Regional e-Notes ~ July 2013 Vol 5, Issue 7

Letter from the Director

Aloha and summer greetings,

As the end of July approaches, I am happy to report that the FY13 development process is moving forward to the full proposal stage. This month, we held three meetings with our Technical Committee and Industry Advisory Council to discuss the 27 pre-proposals CTSA received in response to our April RFP-P. At these meetings, the IAC and TC selected eight pre-proposals to move forward, and determined questions and suggested changes for Principle Investigators to address in the full proposals.

We are excited about this next round of projects that CTSA will support, but want to take this opportunity to remind you that CTSA is not a large funding organization. Our program must distribute its limited funds to those projects that demonstrate the most need and greatest potential for impact, even if that means that some islands in the region do not receive consistent support from CTSA. That being said, a significant amount of pre-proposals this year were from institutions and organizations in the Western Pacific. Several successfully demonstrated the need for local aquaculture efforts and are moving forward to the next stage in the CTSA development process.

Our IAC, TC, and administrative staff are excited to see what will come from the FY13 projects. We encourage researchers who submitted pre-proposals that will not move forward in this cycle to continue submitting their ideas in the future. It takes a lot of thought and energy to prepare a pre-proposal, and we appreciate the effort you put into it.

As always, please let us know if you have any questions, comments, or concerns.

Mahalo,

Cheng-Sheng Lee
Executive Director, CTSA

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7/31 - 4pm
8/1 - 8:30am
8/2 - 12pm (channel FOCUS 49)

CTSA Project Summary & Pacific Islands Spotlight: Collection and Health Certification of Coralgrouper Broodstock in Guam

Hui Gong and John W. Brown
College of Natural and Applied Sciences, University of Guam, Mangilao, GU 96923

Introduction
The coralgroupers are among the most sought after and valuable food fish of the coral reef habitat worldwide. They are some of the highest priced fish in the live food fish markets in Hong Kong, where they are imported from across vast regions of Asia and Oceania.

As such, many coralgroupers are listed in the IUCN Red list. *Plectopomus areolatus* is listed in the threatened category, because they suffer from over fishing throughout their range. On Guam, they have become extraordinarily scarce. Under such circumstances, a program geared towards restoration of the natural stocks of this species should be useful for the local reef ecology. On the other hand, the aquaculture industry of Guam has a strong desire to develop a local, high end product that can be marketed as a live, in-restaurant product for the tourist trade. The relatively large dark-edged circular blue spots on reddish gold background and truncated tail with a narrow white posterior margin of the *P. areolatus* grouper make them an ideal product for a premium live fish dish for a prestigious dinner in any white table cloth Asian restaurant.

Virus infections plague the grouper industry in Asia. Chief among the culprits are two viral diseases, viral encephalopathy and retinopathy (VER)/ viral nervous necrosis (VNN) and the grouper iridovirus diseases. The lack of wild coralgrouper broodstock, its questionable

Fig.1. Health inspection and sampling.

health status, and the widespread presence of viral infections in commercial grouper hatcheries means that there may be a market ready for SPF coralgrouper fry or broodstock in Asia.

The project was conducted to establish a domesticated, high health population of indigenous coralgroupers of Guam at the Guam Aquaculture Development and Training Center. The project had three objectives: 1. to capture sufficient numbers of two species to establish a breeding population, 2. to test all fish for viral infections and maintain the fish in a secure, high heath environment and, 3. to raise the fish to the point where we have sufficient numbers of mature fish both sexes to be ready to begin reproduction trials.

Collection of wild stock for *P. areolatus*

The collection of the indigenous live coral grouper proved to be a long and challenging process. In the beginning stage of the proposal, we were focusing on two coral grouper species, *P. leopardus* and *P. laevis*. After consulting with the fish biologist in Marine laboratory at UOG and local fishermen, we determined that *P. leopardus* is not an indigenous species of Guam, and *P. laevis* is a very uncommon species. Therefore, we focused on *P. areolatus* because of its indigenous status and the history of induced spawning of the species by a researcher of GADTC in the 1990s. John Tucker of Harbor Branch and William Fitzgerald of the Guam Aquaculture Development and Training Center (GADTC) reported the first induced spawning and partial rearing of *P. areolatus* (1994). It was done at the Micronesian Mariculture Demonstration Center in Palau in 1991 using human chorionic gonadotropin.

We contacted the University of Guam Marine Laboratory to inquire about their observations of the abundance of coralgroupers and the locations where they were observed during the fish census transects around the island. We were told that while indeed coralgroupers were quite scarce on Guam, they had been observed in very limited numbers, and the Laboratory provided us with some of the locations where individual fish had been observed (personal communication - Dr. Jennifer McIlwain).

We made contact with numerous people on Guam about the availability of a bounty of $200.00 per live coralgrouper as outlined in the original proposal. Among those contacted were the only two active tropical fish collectors on island and several highly active commercial and semi-commercial fishermen. In addition, we arranged for two sets of directed fishing effort. The first effort was for Dr. Brown and Mr. Frank Alig, the GADTC manager, to bottom fish weekly for coralgroupers during the morning, low wind hours. As a result, five fishing trips were completed. The second directed fishing effort was to contract Mr. Jesse Rosario, the Administrative Officer of CNAS who is also a highly active semi-commercial fisherman, to fish on an overtime basis at night and on weekends. He has completed three fishing trips. These trips covered the Western near shore of Guam plus Apra Harbor with the exception of the Marine Preserves.

In addition to continuously contacting the professional fisherman for live coralgroupers, we started a cooperative effort with Underwater World, the commercial aquarium on Guam, to join their live collecting expeditions on a weekly basis as the senior technician felt that it may have been possible for them to obtain some coralgroupers in the Spanish Steps area south of Apra Harbor. A total of seven trips were made without sighting of a *P. areolatus*. All of our efforts on Guam failed to produce a single live fish or even a sighting of a coralgrouper in the wild.

We then investigated the possibility of cooperating with the Palau Community College Land Grant

aquaculture program and the Palau Division of Marine Resources, as both groups indicated their willingness to assist us in our efforts to obtain the coralgroupers needed to complete this project. The coralgroupers are aggregate spawners. In Palau, the *P. areolatus* spawn in West Pass in May. The fishery closes on April 1st for protection of the spawning. The fish migrate into this area during February, March and April and disperse widely after the spawning season. There are reports of spawning migrations of up to 20 miles. We fished for them in February and March. Both trips were successful, but the late march effort was much more productive. Thus, we successfully obtained a 1st shipment of 10 live *P. areolatus* to Guam in April 2012. The fish intended for the 2nd shipment from Palau died during the pre-shipment, holding period. Dr. Brown visited Palau in mid-November 2012 to try to obtain some additional fish and the trip was unproductive. Plans were made with Thomas Taro to restart our efforts as the fish started to return to the spawning grounds. However, on December 3, typhoon Bopha struck Palau that put the collection effort in halt. In January 2013, our collaborators in Palau tried several times for catching the fish, and no avail. Eventually, the collection efforts in February and early March was successful and resulted in a 2nd shipment comprised of 5 female and 5 male sexually mature groupers to Guam on March 28, 2013.

During the searching effort of the local fish vendors for coralgroupers for the laboratory work, we found out that *P. areolatus* was being shipped to Guam from Chuuk. Through the help of Mr. Kind Kanto, the Dean of the community college campus there, we were able to obtain 15 *P. areolatus* in two shipments that were captured from Chuuk Lagoon. The first shipment of 7 groupers was made on February 7, 2013, and training was provided to the people in Chuuk on packing. The second shipment of 8 groupers arrived Guam on February 26, 2013.

Overall, the four shipments ended up in bringing a total of 35 live *P. areolatus* to Guam.

Disease Diagnosis

Bondad-Reantaso (2002) reviewed grouper disease and health management issues for a regional workshop in Medan, Indonesia. Two families of viruses were singled out as causing the most severe problems in grouper culture: the Nodaviridae and the Iridoviridae.

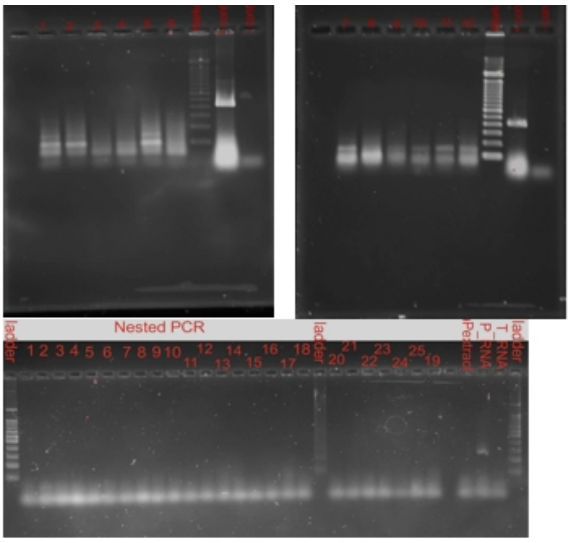

Fig. 2. Gel pictures for VNN detection: top two pictures are from 1st step RT-PCR and bottom one for nested PCR.

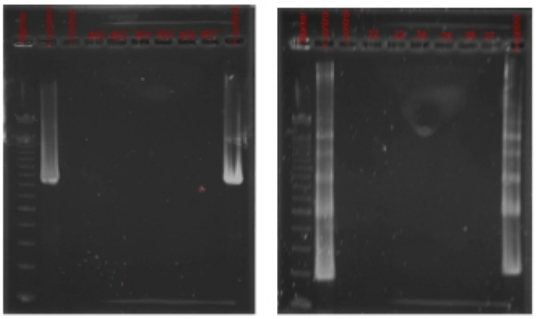

Fig. 3. Gel picture of 1st step PCR (left) and nested PCR (right) for the Iridovirus

In grouper culture, VER/VNN is sometimes called spinning grouper disease, is one of the most significant viral diseases distributed globally. It is primarily a disease of larval and juvenile fish, although adult fish are also affected (OIE Aquatic Manual 2012). It is caused by a small, non-enveloped, bi-segmented, singled-stranded, positive-sense RNA virus. The viruses have a diameter of 25-30 nm and belong to the genus Betanodavirus (Lee et al. 2002). Phylogenetically, the viruses have been separated into four clusters based on the nucleotide sequences of the coat protein gene (Munday et al. 2002). The transmission of VER/VNN is thought to be both vertical and horizontal. Reverse transcription polymerase chain reaction (RT-PCR) was to detect the coatprotein gene (RNA2) of betanodavirus with a primer set (F2 and R3) (Nishizawa et al., 1994), which were available to amply T4 region of any genotypes of

betanodavirus. RT-PCR amplicons were then put through a nested PCR step using another set of primers to identify the presence of the viral infection.

Red sea bream iridoviral disease (RSIVD) is a significant cause of mortality in farmed red sea bream (Pagrus major) and more than 30 other species of cultured marine fish (Kawakami & Nakajima, 2002; Matsuoka et al., 1996), and the causative viral agent is a DNA virus of the family Iridoviridae. PCR is the robust and efficient method of detecting VER/VNN and iridovirus infections in asymptomatic animals.

Individual specimens were held separately until they were sampled for viruses and visually inspected for other disease issues. Once the fish had recovered from the trauma of capture and stress of shipment, a 200L anaesthetizing tank (AT) and a 35L shallow holding tank (HT) were used for grouper inspection examination for lesions and parasites. The concentrations of MS-222 in AT and HT were 70ppm and 35ppm respectively. The fish were weighed and measured to the nearest inch in total length. Gill clippings were taken from each live fish, followed by directly applying iodine disinfection on the gill wound. Fish went through 50ppm iodine dipping tank for 10 seconds and then to oxygenated recovery tank prior to releasing in the rearing tank. Bioteke high purity RNA extraction kit was used for RNA extraction used, DNAzol method was adopted for DNA extraction. RT-PCR for VER/VNN and PCR for RSIVD were performed thereafter. The primers sets used for VNN were 5'-CGTGTCAGTCATGTGTCGCT-3' and 5'- CGAGTCAACACGGGTGAAGA-3' for the 1st step RT-PCR, 5'- ACCTGAGGAGACTACCGCTC-3' and 5'-CAGCGAAACCAGCCTGCAGG-3' for the nested PCR. While we adopted the primer sets developed by Kurita et al. (1998) for RSIVD were 5'-CTCAAACACTCTGGCTCATC-3' and 5'-GCGTTAAAGTAGTGAGGGCA-3' for 1st step PCR, 5'-TACAACATGCTCCGCCAAGA-3' and 5'-GCACCAACACATCTCCTATC-3' for the nested PCR.

PCR and RT-PCR results showed that the neither of the RSIVD nor VNN was detected from grouper samples. Other than the viral diseases, bacterial infection, such as fin rot disease was identified as the major problem encountered throughout the domestication of the groupers and appropriate treatments were sought after. As for the live fish, minor ventral fin lesions were observed from time to time due to water quality problems. The fish from Palau and Chuuk were kept separately in two 20-ton fiberglass tanks. During the project period, all fish had been continuously and carefully monitored for any abnormal signs of disease infection.

Maintenance of groupers

During the domestication of the 1st shipment of coral grouper from Palau, heavy mortality occured due to the infection of fin rot diseases. Fin rot disease was caused by Flexibacter maritimus, and a secondary Vibrio infection worsened the fish condition when it was not treated properly in a timely manner. Because of our inexperience of handling this coral grouper, the intake water wasn't filtered and algae blooming hadn't been controlled properly, 8 out of 10 fish were lost as a result of severe bacterial infection of fin rot disease even though the attempts of treating the fish with freshwater bathes and the antibiotic of 5.26ppm erythromycin for 15 hours were made.

Learning from the bad experience of 1st shipment, we modified our protocol of handling fish: covered the tanks with double layered black shade cloth, filtered the intake water down to 5 mm, applied freshwater bath, and antibiotics treatment for preventative and post-infection treatments. The remaining fish were in reasonably good conditions.

By the end of the project, 11 fish from Palau were maintained in one 20-ton fiberglass tank, and 15 fish from Chuuk were held in another 20-ton fiberglass tank. The fish had been trained to consume frozen squid. Every month, the fish were put through a freshwater bath prior to re-stocking into a newly cleaned/disinfected tank.

The milestone of this objective of having eleven nearly mature, mixed sex and apparently virus free broodstock *P. areolatus* and fifteen younger fish in the maturation tanks at the GADTC was achieved by the end of this project.

Summary

Through this project, we were able to obtain 35 live *P. areolatus* from 4 shipments, which originated from Palau and Chuuk. This population was given health examinations, including PCR diagnosis of VNN and Iridovirus, and we gained knowledge of rearing and handling this specific species. By the end of this project, we had 26 live *P. areolatus* ranging from 202g to 1790g. VNN was not detected in the gill samples of the live fish. Thus, they could be used to establish a broodstock base to generate high health offspring upon further testing by PCR methods.

This coralgrouper offers the possibility of serving three markets: providing fish of the restoration of natural reef populations, providing fry for local farmers and providing SPF fry or broodstock to the Asian aquaculture industry.

Acknowledgments
The authors wish to thank Dr. Leobert de la Pena from the Southeast Asian Fisheries Development Center, Dr. Ruth Garcia Gomez, SPC Aquaculture Section for their technical support in finfish health evaluation, Mr. Thomas Taro, Miguel delos Santos, and Kind Kanto for their kind efforts and assistance in collecting the coral groupers for the project. Our thanks also go to Mr. Frank Alig and GADTC staff for daily maintenance of the groupers.

AquaClip ~ Offshore Aquaculture in Hawaii

Oceansphere: The next wave of sustainable fish farming

By Elga Reyes, www.EcoBusiness.com, July 25, 2013

Honolulu-based aquaculture company Hawaii Oceanic Technology recently received a Canadian patent for its open ocean farming system and the company is expecting to receive more from the European Union, Japan and Australia.

Called the Oceansphere, the fishing system is an automated positioning and submersible open ocean platform that can grow yellow fin tuna and other pelagic species in an efficient and sustainable manner.

Currently, farmed fish provides half of all seafood consumed in the world. However, according to the United Nations Food Agriculture Organization (FAO), by 2030 an additional 62 million metric tons of seafood needs to be produced annually to meet the growing demand without depleting wild ocean stocks.

For Hawaii Oceanic Technology, the solution is in Oceansphere. Bill Spencer, the firm's chief executive officer told Eco-Business, "The Oceansphere is designed to be environmentally responsible and economically sustainable." It utilises proven off-the-shelf technologies that are well suited for large-scale deep ocean fish farming, which can satisfy future demands.

"The idea is to move fish farming off-land, away from industrialised near shore waters, bays and estuaries, out into the deep ocean, where it is a desert-like environment," said Spencer. There, the farming system will be isolated and submerged so it will not be affected by surface activities like high winds or typhoons.

The Oceansphere is what the FAO deems as an aquaculture type of high technology closed system. It is a spherical cage with an exoskeleton made of neutrally buoyant materials with netting that is stronger than Kevlar. There is an automated buoy on the topside and this can hold 100 tons of fish feed that will automatically dispense to the fish contained inside the Oceansphere.

Spencer explained that employing this system, though not tethered to the ocean floor, enables natural processes to occur. This is better for the fish and it results in low food conversion rations, better health and faster growth.

However, the company founded in 2006 has yet to deploy an Oceansphere. Spencer detailed that

they have been obtaining permits for the past seven years.

Click here to read the original article.

National Geographic Article: Sustainable Ancient Aquaculture

By Mark Spalding, National Geographic Ocean Views. July 11, 2013

Phrases like "lessons from the past" or "learning from ancient history" are apt to make our eyes glaze over, and we flash to memories of boring history classes or droning TV documentaries. But in the case of aquaculture, a little historical knowledge can be both entertaining and enlightening.

Fish farming is not new; it has been practiced for centuries in many cultures. Ancient Chinese societies fed silkworm feces and nymphs to carp raised in ponds on silkworm farms, Egyptians farmed tilapia as part of their elaborate irrigation technology, and Hawaiians were able to farm a multitude of species such as milkfish, mullet, prawns, and crab. Archaeologists have also found evidence for aquaculture in Mayan society and in the traditions of some North American native communities.

The award for oldest records about fish farming goes to China, where we know it was happening as early as 3500 BCE, and by 1400 BCE we can find records of criminal prosecutions of fish thieves. In 475 BCE, a self-taught fish entrepreneur (and government bureaucrat) named Fan-Li wrote the first known textbook on fish farming, including coverage of pond construction, broodstock selection and pond maintenance. Given their long experience with aquaculture, it's no surprise that China continues to be, by far, the largest producer of aquacultural products.

Click here to read the original article.

The Center for Tropical and Subtropical Aquaculture (CTSA) is one of five regional aquaculture centers in the United States established and funded by the U.S. Department of Agriculture's National Institute of Food and Agriculture (NIFA) under grants 2007-38500-18471, 2008-38500-19435, and 2010-38500-20948. The regional aquaculture centers integrate individual and institutional expertise and resources in support of commercial aquaculture development. CTSA was established in 1986 and is jointly administered by the Oceanic Institute and the University of Hawaii.

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