ADVANCES IN DIAGNOSIS AND MANAGEMENT OF AMYLOODINIOSIS IN INTENSIVE FISH CULTURE

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Amyloodinium ocellatum is a parasitic dinoflagellate that infects a wide variety of warmwater marine and estuarine fishes and causes one of the most serious diseases in warmwater marine fish culture. The life cycle of the parasite is direct and consists of three stages – the trophont which is parasitic primarily on the gills, the free-living reproductive tomont, and the infective dinospore. The parasite is particularly troublesome for two reasons. First, current diagnostic methods rely mainly on the microscopic identification of parasites on the skin or gills; therefore, infections often go undetected until massive mortality occurs. Second, the life cycle is direct and each tomont can produce up to 256 infective dinospores resulting in the rapid buildup of heavy infections in recirculating systems. Although the general course of infection is understood, no quantitative understanding of the survival and reproductive rates of the parasite that might lead to improved control is known.

Our program has focused on strengthening our ability to address these shortcomings in diagnosis and management of amyloodiniosis. With respect to diagnosis, we developed a novel, highly sensitive and specific diagnostic tool based on the Loop Mediated Isothermal Amplification (LAMP) reaction to detect free-swimming stages of *A. ocellatum* in water samples. The advantages of LAMP reaction compared to the PCR are that is quicker and does not require sophisticated equipment or skilled personnel. No amplification was detected using DNA from related dinoflagellates species, demonstrating the specificity of the assay. The LAMP assay also proved to be more sensitive than the PCR for the detection of *A. ocellatum* in water samples. The established LAMP assay provides a useful tool for the rapid and sensitive detection of *A. ocellatum* in water samples, which could assist in the early detection and control of *A. ocellatum* infections in rearing systems.

With respect to management, we experimentally estimated the vital rates that determine the parasite population's growth and used that information to develop a population model for evaluating the relative effects of changes in vital rates on the population growth rate. A schematic representation of the parasite's life cycle was developed and a laboratory challenge model was used to quantify each process in the life cycle in two species of hosts – the spotted seatrout (*Cynoscion nebulosus*) and the red snapper (*Lutjanus campechanus*). We showed that although there were no significant differences in infection rates between the two species, trophonts grew larger and remained attached longer to red snapper than spotted seatrout and that spotted seatrout could tolerate a higher load of trophonts than red snapper. The model demonstrated that the number of dinospores produced per tomont had the largest effect on parasite population growth. Although the model clearly suggests that control

strategies should continue to focus on limiting the number of dinospores in a system, the model provides a framework for evaluating the relative contribution of various factors to the parasite's population growth rate under a variety of conditions.

PRE-SPAWNING CAROTENOID FORTIFIED DIETS IMPROVE REPRODUCTIVE TRAITS OF CHANNEL CATFISH, *ICTALURUS PUNCTATUS* AND SUBSEQUENT PROGENY PERFORMANCE

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Availability of consistent number of ovulatory competent channel catfish females is a pre-requisite for efficient production of channel catfish ♀ x blue catfish, I.*furcatus* ♂) hybrid in hatcheries. Raising hybrid catfish in production ponds enables the catfish farmer to harness improved growth rates, survival and feed conversion to improve production. Even though hybrid catfish production has been steadily increasing, fingerling production is still insufficient to meet the demand. Hybrid catfish embryo production involves hormone-induced spawning of channel catfish, stripped eggs are fertilized with blue catfish sperm, and fertilized eggs hatched in troughs in the same manner as channel catfish in hatcheries.

High quality broodstock maturation diets are an essential key for successful and sustainable production of hybrid catfish fry from hatcheries. Carotenoid fortification in brood fish diets has been suggested to improve maturation and egg quality in all aquatic animals. However, fish and crustaceans are unable to produce astaxanthin *de novo*, only plants and protists are capable of synthesizing carotenoids.

A 10-week pre-spawning broodfish nutrition study was conducted 2 months prior to spawning in twenty 1.5 m diameter, 760 L plastic tanks supplied with recirculated pond water and continuous air. Each tank was stocked with 10 fully mature 4-year old 'Delta' strain of female channel catfish per tank. This study was conducted to assess the effect of carotenoid fortified diets on reproductive performance of channel catfish during the pre-spawning phase. Four carotenoid fortified diets were prepared by mixing the required quantity of carotenoid in water and sprayed on a 35% protein commercial catfish feed in a concrete blender, followed by a coating of menhaden oil. The carotenoid treatments were: 1) 50 mg/kg Astaxanthin; 2) 100 mg/kg Astaxanthin; 3) 25 mg/kg of Lutein and 25 mg/kg of Zeaxanthin; 4) 50 mg/kg of Lutein and 50 mg/kg of Zeaxanthin and 5) control diet that was sprayed with the same quantity of water and oil. Four tanks were randomly allocated to a treatment.

Broodfish fed 100 ppm of Astaxanthin fortified feed had a higher ($P < 0.05$) percent of gravid females suitable for hormone injection compared to other treatment groups. Gravid females from all the 5 treatments were subjected to hormone-induced spawning procedures to

produce hybrid catfish fry in two spawning trials. Percent of females ovulated in response to hormone injection, fertility and hatching success did not differ ($P > 0.05$) among the treatments. However, ovulatory index, fecundity, and fry produced per kg of female were higher ($P<0.05$) in broodfish fed 100 mg/Kg of Astaxanthin fortified feed during the pre-spawning phase.

The results of the present study suggest that supplemental 100 mg/kg astaxanthin in broodfish diet not only improved fecundity, quality of eggs and fry production, but also enhanced the physiological response of broodfish to induced spawning. Further, progeny derived by Astaxanthin fortification in brood fish diet had a positive effect on growth, increased resistance to ESC disease challenge and had a reduced stress response to low dissolved oxygen.

WITHERING SYNDROME: DISTRIBUTION, IMPACTS, CURRENT DIAGNOSTIC METHODS AND NEW FINDINGS

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Withering Syndrome, WS, is a fatal disease of abalone caused by a *Rickettsia*-like organism (WS-RLO). The causative agent, "*Candidatus* Xenohaliotis californiensis" occurs along the eastern Pacific margin of North America in California, USA and Baja California, Mexico. However, as infected abalones have been transported to Chile, China (People's Rep. of), Taiwan, Iceland, Ireland, Israel, Spain, Thailand, and most recently Japan, and possibly other countries, the geographical range of the etiological agent is suspected to be broad where California red abalones, *Haliotis rufescens*, are cultured or areas where native species have been exposed to this species. Susceptibility varies among species with up to 99% losses of black abalone, *H. cracherodii*, in lab and field studies in the USA to no losses among *H. diversicolor supertexta* in Thailand. Some populations that have suffered catastrophic losses to WS have developed an increased resistance to the disease. In addition, a newly identified phage hyperparasite of the WS-RLO may reduce pathogenicity and dampen losses from the WS-RLO. Diagnosis of WS requires the identification of infection with the pathogen (WS-RLO via *in situ* hybridization or via histology coupled with PCR and sequence analysis)

accompanied by morphological changes that characterize this disease (e.g. pedal and digestive gland atrophy, and digestive gland metaplasia). A real-time PCR (qPCR) assay has been developed and may be useful in quantifying amounts of pathogen DNA. Confirmation of infection by the WS-RLO cannot be done by PCR analysis alone as this method only detects pathogen DNA but can be used as a proxy for infection is areas where the agent is established. Control measures include avoidance, culling infected animals and, as per federal regulations, oral or bath treatment with oxytetracycline. Avoidance is best accomplished by the establishment of a health history and multiple health examinations prior to movement of animals. Although histology or *in situ* hybridization are required to confirm infection, qPCR is able to detect small amounts of pathogen DNA and is advised to be included as part of health examinations.

FEEDING HATCHERY-PRODUCED LARVAE FROM THE GIANT GROUPER *EPINEPHELUS LANCEOLATUS*

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Research on the larval rearing of giant grouper *Epinephelus lanceaolatus* was carried out for the first time at the Pacific Aquaculture and Coastal Resources Center (PACRC), University of Hawaii at Hilo. Fertilized eggs from captive broodstock were obtained from Kampachi Farms, Kailua-Kona, Hawaii. Approximately 36,000 viable eggs were stocked in three round tanks with central drain and conical bottom each with one m^3 of seawater. Fertilized eggs had a diameter of 0.89 ± 0.01 mm and hatched 30 h post fertilization. At 2 days post hatch (DPH) yolk sac larvae measured 2.4 ± 0.2 mm, had the mouth and anus closed, and half of the yolk was resorbed. Microalgae *Isochrysis galbana* was supplied to all tanks from DPH 1 to 20 at a density from 30,000 to 45,000/ml. Two feeding strategies were tested. In one tank, enriched trocophore larvae of the Pacific oyster (*Crassostrea gigas*) were fed in DPH 2 and 3, shifting the feeding at DPH 4 to enriched S-type rotifers (*Brachionus rotundiformis*) at a density of 20 per ml. In the second and third tank fish first food consisted of a mix of calanoid copepods (*Parvocalanus crassirostris*) (0.5 to 3.0/ml) and enriched S-type rotifers (10/ml), both were fed to the fish twice every day. At 4 DPH the yolk sac in larvae measuring 3.0 ± 0.0 mm was almost completely resorbed, however, food was already present in the gut in the mixed copepod/rotifer feeding. At 6 DPH massive mortality occurred in the rotifer treatment as apparently the fish were not able to ingest them. By 8 DPH mortality was total in this tank. In contrast, fish in the mixed feeding regime showed constant

feeding behavior with gut fullness confirmed visually under the microscope. At 10 DPH a co-feeding treatment was initiated in one of the two remaining tanks with a microdiet of 200-300 μm particle size and shifted to 300-500 μm in DPH 14 at a fish larvae length of 5.1 ± 0.3 mm. Despite being fed the same mixed rotifer/copepod ration, growth of larvae was higher in the co-fed treatment. Thus, at DPH 17 co-feeding was also applied in the other tank. Starting at 14 DPH instar I nauplii of *Artemia* was supplied to the larger fish tank followed by a mix of instar I nauplii and enriched metanauplii two days later. The same was applied at DPH 17 in the smaller fish. *Artemia* was fed two times per day at a density of 1-3/ml. At DPH 20 the supply of rotifers and copepods to the tanks was terminated. Grouper larvae presented high growth rates at the *Artemia* feeding stage. Metamorphosis started at 25 DPH and at 35 DPH it was not yet completed with larvae remaining pelagic. Weaning was initiated at 35 DPH. Survival from hatched to pre-weaned larvae under the described conditions was estimated at 2.1%. Photoperiod was maintained at 24L:0D from1 to 20 DPH, then changed to 12L:12D. Water temperature and salinity were 27.6 ± 0.2 °C and 29.5 ± 0.3 ppt respectively.

STUDY ON HIGH INCIDENCE OF DEATH DUE TO COLLISION OF HATCHERY-REARED PACIFIC BLUEFIN TUNA *THUNNUS ORIENTALIS* JUVENILES IN NET CAGES

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Bluefin tuna has been cultured in many countries and regions, such as the Mediterranean Sea, Mexico, Australia and Japan, because of its high commercial value. The decline of wild tuna stocks and the recent controversial issues of tuna capture-based aquaculture catalyzed the need for a stable supply of artificially-reared tuna juveniles for aquaculture purposes. The reasons for that is two-fold; first, to reduce the negative impacts on wild-stocks, and second, to promote the aquaculture industry. In 2002, Kinki University succeeded in the reproduction of Pacific bluefin tuna *Thunnus orientalis* (PBT) in captivity. Various studies to apply artificially-reared juveniles to mass-culture system have been carried out actively since then, however a high mortality of hatchery-reared juveniles is frequently observed after transfer from indoor tanks to net cages at around 30 days post hatch. The mortality is likely due to collisions with the cage nets at dawn. Techniques are being developed to prevent these collisions as the survival rate is much lower than that of other teleosts. In order to reduce the mortality in net cages, it is important to elucidate the developmental stages of reared PBT when the collisions occur in net cages.

Bone injuries can be considered an index for obvious collisions due to the damage caused by physical impact. In the present study, bone injuries of dead juveniles were

investigated in order to examine the prevalence of net collisions. Juvenile PBT were reared in 3 net cages (20 m in diameter) for 90 days after being transferred from indoor tanks at Amami Station, Seikai National Fisheries Research Institute, Fisheries Research Agency, Japan. Dead fish were sampled and examined by clearing and staining method or by dissection to detect injury to the vertebral column and parasphenoid. Results showed that dislocation and fractures of the vertebral column usually occurred between the first and the 15th vertebrae. Up to 30 days after being transferred, the prevalence of injuries of the vertebral column and parasphenoid was low (0.0-12.0%); with fish size being 5.5 to 15.2 cm in total length (TL). From 31+ days after transfer, the injuries drastically increased to 17.8-78.0% (21.0 to 39.2 cm in TL). These results suggest that the mortalities of juvenile PBT larger than 20 cm in TL in cages were caused by net collision. Further studies are required to determine the reason for high incidence of collisions of juvenile PBT larger than 20 cm TL and to reduce collision deaths for a consistent and stable supply of the seedlings for aquaculture.

SUCCESS OF SEED PRODUCTION OF HUMPHEAD WRASSE *CHEILINUS UNDULATUS* WITH IMPROVEMENT OF SPAWNING INDUCTION, FEEDING, AND REARING CONDITION.

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Humphead wrasse, *Cheilinus undulatus*, is the largest labrid fish distributed in tropical and sub-tropical regions of the Indo-Pacific and occur around coral reefs. Increasing fishing pressure since the early 1990s for the live reef-fish trade, caused the number of this species to drop in many countries, finally resulting in its listing in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 2004. Currently, international trade is government-controlled or the catch of humphead wrasse banned in some of the countries. In Japan, the fishery of humphead wrasse is found in Ryukyu Islands, and the annual catch was 6.4t in 1990, but decreased to approximately half that during the last two decades.

Attempts of aquaculture are becoming important for the sustainable utilization of humphead wrasse. Successful seed production (120 juveniles) was achieved once in Indonesia in 2003, however, reproducible production has not been established. Therefore, we examined the method of spawning induction and initial food and rearing conditions of larvae. Spontaneous spawning was found when the water temperature was greater than 28°C, and

within 1 week of the new moon during June through September. Fertilization rate was 10 – 25% with spontaneous spawning, but was successfully induced 100% with a drawdown of seawater in rearing tanks of the broodstock. The mouth diameter of humphead wrasse larvae is small (133 μm), therefore we also examined initial feeding. Two different types of monogonont, SS-type rotifer (*Brachionus rotundiformis* Thai-strain) and more minute Proalid rotifer (*Proales similis*), were used as candidates of live food and boiled chicken yolk and powdered milk were also examined. Larvae preferred *Proales similis* than any other diet during the first 7days after initial feeding, and thereafter, preferred SS-type rotifer. The survival rate of larvae was highest when fish were reared in 0.5 kL circle tanks with 20 ml/min aeration at 30 °C, with addition of a layer of surface oil to prevent surface death of larvae. By using *Proales similis* as the initial live food, optimizing aeration rate and adding feed oil, we produced 22 juveniles at 50 days post hatch [dph] (survival = 0.25%, TL= 9.0 mm) on August 2011. In September 2011, we produced 537 juveniles at 50 dph (survival= 10.7%, TL= 9.1 mm) with the use of concentrated oxygen for aeration, indicating that examined rearing method was reproducible for the seed production of humphead wrasse.

WITHERING SYNDROME IN ABALONE IN JAPAN

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Withering syndrome (WS) is known as a chronic wasting disease in abalone caused by *Candidatus xenohaliotis californiensis*, a Rickettsia-like organism (RLO) and has been prevalent on the west coast of the United States since the mid-1980s. WS is listed as notifiable disease in the International Aquatic Animal Health Code of the Office International des Epizooties (OIE). In Japan, the WS-RLO was first detected in 2011 in farmed, juvenile Japanese black abalone (*Haliotis discus discus*; 14 – 34mm in shell length). Those abalone experienced monthly mortality rates from 3 to 10% with a cumulative mortality of 32.8%, and histopathological changes and pathogens were not found except for basophilic, intracellular bacterial colonies within the epithelium of the digestive tract. Morphological characteristics and target tissues containing the bacterial inclusions were consistent with those of the WS-RLO. High intensity of infection by the RLO was observed in the epithelium of the posterior portion of the esophagus, the intestinal epithelium and, to a lesser extent, in the epithelium of transport ducts in the digestive gland. The RLOs observed were conclusively

identified as the agent of withering syndrome by the additional investigation of PCR and sequence analysis. Preventive measures against WS implemented in Japan will be shown.

MARINE FISH HATCHERY TECHNOLOGY AND RESEARCH AT THE THAD COCHRAN MARINE AQUACULTURE CENTER

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The Thad Cochran Marine Aquaculture Center is composed of 7 new laboratory buildings dedicated to the development of Gulf of Mexico marine fish aquaculture. The buildings include live feed production systems, hatchery, broodstock, juvenile and adult production systems, as well as, wet and dry laboratories for genetic, disease, nutrition and behavioral research. In addition, several buildings are used for teaching and public educational purposes. The primary objectives of the Center are to enhance Mississippi Gulf waters with commercially and recreationally important species and develop aquaculture technology for commercial transfer. The immediate goals of the Center are to develop the methods to capture, maintain and spawn broodstock of marine fish in order to facilitate production, rearing, tagging, releasing and assessing of juveniles in a stocking program. The facility employs cutting-edge technology, peer-reviewed research, and hands-on testing to grow fish in an environmentally sustainable and economically feasible manner. Research is funded through partnerships with state and federal agencies, as well as by private companies. The facility is unique in that all hatchery and production tanks are closed, recirculating seawater systems filled with artificial seawater, insuring biosecurity between tanks and the environment. The research and facilities can be divided into three programs, Spotted Seatrout Program, Red Snapper Program and Demonstration Program for new species. Over the last few years, more than half a million Spotted Seatrout (*Cynoscion nebulosus*) have been stocked into Gulf waters. Seatrout have been tagged and released and an assessment program is being developed in collaboration with the Mississippi Department of Marine Resources. The second major project focuses on Red Snapper (*Lutjanus campechanus*); several thousand have also been released in recent years. The Red Snapper Program has been significantly expanded during the last 2 years with the construction of the new Red Snapper Hatchery and Grow-out buildings. Improvements in live feed production have been key to the success of these expanding hatchery production projects. Additional species have been the focus of the Demonstration Project research, including Cobia (*Rachycentron canadum*), Tripletail (*Lobotes surinamensis*) and Atlantic Croaker (*Micropogonias undulatus*). The Center has led an effort to establish a Gulf of Mexico Marine Fish Hatchery Consortium that would link a number of research and private scientific institutions on the Gulf coast, improving

communication and coordinating joint research.

EFFECTS OF DOCOSAHEXAENOIC ACID AND TAURINE LEVELS IN ROTIFERS ON GROWTH AND SURVIVAL OF LARVAL AMBERJACK *SERIOLA DUMERILI*

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The amberjack *Seriola dumerili* is a commonly cultured species in southwest Japan and aquaculture production, together with yellowtail *Seriola quinqueradiata,* dominates that region. Although a stable supply of amberjack juveniles artificially produced in Japan is urgently needed, the current technology for mass seed production is still incomplete. Successful seed production of marine fish requires fortification of live foods that have insufficient essential nutrients. The docosahexaenoic acid (DHA) and taurine contents of wild amberjack larvae were higher than cultured larvae. These nutrients in *Artemia nauplii* and especially rotifers, which are used as live foods for amberjack larvae during seed production, were markedly lower compared to wild zooplanktons. These findings suggest that DHA and taurine contents in rotifers used for seed production of amberjack are insufficient for the requirements for this species.

First, we investigated the effect of DHA on growth and survival of larval amberjack. Larvae at 3 days post-hatch (dph) were fed rotifers enriched with commercial DHA supplements at 4 levels, and reared for 7 days. DHA enrichment of rotifers was effective to improve growth, survival rate, and swim bladder inflation of amberjack larvae. Next, we investigated the effect of algae with different DHA contents on growth performance, survival, and swim bladder inflation of larval amberjack. The algae were used for rotifer enrichment and also supplemented to the larval rearing tanks in static condition. Feeding trials were conducted from 1 to 10 dph. Rotifers enriched with *Nannochloropsis* (EPA rich rotifers) were effective to enhance growth and survival, but DHA was essential to improve the swim bladder inflation in amberjack larvae. Furthermore, the effect of feeding rotifers enriched with taurine on the growth performance and survival of larval amberjack was investigated. Amberjack larvae at 3 dph were fed rotifers enriched with a commercial taurine supplement at 4 levels (0,

200, 400 and 800mg/l), and reared for 7 days. Growth and survival of fish fed rotifers enriched with taurine supplement at 800mg/l were improved compared to fish fed the rotifers without taurine enrichment. Taurine enrichment of rotifers is effective to improve the survival of amberjack larvae.

The results of these studies indicate that enrichment of rotifers with DHA and taurine is essential for the growth and survival of larval amberjack, and the requirements of DHA and taurine in rotifers are estimated at 1.5 and 5.3 mg/g on a dry matter basis.

APPLICATION TO THE GENETIC BREEDING USING GENOMICS INFORMATION IN YELLOWTAIL (*SERIOLA QUINQUERADIATA*)

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The capture marine products industry has developed as the majority of the fishery industry, directly using aquatic resources. Only recently has the breeding of marine fish been considered important research, due to the decrease of available aquatic resources. Beyond the depletion of aquatic resources, the expectation of aquaculture research is getting higher. Genetic improvement of economic traits is needed to improve phenotypes suitable for captive breeding, improving with every generation. We are researching practical application for selection of economically important traits from natural genetic resources of yellow tail (*Seriola quinqueradiata*).

In our study, we identified sex-linked markers for the genetic-sex of yellowtail as a representative binary trait, which can be useful for controlling sex in yellowtail breeding. When an efficient sex detection method becomes available for yellowtail, it will be possible to select the fastest-growing sex to increase food production. The sex-determining locus is located in Squ12 Genetic linkage map, and the sex-linked alleles were inherited from the female parent. This result suggests that yellowtail has a ZZ–ZW sex-determining system, and that it would be possible to use these sex-linked markers to discriminate between sexes.

We also targeted disease resistance for important traits. Especially, Benedenia disease caused by the ectoparasite *Benedenia seriolae* and is difficult to prevent in marine aquaculture systems. This is a serious parasitic disease in yellowtail aquaculture, leading to secondary viral or bacterial infections. Because fish rub their bodies against the fish cage to

remove the parasite, mortality is quite high especially juveniles. The common way of removing the parasite is to soak the fish in a freshwater bath. However, this method requires a lot of time, cost, and effort. To evaluate the genetic basis of Benedenia disease resistance in yellowtail, a genome-wide and chromosome-wide linkage analysis was initiated using F1 yellowtail families. Two major quantitative trait loci (QTL) regions on linkage groups Squ2 and Squ20 were identified and then confirmed in F1 families. These QTL regions explained 32.9– 35.5% of the phenotypic variance. This is the first genetic evidence discovered that contributes to detailing the phenotypic resistance to Benedenia disease, and the results will help resolve the mechanism of resistance to this important disease of yellowtail.

Advanced research is progressing. DNA marker-assisted selection "MAS" program were performed for linkage groups Squ2 and Squ20 QTL significant region using the F2 family. F2 QTL congenic line had a significant phenotypic difference in the number of parasites, when comparing resistant-parents with susceptible-parents in parental genotype combinations.

The next step is to apply MAS breeding to other target aquatic species. The effective utilization of natural genetic marine resources in aquaculture has more advantages compared because wild species are not selected and still maintain high genetic diversity.

THE COOPERATIVE CULTURE OF SEAWEED IN NEW ENGLAND—HOW RESEARCH, INDUSTRY, AND EXTENSION ARE CULTIVATING A NEW FIELD IN AQUACULTURE

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A partnership of research, industry, and extension in the Northeast has resulted in successful commercial culture of native seaweed species in Maine and Long Island Sound. Development of nursery culture and grow-out technologies for sugar kelp (*Saccharina latissima*) in the Seaweed Biotechnology Laboratory at the University of Connecticut and the lab and farm of Ocean Approved, LLC, of Portland, ME has allowed for the establishment of the first commercial kelp farm in the United States and an educational and research farm in Long Island Sound. The technology was shared and further developed along the coast of Maine by integrating kelp lines on six different shellfish farms through a Maine Sea Grant extension-led collaborative research project. The first regional seaweed aquaculture workshop was held as a result of this work, bringing together members of the seaweed industry, potential new farmers, researchers, extension, students, and entrepreneurs. This emerging new field continues to grow in the Northeast with the sharing of ideas, technologies and information through the open and collaborative relationships of extension, research, and

industry.

INTENSIVE JUVENILE PRODUCTION OF YELLOWTAIL AMBERJACK (*Seriola lalandi*) IN SOUTHERN CALIFORNIA

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Hubbs-SeaWorld Research Institute (HSWRI) has been culturing yellowtail amberjack (*Seriola lalandi*) at its laboratory in San Diego, CA since 2003. Beginning in 2007 intensive-level production began at the Mission Bay research facility in order to refine methodologies at a commercial scale. Initial results yielded survival levels to 1 gram juvenile between 0.2 to 5.0% with varying degrees of skeletal malformation (often greater than 40%). Moreover, larval swim bladder inflation rates have been highly variable; an issue that poses potential problems for the cage-based production of the species. In 2012 live feed quality and larval rearing microbial management improvements were made to production protocols and systems in order to increase juvenile quantity and quality. Many of these changes were based on observations made during HSWRI larval rearing trials coupled with advancements in the global aquaculture industry.

Live feed quality was enhanced through the improvement of the water quality supplying both the rotifers (*Brachionus plactilis*) and *Artemia* (*Artemia franciscana*), as well as limitation of bacterial loading in the live feeds culture systems. The water quality supplying live feeds systems was improved by adding high-capacity 1 μm pleated filters and high-intensity ultraviolet sterilizers. Furthermore, *Artemia* protocols were modified to decrease *Vibrio* spp loading through the use of INVE Separt systems, INVE Hatch Controller, improved sanitation and automated cold storage. Rotifer systems were modified to promote more stable microbial communities through the use of recirculation technology and improved cold storage.

Larval rearing systems were improved through the addition of a dedicated, high-capacity 1 μm pleated filter, ultraviolet sterilizer and heaters. In order to remove accumulated bio-film from tanks, plumbing and equipment, a routine total-system disinfection protocol was established in between production runs. Moreover, quantitative bacterial screening methods were put into place to confirm cleaning and disinfection protocols. With these systems in place, various larval rearing strategies were trialed at intensive levels in "commercial-scale" larval tanks (volumes between 1.6 – 8.0 m^3) based on previous experimental results. In most trials, rotifers and turbidity (algae paste or clay) were administered to larval tanks continuously via an automated temperature controlled cold-storage unit. Although clay-based turbidity protocols were trialed with relatively poor

success, algae-paste turbidity protocols coupled with larval passive-transfer methods yielded the most productive results.

Ultimately, utilizing improved systems and protocols, two *Seriola lalandi* production runs were completed in the summer of 2012 and one is currently underway. The first run yielded more than 200,000 weaned 0.75 gram juveniles (30% survival from egg); the second produced more than 45,000 weaned 1.3 gram juveniles (7% survival from egg) and the third is currently still in its late larval stage but appears to be following a similar successful trend. Skeletal malformation rates have been relatively very low (< 5.0%) compared to previous years production. Although these results have been encouraging, larval swim bladder inflation rates have been very problematic, even compared to previous years, with the first two runs demonstrating inflation rates of < 1.0% and 23.0%, respectively.

Current research and development efforts include further refining systems and protocols, reducing variability in larval survival and improving swim bladder inflation.

IN SITU SWIMMING AND SETTLEMENT BEHAVIOR OF CULTURED SERRANID LARVAE, *PLECTROPOMUS LEOPARDUS* AND *EPINEPHELUS MALABARICUS*

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Cultured serranid larvae, *Plectropomus leopardus* (n=81) and *Epinephelus malabaricus* (n=61) were released during day time in June and July of 1997 and 1998 at various locations along a fringing reef at Ishigaki Island, southern Japan, and their behavior observed by divers. At offshore (1 km) deeper sites (30 m), released larvae swam at an average depth and swimming speed of 10.2 m and 4.9 cm/s for *P. leopardus* and 7.5 m and 6.1 cm/s for *E. malabaricus*. When released at shallower sites (7 m) close to the reef edge (3 m), both species of larvae swam directionally to open water to avoid predators. At sites 400 m offshore and 18 m deep, released *P. leopardus* and *E. malabaricus* larvae settled on the slope at average depths of 16.4 m and 15.4m, respectively. However, there were no significant differences between the two species in settlement depth, time to settlement, swimming speed and swimming distance. When released 3m from the slope and 3m above the rubble bottom at sites 400 m offshore and 16 m deep, 73 % of *P. leopardus* larvae settled underneath prominent live or dead coral on the slope, and 23 % settled underneath rocks on the bottom.

EFFECTS ON GROWTH OF FLATFISH JUVENILE BY ARTIFICIAL LIGHTENING CONDITIONS: INTENSITY, PHOTOPERIOD AND WAVELENGTH

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Mechanisms that respond to light are deeply involved in life history of fish and are widely known. Light control technology has been essential for control of the spawning cycle in broodstock management. On the other hand, in hatcheries for seed production, light environment can significantly affect the first-feeding success in various fish species. Rearing techniques that match the optimal light environment conditions of the target species is essential to efficient and healthy seed production.

Therefore, in the present study, three flatfish species of importance for fisheries in northern Japan, spotted halibut *Verasper variegatus*, slime flounder *MIicrostomus achne*, and Japanese flounder *Paralichthys olivaceus* juveniles were studied to elucidate the necessary artificial lighting conditions: intensity, photoperiod and wavelength. We conducted rearing experiments for each developmental stage, growth, survival, and feeding status (feeding incidence, average number of rotifer in digestive tract) as an indicator of the optimal light environment.

Light intensity: In spotted halibut that live in very shallow seas, feeding status was better, and growth and survival rate was improved in light conditions (>12 μmol･m^{-2} s^{-1}). In addition, they fed even in the dark conditions, showing increased growth. In slime flounder that live in the deep sea, feeding status was better and growth and survival rate was been improved in dark conditions (<0.2 μmol･m^{-2} s^{-1}).

Photoperiod: In spotted halibut and Japanese flounder, feeding status was better and growth and survival rate was improved in continuous lightening (24 h). Photoperiod had no effect on slime flounder.

Wavelength: As a result of rearing juveniles under various wavelengths in spotted halibut and flounder, green light (518nm) feeding status was better compared with white light. Result of electro-intraretinal-retinogram in adult fish of these flatfish, the peaks of spectral sensitivities of spotted halibut were 521 nm, Japanese flounder were 511nm, thus supporting the results of rearing experiments.

As a result of incorporating optimal light environment obtained in the above experiments, seed production technology of spotted halibut has been improved dramatically (average survival rate: improved to 50% from 20%, average normal rate: improved to 80% from 50%).

LARVAL REARING ADVANCEMENTS FOR YELLOWTAIL AMBERJACK (*Seriola*

***lalandi*) IN SOUTHERN CALIFORNIA**

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Hubbs-SeaWorld Research Institute (HSWRI) has been culturing yellowtail amberjack (*Seriola lalandi*) experimentally at its laboratory in San Diego, CA since 2003. In 2007, we began to intensively rear juveniles, and between 2007 and 2011, we achieved survival rates as high as 5.0% from egg to approximately 50 days post hatch (dph). In 2012, we continued to conduct manipulative larval rearing studies to improve overall larval performance. These studies targeted optimizing the live prey feeding regime and reducing the bacterial load (*Vibrio* spp.) in the larval culture tanks.

First, we attempted to further optimize our larval live prey feeding procedures. The traditional HSWRI feeding regime for yellowtail amberjack began with offering rotifers (*Brachionus plicatilis*) at 2 dph through 7 dph, 1st instar *Artemia* (*Artemia franciscana*) were offered from 6 through 8 dph, and 2nd instar *Artemia* are were offered from 7 through 35 dph. In the first trial, larvae were co-fed rotifers, 1st instar *Artemia*, and 2nd instar *Artemia* from 3 through 20 dph in order to gain a better understanding of appropriate weaning times, as well as ingestion levels of each feed over time. Results from this trial showed that the larvae selected 1st instar *Artemia* as early as 3 dph (4.5 mm notochord length) and 2nd instar *Artemia* as early as 5 dph (4.6 mm notochord length). The second trial attempted to remove 1st instar *Artemia* from the current feeding regime because 1st instar cannot be enriched. This trial consisted of three treatments: 1) rotifers to 2nd instar offered at 5 dph, 2) rotifers to 2nd instar offered at 7 dph, and 3) rotifers to 1st instar to 2nd instar (i.e. traditional HSWRI protocol). Results showed no significant differences in growth or survival between any of the treatments, indicating that larvae can be weaned from rotifers directly onto 2nd instar *Artemia* at 5 dph.

Second, we wanted to reduce bacterial load levels, specifically *Vibrio* species, in the larval culture tanks. The bacterial load in larval rearing environment is heavily influenced by the inputs introduced to the rearing tanks (e.g. algae cells and live prey), as well as other factors like water treatment and temperature. We conducted two trials to evaluate methods to minimize the bacterial load (*Vibrio* spp.) in the water, while maintaining good larval performance. The first trial examined the use of Bentonite clay as a turbidity agent to replace algae in a greenwater-type environment. This study consisted of three treatments: 1) clay with continuous feeding, 2) clay with batch feedings, and 3) algae paste with batch feedings. The results showed that both clay treatments had significantly fewer *Vibrio* colonies in the water column (377 ± 120 CFU) than the algae paste treatment (5,692 ± 2,396 CFU) after 14 days of culture. Growth was not significantly different among the treatments, however survival was significantly higher in the clay treatment with continuous feeding (14.1 ± 2.6%) than either

the batch fed clay (2.3 ± 0.5%) or algae paste treatments (2.8 ± 1.5%). The second study attempted to limit bacterial loading in the larval rearing culture tank by siphoning the larvae into adjacent, clean tanks at 1, 5 and 9 dph during the first two weeks of culture. The results from this trial showed that the water in the transfer tank had fewer *Vibrio* colonies (1,025 ± 541 CFU) than the water in the control tanks where larvae were not moved (1,962 ± 1,415 CFU). Also, survival was significantly higher among larvae that were transferred (43.9 ± 13.5%) than in the control tanks (23.1 ± 6.3%).

Based on these results, we applied these same experimental methods on three commercial-scale production runs in 2012 that yielded survival rates as high as 30% from egg to juvenile fish. This work demonstrates the value and transferability of laboratory-scale experimental results to a production scale. Additional experimental work is required to improve the overall consistency of larval survival, including an improved understanding of variability among batches of eggs.

EXPERIMENTAL STUDY ON BROODSTOCK MANAGEMENT OF BARFIN FLOUNDER UNDER THE CONCEPT OF MINIMUM KINSHIP SELECTION

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A large flatfish, the barfin flounder *Verasper moseri*, is distributed from the southern Sea of Okhotsk to northern Japan. This fish is the highest-priced righteye flounder in Japan, but fishery yields have been endangered since the 1970s by its severe depletion. This reduction has rendered barfin flounder a rare species. To correct this situation, a stock enhancement program with annual release of approximately 100,000 seedlings has been promoted since 1987. This program is inferred to be effective because released seedlings are recaptured every year. However, the risk of losing genetic diversity from the base population is ever-present: the proportion of hatchery born fish in broodstock has been increasing because obtaining wild barfin flounder is almost impossible. For this reason, optimal broodstock management is necessary to avoid inbreeding and loss of genetic variation in future generations.

In this study, we applied the concept of minimum kinship selection to broodstock management of barfin flounder using three microsatellite DNA markers. The breeding plan was designed based on the kinship value, which was calculated among the broodstock individuals using microsatellite DNA markers. Moreover, individual identification and artificial insemination techniques were developed to support the breeding plan. The broodstock consisted of 37 wild and 41 hatchery-reared barfin flounders. Each individual was

identified using a PIT tag. The sex was recognized according to the gonad shape, as analyzed by sonography. Artificial fertilization was performed based on the factorial mating design, excluding closely related individuals. Furthermore, the numbers of larvae used for seedling production were equalized among the pairs to maximize the effective population size. Using this procedure, we produced seedlings with high genetic variation that was comparable to that of the broodstock's, showing that the concept of minimum kinship selection is useful for genetic conservation in barfin-flounder stock enhancement.

IMPROVING THE HATCHERY OUTPUT OF THE HAWAIIAN PINK SNAPPER, *PRISTIPOMOIDES FILAMENTOSUS*

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The opakapaka, one of the highly prized "Deep Seven" bottom fish species, has been branded with an "over fishing" status. This situation has mandated that Hawaii develop a fishery management plan for the bottom fish fishery in the Main Hawaiian Islands. Part of this plan was the development of hatchery protocols where initial efforts have resulted in the only spawning broodstock maintained in captivity. This has offered the opportunity to develop hatchery techniques for their artificial propagation.

To insure that the nutritional requirements of opakapaka larvae are being met, fatty acid profiles of spawned opakapaka eggs were determined. Total fatty acid content in the opakapaka eggs matched that of wild caught copepods. While the total fat content may be relatively low the percent composition of the essential fatty acids, DHA and EPA (26.9% ± 4.3% and 2.7% ± 0.4%, respectively) are consistent with other reports indicating that both essential fatty acids are critical for survival and growth of the opakapaka larvae. In addition, the composition of essential fatty acids for the various live food organisms were obtained and with the exception of Nanno-fed rotifers, the live feeds employed, have similar profiles from a percent composition standpoint and indicates that protocols that were developed can be adjusted to meet the nutritional requirements of the opakapaka larvae.

Using changes in total length between fed and unfed larvae and monitoring temporal changes in gape size, it is clearly evident that introduction of live food organisms must take place no later than the third day post-hatch. When larvae were presented with a variety of live food organisms either alone or in combination, the results indicate that a copepod nauplius-only diet is a superior feeding regime over the all others tested.

Since adult copepod and nauplius production is dependent on cell density of the phytoplankton being used, the team used these findings to consistently time production of *Parvocalanus sp.* nauplii at densities ranging between 5-10 individuals/ml in a 3,000-L

rearing tank by the third day post-hatching. This was achieved by "conditioning" copepod cultures using *Isochrysis galbana* (Tahitian Strain) at cell densities of 4 x 105 cells/ml prior to stocking into the larval rearing tank. Employing this strategy resulted in unprecedented larval survival with a mean of 80% passing the first feeding stages up until 10-12 day post-hatching.

Utilizing video recordings of the foraging behavior of opakapaka larvae revealed that the evasive abilities of adult and copepodite stages of calanoid copepods allowed them to avoid being captured by the larvae. While rotifers could be easily captured the growing body of evidence suggests that the ubiquitous rotifer is not a suitable live food for opakapaka larvae and another transitional live food is needed.

UNDERSTANDING *ARTEMIA* BIOGEOGRAPHY RELATED TO HATCHERY PRODUCTION AND JUVENILE QUALITY

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The culture of fish, mollusk, and crustacean larvae is generally carried out under controlled hatchery and nursery conditions, but the failure and high cost to garner high yields is partly due to the lack of ecological studies into live food resources such as the brine shrimp *Artemia*. Developing larvae are usually very small, extremely fragile, and generally not physiologically developed. Proper larval nutrition is one of the major bottlenecks in commercial aquaculture. The best sources of nutrition continue being the development and use of a succession of live food organisms as feed for the developing larvae. *Artemia* is one of the most important live foods in commercial aquaculture. *Artemia* can ingest small food particles ranging from 1 to 50 μm in size including diverse species of live microalgae, baker's yeast, dried microalgae, probiotics, and waste products from the food industry. The non-selective consumption of particulate food by the brine shrimp enables its use as a vector or "biocapsule" for delivering specific nutrients and biological protectors to marine and fresh water larvae, guaranteeing healthy juveniles. These nutrients and immune protectors are not stored in appreciable amounts in the larvae body, so signs of deficiency and sickness usually appear within weeks in young, rapidly growing fish. In many countries products from the processing of plant and animal products from human foods are the primary ingredients available for aquatic animal feeds (rice bran, corn bran, soybeans, whey powder, etc.). Most of these ingredients have limited nutrient levels, or even introduced anti-nutritional factors. Even dough these inert foods could be enriched the active predator larvae cannot take it easily. There is no artificial replacement for natural food sources, and such synthetic feed can increase water eutrophication and stunt larvae development. *Artemia* is considered now more than ever an important source of nutrients not just for its natural nutritional properties but also as an important "Biocapsule" of essential nutrients, vitamins, pigments, antioxidants, active enzymes, probiotics, antimicrobial agents, and vaccines. Considering the costs and difficulties

in the formulation of non-live food with the adequate amount of key nutrients, *Artemia* has several advantages. For all these reasons further studies of *Artemia* biogeography and its nutritional qualities are increasing in recent years. These field and laboratory studies of brine shrimp strains are demonstrating *Artemia's* nutritional value, as well as an indicator of natural ecological condition. In brief, *Artemia*'s use as live aquaculture feed is advantageous over existing inert food sources; as a result several new studies of *Artemia* biogeography and ecology are emerging to evaluate the quality and the production of cysts of different *Artemia* population's worldwide.

CONDITIONING TECHNOLOGIES FOR FLATFISH STOCK ENHANCEMENT: GLOBAL PROGRESS AND PITFALLS

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A successful stocking program requires survival of released fish, and to achieve this, fish must be able to adjust to their new environment, feed successfully, and avoid predation. However, hatchery-reared flatfishes (e.g., flounders, halibuts, soles) often exhibit irregular swimming, feeding, and cryptic (burying and color change) behavioral patterns compared with wild conspecifics, and these behavioral "deficits" are assumed to lead to increased predation risk once fish are released. Conditioning flatfish to natural stimuli before release may offer fish an opportunity to refine these behaviors, which may increase survival in nature and subsequent recruitment to the fishery.

Examples of conditioning technologies that have been applied to flatfish in the hatchery include providing sediments in rearing tanks, live (or life-like) feeds, or predator cues. Marbled flounder, *Pseudopleuronectes yokohamae*, reared for stock enhancement at Hyogo Prefecture Hatchery in Japan, are fed a mixture of minced frozen mysids with the addition of formulated, pellet feed to boost nutritional content. However, pellet feeding is suspended approximately 2 wk before release to focus fish on the more natural feed. In the US, providing hatchery-reared winter flounder, *Pseudopleuronectes americanus*, with live feeds (oligochaete worms and amphipods) during rearing conformed post-release feeding performance closer and more quickly to that of wild fish[1]. Cost and effort, however, are still the biggest impediments to implementing a prolonged, live-feed strategy in the hatchery.

Technologies that can be applied to ease the transition to the wild at, or near, the release site include, conducting "operant conditioning" on fish to respond to light or sound cues for supplemental food provision during the first few days or weeks post release, or short-term release into predator-exclusion cages before full release. Cage conditioning allows hatchery fish to experience natural substrates and sediments, wild (live) food sources, and "safe" predator exposure before actual release. Since 2004, Danish scientists have been cage

conditioning reared Atlantic turbot, *Psetta maxima,* before release, and this practice has resulted in a much lower post-release mortality.[2] Obama Laboratory, National Center for Stock Enhancement, Japan, has been conducting pre-release, experimental cage conditioning for Japanese flounder, *Paralichthys olivaceus,* since 2008 and found that conditioned fish have significantly higher recapture rates in the fishery as well as enhanced burying and feeding skills[3]. In both Danish and Japanese studies, evidence has been detected that intensive researcher recollection efforts at, or near, the release site may disproportionately sample weaker (i.e., non-feeding or non-moving) fish. At the University of New Hampshire in the US, protocols for releasing winter flounder have included cage conditioning since 2004, however, evidence arose that cages themselves attract 'structure-philic' predators, mostly crabs[4] ,so cage design has been modified in recent years.

Successful conditioning of stocked fish before release can increase post-release survival and recapture. However, choosing a location that can be monitored adequately may be just as important if the success of a stocking effort will influence future efforts (i.e., funding, resources, support).

CHARACTERISTIC EVALUATION METHOD RELATING TO BENEDENIA DISEASE OF YELLOWTAIL (*SERIOLA QUINQUERADIATA*)

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Yellowtail (*Seriola quinqueradiata*) is one of the most important species in marine fishery resources and aquaculture in Japan. The production of yellowtails occupies more than 50% in marine finfish aquaculture and reaches about 15,000 tons/year. At present, most yellowtail aquaculture production uses captured wild juveniles, but artificial rearing of juveniles is needed to sustain aquaculture production.

Benedenia is a parasitic disease caused in *Seriola* species by *Benedenia seriolae*. This parasite can cause growth reduction and external injuries in yellowtail, increasing the risk of secondary viral or bacterial infection. The major way of removing the parasite is to soak the fish into a freshwater bath. However, this method requires a lot of time, cost and effort.

Under this situation, we have been studying DNA maker-assisted selection "MAS" breeding, in order to use artificial seeds holding resistance to Benedenia disease in

aquaculture production. Three components ("Reproduction technology", "Character evaluation", and "DNA analysis") are critically important to promote "MAS" breeding certainly.

Here I would like to introduce one of the key components, "Characteristic evaluation method relating to Benedenia disease in the present species".