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# Improved Stocks and Management Practices for Commercial Tilapia Culture in Hawaii and the Pacific Region — Hawaii Component

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## General Information

*Reporting Period*                      September 1, 2005–August 31, 2006 (final report)

*Funding Level*                      Year            Amount  
  1                \$53,950

*Participants*

**James P. Szyper**, Ph.D., Extension Specialist  
Sea Grant College Program, University of Hawaii at Manoa

Kevin D. Hopkins, Ph.D., Interim Director and Professor  
Pacific Aquaculture and Coastal Resources Center, University of Hawaii at Hilo

Cedric Muir, Ph.D.  
College of Natural Sciences, University of Hawaii at Hilo

Clyde S. Tamaru, Ph.D., Extension Specialist  
Sea Grant College Program, University of Hawaii at Manoa

## Objectives

The overall goals of this project are to enhance commercial tilapia production in Hawaii by establishing tested breeding stocks, producing fingerlings and comparing their growth with local stocks, and demonstrating breeding and production management protocols that are widely communicated. Most of the project activities take place at the University of Hawaii at Hilo (UHH) aquaculture program's farm facility.

1. To establish a tilapia quarantine and project working area at the UHH aquaculture program facility.
2. To obtain permits and import:
  - a. fingerling blue tilapia, *Oreochromis aureus* (a state-permitted species on list C: permitted for commerce) from a suitable commercial farm source that will guarantee disease-free stock.
  - b. fingerling Nile tilapia, *Oreochromis niloticus* (recently approved for list A: restricted, research) from a similar commercial source.
3. To quarantine the imported stocks (both species, if permits are granted) pending examination, and to obtain certification of disease-free status by the state fish health management program.
4. To grow and mature breeding stocks from the imported population(s), both species if permits are granted.
5. To produce offspring from the new breeder stocks of *O. aureus*, and to use them to institute a breeding management program.
6. To obtain three existing commercial tilapia stocks from within the state, and to perform the first growth comparison trial between in-state and imported stocks (both species if permits are granted).
7. To characterize the imported (both species) and comparison stocks for genetic identification by microsatellite DNA markers.
8. To communicate the first year's results (broodstock management protocols, growth comparisons, draft of good management practices) in public workshops and in widely accessible written materials.

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## Principal Accomplishments

***Objective 1: To establish a tilapia quarantine and project working area at the UHH aquaculture program facility.***

We established a tilapia project working area at the farm, including shared space in the laboratory and greenhouse. We outfitted four outdoor tanks of 5.5 m (18 ft.) in diameter with shade cloth covers for moderation of diel temperature cycles, supported by pipe frames of geodesic dome design. We built four floating net pens for use within the tanks for separating groups of fish. This facility was informally examined by members of the UH Manoa Institutional Animal Care and Use Committee (IACUC) during a Big Island visit. The committee commented that the facility looked good and appropriate. Student assistants from UH Hilo have been hired and trained in maintenance and record keeping.

***Objective 2a: To obtain permits and import fingerling blue tilapia, *Oreochromis aureus* (a state-permitted species on list C: permitted for commerce) from a suitable commercial farm source that will guarantee disease-free stock.***

We obtained a state import permit for juvenile blue tilapia *Oreochromis aureus* and imported a stock of about 750 juveniles of about 5 g weight from a fish farm in Florida. We chose to work with *O. aureus*, because it is a long-standing good choice for culture, but more important, because it is specifically permitted for importation in Hawaii and, thus, raises no impediment to work progress. At present, however, there is little interest in or demand for this species in the United States, and it is now somewhat rare. Most hatcheries and production facilities are focused either on pure *O. niloticus*, the world's preferred and best-researched species, or on a few particular hybrids. We encountered some difficulty in arranging a shipment. The first supplier arranged was unable to perform after some delay; another was found and the successful shipment made.

***Objective 2b: To obtain permits and import fingerling Nile tilapia, *Oreochromis niloticus* (recently approved for list A: restricted, research) from a similar commercial source.***

We have prepared and submitted a permit application for importation of *O. niloticus*. The permit is in process by the state Department of Agriculture's Plant Quarantine Branch, but it has not been issued as of date of this report.

***Objective 3: To quarantine the imported stocks (both species, if permits are granted) pending examination, and to obtain certification of disease-free status by the state fish health management program.***

We submitted and had approved a required animal care protocol to the appropriate UH organization (IACUC). The time required for revision of the protocol in response to numerous requests for changes created a minor delay in ordering the

fish from out of state. The stock was quarantined at the UHH farm in the facility described previously.

A disease screening program was devised in collaboration with the state Aquaculture Development Program Disease Prevention (ADP-DP) laboratory, as specified in the project proposal. A sample of 22 fish was shipped alive to the ADP-DP laboratory at the Anuenue Fisheries Research Center at Sand Island, Oahu. All fish arrived alive, were examined alive, and then sacrificed for various analyses. The report of results closes with a statement that "... it is our assessment that these representative fish are free of clinical disease" (see the full letter as an appendix at the end of this report).

**Objective 4: To grow and mature breeding stocks from the imported population(s), both species if permits are granted.**

The *O. aureus* stock has suffered little mortality and showed immediate increases in weight at approximately monthly sampling intervals. Growth rates were slow compared with our expectations, because of the cool well water in the refurbished UHH facility. Despite some moderation of temperature cycles by the tank coverings, the winter and spring in Hilo were also unusually cool. Summer temperatures were normal, and the original stock of *O. aureus* has now grown to the age and size of first reproduction, which in this case, was between 100 and 200 g/fish.

**Objective 5: To produce offspring from the new breeder stocks of *O. aureus*, and to use them to institute a breeding management program.**

We divided stock among three cylindrical, plastic outdoor tanks of 5.5 m (18 ft.) in diameter. All three groups had begun to produce fry by the end of the project year. Fry from the three groups are to be kept as families, which will be cross bred and assessed for genetic change during Year 2 as part of a breeding demonstration program.

**Objective 6: To obtain three existing commercial tilapia stocks from within the state, and to perform the first growth comparison trial between in-state and imported stocks (both species if permits are granted).**

Several potential commercial stocks were identified and collaborative work has begun to be arranged for the proposed growth comparisons, which will begin early in Year 2.

**Objective 7: To characterize the imported (both species) and comparison stocks for genetic identification by microsatellite DNA markers.**

Support was established for a UH Hilo graduate student in the UHH laboratory of Cedric Muir. Supplies were ordered in support of the collaborative work (genetic characterization of fish stocks) with this lab, and arrangements and accounts for analytical services at UH Manoa have been established.

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Initial work established methods and markers in the *O. aureus* stock that are useful for comparison of the original stock to later generations and to other stocks.

Work has begun on two stocks, as specified in the proposal. The long-held stock of tilapia at the UHH farm (an unspecified hybrid of *Oreochromis* species, long inbred) was sampled for analysis by taking small fin clips from 30 individuals, all of which survived and were returned to the population. This stock was then eliminated from the farm, mainly by donation to interested parties, to preclude unintentional hybridization with the new stocks. This was done before the importation of the new *O. aureus* stock. Similarly, 30 of the *O. aureus* stock were sampled by fin clipping; all survived and were returned to the population.

Nuclear DNA was extracted from each individual's tissue and sent for analysis of several microsatellite loci using labeled primers and a Beckman CEQ-8000 sequencer. All 30 of the *O. aureus* are homozygous at two of the microsatellite loci examined with two of the several primer sets developed for tilapia by Dr. Thomas Kocher. Samples from two individual fish were sequenced for one of the loci and found to be monomorphic (indistinguishable), having an equal number of tandem repeats. These results indicate a potentially useful degree of uniformity among the 30 individual fish sampled, and, therefore, a statistical indication of uniformity among the stock itself. It cannot be known without sampling other stocks, whether these analyses will permit ready distinction among stocks.

As a second approach to characterization/distinction, a 690 base pair region of mitochondrial DNA, locus cytochrome oxidase II (COII), was sequenced for 10 individual fish from the old farm stock and 10 of the *O. aureus* stock. Individuals of the two stocks uniformly differed at 41 sites (base pairs) and were identical at the remainder of the 690. This suggests, but does not confirm without further comparisons (to be done during Year 2), that this analysis will be effective for distinguishing stocks and populations.

***Objective 8: To communicate the first year's results (broodstock management protocols, growth comparisons, draft of good management practices) in public workshops and in widely accessible written materials.***

The animal care protocol approved by the UHM IACUC will be part of the stock management protocols to be communicated. No other progress has been made on this objective.

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## Impacts

The importation of the *O. aureus* stock provides a recent precedent and an example of a successful permit application, which will facilitate future permit applications by farmers. The same will be true for the *O. niloticus* permit and importation action during project Year 2. The production of fry by the imported stock during Year 1 makes possible the proposed growth comparison trials for Year 2, which will allow farmers to evaluate the status and quality of their existing stocks. They will be able to make decisions about renewal of stocks or expansion of their old ones, which in turn will lead to continued or increased profitability. This impact is difficult to quantify without detailed production and market data, but, since tilapia is the state's food fish of greatest production and sales, the potential benefit is significant.

The successful establishment of genetic analyses that will be useful for distinguishing tilapia stocks and populations will similarly contribute to farmers' ability to evaluate their stocks and potential actions to expand or upgrade them. The developing stock management protocol will facilitate the maintenance of stock quality into the future.

When this project is completed, producers will have improved production from better stocks, and will be spared the time and expense of sorting out the possibilities and arranging access. The industry will be more sustainable with research-based protocols for importation, quarantine, and transfer (minimizing disease import and transmission), and for maintenance of the genetic quality of breeders. Entry into the business of supplying fingerlings to other producers will be encouraged by the experimental results, the protocols, and their demonstration, as well as by the active dissemination of this information. Potential new entrepreneurs will have enhanced information available for consideration.

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## Recommended Follow-Up Activities

Year 2 work will include execution and genetic analysis of the effect of the proposed stock management protocol. Results of growth comparison trials will be obtained and communicated.

We expect to extend and complete further genetic comparisons. We are emphasizing growth comparison trials at the time of this report; we will pursue distribution of small numbers of stock to interested parties as the project progresses. We will pursue growth comparisons and genetic analyses with Nile tilapia when the import permit is granted and a stock is obtained from out of state.

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

None to date.

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# Improved Stocks and Management Practices for Commercial Tilapia Culture in Hawaii and the Pacific Region—Guam Component

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## General Information

*Reporting Period*                      March 1, 2007–September 30, 2007

*Funding Level*                         \$36,050

*Participants*                         **David P. Crisostomo**, Extension Agent and Program Leader  
Cooperative Extension Service, University of Guam

**Note:** This report on the Guam component was submitted by John W. Brown, Ph.D., researcher at the Guam Aquaculture Development and Training Center (GADTC) of the University of Guam, who began overseeing completion of this component's work plan once Crisostomo retired on September 28, 2007.

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## Objectives

1. Produce offspring from breeder stocks developed during Year 2 work of the previously funded tilapia project (two best types for reproductive capacity), and institute a breeding management program to preserve their genetic quality into future generations. It may be necessary to import additional broodstock of these two types. This breeder management program will be analogous to that for Year 1 of the Hawaii component, with modification for Guam site conditions.

2. Send samples of fish from each of the lines established for breeder management to Hawaii for genetic testing.
3. Distribute modest numbers of fingerlings to the community for examination and testing on farm sites, subject to protocols and local shared agreements to avoid interference with markets.
4. Evaluate and improve production protocols established in Year 2 of the previous CTSA tilapia project.

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## Anticipated Benefits

The more rapid growth rates of improved strains of tilapia will provide farmers a choice of either harvesting their fish more quickly or growing fish to a larger size before marketing them. Either choice will mean increased productivity from existing facilities and improved profitability for local farmers.

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## Work Progress and Principal Accomplishments

The Guam principle investigator, David Crisostomo, retired unexpectedly at the end of September 2007, and management of the project was locally assigned to University of Guam researcher John W. Brown, Ph.D., pending formal approval by CTSA. Two limited-term employees on the project have remained in place, and they have continued to manage day-to-day operation of this project while Dr. Brown familiarizes himself with this project. The transition between principal investigators has been relatively smooth, and it has not created extra challenges.

***Objective 1. Produce offspring from breeder stocks developed during Year 2 work of the previously funded tilapia project (two best types for reproductive capacity), and institute a breeding management program to preserve their genetic quality into future generations. It may be necessary to import additional broodstock of these two types. This breeder management program will be analogous to that for Year 1 of the Hawaii component, with modification for Guam site conditions.***

We have the Philippine origin fish in two small raceways. One raceway contains a mixture of the Philippine Select strain and the GIFT strain. There are 45 males and 150 females in this raceway. The second raceway contains only the GIFT strain. There are 30 males and 100 females in the raceway. The Chitralada strain is in a large, 200-m<sup>2</sup> raceway. There are 387 females and 120 males. The breeding lines have not yet been established nor has the formal protocol been put in place.

***Objective 2. Send samples of fish from each of the lines established for breeder management to Hawaii for genetic testing.***

The Hawaii component's principal investigator, James Szyper, was contacted to establish the status of this objective. His response was "...analysis of Guam fish can't happen any longer, because of the time discrepancy between the two projects."

**Objective 3. Distribute modest numbers of fingerlings to the community for examination and testing on farm sites, subject to protocols and local shared agreements to avoid interference with markets.**

We have provided several farmers on Guam and Saipan with small numbers of tilapia fry.

**Objective 4. Evaluate and improve production protocols established in Year 2 of the previous CTSA tilapia project.**

The raceway system is working well, and the two lines are producing substantial number of eggs on a weekly basis. Numbers are summarized in Table 1 and Table 2 at the end of this report. It appears that the percentage of females producing eggs each week is higher for the GIFT/Philippine Select lines than for the Chitralada line. However, the average number of eggs per female with eggs is roughly similar. This result would seem to indicate that the fish of Philippine origin will produce a larger number of eggs for an equal size stock of spawners.

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## Work Planned

We will design and implement breeding protocols to preserve the genetic benefits of improved lines during the remainder of this project. We will investigate alternatives to sending fish to Hawaii for genetic testing. We continue to distribute tilapia fry to local farmers. We will continue to refine the raceway system used for egg production and hatchery rearing of the fry.

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## Impacts

Local farmers are beginning to utilize our locally produced fry, and they now have access to fry in the winter months when tilapia fry have not been normally available from Taiwan. Also, decreased imports of fry will reduce the risk of introduction of non-native species and diseases.

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## Publications in Print, Manuscripts, and Papers Presented

Crisostomo, David. 2007. Tilapia variety evaluation and hatchery expansion for local fry production in Guam. *CTSA Regional Notes* 18(3):4–5.

## Appendices

TABLE 1. The Chitralada tilapia stock is one of two lines in the Guam component that are producing a substantial number of eggs on a weekly basis.

### CHITRALADA 387 FEMALES

Week #	Week of	females	percent	eggs	eggs/female
1	12-Aug-07	29	7.49	8,850	305
2	19-Aug-07	39	10.08	8,550	219
3	26-Aug-07	76	19.64	24,450	322
4	02-Sep-07	61	15.76	23,361	383
5	09-Sep-07	75	19.38	28,461	379
6	16-Sep-07	72	18.60	26,805	372
7	23-Sep-07	62	16.02	20,177	325
8	30-Sep-07	73	18.86	33,122	454
9	07-Oct-07	49	12.66	17,978	367
10	14-Oct-07	68	17.57	34,027	500
11	21-Oct-07	70	18.09	35,244	503

TABLE 2. The GIFT/Philippine tilapia stock is one of two lines in the Guam component that are producing a substantial number of eggs on a weekly basis.

### GIFT & PHILIPPINE SELECTS 250 FEMALES

Week #	Week of	females	percent	eggs	eggs/female
1	12-Aug-07				
2	19-Aug-07				
3	26-Aug-07	5	2.00	1,200	240
4	02-Sep-07	7	2.80	4,200	600
5	09-Sep-07	10	4.00	5,373	537
6	16-Sep-07	15	6.00	8,605	574
7	23-Sep-07	22	8.80	6,238	284
8	30-Sep-07	62	24.80	28,050	452
9	07-Oct-07	22	8.80	7,150	325
10	14-Oct-07	78	31.20	24,961	320
11	21-Oct-07	69	27.60	33,133	480
12	28-Oct-07	92	36.80	35,861	390