Transitioning Hawaii’s Freshwater Ornamental Industry, Years 1–3

General Information

Reporting Period
January 1, 2000–December 30, 2001 (Year 1)
October 1, 2001–March 31, 2003 (Year 2)
October 1, 2002–March 31, 2004 (Year 3, termination report)

Funding Level

<table>
<thead>
<tr>
<th>Year</th>
<th>Amount</th>
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<tbody>
<tr>
<td>1</td>
<td>$100,000</td>
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<tr>
<td>2</td>
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<td>3</td>
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</table>

TOTAL $245,000

Participants

Clyde Tamaru, Ph.D., Extension Specialist
Sea Grant Extension Service

Kathleen McGovern-Hopkins, Extension Agent
Sea Grant Extension Service

Harry Ako, Ph.D., Professor
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Jennifer Olson, Matt Lyum, and Lena Asano, Graduate Students
University of Hawaii at Manoa

Eri Shimizu, Graduate Student
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Year 1 only

Year 1 and 2 only
Year 2 only
Brandon Avegalio and Kelly DeLemos, Graduate Students
University of Hawaii at Manoa

Years 2 and 3 only
James Szyper, Ph.D., Extension Specialist
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Justin Iwai, Undergraduate Student
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Objectives

Year 1
1. Conduct laboratory and field studies on sex determination in livebearers.

2. Demonstrate live feeds production systems to practicing freshwater ornamental aquafarmers.

3. Investigate the role of highly unsaturated fatty acids (HUFAs) in the culture of freshwater ornamentals.

4. Provide technical assistance in the form of verbal consultations, written information, site visitations, workshops, manuals, and production handouts.

Year 2
1. Invite industry expert(s) to participate in a conference on marketing trends in the freshwater ornamental fish industry.

2. Investigate use of carotenoids for color enhancement.

3. Validate techniques for artificial insemination of livebearers.

4. Determine minimum effective dosage of Ovaprim for induction of spawning.

5. Validate factors affecting the sex ratio in swordtails.

6. Conduct technology transfer activities.
Year 3

1. Produce a position paper summarizing the current status of Hawaii’s freshwater ornamental fish industry.

2. Field test the artificial insemination technique to increase efficiency in the commercial production of lyretail swordtails.

3. Demonstrate the cost-effectiveness of specialization in the production of freshwater ornamental fishes.

4. Provide technical assistance in the form of workshops, verbal communication, written material, and site visits.

Reason for Termination

CTSA prefers not to fund the same priority area for longer than three years. This project has focused on and addressed specific aspects of freshwater ornamental aquaculture for three years, and the majority of the objectives have been completed. However, there is still a need for continued support of the freshwater ornamental fish industry in Hawaii, and it is hoped that funding agencies and researchers will continue to help this growing industry.

Principal Accomplishments

Year 1

Objective 1: Conduct laboratory and field studies on sex determination in livebearers.

Laboratory trials were conducted using 10-gallon aquaria and two varieties of swordtails (red velvet and pineapple). Average body weight was found to be negatively correlated with stocking density, and with regard to the resulting sex ratio, the data was found to be extremely misleading if one were to simply score the number of females versus males based on the presence or absence of the gonopodium. A preponderance of females resulted in all groups because all fish that were smaller than 30 mm in total length were scored as females irrespective of the stocking density. If the diagnostic characteristic of males was the presence of the “sword” on the caudal fin, individuals with this trait were found only in size classes of fish that were greater than 30 mm in total length. If the individuals that
were smaller than 30 mm in total length were removed from the scoring of the sex ratio, statistically there were no differences in the sex ratio in response to stocking density. Based on the results of these experiments, it would appear that stocking densities between 1 and 3 fish/L would be the highest ratio one would use for the culture of swordtails in a recirculating system.

**Objective 2: Demonstrate live feeds production systems to practicing freshwater ornamental aquafarmers.**

Grow-out trials for *Moina macrocopa* using live *Chlorella vulgaris* purchased from Japan were found to produce the highest densities yet recorded. However, the costs of importing the algae, its limited shelf life, and the permitting needed to import the algae into Hawaii make it impractical for use unless it is used at a large enough scale. Similar results were also achieved with the use of a relatively new product (*Nannochloropsis oculata*) from Reed Mariculture, San Diego, California. The product comes in a slurry and can be frozen for up to a year. It is also relatively expensive, but can be stored frozen and used in times of emergencies or to boost the nutritional quality of the *Moina*. An alternative feed that also produced similar results was with the standard “green water” (tilapia, guppy, and recirculating systems) culture systems. The quantity of “green water” is the limiting factor, and larger-scale grow-out fish systems (300 gallon/1200 L or greater) need to be available to culture high densities of *Moina* using “green water” as the feed.

**Objective 3: Investigate the role of highly unsaturated fatty acids (HUFAs) in the culture of freshwater ornamentals.**

Investigations demonstrated that there are similarities in essential fatty acids for various live maturation or conditioning feeds used in the freshwater ornamental fish industry. However, arachidonic acid appears to play an essential role in reproduction of freshwater ornamentals as opposed to docosahexanoate (DHA), which is in striking contrast to what has been reported for marine fishes.

**Objective 4: Provide technical assistance in the form of verbal consultations, written information, site visitations, workshops, manuals, and production handouts.**

Kathleen McGovern-Hopkins was hired as an assistant extension agent with the University of Hawaii Sea Grant Extension Service in September 2000. The repairs to the Windward Community College (WCC) aquaculture complex were completed, and the facility is in compliance with the University of Hawaii’s Animal Care and Use Committee and Environmental Health and Safety Office.

A total of ten workshops and six presentations were held on various islands.
Year 2

Objective 1: Invite industry expert(s) to participate in a conference on marketing trends in the freshwater ornamental fish industry.

This project, in collaboration with the USDA’s Initiative for Future Agriculture and Food Systems project, the University of Hawaii Sea Grant Extension Service, and the Hawaii Aquaculture Development Program, organized a workshop series that focused on the marketing of aquatic products. It was held at the University of Hawaii’s Manoa campus and at Windward Community College on September 12–14, 2002.

The distinguished panel consisted of three experts: Mr. Mark Taylor (African Northwest), Mr. Ron England (Worldwide Aquatics), and Dr. C. L. Cheshire (University of Hawaii Pacific Business Center). A summary of the workshop was also disseminated in CTSA’s Regional Notes newsletter and in the Hawaii Aquaculture Association newsletter.

It would appear that Hawaii’s freshwater ornamental producers have begun to make some market penetration that can definitely be built upon. A sound growth strategy for producers is to ensure a higher quality product, an increase in variety, and an increase in volume. This should ultimately equate to more sales, revenue, and net profit (i.e., expansion of the industry). The major take-home message for Hawaii’s players is to understand where in the freshwater ornamental fish business they should be (e.g., either producing or marketing) and to not try to do all things.

Objective 2: Investigate use of carotenoids for color enhancement.

In a collaborative effort, both Sanders Brine Shrimp Co. and Rolf C. Hagen Inc. incorporated 2% of Spirulina Pacifica™ and 1% NatuRose™ into flake feeds. The amount of the two algae used were characterized as acute dosages where an effect would be noticeable within two to three weeks. These were tested with a variety of freshwater ornamental fishes courtesy of our freshwater ornamental fish growers in Hawaii. Some of the fishes that were tested were: red velvet swordtails, rainbowfish, 24K mollies, topaz cichlids, neon swordtails, discus, rainbow sharks, pink gouramis, and rosey barbs. Only a subjective test of whether the treated feeds worked was used, and in all cases, the treated feeds resulted in a noticeable change in color in fish provided the feed. However, it is also clear that what colors appear in a fish are dependent on the species, sex, and state of maturity. In other words, the prepared feeds enhance the natural coloration of the target fish and do not add color as with dyeing a fish (e.g., glass fish).

Promising results were achieved using prepared feeds for the majority of the fish that were tested.

Tropical Ponds of Hawaii and breeders of Siamese fighting fishes, Betta splendens, expressed interest in the use of the color-enhanced feed produced by the project.
work group and provided it on a trial basis. Elizabeth Hahn, an avid breeder, utilizes the feed to enhance the color of her fishes, which she enters in the International Betta Congress shows and which have done very well. Her Web site is at http://planet-hawaii.com/liz/betta/.

**Objective 3: Validate techniques for artificial insemination of livebearers.**

According to the Mendelian law of segregation, of a dominant (lyretail) and recessive (common) gene, the expected ratio of a mating between a common swordtail to a heterozygous lyretail individual is 1 common : 1 lyretail. This was statistically confirmed where the observed ratio was consistent with the expected 1:1 ratio (chi square = 0.06, P>0.50). Using the artificial insemination technique, male lyretail and female lyretail crosses were made between November and December of 2001, and offspring were produced during January through March 2002. These were grown out to be large enough so that their phenotypes could be scored confidently. Four clutches from four separate crosses were found to have ratios of lyretail to common swordtail phenotypes that were consistent with the expected 3:1 ratio. Equally important was that 25% of the lyretail individuals should possess a homozygous genotype for the lyretail trait, which would mean the possibility of producing progeny that would be 100% lyretail. This was achieved by utilizing progeny testing. The results obtained confirm and validate the inheritance of the lyretail trait and the use of the artificial insemination technique for livebearers as a means to generate the homozygous individual. While the inheritance of the lyretail trait has long been proposed to follow the dominant-recessive model, to our knowledge, the generation of actual individuals homozygous for the trait has never been reported. The three females that were determined, by progeny testing, to be homozygous for the lyretail trait mark a milestone in fish breeding.

**Objective 4: Determine minimum effective dosage of Ovaprim for induction of spawning.**

Redtail sharks and rainbow sharks were found to be sexually mature beginning in June 2002. At that time, all females possessed oocytes of the same size (approximately 1 mm in diameter), and expression of milt from males could be accomplished by applying slight pressure to their abdomen. Induction-of-spawning trials were initiated in August 2002, and the experimental design for the spawning trials consisted of initially anesthetizing the individual and obtaining an ovarian biopsy using a polyethylene cannula. Only females that possessed oocytes that averaged 1 mm in diameter and in which the germinal vesicles were centrally located were used in the spawning trials. Likewise, only males from which milt could be extruded after applying slight pressure to the abdomen were used in the spawning trials. Females were administered varied dosages of Ovaprim, and the percentage of females that ovulated and also spawned was recorded. The manufacturer’s
Results indicate that lower stocking densities would result in a higher percentage of males with the caudal sword.

**Objective 5: Validate factors affecting the sex ratio in swordtails.**

Year 1 activities focused on small-scale (38-L aquaria) trials conducted in the laboratory. Year 2 activities were conducted in large-scale (2000-L) outdoor rearing trials. Sex ratios were consistent with those reported for the laboratory trials where the sex ratio was apparently related to the size of the fish. The ratio of males to females present in groups of fish that were greater than 45 mm in total length approached 1:1. The implication of the results were that the grow-out systems for swordtails will need to maximize growth to result in suitable quantities of males.

**Objective 6: Conduct technology transfer activities.**

A total of 11 newsletter articles, one manual, three presentations, and one workshop were completed.

**Year 3**

**Objective 1: Produce a position paper summarizing the current status of Hawaii’s freshwater ornamental fish industry.**

The Year 3 project closed before a full assessment of the status of the industry could be completed. This activity is currently being carried out in collaboration with Dr. PingSun Leung and graduate student Ms. Lotus Kam under the auspices of another CTSA-supported project titled “Economic Feasibility for Freshwater Ornamental Fish Growers in Hawaii to Market their Products Directly to West Coast Retailers.”

**Objective 2: Field test the artificial insemination technique to increase efficiency in the commercial production of lyretail swordtails.**

The results to date validate the mode of inheritance of the lyretail trait in swordtails and also demonstrates that the use of the artificial insemination technique is a means to generate a higher percentage of lyretail offspring and also produce the homozygous lyretail genotype. F1 lyretail males that were part of the same brood that produced the homozygous females were used to artificially inseminate lyretail females (i.e., progeny testing of lyretail males). Artificially inseminated females were then monitored for the production of fry, and these were then grown out until they...
Output would have to be very high for a hatchery to become cost-effective. To date, there have been ten crosses that have resulted in the production of fry, and of these, three cohorts are all lyretails indicating that the males used to artificially inseminate the females were homozygous. If the trait is inherited in Mendelian fashion, then we would expect at least three males to be homozygous out of the ten that were monitored. A major difference in the results when conducting progeny testing of the male lyretail is that lyretail females were being artificially inseminated. In this case, when a homozygous male had been identified, 50% of the progeny were homozygous providing for a means to amplify the number of homozygous individuals quickly. In addition, even when a heterozygous male lyretail was identified, 25% of the progeny were expected to be homozygous. In short, the project work group was about one generation away from achieving the production of a population of homozygous lyretail swordtails through traditional breeding.

**Objective 3: Demonstrate the cost-effectiveness of specialization in the production of freshwater ornamental fishes.**

Research results obtained on the use of a commercial diet exclusively for first feeding and on the induction of spawning using Ovaprim as the inducing agent form the basis for the estimates to be described. A summary of the costs and estimated returns at various prices of two-week-old rainbow shark fry are presented in Table 1. The gross revenue is based on the production of 417,000 two-week-old fry over a period of four months and has been calculated for a range of values between $0.05 and $0.02 per individual. The capital costs for a hatchery facility capable of producing the two-week-old fry that would fit on a 100-m² footprint was estimated to cost approximately $1,200 amortized over five years. Feed and other supplies were estimated at $800 per year, and the estimated labor in hours per year for construction and maintenance were 50 and 300, respectively. Using a spread of between $0.05 and $0.02 per fish, we can see the estimated gross revenues and what the return in terms of wages ($/hour) are like for this enterprise.

<table>
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<th>Price ($)</th>
<th>Gross Revenue ($)</th>
<th>Return on Labor ($/hr)</th>
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<tr>
<td>0.05</td>
<td>20,850</td>
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<td>0.04</td>
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<tr>
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Note: For production of 417,000 fry per year in a 100-m² facility using an estimated 50 hours per year for construction labor, 300 hours per year for production labor, $1,200 for capital costs amortized over five years, and $800 per year for feed and other supplies.
It is fairly clear that having a specialized hatchery output for just one species can work as a supplemental income for a person, or it would also work as a single output from a multi-species hatchery enterprise.

**Objective 4: Provide technical assistance in the form of workshops, verbal communication, written material and site visits.**

In total, eight workshops were held, seven presentations were given, and four articles were published.

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### Impacts

- Between 1992 and 2002, Hawaii’s ornamental aquaculture industry grew at an average rate of 15% (Kam et al., in press).

- In 2003, freshwater ornamental production reportedly accounted for approximately $500,000 in sales (Hawaii Aquaculture Development Program, personal communication).

- There are an estimated 30 “farm-level” producers with an additional 40 backyard or garage producers in Hawaii (Natarajan, 2002).

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### Recommended Follow-up Activities

The principal investigator is to work collaboratively with Dr. PingSun Leung to assist in the completion of the CTSA-supported project “Economic Feasibility for Freshwater Ornamental Fish Growers in Hawaii to Market their Products Directly to West Coast Retailers.” It is anticipated that this project will provide the necessary information that will give a fairly rigorous assessment of the status of Hawaii’s freshwater ornamental fish industry.

A manual on the culture of the freshwater cladoceran *Moina macrocopa* is to be completed and printed during 2004–2005 utilizing funds from the University of Hawaii Sea Grant College Program and the Hawaii Aquaculture Development Program. In addition, the genus *Moina* is to be petitioned for inclusion on the list of species that can be legally imported into Hawaii. Lastly, three workshops are to be held on the islands of Oahu, Maui, and Hawaii on the use of *Moina* in freshwater ornamental fish culture.
Work is to continue on identifying a gene marker for the lyretail trait as well as the development of a simple biochemical test that will be able to distinguish between homozygous and heterozygous lyretail individuals. In addition, there will be a focused effort to establish at least five strains of homozygous lyretails. Lastly, investigations will commence on sex reversal of swordtails in order to be able to produce viable all-female populations. In combination with the production of homozygous lyretail populations, the ultimate goal will be to produce all female homozygous lyretail swordtails that can be used by Hawaii farmers to increase the production of lyretails or to be sold as broodstock as a value-added product.

In collaboration with the private sector, investigations will be done on maturation and spawning of a variety of freshwater ornamentals. In particular, the role of arachidonic acid on reproduction in these fishes will be investigated.

The Hawaii Department of Agriculture Plant Quarantine Branch should be petitioned to include the Asian Arrowana on the list of species allowed to be imported into the state. Minimum listing is on Appendix B.

Technology transfer has always been a hallmark of the Sea Grant College Aquaculture Extension Program, and that will not change. Extension agents and specialists will continue to be a conduit of information to researchers as well as to the private sector.

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**Publications in Print, Manuscripts, and Papers Presented**

**Year 1**

**Publications in Print**


**Papers Presented**


**Year 2**

**Publications in Print**


**Papers Presented**


**Year 3**

**Publications in Print**


Papers Presented


Literature Cited
