photoperiod and temperature regime and varied broodstock diet. This approach enabled the rapid onset of reproductive activity, continuous daily spawning, and record levels of egg production from captive flame angelfish stocks. Installation of multiple broodstock holding units based on this design generated sufficient flame angelfish larvae to begin small-scale hatchery rearing trials.

Several colonies of captive yellow tang were established during project Year 1. These stocks began releasing unfertilized eggs that year and subsequently proceeded to produce fertile spawns, enabling the first description of yellow tang yolk sac larvae. Examination of spawning rhythmicity indicated a lunar spawning cycle for this species. Yellow tang stocks were expanded to eight tanks during Year 2, however, low egg fertilization rates were problematic, and larvae supply was sufficient to carry out only preliminary rearing trials.

A breakthrough occurred in Year 1 when a feeding strategy was identified that kept flame angelfish larvae alive for 19 days post-hatch.

In an effort to identify suitable diets for small marine ornamental fish larvae, a series of replicated experiments was carried out at OI during project Year 1, comparing the survival and growth rates and feed uptake of flame angelfish larvae offered different zooplanktonic prey types. None of the tested diets (ss-type rotifers, oyster trochophores, and dinoflagellate/protozoa combinations) enabled survival beyond yolk exhaustion. Indeed, the angelfish larvae appeared to be starving despite apparent ingestion of experimental diets. Better progress was made by applying a semi-intensive feeding strategy to rear flame angelfish larvae in 1,500-L tanks. Using a combined diet of dinoflagellates, ciliated protozoa, and rotifers, angelfish larvae were reared to 19 days post-hatch, which represented a breakthrough at the time.

During Year 1, collaborators from the Guam Aquaculture Development and Training Center (GADTC) collected adults of several ornamental fish species, including clown coris, but were unable to obtain spawns. Due to changes in personnel at GADTC, this collaboration was dropped in subsequent project years.

Planned experiments to compare intensive versus semi-intensive "green water" rearing methods for a variety of fish larvae were not accomplished during project Year 1 (University of Hawaii and UH Sea Grant Extension Service). Owing to the departure of the relevant investigator, Year 1 research to develop culture methods for feather duster worms (University of Hawaii and UH Sea Grant Extension Service) was transferred out of the CTSA Marine Ornamentals project.

During project Year 2, larvae research at OI focused on examining the effects of different microbial conditions on flame angelfish rearing performance. A small-scale rearing system was established, allowing rigorous testing of multiple experimental treatments. This system was used to develop a disinfection technique that reduced surface bacterial loading of angelfish embryos by more than 99%, involving immersion in a 3% solution of hydrogen peroxide for five minutes.

Studies were also carried out at OI during Year 2 to examine the effects of water source and microalgae type on the survival rate of flame angelfish yolk sac larvae. Larvae were reared in "mature," UV-sterilized water, or in water that had only received mechanical filtration, with or without addition of microalgae. Highest mean survival rate (70% to day-4 post-hatch) was obtained in groups of larvae receiving matured water plus T. *Isochrysis* sp. microalgae. *Nannochloropsis* sp. algae was not beneficial to early larval survival, while larvae reared in "clear," mechanically filtered water exhibited much lower mean survival (23%) than all other groups. Based on these findings, the use of matured water plus T. *Isochrysis* sp. was adopted as standard for first-feeding flame angelfish larvae.

In Year 2, progress from CTSA- and NOAA-funded projects led to the production of the world's first captive-reared juvenile flame angelfish.

In parallel to these Year 2 CTSA studies, a breakthrough was made within a NOAA-funded project at OI in culturing a suitable zooplanktonic diet (calanoid copepod) for flame angelfish and other tropical marine fish species that produce very small larvae. The world's first captive-reared juvenile flame angelfish were produced during 2001 using this diet. Due to the combined progress in flame angelfish larviculture within the CTSA- and NOAA-funded projects during 2001–2002, it has been possible to direct Year 3 CTSA funding towards scale-up of egg production and the development of pilot-scale rearing methods for this desirable ornamental fish species.

Progress in rearing new marine ornamental fish species was also made by WAQ researchers during 2001, with the first ever production of juvenile masked angelfish, *Genicanthus personatus*, using cultured prey. WAQ researchers also provided a zooplankton identification service for the GADTC during project Year 2, of samples collected in Guam during Year 1.

Year 3

Objective 1: Maintain and expand centralized broodstock populations of flame angelfish (Centropyge loriculus) and yellow tang (Zebrasoma flavescens).

Extensive system commissioning was undertaken to allow for expansion of both flame angelfish and yellow tang broodstock populations.

Flame angelfish broodstock populations were expanded, which proved to be a challenging endeavor, and the broodstock system was converted to water reuse to mitigate the problems.

The flame angelfish broodstock population was expanded from four tanks to 19 tanks, 13 of which were used for CTSA-funded research. With expansion of flame angelfish broodstock populations came a series of unexpected challenges that severely limited what was envisioned to be a relatively straightforward objective. The first challenge was the poor survival and rapid deterioration of newly stocked broodstock in OI saltwater. After considerable efforts to troubleshoot the problem, we determined that the low pH (approximately 7.6) of water from the OI saltwater well was likely detrimental to the fish, resulting in rapid loss of coloration and high incidence of mortality. This was a surprise finding in that many marine aquaculture systems for foodfish production operate at system pHs in the mid to low 7 range without apparent detrimental effects on the animals. However, these coral reef species appear much more sensitive to changes in water chemistry than previously cultured foodfish species. Attempts to degas CO_2 from the system were unsuccessful due to the higher flow created by expansion in the number of tanks. Commercial degassing columns were only partially successful in removing CO_2, increasing pH by less than 0.1 pH unit. Therefore, we decided to convert the replicated flame angelfish broodstock system to water reuse, which would provide greater opportunity to remove ambient CO_2 through degassing and restore water pH to more normal levels for coral reef species. The system installation included a degassing column on incoming water, a fluidized sand-bed biofilter, large (oversized) protein skimmer, and UV sterilizer (Figure 1).

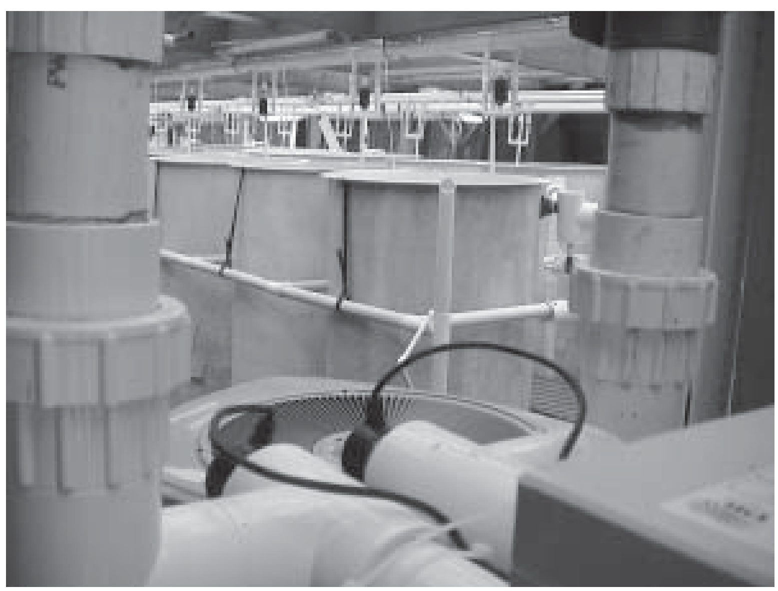

FIGURE 1. *Photograph of new replicated flame angelfish broodstock system showing several of the broodstock tanks in the background and heat pump and UV sterilizer in the foreground.*

This new system appears to have prevented the pH-induced changes in broodstock coloration when operated at very high water reuse rates (99% or higher), but the newly stocked fish did not initiate spawning activity as expected and continued to demonstrate unusually high mortality rates due to *Amyloodinium* infestation. It is unclear whether the high incidence of *Amyloodinium* is related to higher water temperatures (>29°C) experienced in the water reuse system, or represents contamination of stocks obtained from the local wholesaler. Therefore, we have made adjustments to both, including the addition of a 1.5 hp heat pump to tightly regulate water temperatures in the broodstock system and sourcing broodstock from a new transshipper. The new broodstock system appears to be performing well, and a new stock of flame angelfish broodstock from a new supplier should arrive in the next few weeks.

Yellow tang broodstock populations were also expanded and maintained.

The yellow tang populations were also expanded under the CTSA project to eight broodstock tanks to increase potential egg production and to examine the effect of broodstock population size (see Objective 2).

Established populations of yellow tang and flame angelfish continue to be maintained for seed production using current best husbandry practices. Daily measurements of fecundity and egg fertilization rates are made for each spawning stock at OI, and developing embryos are supplied for hatchery rearing trials as required.

FIGURE 2. *Photograph of a "normal" flame angelfish (left panel) and flame angelfish exhibiting severe coloration changes on the head (right panel). The "disease" has been attributed to the high* CO_2 *concentrations in water sourced from saltwater wells.*

Objective 2: Evaluate the effects of harem size on reproductive activity and spawning performance of angelfish broodstock.

The flame angelfish harem size experiment was stocked twice, with each experiment running for six months prior to termination. In each case, the slow onset of spawning activity, poor egg fertility rates, and escalating rate of mortalities and *Amyloodinium* infestation compromised the experiment. As fully discussed under Objective 1, we have made substantial changes to the broodstock holding system in response to encountered problems and hope to complete this trial under the Phase II, Year 1 project.

Objective 3: Determine appropriate stocking densities and sex ratio for yellow tang broodstock.

The new yellow tang broodstock tanks were successfully commissioned allowing stocking of the stocking density trial early in 2004. However, extensive wind and storm damage to the Doherty hatchery and broodstock facilities forced termination of the trials to allow for roof repairs over experimental tanks. The repairs have just been completed, and the tank systems will immediately be restocked with yellow tangs as described under the experimental work plan to complete the proposed studies.

Objective 4: Test pilot-scale rearing system for mass production of angelfish fry.

During Year 3, trials were carried out to compare semi-intensive versus intensive rearing of flame angelfish larvae. Larvae were reared either semi-intensively in 4,000-L tanks, or intensively in 200-L tanks (two tanks per method). For the semi-intensive approach, the rearing tanks were inoculated with T. *Isochrysis* sp. microalgae and cultured calanoid copepods (all developmental stages), before stocking with two-day old flame angelfish larvae at a density of 0.7 to 0.9/L. Larvae grazed on the tanks' endogenous copepod populations, and rotifers were introduced only when copepod supplies were becoming exhausted. In contrast, the intensive 200-L tanks were stocked at a higher density (~10 larvae/L), and the angelfish larvae were fed a defined copepod ration each day. Rotifers were introduced to the intensive rearing tanks from day-10 post-hatch. Survival rate to 3–4 weeks post-hatch was higher (2.1%, 6%) in the intensive rearing tanks than in the semi-intensive rearing tanks (0%, 1.4%). Fish from both groups were reared to metamorphosis, although chronic mortality during the late postlarval phase was problematic.

Subsequent larval rearing experiments were delayed while the new broodstock populations were established and conditioned to generate sufficient numbers of eggs to meet experimental requirements and while the Doherty hatchery facility was repaired from storm damage in December 2003 and January 2004. Mass production systems will continue to be developed and evaluated under Phase II studies once broodstock egg production meets the necessary output for mass production studies.

Objective 5: Determine uptake rate, survival and growth of angelfish and yellow tang larvae, and/or other available species fed wild zooplankton collected from in-shore waters in South Oahu.

At WAQ, *Genicanthus personatus* stocks produced only very small batches of eggs, preventing rearing trials from being carried out. Small spawns of *C. fisheri* and *C. multicolor* were also obtained. Trials were conducted with *C. fisheri* larvae supplied by Frank Baench, using rearing methods developed in 2001. None of these larvae survived for more than 10 days. Wild plankton was collected in offshore tows on three occasions and by hand from near-shore reef areas on three other occasions. These samples were screened, sorted, and identified, and cultures were started with several of the smaller copepod species. Cultures were maintained for up to two weeks. A starter culture of *Oithona* sp. was also obtained from the Hawaii Institute of Marine Biology, and culture trials are ongoing.

Work Planned

The following research work is planned under Phase II of the project:

- Continue husbandry of adult flame angelfish for seedstock production.
- Continue harem size experiment.
- Continue flame angelfish pilot-scale rearing trials, with emphasis on improving postlarval survival rates.
- Scale-up of larval rearing production methods.
- Technology transfer through CTSA-sponsored workshops and publications.

Impacts

The ultimate goal of this project is to assist in the development of a marine ornamental aquaculture industry in Hawaii and the Pacific. This represents a key economic opportunity for farmers in Hawaii and Pacific Island affiliates, such as Guam, for several reasons. Firstly, there is a worldwide void in aquaculture production of marine ornamental species. It is estimated that less than 5% of all marine ornamental species traded on the open market are aquaculture-raised, and that the actual numbers of cultured fish traded is miniscule compared to those traded by collectors. This is unlike the situation currently faced by freshwater ornamental farmers in Hawaii, who compete in markets with well-established foreign and other domestic producers. Secondly, it is well known that the health of coral reef ecosystems around the world is being severely degraded and that wild collection practices are likely unsustainable unless alternatives are sought. Moreover, the Hawaiian Islands are home to over 85% of the coral reefs in the United States, positioning the region well for development of an aquaculture-based industry. Success of this project will not only provide new economic opportunity to farmers, but will also help ensure the long-term sustainability of the marine ornamental trade by providing alternatives to wild collection practices and a means to practice resource conservation.

Publications in Print, Manuscripts, and Papers Presented

Invited presentation at Marine Aquarium Council of North America XV on "Research development on yellow tang and pygmy angelfishes at the Oceanic Institute in Hawaii." September 5–7, 2003, Louisville, Kentucky.

Laidley, C.W., A.F. Burnell, R.J. Shields, A. Molnar, and T. Kotani. 2004. Marine ornamentals: captive culture progress at the Oceanic Institute. Global Aquaculture Advocate 7(2):53–54.

Laidley, C.W., A.F. Burnell, R.J. Shields, A. Molnar, and T. Kotani. 2004. Research and development of marine ornamentals at the Oceanic Institute in Hawaii. Marine Ornamentals '04. March 1–4, 2004, Honolulu, Hawaii.