Improved Stocks and Management Practices for Commercial Tilapia Culture in Hawaii and the Pacific Region—Hawaii Component, Year 2

General Information

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Funding Level

Year	Amount
H1	$53,950
H2	**$52,730**
TOTAL	$106,680

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 (PACRC)
University of Hawaii at Hilo

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

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

Objectives

The overall goal of the Hawaii component is to enhance opportunity and options for commercial tilapia production in Hawaii by examining newly imported pure stocks, comparing them with some existing stocks, and drawing conclusions about possibilities and prospects for improvements to production and profitability.

1. Complete the first growth comparison trials, begun in Hawaii Year 1 (Objective 6 in Year 1), with extension to approx. 400 g final fish size. Also, send fingerling *O. aureus* to Oahu for comparison trials there.
2. Obtain at least two additional in-state stocks, and perform further experimental comparisons of growth potential.
3. Distribute fingerlings of imported *O. aureus* stocks (*O. niloticus* is not permitted for distribution from the farm) to interested businesses for on site testing, under terms to be established with community input, avoiding interference with markets.
4. Integrate the work on importation, quarantine, growth comparisons, and stock maintenance protocols into a manual of best management practices, and offer public workshops including this information during the last half year of the project.

Anticipated Benefits

Hawaii

This project will address constraints to commercial tilapia production and business profitability by making experimental determinations of the growth potential of selected local stocks compared with one or more disease-free, permitted imports on a secure university site. Genetic analyses will be used to characterize the stocks and provide a baseline for further comparisons, including detection of changes, if any, in stocks under breeding management. Results of these relatively straightforward determinations will be used to address the hypotheses that Hawaii's demonstrably introgressed stocks (Szyper et al. 2000) have detectable genetic differences from good commercially available stocks and demonstrable disadvantages in production qualities, such as growth rate in production systems and survival rates of offspring. Another hypothesis to be tested is that the modest-cost breeder management program will result in much smaller genetic differences during the term of the project than the differences among stocks (some of which have been documented in Szyper et al. (2000) at the beginning. This project also will offer modest numbers of fingerlings to the community during the two-year term of this project, subject to protocols and agreements to avoid interference with markets and subject to quarantine and transfer controls with the assistance of the Hawaii state fish health management program.

Work Progress and Principal Accomplishments

Objective 1. Complete the first growth comparison trials, begun in Hawaii Year 1 (Objective 6 in Year 1), with extension to approx. 400 g final fish size. Also, send fingerling O. aureus to Oahu for comparison trials there.

Objective 2. Obtain at least two additional in-state stocks and to perform further experimental comparisons of growth potential.

Progress reporting for Objectives 1 and 2 are combined here. We have performed two growth comparison trials on the island of Hawaii at the University of Hawaii at Hilo (UHH) aquaculture facility, and one on Oahu at the Windward Community College facility. These trials compared short-term growth performance of tilapia stocks obtained from farmers with that of this project's imported stock of blue tilapia, *Oreochromis aureus*, which we imported during Year 1. Results of those growth comparison trials are detailed in Appendix A of this report.

Results of these experiments do not support the hypothesis that existing Hawaii farm stocks are characterized by suboptimal growth. Three farm stocks grew equally or more rapidly than the imported *O. aureus*. However, neither do these brief results establish that no advantage can be gained for Hawaii business by use of better stocks. It remains possible that the improved strains of Nile tilapia, which outperform the unselected stocks of that species (Eknath et al. 1993, Ridha 2006), would do better here.

Two observations, discussed in Appendix A, suggest that the imported stocks adapted to Hawaii conditions after a time and then performed more normally, similar to general expectations and similar to currently produced local farm stock. Collaborating farmers have informed us that they are in general satisfied with the performance of their stocks but would be open to trying stocks that might perform better. Greater growth rates create the potential for greater profits. For example, among other things, reducing production costs, such as utilities and labor through time, associated with bringing batches of fish to a target market size could increase farm profits.

Note: These trials were conducted with the second-priority species for the project. The *O. aureus* were named in the proposal and imported so that work could proceed if there were delays in obtaining a state importation permit for the first priority species, the Nile tilapia, *Oreochromis niloticus*. As of the date of this report, we have not yet received an import permit. Before the initiation of Year 1 of this project, *O. niloticus* was approved by the Hawaii state Board of Agriculture for list A: restricted, research. Our permit application was submitted during Year 1. Following the listing approval by the Board of Agriculture, the regulating agency prepared a major revision of the complete listing of permitted and restricted species. This revision was sent through a lengthy sequence of public hearings, followed by reflections among various government entities, including the governor's signature, with some abnormal delays reported during the process. We maintained contact and made frequent inquiries during this process but could not affect its timing. We have arranged a supplier for Nile tilapia, research colleagues at the University of Arizona. Working with the state aquatic animal health program and state aquatic veterinarian Dr. Allen Riggs, we have had that potential stock screened for its health status in Arizona, and we have communicated the negative test results to the Hawaii Deptartment of Agriculture Plant Quarantine Branch. We remain hopeful of receiving a permit with useful time remaining in this project.

Objective 3. Distribute fingerlings of imported* O. aureus *stocks (*O. niloticus *would not be permitted for distribution from the farm) to interested businesses for on-site testing, under terms to be established with community input, avoiding interference with markets.

We have made no distributions of our imported *O. aureus* stock. Because growth trials showed that this stock did not perform better than in-state stocks, we did not want to promote its adoption commercially. This outcome of this project's growth trials represents a well-conducted technical examination of the hypothesis that state stocks may have deteriorated in terms of growth. The evidence does not support that hypothesis. This outcome is encouraging because at least collaborating farmers may retain confidence in their stocks and because we have not yet been permitted to examine the best prospect, namely *O. niloticus*, for better performance in any case.

Objective 4. Integrate the work on importation, quarantine, growth comparisons and stock maintenance protocols, into a manual of Best Management Practices, and to offer public workshops including this information during the last half year of the project.

We have not been able to address this objective. This objective was a reasonable request of reviewers, and we agree that practical investigations of this sort should target such a product. However, because imported stock grew and reproduced slowly in the cold source water of the refurbished UHH farm, we produced only the F1 generation of offspring and could not examine further generations for effects of inbreeding and drift, or lack of same, as originally projected. Furthermore, because we do not have stocks of the priority species for improved production, protocols for management of the alternative species would be of limited commercial value. Although the two species are closely related by taxonomists based on external characters, they differ significantly in the genetics of their reproduction and might, therefore, show very different results of long-term breeder maintenance.

DNA analyses produced potentially valuable results, discussed in Appendix B. The two techniques used are state-of-the-art analyses capable of characterizing groups of fish and of distinguishing degrees of relationship among groups. Microsatellite analysis of nuclear DNA and sequencing of a selected subset of mitochondrial DNA showed that the imported stock was clearly distinguishable from the hybrid UHH farm stock that was held before and until this project began. Also, F1 offspring of the imported stock were for growth trials clearly distinguished from the hybrid provided by a farmer on the island of Hawaii.

Reference

Szyper, J. P., K. D. Hopkins, W. Malchow, and W. Y. Okamura. 2000. History and prospects of tilapia stocks in Hawaii, U.S.A. In *Tilapia Culture in the 21st Century—Proceedings from the Fifth International Symposium on Tilapia Aquaculture*, eds. K. Fitzsimmons and J. Carvalho Filho, pp. 663–672, Rio de Janeiro, Brazil.

Work Planned

Genetic analysis comparing the parental and F1 generations of imported stock is in progress and will be discussed in a later report. There is literature that describes use of these analyses with *O. niloticus* but apparently not with *O. aureus*. Therefore, the results are an original application.
Working group members are discussing production of a journal article on this work.

Because imported stock appears to have adapted to our conditions and grown normally and comparably to farm stocks after acclimation, we will consider sharing limited numbers with interested farmers during and after the remaining term of this project. In addition to attaining growth rates similar to a farm stock on Oahu (Appendix B), the holding population of imported stock has apparently grown more rapidly (not quantified) during its second year at the UHH farm.

Impacts

Hawaii

This project revealed that some local tilapia stocks perform as well or better than the imported stock in terms of growth as juveniles, a key finding that can spare farmers the time and expense of searching for improvement along the lines of new stocks, within the limitations of these results. It remains possible that access to improved stocks of Nile tilapia could be helpful, but measures that fall short of this presently unavailable option can be omitted.

In collaboration with the disease management program of the Hawaii Department of Agriculture's Aquaculture Development Program (ADP), we have established procedures for assessment and importation of tilapia stocks with proper quarantine and disease assessment procedures. This project outcome spares businesses the time and expense of developing a sequence of activities on their own, which in turn helps to prevent future losses to disease. These impacts are of practical value, but they are difficult to assess in economic terms because they deal in contingencies.

Analysis and interpretation of our genetic assessments of stocks, when it is completed, will have future value for the establishment and evaluation of broodstock management protocols and other purposes of fish stock comparisons. For example, they could help compare different potential sources of future imports or exports.

Publications in Print, Manuscripts, and Papers Presented

None to date.

Appendices

Appendix A. Tilapia Growth Comparison Trials

Overview. Three growth comparison trials were done during this project. All involved F1 juvenile offspring of the project's imported stock of *Oreochromis aureus*. It has been mentioned repeatedly in this and other project reports that this species served as the project's second-choice, "backup" organism. The preferred species is the Nile tilapia *O. niloticus*, but as of this reporting date, the state has not issued the importation permit. *O. aureus* was included in the project planning so that some work such as this could be done in the absence of a permitted importation of *O. niloticus.*

O. niloticus had been approved for listing as "Restricted A-Research" by the state Board of Agriculture before the project began, but various administrative delays in the permit process persist. We retain some hope of making the importation before the project's no-cost extension ends on February 28, 2008.

It has also been noted earlier that the imported stock of *O. aureus* grew and matured more slowly than had been expected, mainly because the University of Hawaii at Hilo (UHH) farm's new water supply, put into use shortly after initiation of this project, was quite cold for tilapia keeping in general. The necessity of tank maintenance by water flow-through kept the ambient temperature near and below 22°C most of the time. The imported stock grew slowly during its first year, maturing near individual weight near 300 g/fish, and producing fry by late August 2006 following its arrival as small fingerlings in November 2005. Once fry became available, the growth comparison trials were planned and carried out.

The purpose of the trials was to provide a partial test of a hypothesis implied in the project's original rationale, namely that existing farm stocks of tilapias in Hawaii may have suboptimal growth rates because stocks have been allowed to drift and inbreed for years, without active on-farm management. An earlier study (Szyper et al. 2000) discussed a survey of some Hawaii stocks and analysis of characteristics (not including growth) related to inbreeding and genetic drift.

Summary Results. The results of these experiments do not support a hypothesis that existing Hawaii farm stocks are characterized by suboptimal growth. Three farm stocks grew equally or more rapidly than the imported *O. aureus*. However, neither do these brief results establish that no advantage can be gained for Hawaii business by use of better stocks. It remains possible that the improved strains of Nile tilapia, which outperform the unselected stocks of that species (Eknath et al. 1993, Ridha 2006), would do better here.

We note that our imported stock did not come with any claim to selective advantage in growth; rather the concept for comparison is that the stock is presented as a commercial-farm-managed, presumably relatively pure strain that has not been subjected to the conditions of unmanaged propagation presumed for Hawaii in the hypothesis under discussion. Furthermore, although the growth experiments were well conducted, the results may well have been compromised in part by the cold water conditions. Despite the reputed relative cold tolerance of *O. aureus* , the temperatures were still low for tilapias in general.

Two observations, discussed below, suggest that the imported stocks adapted to Hawaii conditions after a time, and then performed more normally, similar to general expectations and similar to an actively produced local farm stock.

Trial Descriptions and Results. Trials I and II were done at the UHH aquaculture facility at the agriculture farm in the Panaewa area near Hilo; Trial III was done at Windward Community College on Oahu by Clyde Tamaru and Kathleen McGovern-Hopkins. All involved F1 juvenile offspring of the project's imported stock of *Oreochromis aureus*. All consisted of a direct growth comparison of F1 generation fingerling juveniles of the imported stock, labeled UHHF1, with stocks obtained from collaborating local farmers. Table 1 (Appendix A) describes the conditions of the three trials. Fish were fed approximately ad-lib amounts of feed (presumed by observation of leftovers), in one or two feedings per day. Therefore, no estimates of FCR can be made. Water quality was maintained by flow-through in tanks containing low biomass densities, with aeration provided.

In Trial I, fry grew from about 0.2 to 0.6 g/fish during 30 days (Figure 1, Appendix A). The UHHF1 fish began slightly heavier than farm stock A (FSA, a long-held stock of presumed *O. aureus*). There was no significant difference between the two stock treatments in weight gained during the trial ($P >> 0.05$). The mean growth rate of 0.014 g/fish/day is only marginally less than the 0.018 figure achieved by the "unselected" Nile tilapia fry grown at 29°C in Kuwait by Ridha (2006), in which study the "improved" strains grew 30–60% more rapidly. Survival among the 6 tanks cohorts was highly variable, with a mean of 61%. Young fry in general suffer greater mortality than older juveniles, and a few were lost during weekly weighings and tank transfers.

In Trial II, stock UHHF1 and farm stock B (FSB, a long-held stock that resembled *O. aureus*, from a different farmer) began at about 5 g/fish, and grew scarcely at all (making up small losses during the first 2 weeks) during 35 days, in four of the same six tanks used for Trial I, under nearly-identical conditions (Figure 2, Appendix A). The fish reacted to feed offered. Average survival was 96%. We have no explanation for these results other than the cold temperatures of 21–22°C. The UHHF1, held in a 3 m diameter tank in the UHH greenhouse after the experiments, grew slowly during the subsequent months to 300 g and more but did not produce fry.

In Trial III on Oahu, stock UHHF1 grew substantially more slowly than farm stock "Mokuleia," a hybrid resembling *O. aureus*, during a 65 day period in warmer water of about 25°C, with both stocks beginning at a size of about 4 g/fish. UHHF1 fingerlings grew at a rate of 0.067 g/fish/day, while the Mokuleia fish grew at 0.30 g/fish/day, exceeding the warm water rate recorded in Kuwait by Ridha (2006) for "unselected" Nile tilapia (and again the selected strains well exceeded these rates). Survival was high in Trial III, as it was in Trial II.

Trial III continued for 45 more days in the same tanks, with the UHHF1 growing more rapidly (0.62 g/fish/day), though still lagging the other group (1.09 g/fish/day). This indicates adaptation by the UHHF1 to the warmer temperatures and/or other conditions. It must also be noted that the fish began at larger individual weights for this period (7 and 20 g/fish, respectively), that larger absolute daily gains are therefore to be expected, and that these results are no longer comparable to the cited warm water study of shorter duration.

At the end of the replicated trial at 110 days, the 30 largest fish from each group were placed into separate larger tanks, and fed for 90 days more. During this period, the UHHF1 grew nearly as rapidly as the Mokuleia fish, the rates being 1.22 and 1.26 g/fish/day, respectively. The complete 200 day trial period is represented in Figure 3.

Conclusions. These trials, while they do not constitute an exhaustive test of the project's underlying hypothesis about Hawaii stocks, do deny that hypothesis by counterexample. No farm stock grew more slowly than the imported stock, and in the Oahu experiment, much more rapidly at first, and equally in the later period. However, because the imported stock was not of the world's preferred and most developed species, it remains possible, in fact, likely, that Hawaii tilapia farmers would benefit from access to the selected strains of Nile tilapia that are referenced briefly in literature here. These stocks perform in general 50% better, sometimes more so, than unselected Nile tilapia stocks. The project hopes to import Nile tilapia before its expiration date, and the working group will consider arranging further growth comparisons outside this project.

Appendix B. Tilapia Stock Identification and Comparison by DNA Analyses

Overview. One of this project's goals was to develop a broodstock management protocol to minimize potential effects of inbreeding and genetic drift during extended holding of fish derived from a single source. This goal, possibly overly ambitious from the start, has been compromised by the small delays in permitting and importation of the first stock, and by the slow growth and reproduction of that stock. Multiple generations of offspring were to be analyzed for genetic comparisons, but only the F1 generation has been produced.

We have, however, established and conducted DNA analyses of four fish stocks: the imported stock of *O. aureus*, its first-generation offspring (F1), and two other tilapia stocks, one from a collaborating farmer (stock FSB as discussed regarding growth trials), and finally the long-held hybrid stock at the UHH farm, which was eliminated from that facility before the importation of new stock for this project. Analyses were conducted by the laboratory of project working group member Cedric Muir of the UHH biology department. Project funds supported materials, supplies, and collaborating UH laboratory fees, as well as the stipend of graduate student Laura Crane, who participated in fish sampling and performed most of the UHH laboratory work. Some of the text of this section is taken from reports and background provided by Muir and Crane, without specific attribution; principal investigator, James Szyper, is nonetheless responsible for the substance of all statements in this report regarding the Hawaii component of this project.

Analyses completed to date constitute an original characterization and comparison of this set of stocks by these techniques. We are discussing preparation of a journal article describing these results. As noted, we still hope to import *O. niloticus* if we receive our state permit with sufficient time remaining in the project period.

Summary Results. Results demonstrated, as would be expected, that these techniques readily distinguished the imported *O. aureus* stock from the old UHH farm stock. Similarly, the F1 offspring of the imported stock can clearly be distinguished from the collaborating farmer's stock FSB. A comparison of the imported stock with its F1 offspring is in progress and will be completed by the end

of this project. The sampling and analytical techniques chosen and developed during this project have detected small differences among these stocks, which means that they would be effective in tracking changes in seed produced by long-held breeder groups, as originally envisioned for this project.

Work done under this project appears to be an original characterization for *O. aureus*; there are literature cases of similar work on *O. niloticus*.

Sampling and Analytical Methods. DNA was extracted from small scissor clippings (approximate triangles about 5 mm on a side) from the caudal fins of anesthetized fingerling tilapias. Samples were taken at the UHH farm; preparation of materials and analyses were performed at UHH and UHM laboratories.

Two approaches were used for this work. Microsatellite analysis examines regions of an organism's chromosomal DNA (microsatellites) that appear not to be involved in coding for life processes but rather contain repeated short sequences of nucleotide bases, which are annotated by the initials designating bases, for example "CA." The number of repeats carried by an individual in a target region of its DNA is subject to mutation and varies among individuals and groups of individuals. Research has identified such regions of DNA strands likely to be useful for distinguishing relationship with particular organisms, including tilapias. The small amount of DNA contained in tissue samples is amplified (replicated to produce amounts large enough for handling) by PCR, using primers (prepared DNA sequences) that direct the process to the proper regions. The primers used for this work targeted known DNA regions that have been developed by others to distinguish differences among tilapia individuals and groups.

The Year 1 final report (published in the 2006 Accomplishment Report) of this project stated, "All 30 of the (originally imported) *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." However, this statement is conservative and useful capability to distinguish stocks is very likely. As noted above, this stock was clearly distinguishable from the old (hybrid, very different) UHH stock in these terms.

The second technique was sequencing a portion of mitochondrial DNA to look for differences among individuals and groups in single nucleotides along the sequence. As with the other technique, PCR was used to amplify the targeted DNA sequence, in this case, a locus labeled COII. This locus has been used in hundreds of studies of fishes, including Nile tilapia. From the Year 1 final report: "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." A similar clear distinction was found between the F1 offspring of the imported stock and the (hybrid, very different) FSB. Comparison of the parental

imported stock and the F1 offspring in terms of this analysis will be informative with regard to the statistical ability (and sampling necessities) for distinction among closely related stocks. This work is in progress and will be completed by the end of this project.

Conclusions. The collaboration with UHH for these analyses established effective sampling and analytical procedures to distinguish among tilapia stocks for purposes of this project. Some of the results are original for the stocks used, and a journal publication is being planned. This system is a powerful set of techniques for both the project's stated purposes and others. As noted, this project has been limited by the slow progression of generations and by delays in permitting for the primary target fish species.

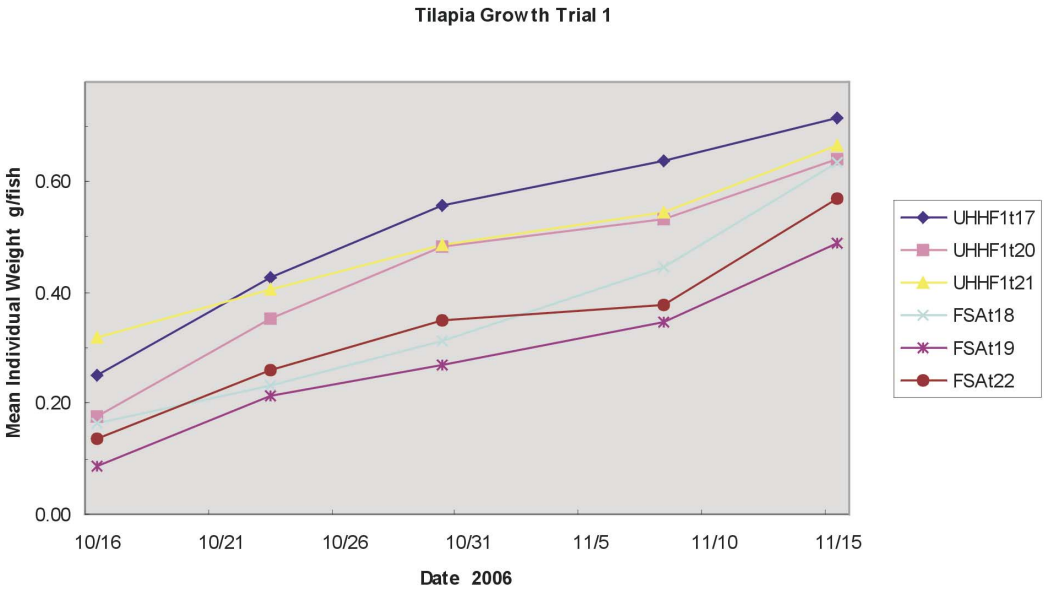

FIGURE 1. Growth of juvenile tilapia during Growth Trial I at the University of Hawaii at Hilo farm. The project-imported stock (UHHF1) is represented by groups of fish held in three tanks (t17, t20, t21), and the compared farmer's stock A (FSA) is represented by groups held in three tanks (t18, t19, t22).

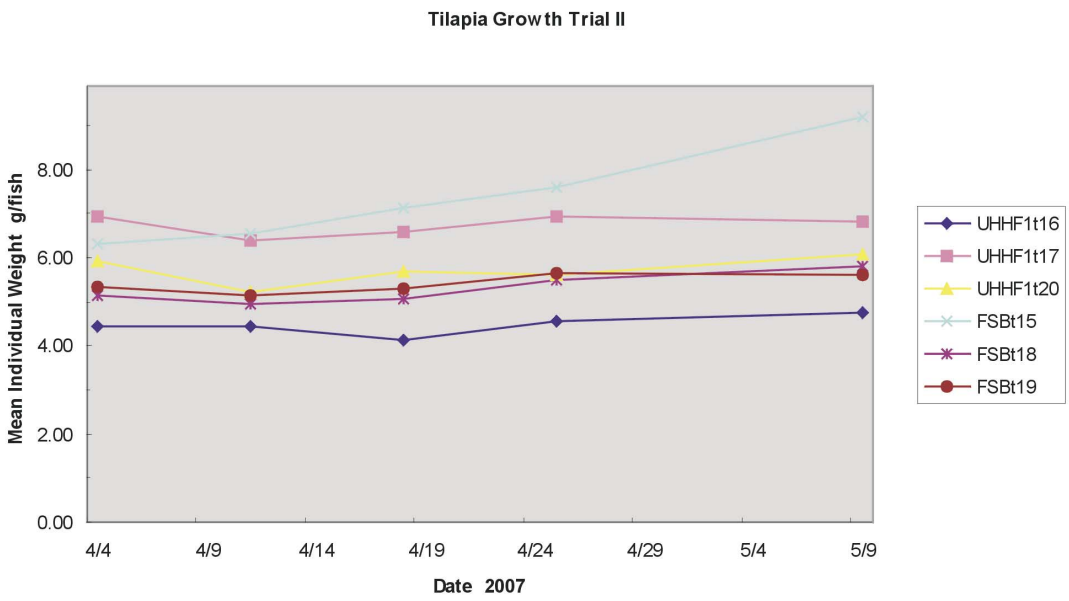

FIGURE 2. Growth of juvenile tilapia during Growth Trial II at the UHH farm. The project-imported stock (UHHF1) is represented by groups of fish held in three tanks (t16, t17, t20), and the compared farmer's stock B (FSB) is represented by groups held in three tanks (t15, t18, t19).

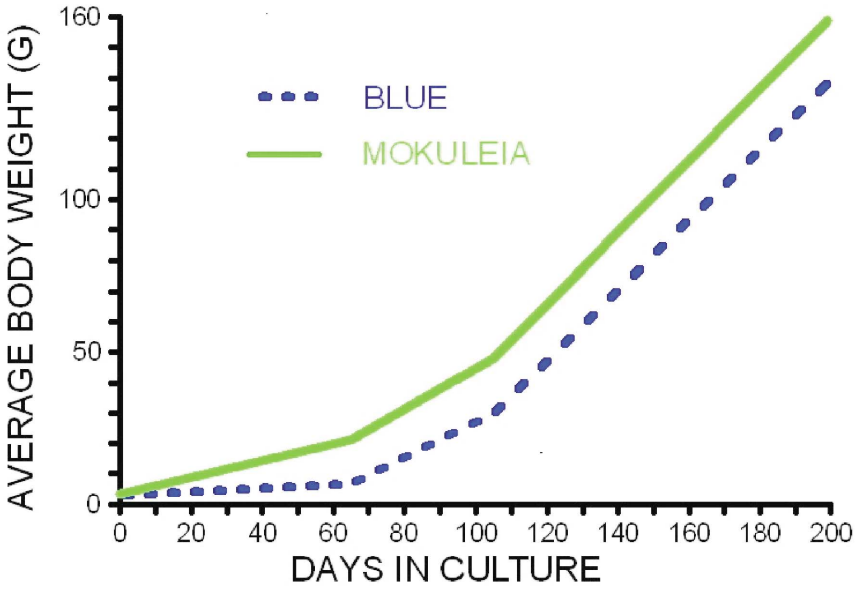

FIGURE 3. Growth of juvenile tilapia during Growth Trial III at Windward Community College on Oahu. The project-imported stock (UHHF1) is represented by groups of fish held in three tanks (combined and labeled BLUE), and the compared farmer's stock is represented by groups held in three tanks (combined and labeled MOKULEIA).