



Letter from the Director

Aloha!

This month's issue of e-notes features an article on the ongoing CTSA project to establish Coral grouper aquaculture in Palau. I am excited about the promising results that this project has obtained thus far, and look forward to the establishment of grouper culture technology in the small island nation. Thank you, Miguel de los Santos.

Grouper is a highly desired food fish worldwide, and the farming of Coral grouper has multiple benefits. Aside from increased food and economic security, the Coral grouper project (and other similar aquaculture activities such as Mangrove crab farming) can also help to preserve species that are threatened by overfishing in Palau. Coral grouper used to be abundant in the region but wild populations are now scant. While it may take a few years for the culture technology to be applied commercially, fingerlings produced through the project can be used immediately to replenish dwindling stocks and increase biodiversity. This is especially fitting for conservation-minded Palau.

Even though our region does not have a large volume of aquaculture production, we can certainly make a unique and resounding impact within our communities and beyond by capitalizing on our understanding of the importance of all efforts to keep our ocean biodiverse.

On a related note, I would like to express my sincerest appreciation to my friends and colleagues Dr. Harry Ako and Dr. Clyde Tamaru, who have dedicated countless hours to advancing our industry and improving the lives of others through responsible aquaculture. At their joint retirement banquet earlier this week, a review of their spectacular professional careers reminded me of the importance of teamwork. I look forward to reading this year's pre-proposals and seeing the all of the new collaborative ideas to address the problems facing our industry.

Mahalo,
Cheng-Sheng Lee
Executive Director, CTSA

REMINDER! CTSA FY16 Pre-Proposals Due June 1

The Center for Tropical and Subtropical Aquaculture (CTSA) requests pre-proposals for applied research and extension that addresses problems and opportunities in the regional aquaculture industry. CTSA stakeholders have identified several strategic areas and species as the top aquaculture development priorities, listed in the full announcement. Pre-proposals that target these

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strategic areas and priority species will receive highest preference. However, pre-proposals that do not fall under specific priority areas but address CTSA's mission will be considered in our development process. Our focus is on funding projects that will have immediate, positive impacts on the regional aquaculture industry.

[Click here to view the full announcement](#), including submission instructions, on the CTSA website. Please contact mbrooks@ctsa.org with any questions.

CTSA Project Update: Establishing Coral Grouper (*Plectropomus leopardus*) Production in Palau through the Application of Intensive Copepod Production Technology

by Chad Callan, Ph.D. and Miguel de los Santos; edited by Meredith Brooks, CTSA

The culture of high-value marine fish (such as grouper) is rapidly expanding in Asia. In particular, the high demand for live grouper in the Hong Kong market commands wholesale prices of up to US\$100 per kilogram. Groupers have been cultured in South East Asia for over 30 years, yet current culture methods still face very low hatchery survival (~1%) in some of the most commercially important species, such as the coral grouper (*Plectropomus leopardus*), largely due to inadequate larval feed items. Copepods have been found to significantly improve larval survival in many grouper species but, due to their inherent production challenges, have failed to become integrated in most grouper rearing protocols.



A female coral grouper broodstock being held at PCC CRE Hatchery showing a full abdomen of hydrated eggs.

To address this issue, CTSA is supporting the project "Establishing Coral Grouper (*Plectropomus leopardus*) Production in Palau through the Application of Intensive Copepod Production Technology," now in its second year, to apply and expand intensive, copepod-based larval rearing technology developed at the Oceanic Institute (OI) at Palau Community College (PCC) hatchery facilities. The goals of Year One of this project were to first establish coral grouper broodstock at the Palau Community College (PCC) CRE Hatchery, establish copepod production capacity at the hatchery, and to identify suitable species of copepods for the culture of coral grouper larvae. We are pleased to report that we have made significant progress towards achieving all these

goals, in addition to progress on goals related to spawning and larval rearing.

In late 2014, coral grouper were successfully recruited and maintained at PCC hatchery facilities, where 30 fish currently remain. We identified that external parasites are a particular concern for this species and have identified an effective treatment remedy (formalin). In June 2015, OI staff traveled to Palau to assist with spawning of the grouper, as their natural spawning season is June-August. At that point, the grouper that were held at PCC had not spawned naturally. We concluded that it may be necessary to induce the grouper and/or attempt to utilize the eggs from a different species (if they were available). This is why we decided to also bring in *P. aeorolatus*. We used LHRH-a (as HCG was not available on island at the time) to induce several females from each species that were showing signs of hydrated oocytes. The fish that were induced (four females and 2 males) were placed in a 6-ton tank and held overnight. A mesh bag was placed over the effluent pipe to catch any eggs that were produced.

The next morning we were pleased to see that the *P. aeorolatus* spawned and produced nearly 1M eggs, but the *P. leopardus* did not spawn. However, after inspection of the eggs under the microscope it was determined that the eggs were not fertilized. We assessed the fish again in the tank and upon pressing the abdomens of the males could see the good expression of sperm, so we knew they were mature. We concluded that they may not be spawning for other reasons, so we tried to strip hydrate eggs from females that did not spawn and sperm from those males.

Unfortunately, this method was not effective and we were not successful in obtaining fertilized coral grouper eggs during that attempt.

A second induced spawning was attempted in December 2015 utilizing HCG (which the Co-PI has used effectively in the past). In that attempt, five *P. leopardus* females from the large tank (avg. 800 g) and five *P. leopardus* females from the smaller tank (avg. 700 g) were injected with 700 IU HCG on the day of the new moon and the day after. Additionally, four of the *P. aeorolatus* females (avg. 1.2 kg) were injected as well. Unfortunately, none of the fish spawned, despite showing swollen abdomens (presumably from hydrated oocytes) and displaying courtship behaviors. We also were not able to get sperm expressed from the males at that time, so we suspected many of the males may not have been fully running ripe.

We are very pleased to report that in March 2016, the *P. leopardus* broodstock started to spawn naturally. From the larvae that hatched, we were able to conduct preliminary larval rearing trials using a 6-ton capacity rectangular concrete larval rearing tank. In our first run, out of 66,000 larvae we only produced 102 fry over 30 days old, while in our second run we were able to produce about 12,000 fry (over 30 days old) out of 158,000 larvae. The total number of eggs collected for the three consecutive spawning months was about 3M eggs, and the average percentage of good eggs was about 58%.



Coral grouper larvae at 21 days post-hatch

A large part of the success we are experiencing with larval rearing is due to the establishment of copepod cultures at PCC hatchery. In November 2014, copepods were collected from the waters adjacent to the PCC hatchery and sorted to identify a suitable species for culture. *Parvocalanus* sp. were found in the region and were selected for scale-up along with a few other, yet to be identified, species. The methods developed at OI for the culture of *Parvocalanus* copepods in Hawaii proved very adaptable to the conditions in Palau and populations were quickly scaled-up to 400L culture volume. At the same time, considerable improvements were made to the PCC algae production lab.

Cultures of *Tisochrysis lutea* and *Chaetoceros mulleri* were established and monitored for growth and production potential. Over the reporting period, weekly transfers have been performed and cultures have yielded comparable production to the systems at Oceanic Institute's upgraded lab. Initially, daily egg and nauplii export from a single 400L copepod culture tank were subsequently recorded over 14 production cycles (~100 days) yielding an average daily egg and nauplii production of 1.5M nauplii per day (min =0, max =7.1M). In June of 2015, a second production tank was established to increase the numbers of daily nauplii produced in anticipation of grouper larval rearing commencing. The addition of this second tank did increase copepod nauplii production, with daily peaks over 8M per day recorded. However, harvest numbers fluctuated greatly from day to day, with the daily average being closer to 3M nauplii per day.



Improved lighting and aeration has dramatically improved the production of live microalgae at PCC CRE Hatchery

Higher and more consistent production numbers are expected as PCC hatchery staff become more familiar with the culture management of *Parvocalanus*. Also, a modified copepod maturation protocol is being implemented now, which should alleviate some of the daily fluctuation in nauplii production numbers. As a result of implementation of copepod production capacity at PCC Hatchery, we were able to successfully test the copepod nauplii as a first food compared to rotifers in 2015 using grouper larvae (reared from eggs collected from the wild, since our fish had not yet produced viable eggs). Grouper larvae fed with copepod nauplii had significantly higher ($P < 0.05$) survival rate of $64.25 \pm 2.81\%$ compared to

those that were fed with rotifers which was only 7.13 ± 3.69 %. This result confirms that copepod nauplii are essential in the successful production of grouper and that the scaling up of copepod production capacity is an imperative area of future focus. Indeed, this is the primary objective of Year Two of this project, where we plan to implement and test scaled up production methods for both algae and copepods. The goal would be to produce 20-30 million nauplii per day (roughly ten times the current production) in order to effectively supply a 4,000 L larval rearing tank capable of producing thousands of fingerlings.

Our results have thus far been shared with at least one local industry stakeholder, Biota Inc., as we performed our larval feeding trial in parallel with them at their own facility. Furthermore, technicians from that facility have come to PCC to observe and learn about the improved algae and copepod production methods implemented at the PCC Hatchery. In addition, as a direct result of this project, the Co-PI now has two highly trained technicians who can effectively run all aspects of hatchery operations in his absence. This is a huge increase in aquaculture capacity in this region and will surely increase the likelihood of overall project success.

Retirement Banquet Honors Dr. Clyde Tamaru and Dr. Harry Ako for their Dedication to Serving Others through Teamwork



L to R: Dr. Clyde Tamaru, Dr. Harry Ako, Dr. Cheng-Sheng Lee

On May 24th, the Hawaii Aquaculture and Aquaponics Association (HAAA) hosted a banquet at Kapi'olani Community College to honor the work of Dr. Clyde Tamaru and Dr. Harry Ako. Dr. Tamaru, who served as PI of several CTSA projects, and Dr. Ako, who serves as the Chair of the CTSA Technical Committee, recently retired from the University of Hawaii at Manoa.

At the banquet, both gentlemen were presented with Proclamations from the HAAA and 'Certificates of Appreciation' from CTSA, honoring their dedication to public service in support of the Hawaii Aquaculture and Aquaponic Industry. Dr. Tamaru also shared a heartfelt presentation highlighting his and Dr. Ako's storied careers, and acknowledging their colleagues and others who have helped them achieve lasting impacts.

"Working with my colleagues to help people has been the best part of the job for me," stated Dr. Tamaru during his presentation. "The highlight of my career was the work we did to help establish backyard milkfish farms in Indonesia. It was very rewarding because it resulted in the alleviation of poverty for participating farmers."

CTSA Executive Director Dr. Cheng-Sheng Lee reiterated that sentiment as he reminisced about many of the stories Clyde shared about their efforts to improve aquaculture in the Pacific region. "Teamwork is essential, and also enjoyable" exclaimed Dr. Lee, who has collaborated with both researchers for over thirty years. "Clyde joined my team at Oceanic Institute in 1984, followed shortly after by Harry, who brought a unique set of biochemical analytical skills to investigate many unknowns. We worked together across the globe to perpetuate aquaculture and address problems facing farmers. One of our most notable accomplishments, the development of the world's first homemade hormone pellet, would not have been possible without everybody working together."

More than 50 people attended the event to share their gratitude and personal stories of how the researchers have impacted their lives. "These two gentlemen are amazing and have dedicated their careers not only to scientific research but to helping the next generation of scientists and leaders," stated Manning Taite of NALU Studies. Both Dr. Ako and Dr. Tamaru encouraged Mr. Taite to establish a science education program for at-risk youth, and provided mentorship and technical support along the way. "They are tireless in their commitment to the students, especially the ones that don't normally have those opportunities. We are extremely grateful for the difference they have made in our lives and in our students lives, and we cannot thank them enough."

The event was a reminder of what we as industry stakeholders can achieve if we work together, from research breakthroughs to meaningful community development. "Both Harry and Clyde are doers who make things happen," added Dr. Lee. "It's never been important who gets the credit. Let's use their example and keep working together to get more done."

Up next for Dr. Tamaru is a retirement filled with much deserved relaxation and quality family time with his wife, children, and grandchildren. Dr. Ako is anticipating a "working retirement," as he will continue consulting in the aquaculture and agriculture industries. CTSA congratulates both gentlemen on their four decades each of research, education, and extension efforts to improve aquaculture technology and help people. We sincerely appreciate their contributions to the development of aquaculture in Hawaii and the Pacific Islands.

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AquaClip ~ Investor takes Cyanotech to task over astaxanthin supply

by Hank Schultz, Nutraingredients-usa.com. May13, 2016.

An investment firm that now owns almost 13% of the stock of Cyanotech is announcing an effort to push the company to speed up the process of bringing additional astaxanthin supply capacity online.

[Read full 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 2012-38500-19566 and 2014-38500-22241. 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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