Marine Food Fish Seedstock Production, Years One, Two & Three

Reporting Period

January 1, 1999 - September 30, 2002 (termination report)

Funding Level

Year 1	$110,000
Year 2	$135,000
Year 3	$135,000
TOTAL	$380,000

Participants

Year 1

Anthony C. Ostrowski, Ph.D., The Oceanic Institute

James A. Brock, Ph.D., Aquaculture Development Program, State of Hawaii

Christopher Kelley, Ph.D., Hawaii Institute of Marine Biology, University of Hawaii

PingSun Leung, Ph.D., University of Hawaii at Manoa

Year 2

Brad J. Argue, Ph.D., The Oceanic Institute

Anthony C. Ostrowski, Ph.D., The Oceanic Institute

James A. Brock, Ph.D., Aquaculture Development Program, State of Hawaii

Christopher Kelley, Ph.D., Hawaii Institute of Marine Biology, University of Hawaii

Charles W. Laidley, Ph.D., The Oceanic Institute

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Year 3

Anthony C. Ostrowski, Ph.D., The Oceanic Institute

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Reason for project termination

The CTSA-funded "Marine Food Fish Seedstock Production" project has now closed, having successfully met its major goal of assisting in the establishment of a marine food fish industry in the Pacific region. To this end the project has successfully:

- Established a year-round supply of fertilized Pacific threadfin eggs and transferred broodstock technologies to local industry.
- Completed threadfin hatchery technology refinement, including development of an economic model, and transfer to commercial operations. Transfer was completed under a phased-out program that delivered 273,449 fingerlings in year one, 215,896 fingerlings in year two, and 178,514 Pacific threadfin fingerlings in year three of the project. A commercial operator is now supplying fingerlings to the local industry.
- Established multiple small-scale growout operations on the islands and enabled large-scale, offshore production of threadfin to be initiated.
- Initiated health assurance and disease monitoring programs.
- Generated captive farmed threadfin products that are now appearing in local restaurants, retail markets, and are being sold both to mainland and international markets.

Efforts to diversify with the milkfish and crimson snapper did not progress to the same degree as the Pacific threadfin. There is still interest in establishing a commercial hatchery for milkfish on the island of Molokai although economics of this lower value species has slowed its commercial development in Hawaii. Efforts to resolve critical bottlenecks in the life cycle of the crimson snapper have proved more challenging and now form the basis of a DLNR-funded bottom-fish project being conducted at HIMB.

Objectives

Year 1 (January 1999-June 30, 2000)

1. Maintain broodstock and distribute a total of 250,000 Pacific threadfin fingerlings to qualified, participating farmers.

2. Maintain broodstock and provide 100,000 milkfish fingerlings to qualified farmers.

3. Initiate a health assurance program for Pacific threadfin and milkfish.

4. Initiate and maintain a broodstock development program for crimson snapper (*Pristipomoides filamentosus*).

5. Determine the size of a Pacific threadfin hatchery and costs required for various levels of production.

6. Initiate a phased payment schedule for Pacific threadfin and milkfish seedstock distributed to farmers statewide, and provide technical advice and assistance for growout operations.

Year 2 (October 2000-December 2001)

1. Maintain Pacific threadfin, milkfish, and crimson snapper broodstock.

2. Produce 250,000 threadfin and 100,000 milkfish fingerlings for distribution to qualified farmers.

3. Refine disease-free certification program for finfish larvae and improve fry quality.

4. Begin to domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.

5. Determine production cost structure and profitability of milkfish growout in Hawaii.

6. Continue phased fry payment schedule and expand program to other areas of the Pacific taking into account site-specific, economic, and genetic considerations.

7. Evaluate other bottom fish species as potential aquaculture candidates and develop broodstock capabilities as money and fish become available.

Year 3 (April 2001-September 30, 2002)

1. Improve threadfin fry quality by use of rotifer enrichment methods.

2. Determine role of enhanced natural productivity in semi-intensive production ponds on improved survival and growth of larval milkfish.

3. Continue the disease-testing program to insure the disease-free status of threadfin fry provided to the industry and refine it by establishing a cell line to screen for potential viruses.

4. Develop pair spawning methods for Pacific threadfin for genetic selection work.

5. Domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.

6. Confirm transfer of reliable fry production technologies to commercial hatcheries.

Work Progress and Principal Accomplishments

Year 1

1.0 Supply Pacific Threadfin Seedstock

Objective 1: *Maintain broodstock and distribute a total of 250,000 Pacific threadfin fingerlings to qualified, participating farmers.*

At the request of farmers, the OI hatchery initiated quarterly threadfin fry production runs during year 1, to allow farmers to better manage their growout operations and provide a more even flow of product into the marketplace. OI scheduled hatchery runs in April, June, September, and October 1999. All hatchery runs followed the methods developed by Ostrowski and Molnar (1998).

A total of 273,449 threadfin fingerlings were supplied to eight threadfin farms over the project period. The April 2000 run, (two, 5-ton production tanks) yielded 31,088 fish; the June run (two tanks) yielded 28,400 fish; the September run (three tanks) yielded 87,116; and the fourth run (four tanks) in October yielded 84,078 fish. An additional 42,767 threadfin were supplied to farmers from the OI hatchery from other ongoing research. These fish were raised in the nursery to 30 days of age (D30) and approximately 0.05 g each, packed, and air freighted to participants on Hawaii and Molokai. While shipping at this age reduced costs by allowing more animals to be packed in a box, farmers had to implement protocols required during the Nursery I phase (D25 – D40) to control cannibalism. Local farmers on Oahu received their fish on D40 (1.0 g) to lessen their burden of implementing the Nursery I phase. New packing protocols based on 24-hour pack-out experiments conducted at OI have increased the survival (98%) of landed fry to the outer islands. The new packing regimen includes reduced handling, incorporation of ammonia pillows in each bag, square bottom shipping bags, and reduced transfer/air time.

In addition to fry, 1,190,000 threadfin eggs were shipped to local farmers and researchers from broodstock maintained at OI. The five participants that received eggs included Ben Krause, Ryan Murashige, Romy Aquinaldo, Anuenue Fisheries Research Center (AFRC), and Hawaii Institute of Marine Biology (HIMB). In addition to fry, farmers were given the OI hatchery manual for threadfin production as a guide through rearing.

Results of the on-farm hatchery runs were mixed. Often-cited problems included lowered priority for the run, which lead to neglect in some instances, difficulty in scaling-down of the OI methods, and inconsistency in rotifer supply. In short, successful larval culture from the farmers has been limited due to the lack of experience in this field. Additional on-farm training in larval rearing and live feeds management through this project should improve success.

Follow-up conversations over the course of the year indicated that out of all of the fingerlings sent to farmers, approximately 65% (177,741 fish) would reach market during 1999-2000. Farmers currently sell 0.5 – 1.25 lb fish for prices ranging from $4.50 – 7.00/lb in the round. Given a mode 0.75 lb/fish harvested weight (133,306 lbs total) and a $5.00/lb price, the total economic impact is estimated to be $666,529 in off-the-farm sales. Mortality on growout farm sites is primarily due to catastrophic reasons (e.g. water shut off, oxygen depletion), and the gill parasite, *amyloodinium*. Recently, the use of restricted light to control the growth of the parasite has proved promising on one farm that has had a history of outbreaks.

2.0 Supply Milkfish Seedstock

Objective 2: *Maintain broodstock and provide 100,000 milkfish fingerlings to qualified farmers.*

During 1999, OI initiated research to develop a semi-intensive hatchery method for production of milkfish fry in conjunction with production activities. Semi-intensive methods of milkfish production are commonly practiced in Taiwan and other regions of the world, but the techniques have not been extended to Hawaii. Such methods would significantly lower production costs for fry, and improve profitability of milkfish culture in the state. Last year, six production runs were conducted in 12 ft x 35 ft, concrete ponds (40-ton capacity) that yielded an overall survival rate of 5% from stocked eggs. Problems with gas supersaturation (over 104%) due to high levels of oxygen production from algal blooms were responsible for heavy mortality of larvae around D11. Efforts this year were intended to refine techniques and improve overall survival rates.

Overall survival from four semi-intensive hatchery runs completed between July and November 1999 yielded an average 24% survival from stocked eggs (150,000/tank) to D40. This survival rate was nearly 5 times greater than achieved last season. Reasons for the improvement included better management techniques and more stable rotifer densities in tanks. The use of 80% shade-cloth over tanks to restrict ambient light intensity in the outdoor systems dramatically improved survival of larvae through the first 11-12 days of development. Algae blooms were better controlled with use of the shade-cloth, which prevents large increases in oxygen and subsequently total gas supersaturation levels. Algae densities were maintained at a secchi disk reading of between 20-30 cm. Temperatures ranged from 24.3 – 27.8°C and pH was maintained between 8.1 – 9.0 using water exchange. Rotifer production from the hatchery was also more consistent, yielding better control of live feed densities in tanks during critical periods. Rotifer densities in tanks were consistently maintained between 3-5/ml.

Production from the semi-intensive runs was better than expected (144,071); yielding 44% (44,071) more fry than originally targeted. Fry were distributed to 27 farmers on the islands of Oahu, Molokai, Maui, and Hawaii. Fry were shipped between D38 and D40 (0.05-0.08 g each), and packed at 1,000/shipping bag. Survival rates of fish to outer islands ranged from 96-99%. Approximately 54.5% of the fry went to freshwater facilities (78,571) while 45.5% of the fish went to saltwater farms (65,500). In addition, 300,000 eggs were provided to Ryan Murashige to conduct hatchery runs.

> ***Results at a glance . . .***
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> Established a year-round supply of fertilized Pacific threadfin eggs and transferred broodstock technologies to local industry.
>
> Completed threadfin hatchery refinement.
>
> Established multiple small-scale growout operations in the islands and enabled large-scale, offshore production of threadfin to be initiated.
>
> Initiated health assurance and disease monitoring programs.
>
> Generated captive farmed threadfin products that now appear in local restaurants and retail markets and are being sold to mainland and international markets.

Follow-up conversations indicated that nearly 70% of fish sent to farmers would reach market size during 1999 – 2000. Currently, farmers are able to receive up to $3.50/lb of 5-6" (3-4 oz.) or 1 lb. or greater milkfish. The former can be produced within four months growout. This season, farmers have noted an increase in market demand for the smaller product which is prized by the local Filipino community. Given that demand and a conservative $3.00/lb price of fish, it is estimated that economic impact from the project this year would yield approximately $75,600 in off-the-farm sales.

Results from the production runs indicate that methods of semi-intensive milkfish fry production can be achieved in Hawaii and that fry can be produced reliably. On average, tanks yielded 1 fry/liter (i.e., ca. 42,000 fry/40-ton tank). Preliminary estimates from this and CTSA-funded milkfish research are that it costs between $0.03 – 0.05/D40 fry produced, including broodstock holding costs. This evidence should help support the creation of commercial, Hawaii-based milkfish fry production facilities for industry expansion.

3.0 Design and Implement a Health Status Program

Objective 3: ***Initiate a health assurance program for Pacific threadfin and milkfish.***

Largely because of favorable survival at harvest and the virtual absence of episodes of disease in the hatchery phase, the health status of threadfin and milkfish has not been systematically evaluated for the fingerlings previously produced through CTSA-funded projects. It is now well understood that the provision of healthy and robust fry from the hatchery is a critical component to success of any fish production industry, and that establishment of fry quality must be at the foundation of industry transition. The IAC recommended that more aggressive surveillance of the fish should be initiated to begin to document the health condition of the fingerlings produced by the project prior to being distributed to participating farms.

Threadfin and milkfish fingerlings (30 from each sp.) from each production and research run conducted were delivered to Dr. Jim Brock at the Anuenue Fisheries Research Center (AFRC) for examination. Each group of fish was killed with an overdose of MS-222, weighed, necropsied, and tissues from major organ systems (gill, heart, liver, spleen, caudal kidney, digestive tract and attached abdominal viscera) were collected and preserved in Davidson fixative. The specimens were blocked and processed by routine histopathology methods and slides were prepared. The tissue sections were stained with hematoxin and eosin and inspections were conducted.

According to Dr. Brock, results of necropsy and histopathology found no gross change in the threadfin or milkfish fry that would suggest the presence of an infectious disease problem in the batches of fish. Fatty vacuolation of the livers was found in 33% of the threadfin. This was related to a common microscopic change present in cultured threadfin. Its appearance suggested an excess of lipid in the diet of the fish. The clinical impact of this was considered apparently minimal, based on the relatively high (ca. 85%) survival rate of nursery fish at OI.

4.0 Develop Crimson Snapper Broodstock Population

Objective 4: *Initiate and maintain a broodstock development program for crimson snapper (Pristipomoides filamentosus).*

The original design of this trial was to split remaining fish from the previous growout trial into broodstock groups that would be monitored for maturational development and eventually spawned. Additional fish were to be collected from the wild to supplement stocks. Unfortunately, similar problems encountered with the growout trial precluded set up of the broodstock trial. It was decided it was not warranted to collect additional fish and place them in tanks until remedies for exopthalmia and swim bladder inflation were found. A total of 47 fish remained from the original 184 fish stocked into OI tanks in July 1998. Of these, 21 fish had exopthalmia (popeye) or were blind, and were immediately culled. The remaining 26 fish (448.9 ± 122.8 g, 27.7 ± 1.7 cm, fork length (FL)) were stocked into a 50-ton (37.5-ton working volume) square (6.1m x 6.3m x 1.0 m) concrete tank. Fish were fed combinations of squid, smelt, krill, and supplemental vitamins and minerals at a rate equal to 2-3% body weight daily. An artificial structure comprised on concrete blocks and 6" PVC-pipe was also placed in the center of the tank for refuge. In addition, 95% shade cloth was placed over the tank and around tank sides to minimize light input and disturbance to fish. The purpose was to provide an environment to minimize stress in fish.

Despite changes in holding protocols, fish continued to exhibit exopthalmia and/or swim bladder inflation almost on a weekly basis and died throughout the summer months. After 3 months, only 14 fish remained. These fish were then placed in a 20-ton (7.3 m diameter) circular tank with a 3.0 m inner ring. This donut-shaped tank was covered twice with 80% shade cloth, and supplied high current and water exchange. Fish were held for 9 months in this system, but continued to die. In November 1999, only 4 fish remained.

The remaining four fish were then transferred to a 3 m diameter HDPE tank (8-ton working volume) located indoors in an a photoperiod control lab and placed under short (8 hours light; 16 hours dark) photoperiod regimen. Fish appeared to adapt better to the long photoperiod regimen and no cases of expothalmia occurred. Fish were euthanized in March 2000, weighed and measured. Fish grew to a range of 2.4 – 4.0 kg, and 35 – 45 cm FL. No signs of pairing or reproductive behavior were observed in any fish.

Late during the project period it was decided that onshore holding of fish was not possible. Newly caught adult snapper were stocked into 28m^3 floating cages moored near Coconut Island. Each cage was 3m in depth. Fish appeared to adjust better and the incident of exopthalmia was reduced dramatically. Viable spawns have yet to be recorded.

Results indicate that crimson snapper are a challenging species to domesticate and maintain in onshore tanks. Difficulties remain with the acclimation process of newly captured fish, as well as fish that have been maintained on site for extended periods of time. Problems believed to be stress-related from the change in environment lead to exophthalmia, inflated swim bladders, cessation of feeding, and finally death. Holding of broodstock in nearshore cages appears promising. These holding methods might be linked to onshore techniques of spawning and eventually larval rearing.

5.0 Determine Economics of Hatchery Production

Objective 5: *Determine the size of a Pacific threadfin hatchery and costs required for various levels of production.*

A spreadsheet model was developed by Dr. PingSun Leung and staff over the project period to determine a viable scale for a commercial threadfin hatchery in Hawaii. Dr. Leung and staff met with OI hatchery personnel, OI engineers, and engineers at the Natural Energy Laboratory of Hawaii Authority (NELHA) to establish basic model assumptions. The model characterized finfish production parameters and permitted ease of calculation of the costs associated with variations in production design and scale of production. The base production scheme was modeled after current hatchery practices performed at OI. Production requirements were determined according to demand and the estimates for survival over each phase of production including the spawning (broodstock), larval rearing, and early nursery phases. Site-specific requirements including construction, site survey and preparation were based on estimates from NELHA. The engineering designs for plumbing, drilling and aeration assumptions were based on estimates from OI.

Results indicated that for a hatchery enterprise producing 1.2 million fry per year, the cost associated with raising each 1-g fry is estimated at 20.13¢. The largest variable costs were labor and supplies, which comprised 54% and 10% of the total production cost. The combined annualized fixed costs for development and equipment was approximately 10% of total production costs. At a sale price of 25¢ per fry, the 20-year internal rate of return (IRR) was 47.70%. In comparison to the 20.13¢ unit cost for 1.2 million fry production, analyses of smaller enterprises producing 900,000 and 600,000 fry per year reflected significant size economies with unit costs of 25.25¢ and 35.98¢, respectively. Additional analyses revealed that increased profitability is possible by shortening the nursery period, but at a diminishing rate. Increasing the nursery length exhibits decreasing daily costs per fry. The cost savings resulting from the elimination of live feed production was also calculated and serves as a decision aid for management when considering outsourcing or investing in commercial substitutes.

6.0 Implement Technology Transfer

Objective 6: *Initiate a phased payment schedule for Pacific threadfin and milkfish seedstock distributed to farmers statewide and provide technical advice and assistance for growout operations.*

Prior to the beginning of fry distribution, prospective threadfin and milkfish farmers were contacted to inform them of the phased payment plan. Prices were set at $0.07/threadfin and $0.01/milkfish fry supplied, roughly one-third the estimated OI fry production cost for each species. Farmers were urged to pay for the fingerlings as soon as possible but were allowed to make payments over a period of up to eight months to allow income received from the harvest of fish to accumulate. In addition, outer island farmers were allowed to pay for only 80% of fish sent to them, minus mortality recorded within two days post-shipment, in the interest of parity. The rationale was a concern expressed by outer island farmers that fish they received from the project were still within the cannibalistic phase (ca. D30), while those received by farmers on Oahu were past this stage (D40), and thus less prone to subsequent losses on the growout farm. Outer island farmers also noted that Oahu farmers did not experience mortality due to air shipment. A similar arrangement was made with milkfish farmers.

The income received was used to supply additional threadfin and milkfish fry to farmers, and to increase information exchange efforts and onsite visits. The project this year distributed 9.4% more threadfin and 44.1% more milkfish fry to farmers than was originally targeted as deliverables. A greater exchange of information occurred this year over the Internet, but onsite visits proved valuable to refine methods to preclude problems on existing farms and assist new farms in start-up. Activities coordinated with farmers included system design, feeding strategies, disease, stocking densities, ordering supplies (i.e., identification of sources), fish transport, and handling. An average 2.3 site visits occurred monthly that included 15 outer island visits and 13 visits on Oahu. Staff aided new threadfin and milkfish farmers on the outer islands of Kauai, Maui, and Hawaii. Oahu site visits covered a broader area and new farmers as well in Kahuku, Waipahu, Laie, and Kaneohe.

A key contribution by OI staff has been the coordination of feed shipment with threadfin farmers and the local feed import and storage facility, Land-O-Lakes (LOL), located on the leeward coast of Oahu. The majority of feed used by farmers is the Marine Grower, a diet formulation researched by the project PI, Dr. Ostrowski, and developed in concert with Moore-Clarke, Co. Canada. Current cost of the diet is $0.65/lb FOB, LOL. OI staff met with Moore-Clarke to discuss ways to support industry development. Moore-Clarke agreed to lower costs of the feed with bulk orders, and OI staff coordinated needs with local farmers. Recent bulk orders through LOL reduced the price to $0.58/lb FOB, LOL, and larger shipments are targeted to reduce the price to $0.52/lb. Previous economic work (see draft publication Martinez-Cordero et al. in Appendix 3) indicated feed costs for threadfin growout are the largest variable cost, ranging from 23 – 27% of total operating costs. At a feed conversion of 1.3, a $0.12 reduction in price of feed translates into a 20% reduction in total feed costs.

Research efforts to further reduce feed costs were also coordinated with Ben Krause of Pacific Harvest, Kona, Hawaii, and the largest threadfin producer in the state. A new formulation based on past research of the PI was developed with Moore-Clark. This new formulation incorporated use of a lowered dietary protein level and soybean meal as a fishmeal substitute. The new diet was tested in a single tank of threadfin in growout at Pacific Harvest. Preliminary estimates are that, if successful, the price of feed could drop to near $0.45/lb, FOB, LOL.

OI also provided threadfin eggs to the State of Hawaii's Anuenue Fisheries Research Center (AFRC) to aid their efforts in transition to support industry needs. A recent bill passed in the Hawaii State Legislature, supported by OI, which included provisions for the AFRC to produce fry to support the growing marine foodfish industry in the state. OI staff through the CTSA project will continue to assist the AFRC.

Year 2

1.0 Maintain Broodstock

Objective 1: *Maintain Pacific threadfin, milkfish, and crimson snapper broodstock.*

OI continued to maintain Pacific threadfin and milkfish broodstock for the project. Pacific threadfin spawned on regular monthly intervals throughout the report period. Milkfish began spawning in May 2001. Eggs were collected routinely and many have been distributed to local farmers to attempt mass culture. The distribution of eggs is summarized in Task 2.0 below.

By the time this phase of the project was initiated, there were only four broodstock crimson snapper remaining at OI. It was decided to cull these animals rather than risk any potential disease transference by combining them with established animals at HIMB. Broodstock snapper at HIMB continued to grow and develop well in the net-pens. It was further decided by the Project Work Group not to collect additional animals to avoid overstocking the net-pens. Animals are currently being monitored and plans are to send eggs to the OI hatchery if and when they become available to attempt mass culture. During this period, Dr. Kelley resigned his position at HIMB and currently works for the NMFS in Honolulu.

2.0 Supply Threadfin and Milkfish Seedstock

Objective 2: ***Produce 250,000 threadfin and 100,000 milkfish fingerlings for distribution to qualified farmers.***

The provision of Pacific threadfin and milkfish seedstock to support industry development remains a critical aspect of these emerging industries. OI is the primary supplier of threadfin and milkfish seedstock in the State of Hawaii and supports both growout and baitfish production operations at more than 30 farms on the islands of Oahu, Maui, Molokai, and Hawaii. Since 1996, the OI hatchery has conducted large-scale research during these production runs to improve efficiency and overall fry quality. Last year all milkfish fry were produced using semi-intensive methods by first inoculating outdoor tanks with shrimp effluent water, and supplementing natural phyto-and zooplankton. This season the target was to test the usefulness of a product called AquaMats™ which purportedly improves semi-intensive production methods by providing biologically active surface area and enhancing natural biological processes to grow phytoplankton, detrital bacteria, and sessile zooplankton. In addition, efforts were targeted to improve threadfin fry survival, growth, and overall robustness by testing the usefulness of rotifer enrichment methods. The method originally developed for threadfin fry production included only enrichment of *Artemia*, the second live food item for this species. Rotifer enrichment methods are a new phenomenon and were not available during the hatchery development phase of this project.

The final two threadfin production runs scheduled for this phase of the project were completed in October 2000, and January 2001. In total, 215,896 fingerlings and over 3.6 million eggs were shipped to five facilities across the state. The majority of eggs were shipped to Pacific Harvest, which has established a pilot larval rearing facility. OI staff has assisted with technical advice and visits to this facility to aid hatchery development as well. No other milkfish trials were conducted over this report period due to the lack of spawns. All production and research trials scheduled to yield fingerlings for distribution have been completed as planned.

Results of trials in 200-L tanks demonstrated significantly greater survival rates to metamorphosis for threadfin larvae receiving "Algamac 2000" – enriched rotifers (Aquafauna Biomarine, Inc.) compared to those receiving *Nannochloropsis*-enriched rotifers. However, there was little difference in opercular deformity rates. Rotifer enrichment is recommended to improve survival and overall production of threadfin fry.

A total 14,100 fry were distributed. A drop in algal production at the OI facility that resulted in low rotifer output hampered production efforts. Efforts were directed to correct the situation and additional runs were re-establihed in October. Results of the Aquamat™ trials were inconclusive.

In addition to fry, the OI hatchery also provided threadfin and milkfish eggs to support industry development. Several farmers this year have begun hatchery efforts in earnest in response to increasing demands for fry. During this project phase, the OI hatchery supplied 1.7 million threadfin and 1.34 million milkfish eggs to three growout and research operations on the islands of Hawaii and Oahu.

3.0 Health Assurance Program

Objective 3: ***Refine disease-free certifications program for finfish larvae and improve fry quality.***

Threadfin and milkfish fingerlings (30 from each species) from each production run at OI were delivered to Dr. Brock at AFRC for examination. Each group of fish were killed with an overdose of MS-222, weighed, necropsied, and tissues from major organ systems (gill, heart, liver, spleen, caudal kidney, digestive tract, and attached abdominal viscera) were collected and preserved in Davidson fixative. The specimens were blocked and processed by routine histopathology methods and slides were prepared. The tissue sections were stained with hematoxin and eosin, and inspections were conducted.

The necropsy and histopathology findings showed no gross change in the fry threadfin or milkfish, which disputes any presence of an infectious disease problem in the batches of fish. Fatty vacuolation of the livers was found in 33% of the threadfin. This is a common microscopic change present in cultured threadfin. Its appearance suggests an excess of lipid in the diet of the fish. The clinical impact of this is apparently minimal. Documented results of these findings are in Appendix 1.

Before the project end, Dr. Brock resigned his position as the State of Hawaii's Aquatic Veterinarian. Dr. Robert Bullis from OI assumed temporary responsibility for this task. Due to the lag time in transition, Dr. Bullis did not complete the processing and reading of the remaining cases before the end date of this project.

4.0 Develop Selected Broodstock Lines

Objective 4: *Begin to domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.*

Efforts to implement a selected breeding program for Pacific threadfin have been initiated with the development of a domesticated broodstock group in which 10 females collected from the wild have been stocked with 10 F1 males. These animals reached reproductive condition in September with small spawns in September and October.

Spawns from semi-domesticated broodstock populations were reared through the larval hatchery in February 2001 producing 4,000 fingerlings that were then transferred from nursery to 30-ton growout tanks. These fish are grown to market size at which time control and selected top growers are PIT-tagged and stocked into broodstock tanks. Preliminary tissue samples were sent to Kent Sea Tech, Biotechnology division for preliminary microsatellite marker development. Costs associated with this effort were much higher than originally quoted and the effort was curtailed.

5.0 Milkfish Economics

Objective 5: *Determine production cost structure and profitability of milkfish growout in Hawaii.*

Delays in obtaining information from farmers and write-up caused a request to extend the project deadline. The model developed for milkfish was similar to the one developed for threadfin growout under a previous CTSA project. The synthetic enterprise budget was based on the design and operating parameters (feed conversion, mortality, yield) from over twenty existing farms that receive fry from the project. Budgets were developed for pond and tank systems in fresh, brackish, and saltwater, currently practiced in Hawaii. Capital costs, overhead expenses, and variable operating costs were estimated using the current costs faced by commercial aqua farmers in Hawaii. A computerized budget generator designed for milkfish growout production was developed under the Windows 95 environment for ease of use.

Evaluations of Hawaii milkfish pond and tank production systems were made. Spreadsheet models were developed for each system based on input from Hawaii farmers. Based on the observed practice of milkfish culture as a secondary or tertiary crop, capital costs and several operation costs of the farm were pro-rated to accurately depict the current situation. The study estimated the total production cost for milkfish production at $3.31/lb for the pond system and $3.81/lb for the tank system. At a sale price of $3.00/lb and seed cost of $0.25/fingerling, neither system is profitable under a 20-year project life. Based on the cost structure developed for each system, fixed costs including administrative overhead, equipment depreciation, and insurance comprise an estimated 40% of the total production cost. Discussions with milkfish farmers support the view of milkfish production serving as supplemental revenue to regular farm activities and legitimize the need to focus on variable expenses incurred. When considering variable costs alone, the production cost is $1.79/lb for the pond system and $2.31/lb for the tank system, yielding returns on variable costs of 68% and 30% respectively. The sentiment of Hawaii milkfish farmers is consistent with the results of this study: considering the market conditions for input requirements and product (milkfish) demand in Hawaii, milkfish production is only secondary to core species. Based on the information and assumptions used in this study, projected revenues from milkfish sales are not a premise for investment in dedicated farm infrastructure (i.e. a start-up venture in milkfish production) and the analysis of additional production scenarios.

6.0 Expand Program

Objective 6: *Continue phased fry payment schedule and expand activities to other areas of the Pacific taking into account site-specific, economic, and genetic considerations.*

Seedstock provision to farmers was logged for each shipment. A cost of $0.14/fingerling for threadfin and $0.03/fingerling for milkfish were assessed for each shipment (67% of actual cost for each fry produced from the OI hatchery). Costs to farmers for milkfish were based upon OI's costs of $0.05/fry to raise fry extensively to 25 days of age (D25). Costs to farmers for threadfin were based upon OI's costs of $0.21/fry to raise fry intensively to D25.

Site visits were conducted on the islands of Hawaii and Maui to assist in hatchery and growout set-up. One farmer is currently setting up a hatchery and conducting trial, larval rearing runs using eggs provided by OI. In addition, a total 30 hours were spent by staff on the phone arranging shipments of fry and eggs, and advising on receiving fish, tank design, feed requirements, and stock densities. There were no requests for fry from Pacific island areas other than Hawaii.

7.0 Develop Other Bottom Species

Objective 7: *Evaluate other bottom fish species as potential aquaculture candidates and develop broodstock capabilities as money and fish become available.*

Accomplishment of this task was dependent upon the supply of eggs from the CTSA (Task 1.0 above), DLNR, or NOAA funded projects, and the amount of funds remaining in this project. There were no spawns from any snapper species under any project.

Year 3

Objective 1: *Improve threadfin fry quality by use of rotifer enrichment methods.*

Two sets of experiments were conducted to examine the effects of diet on the quality of Pacific threadfin fry during the year-three project period. The first experiment compared rotifer and *Artemia* enrichments and the second experiment specifically examined the effects of vitamin C supplementation.

Experiment 1: Rotifer and Artemia enrichments. An experiment was conducted comparing the survival and growth rates and morphological characteristics of threadfin larvae receiving two different diet enrichment products (Algamac 2000®, Aquafauna Biomarine Inc; Aquagrow® Advantage, Martek Biosciences Corp.). Both of these DHA-rich products are prepared from dried protist cells (*Schizochytrium* sp and *Crypthecodinium* sp respectively).

The experiment was carried out in 4,000L larvae rearing tanks, 2 tanks per treatment. Each tank was stocked at a density of 40 threadfin eggs/L. Water exchange rates, aeration rates and diet transition regimen followed standard OI procedures. Larvae received the experimental enrichments throughout the rotifer phase, (day 2 to day 15 post-hatch), according to the manufacturers' directions. During the *Artemia* phase (day 12-15 post-hatch), 50% of the daily *Artemia* ration was enriched using the experimental product and 50%

with DHA Selco (Inve Aquaculture NV).

Samples of enriched rotifers and *Artemia* were collected for biochemical analysis while larvae samples were collected at 5-day intervals throughout the experiment for size measurement and biochemical analysis. At the end of the experiment an additional sample of 60 fish per tank was examined for opercular morphology ('normal', 'wrinkled' or 'missing' operculum). This involved close inspection of each fish using a dissecting microscope.

Survival rate of threadfin to day 25 was variable between tanks with no clear relationship to enrichment type. Threadfin from the Algamac group were 18% larger (standard length) on average than those from the Aquagrow group at the end of the experiment. Opercular characteristics were highly conservative within groups. 71.7% of threadfin receiving Algamac 2000 did not exhibit any opercular abnormalities at all compared to just 49.1% of Aquagrow-fed fish. Both 'wrinkled' and 'missing' operculae occurred with higher incidence in the Aquagrow group. Biochemical analyses and intermediate growth measurements are to be completed. While neither of the test products was effective in eliminating threadfin opercular abnormalities, the strong treatment effects provide evidence for a nutritional component to this phenomenon. On a practical basis, the Aquagrow Advantage product is not recommended for threadfin larviculture under the intensive rearing conditions tested.

Experiment 2: Effects of vitamin C supplementation. A second experiment was conducted to specifically examine the effects of dietary vitamin C supplementation on survival, growth and morphology of intensively reared Pacific threadfin larvae. The hypothesis being investigated was that dietary deficiency of vitamin C impairs collagen formation in developing threadfin larvae leading to skeletal abnormalities including malformed operculae.

Rotifers and *Artemia* were supplemented with ascorbyl palmitate (AscP, a fat-soluble ester of vitamin C), delivered *via* lipid emulsion and particulate enrichments. The experiment was conducted in 6 production scale (4,000L) rearing tanks, each stocked at a density of 35 threadfin larvae/L.

Larvae were sampled for size measurement and biochemical analysis at 5-day intervals from day 1 to day 20 post-hatch. Numbers of surviving threadfin in each tank were also counted during transfer to the nursery on day 25 and samples collected for size determination, biochemistry and morphology assessment.

No significant diet-dependent differences in survival or growth rates were observed over the experimental period. However, threadfin receiving ascorbyl palmitate-supplemented rotifers and *Artemia* suffered a significantly higher incidence of malformed operculae relative to those in the control group (mean 71.6% "wrinkled" + "shortened" operculae, versus 44.9%). Interpretation of these results is complicated by the provision of DHA Selco-enriched rotifers only to the supplemented group; nonetheless these findings demonstrate a clear influence of larval diet on threadfin opercular characteristics. It can be speculated from this data that vitamin C deficiency is not the determining factor for abnormal opercular development in threadfin, however further interpretation must await analysis of tissue levels of vitamin C.

Objective 2: *Determine role of enhanced natural productivity in semi-intensive production ponds on improved survival and growth of larval milkfish.*

This objective was switched to a comparison of intensive versus semi-intensive methods of threadfin production, with emphasis on fingerling quality from the different rearing systems. Milkfish fingerlings for distribution to farmers were instead produced in a single intensive hatchery run.

The semi-intensive rearing units for threadfin were located outdoors and consisted of rectangular concrete tanks, 9.6m by 3.7m, with operating volume 20,000L. The intensive rearing units were 4,000L circular black GRP tanks, located indoors. OI's standard threadfin rearing protocols were used for the indoor 4,000L tanks involving daily addition of microalgae and enriched rotifers/*Artemia*. Threadfin eggs were stocked into the 4,000L tanks at a density of 40/L. The 15,000L tanks were filled with seawater and fertilized to obtain 2mg/L total ammonia nitrogen (TAN), then inoculated with *Nannochloropsis* sp (300,000 cells/ml). Once TAN levels had dropped below 1mg/L, s-type rotifers were inoculated at a density of 2/ml and 1 day old threadfin larvae at 5/L. Water exchange was adjusted within the range 5-15% of tank volume per day. After stocking, microalgae and rotifers were added to the 15,000L tanks as required.

A preliminary trial was carried out comparing 3 intensive versus 2 semi-intensive rearing tanks. Threadfin larvae were sampled from each system at 4-day intervals for size measurement and an additional sample of 60 fish per tank was sampled for operculae assessment on day 25.

Mean survival rate from the intensive 4,000L rearing system was 19.6%, equivalent to 24,817 fingerlings per tank. Of the two 15,000L semi-intensive rearing tanks, one did not yield any fish, while the second produced 399 fingerlings (= 0.4% survival). Surviving fingerlings from the 15,000L tank were smaller than those reared intensively (mean standard lengths 8.6mm and 13.9mm respectively). Despite substantial differences in stocking density, feeding regimen and physical environmental conditions, the incidence of opercular deformity was comparable between the two rearing systems. The total opercular deformity rate among semi-intensively reared fingerlings was 52.5% (comprising 21.2% wrinkled plus 31.2% missing), compared to 42.8% for those reared intensively (20.0% wrinkled plus 22.8% missing).

We conclude that the semi-intensive husbandry techniques applied in the 15,000L tanks were sub-optimal for Pacific threadfin larvae. The relatively small size of fingerlings harvested from this system indicates that the larvae's energetic requirements were not adequately met. Unlike their intensively reared counterparts, the outdoor population did not benefit from *Artemia* or from the early introduction of formulated feed. As for the low overall survival rate in the semi-intensive rearing system, it remains to be seen whether physical or biological constraints were the most significant. Despite the low survival rate obtained, this first documentation of semi-intensively reared threadfin provides a valuable new tool for studying fingerling quality issues in this species.

Objective 3: *Continue the disease-testing program to insure the disease-free status of threadfin fry provided to the industry and refine it by establishing a cell line to screen for potential viruses.*

During the report period both Dr. James Brock, long-term state aquatic veterinarian, and Dr. Robert Bullis of OI, who was assisting the State during the transition period, resigned their posts. In the interim, fish from hatchery production runs were supplied to Dee Montgomery-Brock with the State of Hawaii for disease-free certification. During this period there were no positive reports of disease in hatchery-reared fry although one batch obtained as a by-product of the vitamin C supplementation study and stocked in an offshore cage showed an unusually high-incidence of chronic mortality that could not be diagnosed. This reinforces the requirement for disease diagnosis and treatment expertise in the Islands, a need that is made even more critical with the massive scale-up of offshore cage culture activities in the Islands.

Objective 4: *Develop pair spawning methods for Pacific threadfin for genetic selection research.*

Pacific threadfin from OI production tanks with a proven record of "group-spawning" were individually picked for experimentation. Eight pairs (one female and one male) confirmed by biopsy were stocked into separate 5,000-L broodstock tanks in an attempt to get natural pair spawns. Tanks were fed daily to satiation with the standard OI broodstock diet, and tank effluents were monitored for the appearance of fertilized eggs for a period of three months (February through April) and covering two expected spawning periods. During this interval, none of the selected pairs spawned. However, the stock tank from which pairs were derived (i.e., external control) also failed to spawn during this period, likely attributed to the stress of handling and movement.

A second experiment using experimental stocks allocated into twelve 5 m^3 tanks at densities of 2, 4 and 8 fish with equal number of male and females is currently under progress. Fish will be implanted with LHRHa implants (100 ug/kg) and monitored for egg production in the tank effluent.

A final approach using hCG injection and strip-spawning will be adopted as the last resort. These trials were scheduled under the end of year 3 activities and in the beginning of the "Reproduction and Selective Breeding of Pacific threadfin" year 1 activities and will be complete prior to adoption for pair spawning of select and control stocks in late January 2003.

Objective 5: *Domesticate Pacific threadfin for aquaculture and produce a selected line for increased growth.*

The Pacific threadfin genetic selection project is by necessity a multi-year project due to the time requirement necessary to domesticate stocks, select animals and complete life cycles.

The first round of selection was completed in August 2001 from a post-growout population of 604 fish at approximately 6 months of age at a mean weight of 358g. From this population, 50 controls (mean weight of 378g) and 50 "select" fish (mean weight of 516g) were established and stocked into 25m^3 broodstock tanks for growth and sexual maturation.

January 2002 maturation checks (at about 12 months of age) revealed that in addition to maintaining significant size difference between groups (686g vs. 896g), 10 out of 47 control animals had reached the male stage of sexual maturity (Pacific threadfin are protandrous hermaphrodites) whereas 41 out of 50 growth selected animals have entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (730g vs. 980g) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of development with 12 males and 35 females while the control group was slower to develop reproductively with 12 fish remaining immature, 22 as males, and 13 advancing to the female stage.

This data also suggests that size, rather than environmental or behavioral conditions appear to be more important in determining the timing of sexual development and sex change in captive stocks of Pacific threadfin.

Somewhat surprisingly, despite the appearance of females in both the control and select populations, there has not been any spawning activity to date. The original project work plan had anticipated somewhat earlier sexual development and the initiation of natural spawning activity with the appearance of a significant number of female stage animals. This has forced us to abandon natural tank spawning activity as a source of seed stock and increase efforts on hormone induced, and possibly strip-spawning approaches to generate seed stock lines for selection evaluations.

Objective 6: *Confirm transfer of reliable fry production technologies to commercial hatcheries.*

A total of three threadfin hatchery runs and one milkfish run were carried out during the third year of project activities. Qualified farmers were asked to submit requests in advance of each run and the total available fingerlings were then distributed on a proportional basis.

Impacts

The overall project goal of assisting in the development of a sustainable marine food fish industry has clearly been met. Some of the specific accomplishments include:

- An emerging marine food fish industry is now present in Hawaii with both threadfin and milkfish farms having been established under CTSA assistance.
- During the 3-year project, almost 700,000 fingerling threadfin and 170,000 fingerling milkfish were distributed to over 30 farms on the islands of Oahu, Maui, Molokai, and Hawaii to meet industry needs.
- Developments in hatchery technology (now transferred to industry) have enabled the recent establishment of a commercial cage culture operation with production capacities of over 1 million threadfin per annum.
- Captive farmed products now appear in local restaurants, retail markets, and sales are continuing to grow both on the mainland and in international markets. Health assurance and disease monitoring programs have been initiated.
- Industry has begun to examine other marine food fish species in efforts to diversify the market and broaden economic expansion in the Islands.

CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments of new project funding will further assist in securing requisite fingerling supplies to meet the needs of both on-shore and offshore production and support industry diversification.

Recommended Follow-up Activities

The establishment of the Marine Food Fish Industry in Hawaii has opened the door to broad suites of economic opportunity that will not only provide a new source of revenue to the islands, but does so in a manner that is both environmentally and socially appropriate. However, it is important to note that the industry is still very early in its development and will need continued support and nurturing to ensure long-term viability and profitability. Some areas for recommended follow-up include:

- Establish domesticated disease-free stocks with selection for performance-enhancing traits to establish and maintain industry competitiveness.
- Examine production efficiencies at all stages of the production cycle including hatchery, nursery, transport, and growout technologies to allow farmers to increase productivity from available facilities and labor resources.
- Implement water re-circulation technologies to reduce water consumption, gain better control over water quality, and reduce effluent discharge. It is important that the aquaculture industry become good stewards of the environment.
- Implement quality assurance measures for farmed products, to ensure consumer confidence. This is to include research to optimize physical traits (morphologically normal) and nutritional quality of Hawaiian farmed marine fish.

- With industry growth, species diversification and intensification, will also come increasing challenge from diseases. Other areas (and sectors) have been hard hit by catastrophic disease outbreaks and we should be proactive in the areas of disease detection, disease prevention, and treatment. Included in this category is the need to develop, certify, and maintain disease-free broodstock and strict disease prevention protocols statewide.
- Continue development of both on-shore and offshore growout technologies with emphasis on greater intensification. Both approaches to industrial scale-up should be investigated and evaluated since either has benefits and problems and it is likely that both approaches will be part of aquaculture development in the Islands.

Publications in Print, Manuscripts and Papers Presented

Publications in Print (Year 1)

Martinez-Cortero, F.J., P.-S. Leung, A.C. Ostrowski and M.D. Chambers. 2001 Profitability analysis of the commercial growout of moi (*Polydactylus sexfilis*) in Hawaii under different production systems. Journal of Aquaculture in the Tropics 16:101-112.

Manuscripts (Year 1)

Kam, L.M., P.-S. Leung, A.C. Ostrowski and A. Molnar, in press. Economics of a moi hatchery in Hawaii. Journal of the World Aquaculture Society 33(4):xxxxx. (pages not yet designated).

Publications in Print (Year 2)

Kam, L.E.Y.W., P.-S. Leung, A.C. Ostrowski and A. Molnar. 2001. Economics of a Pacific threadfin (*Polydactylus sexfilis*) Hatchery in Hawaii. Center for Tropical and Subtropical Aquaculture. Publication no. 146. 50 pp.

Ostrowski, A.C. and A. Molnar. 1998. Pacific Threadfin *Polydactylus sexfilis* (Moi) Hatchery Manual. Center for Tropical and Subtropical Aquaculture. Publication no. 132. 96 pp.

Publications in Print (Year 3)

None