Amberjack Fingerling Production, Year 2

General Information

Reporting Period July 1, 2006–June 30, 2007 (final report)

Funding Level

Year	Amount
1	$100,000
2	**$100,000**
TOTAL	$200,000

Participants

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Objectives

1. Establish an expanded amberjack broodstock population and determine the optimal stocking density for long-term broodstock health and optimized egg production.
2. Resolve monogenean parasite infestations that are being experienced when amberjack are grown in unfiltered waters associated with open-ocean cages.
3. Conduct workshop and develop a fact sheet incorporating results of this project for rapid transfer to farmers in Hawaii and the Pacific region.

Principal Accomplishments

Objective 1: Establish an expanded amberjack broodstock population and determine the optimal stocking density for long-term broodstock health and optimized egg production.

In February 2007, an outbreak of *Cryptocaryon* wiped out the entire stock of kahala at the Oceanic Institute (OI). This event highlighted the importance of having a backup stock of brood fish and exemplified the importance of this research project.

Broodstock recruitment and conditioning. In collaboration with OI's industry partner, Kona Kona Blue Water Farms (KBWF), a total of 127 F1 fish were sent from KBWF in Kailua-Kona, Hawaii, to OI in Waimanalo, Oahu, between July and December 2006. Prior to shipping, the *kahala*, Hawaiian for the longfin amberjack (*Seriola rivoliana*), were transferred from open-ocean cages to a KBWF land-based site and treated for three weeks for ectoparasite removal. In preparation for shipping, the temperature of the holding water was slowly decreased prior to placement into large shipping bags that were filled with oxygen and chilled water. Fish were placed into large (90-L) picnic-style coolers, which were shipped via Commodity Forwarders Inc. to the Kona International Airport, and, again, from Honolulu International Airport to OI. Preliminary broodstock shipping trials demonstrated that, despite the large size and high metabolic rate of larger kahala broodstock, stocks could be successfully maintained in transport containers for about six hours. Actual success in shipping kahala broodstock proved highly variable, dependent upon cargo space availability with the airlines. Groups of approximately 15 fish (one fish/container) were shipped over a six-month period with on-time shipments yielding excellent survival, with only five mortalities directly attributed to shipping stressors. However, frequent airport delays (transport containers bumped to subsequent flights) in shipment led to very high rates of mortality. In total, 127 kahala (each about 2 kg) broodstock were successfully shipped over the project period, yet approximately 50 additional fish did not survive transport mainly due to transport delays.

Successfully transported stocks also experienced some early challenges, including intolerance to MS222 anesthesia during handling, development of eye pathologies, and repeated parasite outbreaks. Although MS222 anesthesia is the standard method for sedating and handling large marine fish stocks, kahala clearly do not respond well to this form of sedation, suggesting a need to examine alternative anesthetics such as clove oil. Stocks held at OI also experienced a high incidence of exophthalmia and cataracts, likely resulting from high total gas pressure in the OI saltwater supply. In addition, through the early part of this grant period, we continued to be challenged by outbreaks of *Cryptocaryon* that resulted in a loss of 14 additional stock in December 2006 and January 2007. Hydrogen peroxide treatments helped in the short-term to alleviate *Cryptocaryon* infestations, which typically reemerged several months later, indicating incomplete eradication. In April 2007, we re-quarantined kahala stocks and initiated a more comprehensive set of parasite treatments lasting for periods of four weeks in order to cover the complete life cycle of *Cyrptocaryon.* This extended treatment period appears to have stopped the reoccurring parasite outbreaks. We are also attempting to find funding to improve water filtration and degassing incoming water to help with parasite and total gas issues.

Improvements to broodstock tank systems. In preliminary studies we recognized a need to improve egg collection methods for kahala to help reduce the amount of uneaten feed and feces collected along with eggs. We also noted that there was a significant decrease in egg viability with time in the egg collector system due to the high water flow required for kahala broodstock maintenance. Therefore, we contracted Plas-tech Ltd. of Honolulu (the original tank manufacturer) to mold a secondary surface drain and egg collector system to collect floating eggs separate from uneaten feed and feces, which should exit separately via the bottom drain (Figure 1 in this report's Appendix). Additionally, we asked Plas-tech to also retrofit broodstock tanks with window systems to facilitate observation of feeding behavior and behavior related to parasite infestation. These tank upgrades are currently under installation. On-site electrical capacity is being examined to determine whether electrical pumps can be added to individual tanks for a secondary processing loop that includes degassing towers to ameliorate high total gas pressure in the water.

Effect of broodstock stocking density. At the end of June 2007, fish were randomly stocked into four broodstock tanks: two tanks with 14 fish (low density) and two tanks with 30 fish (high density). Unfortunately, there was insufficient number of fish to stock a middle density treatment with 24 fish per tank, as planned in the original proposal. Fish were individually PIT tagged, weighed, and measured for fork-lengths. The average starting weight and length for all fish was 4.8 kg and 58 cm, respectively. Average total biomass/tank was 59 kg for the low density group and 154 kg for the high density group which was equivalent to a density of 3 kg/m^3 and 7.7 kg/m^3, respectively.

Fish were hand-fed daily to apparent satiation with Vitalis SA (Skretting, Canada) 13.0 mm salmon broodstock pellets. This diet, formulated for salmonid broodstock, contains 48% protein and 24% lipid and has been shown to improve reproductive output in kahala at OI in the past (see Year 1 report). Fish were not given raw diets since our previous work has demonstrated positive effects of the Vitalis SA diet on kahala reproduction. On average, the low density group was fed 394 g/day, while the high density group was fed 875 g/day. This feeding was equivalent to approximately 0.6% body weight consumed per day for both treatments (as-is basis). Feeding behavior is quite different between the different densities. The low density group tends to eat slower, while the high density group eats more vigorously. This can probably be attributed to greater competition for food in the higher density group.

Spawning occurred 19 days after stocking for the high density group, while it took 51 days for the low density group to start spawning. The total number of monthly spawns was low in July, when the fish initiated spawning, and the number of spawns has increased in succeeding months (Figure 2). Furthermore, total egg production increased from 600,000 in July to 5,000,000 in October (Figure 3). When expressed on an eggs-per-fish basis (Figure 4), there tended to be more eggs produced by the low density group than the high density group.

As shown in Figure 3, egg quality has been poor with a majority of the eggs being infertile and very few viable eggs being produced. Similarly, Figures 4 and 5 show that egg fertility and viability rates have remained low over the four months of spawning. The poor quality of spawned eggs is not totally unexpected, since the fish are young and "inexperienced" spawners. Additionally, since individual fish mature at different rates, some fish in each tank may be mature enough to produce gametes, while

others may not be reproductively mature. Currently, the ratios of mature to immature fish, as well as sex ratios in each tank, are unknown.

Establish a year-round supply of kahala eggs in support of emerging commercial kahala production. Kahala stocks started spawning at an average weight of 5 kg. The project is currently four months old, and the fish have been spawning throughout each month. We are confident that the kahala will continue to spawn, egg quality will improve as the fish mature, and that there will be a year-round supply of eggs in the future. Once the fish fully mature and more viable eggs are produced, kahala eggs will be available to industry to assist in commercial startups and as backup egg supplies to commercial producers.

Objective 2. Resolve monogenean parasite infestations that are being experienced when amberjack are grown in unfiltered waters associated with open-ocean cages. Project activities initially focused on molecular characterization of the monogenean parasite using archived samples stored in ethanol from the 2004 outbreak in the Kahala Offshore Culture Demonstration Project as well as freshly collected parasites provided by Kona Blue Water Farms. DNA was extracted using a commercial kit (DNeasy; Qiagen, Valencia, Calif.), and PCR was amplified using two different primer sets, both based on 28S ribosomal DNA sequences. The two different PCR strategies both yielded positive amplification of parasite DNA, and in all cases, the sequencing results unequivocally identified the monogenean parasite as *Neobenedenia* sp. Originally thought to be *Benedenia seriolae*, the confirmation of *Neobenedenia* is of concern because this parasite has a broader host range than that described for *B. seriolae*.

In flow-through experiments conducted at the University of Hawaii at Manoa's Hawaii Institute of Marine Biology (HIMB), kahala fingerlings provided by KBWF were maintained in unfiltered, flow-through Kaneohe Bay water conditions to determine whether the parasite would naturally infect the fish. After six months, the fish had not been parasitized by any monogenean trematodes. This result was disappointing since it refuted the assumption that *Neobenedenia* would naturally infect kahala at HIMB in a manner similar to what occurred in the Kahala Offshore Culture Demonstration Project. The fish were very sensitive to *Cryptocaryon irritans* infection while in unfiltered flow-through conditions and confirmed that this ciliated ectoparasite continues to be a pathogen of concern for kahala.

Working in collaboration with KBWF, a number of observations have been made relating to the ease/difficulty in working with *Neobenedenia* that were previously unknown. One, it was determined that live parasites can be transported to HIMB by taking fin clips from infected fish and storing them in filtered seawater for a short period of time (4–6 hours, from time of collection to air transport from KBWF to HIMB). However, parasites cannot currently be maintained *in vitro* for a period greater than 24 hours in the laboratory. Refinement of methods to propagate this parasite in the laboratory are ongoing, as resolving this bottleneck is critical to our ability to conduct the proposed parasite control experiment, since we must have enough of the parasite to replicate challenge dosages of fish in the experiment). Two, dead parasites are much easier to collect than live parasites, as this parasite is exquisitely sensitive to freshwater. Freshwater dip treatment of euthanized infected fish detaches the parasite from the body of the fish rapidly as well as changes the appearance of the parasite from being clear, almost transparent to white and readily apparent. Three, parasites hatch and mature in tank

culture in a more rapid fashion than expected compared to the time it takes for this and related monogeneans to hatch and mature as reported by researchers in Australia and Japan.

Culturing this parasite has proven more difficult than anticipated, and with the identification of *Neobenedenia* vs. *B. seriolae,* there is more concern about doing the proposed experiment at HIMB in close proximity to the Bottomfish/Opakapaka Hatchery.

Objective 3. Conduct workshop and develop fact sheet incorporating results of this project for rapid transfer to farmers in Hawaii and the Pacific region.

Although an industry stakeholder workshop was held on May 25, 2006 in Year 1 of the project, a second workshop was not conducted during Year 2. No fact sheet was developed. An industry workshop and CTSA *Regional Notes* article will be completed when sufficient data from ongoing work has been attained.

Impacts

This is part of an ongoing effort to diversify the Hawaiian aquaculture industry, with emphasis on developing aquaculture technology of high-value species, such as moi and kahala. Hawaii has a select advantage over competing regions, such as Japan, the Mediterranean region of Europe, and even the mainland United States, due to ease of access to pristine waters of the open ocean with relatively constant year-round temperatures. However, commercial companies are in the very early stages of operations and are very vulnerable to the complex array of challenges associated with generating fingerling supplies for cage growout operations. Therefore, this project has made critical impacts by developing culture technology for kahala broodstock, transferring this technology to local industry, and supplying eggs to assist in hatchery operations. Without this research and associated egg supplies, commercial operations would have been severely threatened. Additionally, emerging disease issues for broodstock (*Cryptocaryon* ectoparasites), hatchery (*Epitheliocystis*), and growout (*Neobenedenia*) are proving to be key challenges toward continued culture development of this species and sustainability of developing offshore operations in Hawaii.

Recommended Follow-Up Activities

Toward developing a coordinated research plan that ensures research activities focus on the most immediate concerns of local industry, we have sought industry input. This feedback places a high priority on (1) broodstock management to secure year-round supplies of high quality eggs and (2) monogenean infestations in open-ocean cage settings. These studies are ongoing.

Publications in Print, Manuscripts, and Papers Presented

Laidley, C. W. 2007. Development of captive culture technology for marine fishes of Hawaii. Presentation given at the Sept. 30 meeting of the Hawaii Society of Corporate Planners billed as, "Hawaii's transition to the new global economy, the one that didn't get away: Hawaii's emerging fish farms," Honololulu.

———. 2007. Plenary talk: Tropical and subtropical aquaculture of high-value marine finfish species in Hawaii and the Pacific region. Presentation given at The 4th National Aquaculture Extension Conference, Cincinnati, Ohio.

Laidley, C. W., K. Liu, A. Ellis, C. Demarke, and A. Molnar. 2005. Aquaculture development of the Pacific threadfin, longfin amberjack and bluefin trevally for commercial cage culture in Hawaii. In *Aquaculture and stock enhancement of finfish: Proceedings of the 34th meeting of the UJNR Aquaculture Pane*l, San Diego, Calif. (The UJNR is the United States and Japan Cooperative Program in Natural Resources.)

Laidley, C. W., R. J., Shields, and A. O. Ostrowksi, 2004. Progress in amberjack culture at the Oceanic Institute in Hawaii. *Glob. Aqua. Advoc*. 7(1):42–43.

Lewis, T. D. and J. Kishimori. 2007. Name that monogene. CTSA *Regional Notes* 18(2):7.

Lewis, T. D., J. M. Kishimori, and J. C. Leong. 2007.Identification of *Neobenedinia* and additional ectoparasites from wild kahala *Seriola rivoliana*, a cage culture species in Hawaii. Paper presented at the Asia Pacific Aquaculture 2007 conference, Hanoi, Vietnam.

Appendix

FIGURE 1. Photos of the newly designed surface drain egg collector systems (left) and tank broodstock observation window retrofits (right) to the Oceanic Institute kahala broodstock holding systems.

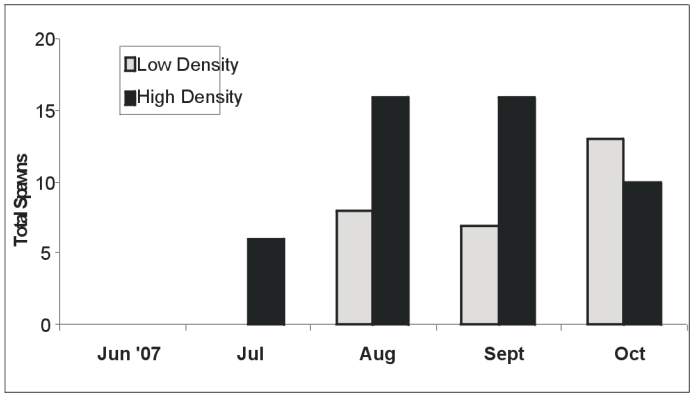

FIGURE 2. Total number of monthly spawns from kahala stocked at low and high densities.

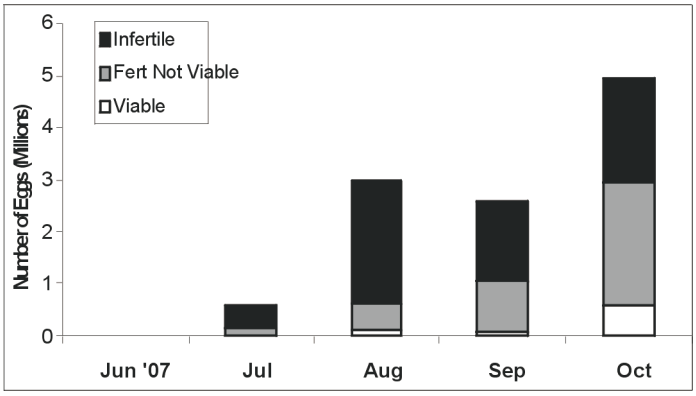

FIGURE 3. Total egg production from all kahala stocks from June through October 2007.

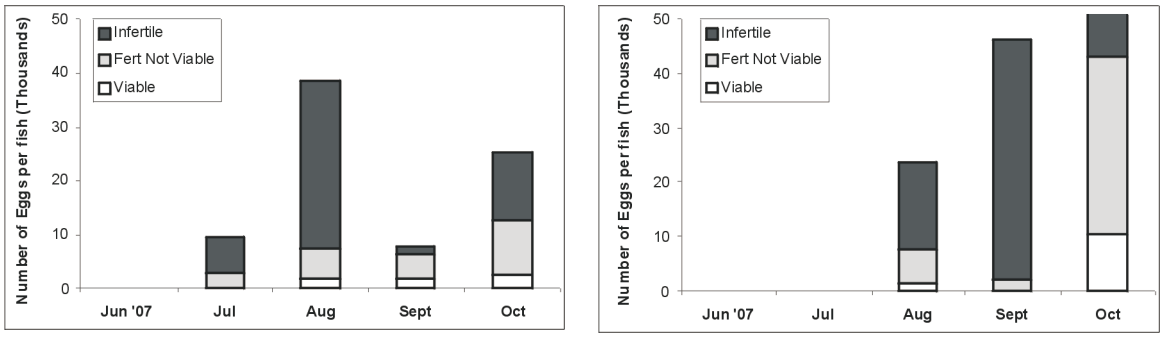

FIGURE 4. Total egg production per fish from the high-density group (left) and the low-density group (right).

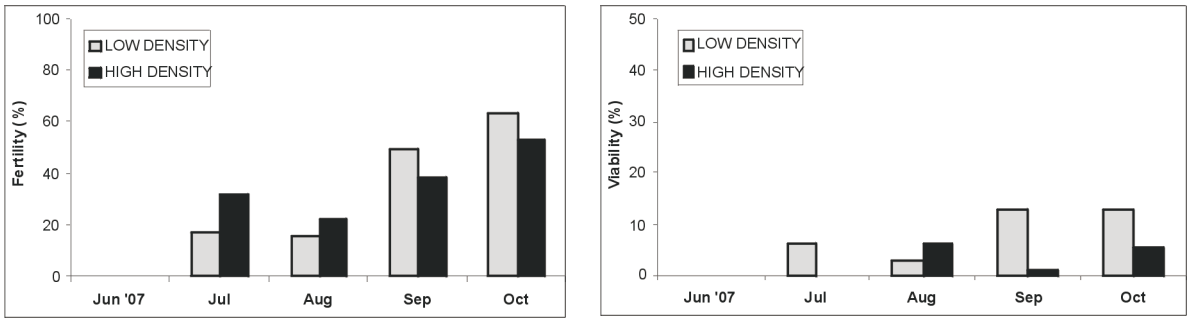

FIGURE 5. Egg fertility (left) and viability (right) rates from eggs spawned from kahala stocked at different densities.