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REGIONAL NOTES
CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

Hawaii's 2nd Open-Ocean Farm Launches Kona Kampachi

One of only two open-ocean aquaculture companies in the nation, Kona Blue Water Farms LLC last fall officially introduced its first product, a fish the upstart has given the trademarked name of Kona Kampachi. "It's tremendously rewarding to have taken this species through the research and development stage and to now see the responses from people as they taste the fish," says Kona Blue president Neil Sims.

The four-year-old Big Island company's version of kahala or amberjack (*Seriola rivoliana*) has seen solid progress early into its market development. Two months after its Sept. 22 harvest-blessing event, Kona Blue was selling its Kona Kampachi to as many as 25 distributors on a weekly basis, says Terry Delaney, vice president of sales and marketing.

Hawaii and the West Coast of the United States are the focus of sales efforts. Still, restaurants in Denver, Chicago, and New York have shown interest in the fish, and Kona Blue has had inquiries from Europe.

Wholesalers report that demand is rising for the sashimi-grade fish. "We've sold more in the last few weeks than the first few weeks," says Bob Fram, president of Garden & Valley Isle Seafood of Honolulu. "Kona Kampachi is not a household name. It's just starting, but there's great capacity and potential for growth."

Photo courtesy of Kona Blue

Kona Blue raises its fish, shown harvested above, for up to nine months in open-ocean cages.

Diamond Head Seafood president Gary Ishimoto concurs, adding that the restaurant and food service markets will realize success more easily than the retail market. Consumers tend to act more thrifty and less adventurous at retail than they typically act at a restaurant, explains Ishimoto.

Photo courtesy of Kona Blue

Each of four growout cages, located a half-mile offshore from the Big Island's Keahole Point and submerged 30 feet below the ocean surface in water 200 feet deep, hold up to an estimated 110,000 fish.

Restaurant chefs play a key role in marketing fish, says Kona Blue's Delaney: "We have to get in front of chefs. They are always looking for new and interesting products." White-tablecloth restaurants make up one of three markets at the center of the company's marketing strategy. Another target is the natural food industry.

The primary market includes Japanese, sushi, and Asian restaurants and supermarkets. Kona Kampachi is similar to popular sushi fish like the Japanese hamachi. "We have sold more as sashimi versus filet," says Ishimoto, referring to his Honolulu-based business and noting that other fish, such as marlin and mahimahi, make for more cost-effective filet products.

The only complaint distributors have about Kona Kampachi is about its high price. Kona Blue's Sims declined to provide specific data in our interview, but he did say the fish typically prices similar to other upscale fish like opakapaka. Wholesalers report that they price a whole fish between $5.75 and $7 per pound.

Upscale is just where Kona Blue wants to be. "We can't compete and we don't want to compete on a dollar value," says Delaney. "For now, we're going to stay focused on the high end." The hope,

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Letter from the director

Hau'oli Makahiki Hou! As we begin a new year, I'd like to review some key changes made in 2005. We leveraged teleconferencing and Internet technology last year more than ever before, making our communications more efficient. Reducing the need for a physical presence to perform duties, CTSA less often requires members of the IAC, TC and Board of Directors to spend time away from their farm or research institution. Most important, we have shortened our project development process.

Meanwhile, we altered the purpose of our annual IAC-TC meeting to focus on project progress. My aim with this shift is to ensure CTSA-supported research continues to provide useful results to the aquaculture industry in Hawaii and the American Insular Pacific. In 2006, we will redouble our efforts to improve the quality of our program. Also, please see the box on Page 9 about important personnel changes.

Cheng-Sheng Lee

REGIONAL NOTES

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AQUA CLIPS

Infected shrimp batch destroyed

By Leila Fujimori, Honolulu Star-Bulletin, November 24, 2005

A batch of 10,000 imported live baby shrimp held for a 120-day quarantine in Hawaii tested positive for the white-spot syndrome virus. The virus, highly contagious to shrimp and other crustaceans, was safely contained, state officials said yesterday.

Hawaii Kuruma Shrimp imported the immature tiger shrimp, highly prized by sushi chefs and fetching up to $30 a pound, from Mie prefecture in Japan, said Janelle Saneishi, spokesperson for the state Department of Agriculture. The same virus occurred at Ceatech on Kauai in April 2004 in an unrelated case, she said.

"The potential for Hawaii aquaculture is tremendous, and the health of the industry relies on prompt reporting and management of any disease problem," said John Corbin, manager of the Aquaculture Development Program. State officials said the incident illustrates the need for Hawaii's animal quarantine system.

NEHLA tenants protest seawater rate hike

By Kevin Dayton, The Honolulu Advertiser, August 27, 2005

The board of directors of the Natural Energy Laboratory of Hawaii Authority (NEHLA) yesterday approved a rate increase for the deep seawater it supplies to its tenants, a change that drew objections from many tenants yesterday. Ron Baird, chief executive officer of NEHLA, has said the rate hike will help to cover increased costs at the 870-acre ocean science and technology park at Keahole Point in Kona, and is a part of a larger effort to make the park self-sufficient.

Gerald Cysewski, president of a tenants' group called the Keahole Point Association, said most of the aquaculture companies in the park are already struggling, and the rate increase will hurt. Cysewski, also president and chief executive officer of Cyanotech, said tenants believe the rate increase will raise considerably more money than the park needs to cover its higher operating costs. For aquaculture companies, the rates would be increased to 14.4 cents per thousand gallons from 11.74 cents per thousand gallons of deep seawater, he said. The hike follows 20 percent water rate increases that were imposed in each of the last two years, he said.

Oceanic Institute president selected for open-ocean aquaculture group

By Staff/Moon Yun Choi, Honolulu Star-Bulletin/MidWeek, August 14/July 6, 2005

Oceanic Institute President Bruce Anderson was recently selected as one of nine members of a national task force created to address issues of open-ocean aquaculture. The Marine Aquaculture Task Force will look at risks and benefits of aquaculture in the sea and publish a report recommending national standards for sustainable aquaculture in 18 months. "My role will be providing the task force with practical knowledge about aquaculture from the standpoint of a state that's actually done it," says Anderson. "Hawaii is the only state in the country that has that experience."

New Ceatech owners make progress at Kekaha

By Andy Gross, The Garden Island, August 6, 2005

The new ownership team of the Kekaha-based Ceatech USA Inc., Sunrise Capital LLC purchased the assets of the bankrupt shrimp farm in late June for $389,000. Ceatech filed for Chapter 11 bankruptcy protection from creditors on March 22. Kelvin Taketa, a principal of Sunrise Capital, said he expected the actual sale to close in September.

The company is formulating plans for restoring the operation and has received approval from the state to assume the leases for the hatchery site and also received a 20-year lease, Taketa said. "Our reason for doing this is to design and create an aquaculture industry. It is our expectation this (enterprise) will be a sustainable, long-term business," said Taketa, adding that he could envision raising other types of shrimp and seafood products for the local market and for export to Asia on the state-owned land in Kekaha.

Stakeholder summit articulates bottlenecks

More than 65 representatives from the aquaculture community gathered at the Hawaii Aquaculture Industry Stakeholder Summit on Sept. 30 to brainstorm and build consensus around ways to unify the state's industry and to take it beyond a total annual product value of $28.1 million. That figure, reported by the Hawaii Agricultural Statistics Service for 2004, denotes steady growth but is dwarfed by aquaculture production in other areas of the world.

"For the first time, we all sat down together," says Clyde Tamaru, one of the event's organizers and an extension specialist at the University of Hawaii at Manoa's Sea Grant College Program. Farmers, government representatives, researchers, and scientists met at the Waimanalo, Hawaii, campus of the Oceanic Institute, one of four sponsors of the summit. The other sponsors were the University of Hawaii, the state's Aquaculture Development Program, and the Hawaii Aquaculture Association.

Photo courtesy of Oceanic Institute

Donna Ching, an extension specialist at the University of Hawaii, facilitated deliberations at the summit.

"We wanted to get stakeholder input on where we want to be in five or 10 years," says Tamaru, who gathered and summarized some of that input or "homework" ahead of the meeting. Counting responses from people who did not attend the actual event, Tamaru states that almost 90 people offered their views on strengths, challenges, and opportunities.

Researchers outnumbered farmers, a dynamic that resulted in research-oriented planning, claim the farmers interviewed about the summit. "The private sector said they didn't get much out of it," says Tamaru, admitting that at least 60 percent of the event attendees came from the research community. "Hawaii still has a research-driven industry, but that's changing."

Also, a few sources expressed disappointment that more people from the investment community were not at the assembly. They would have liked to have discussed, for example, what it takes for a small family farm to attract investors and garner funds that would help grow its aquaculture business.

"It was a first step. We're not going to decide all the ways to move forward at a one-day meeting," says Ron Weidenbach, owner of Hawaii Fish Co. in Waialua, Hawaii, and president of the Hawaii Aquaculture Association. "We've identified issues and the first steps to take. That's where it stands now."

Donna R. Ching, an extension specialist in agricultural leadership at the College of Tropical Agriculture and Human Resources at the University of Hawaii at Manoa, facilitated discourse. After reviewing a summary of homework responses, attendees divided into six groups to determine the next steps in key areas: leadership; coordination between industry, research and government; road map creation; product and marketing; and education.

As simple as it sounds to get a bunch of people in a room, the summit required forethought and structure, Tamaru explains. The homework and the effort to get ideas and participation from as

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UPDATE

Year 19 Plan of Work. CTSA's Board of Directors on Jan. 24 will consider the following 10 proposals for approval. Then, the resulting Plan of Work will go to the USDA for final approval.

- Bioprocessing Pacific Island Byproducts for Production of Value-added Feed Ingredients
- Development of DNA Markers for Pacific Threadfin Aquaculture
- Improving Outputs in the Commercial-scale Production of Swordtails in Hawaii
- Evaluating Alternative Diets for the Harlequin Shrimp *(Hymenocera picta)*
- Risk Assessment to Identify Potential Shrimp Virus Impacts in Hawaii and Development of Biosecurity Protocols
- Developing Bivalve Culture to Diversify and Position Hawaii as a Supplier of Safe, Premium, Edible Shellfish Products
- Seedstock Transportation and Spawning Synchronization in Sturgeon
- Improved Stocks and Management Practices for Commercial Tilapia Culture in Hawaii and the Pacific Region
- National Aquaculture Extension Conference
- Pacific Regional Aquaculture Information Service for Education (PRAISE) and Publications, Year 2

Year 18 Plan of Work approved by the USDA in June 2005:

- Improving Sturgeon Hatchery and Production Efficiency in Hawaii
- Intensive Microalgae Production
- Feasibility Analysis of Shrimp Waste Processing Alternatives
- Determining the Optimal Nutritional Requirements for the Chinese Catfish *(Clarias fuscus}*
- Disease Management in Pacific Aquaculture, Year 12
- Aquaculture Extension and Training Support for the U.S.-affiliated Pacific Islands with a Special Emphasis on Hatchery Propagation of the Black-lip Pearl Oyster (*Pinctada margaritifer*a), Year 16
- Amberjack Aquaculture Development, Year 3
- Improved Stocks and Management Practices for Commercial Tilapia Culture in Hawaii and the Pacific Region
- Publications, Information, and Library

AQUA TIPS

Vaccine development against *Cryptocaryon irritans* – a progress report

I. Misumi, J. L. Keffer, and T. D. Lewis

Hawaii Institute of Marine Biology, School of Ocean & Earth Science &Technology, University of Hawaii at Manoa

This article was written as part of the work for the project titled "Disease Management in Pacific Aquaculture, Year 10," which the Center for Tropical and Subtropical Aquaculture in part funded under a grant from the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service.

Introduction

Vaccine development against *Cryptocaryon irritans* is an ongoing project at the Hawaii Institute of Marine Biology, and this article describes our progress to date.

The project team expects to complete three main objectives over a three-year period. Briefly, these goals include (1) identification of the life stage of *Cryptocaryon* best suited for use in a vaccine protocol and initial characterization of moi antibodies (IgM), (2) vaccination and challenge trials, and (3) identification and characterization of antigens in effective vaccine fractions and characterization of the anti-*Cryptocaryon* antibody response. We performed our initial experiments with tilapia, since commercially produced antibodies are currently available for this species and our efforts to develop a purified anti-moi IgM antibody are still in progress.

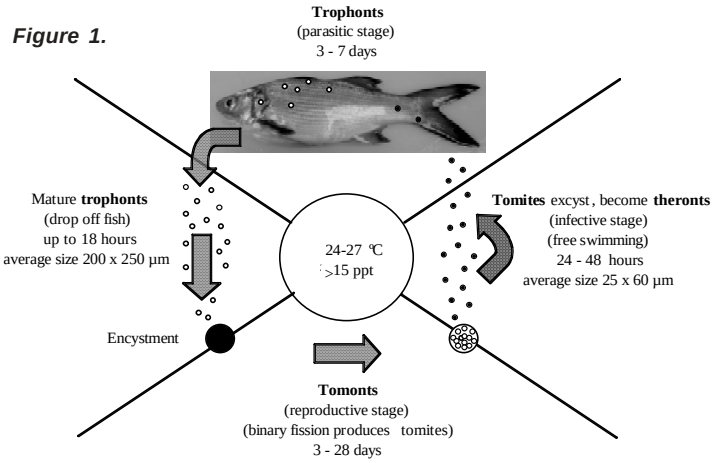
The ciliate *Cryptocaryon irritans* is an obligate parasite responsible for "saltwater ich" or "marine white-spot disease" in warm water marine fishes. This parasite exhibits low host specificity and is distributed globally. In the last two decades, with the increase of commercial marine aquaculture, many cases of mass mortalities of farmed fish due to *C. irritans* infection have been reported.

Indeed, it has become one of the most common and destructive protozoan parasites of marine fish. *C. irritans* has a direct life cycle including three different main developmental stages: trophont, tomont, and theront (Brown 1963, Colorni 1987, Colorni and Diamant 1993) (Figure 1). Amplification of parasite numbers can occur rapidly once the parasite is introduced into a culture system, and vigilance is required when culturing susceptible species.

Despite the increasing occurrence of this epizootic in aquaculture, few methods are available to control it, especially in cultured food fishes (Yoshinaga and Nakazoe 1997). *Cryptocaryon* requires at least 15 ppt saltwater and thrives in warm water temperatures. Currently the most effective control measure involves a surface treatment (generally freshwater/lower salinity water dip or chemical) followed by repeated transfer of fish from tank to tank, a practice that is labor-intensive and extremely stressful to the fish. Treatment in this fashion is meant to interrupt the life cycle of the parasite by removing the parasitic stage from the skin and gills of the fish and the reproductive stage from the tanks.

In studies with thick-lipped mullet (*Chelon labrosus*), an acquired protective immune response against *C. irritans* has been observed in fishes immunized by controlled infections followed by a challenge infection (Burgess and Matthews 1995). Acquired protection against *C. irritans* also has been reported in the mummichog minnow (*Fundulus heteroclitus*) and barramundi *(Lates calcarifer*) (Yoshinaga and Nakazoe 1997; Bryant et al. 1999). *Ichthyophthirius multifiliis*, a related ectoparasite known to cause disease in freshwater fishes, also stimulates a protective immune response in fish. Efforts to identify mechanisms of acquired immunity against *Ichthyophthirius* have been in progress for almost 30 years, stimulated by the observation by Hines and Thira (1974) that sera from convalescent fish immobilized the parasite *in vitro*. Immobilization is caused by antibodies in the

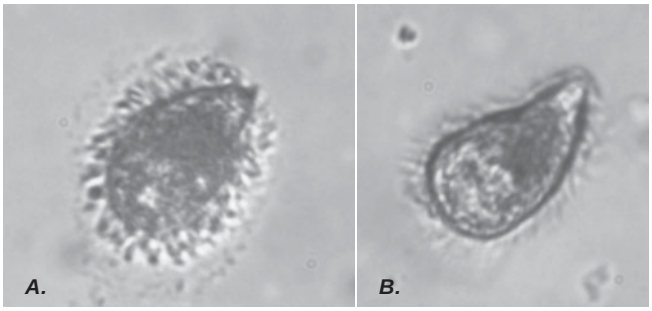
Figure 1.



serum binding to cilia in a way that impedes ciliary motion. If cilia do not function properly, the parasite cannot establish an infection on the fish. The portion of the cilia protein recognized by the antibody is called the immobilization antigen (i-antigen) and the antibody that interacts with it the immobilization antibody (i-antibody).

Immobilization has also been observed in free-living ciliates such as *Tetrahymena* and *Paramecium* (Dickerson et al. 1989). The i-antigens of these free-living ciliates appeared analogous to those of *I. multifiliis* (Lin and Dickerson 1992). Two strains of *I. multifiliis* were identified as possessing different membrane proteins of sizes 48 and 60 kilodaltons (kD) for the first strain and 46 and 56 kD for the second strain (Wang and Dickerson 2002). *Tetrahymena* and *Paramecium* were identified as having proteins of sizes 30 to 58 kD and 40 to 45 kD, respectively (Lin and Dickerson 1992) that possess i-antigen epitopes. Since the proteins were similar in size in these ciliated protozoans, we anticipate that *C. irritans* possesses similarly sized proteins with i-antigen epitopes.

Vaccination against *I. multifiliis*, using intraperitoneal injection of live theronts or isolated cilia proteins, stimulates antibody production in fish blood and mucus (Burkart et al. 1990; Wang and Dickerson 2002; Xu et al. 2002; and Xu et al. 2004). These antibodies in the serum and mucus immobilize theronts *in vitro* (Sigh and Buchmann 2001; Xu and Klesius 2003; and Xu et al. 2004). Fish



Photos by I. Misumi

Figures 2A and 2B. *Immobilization assay results showed theronts with cilia that were either normal (A, control group) or clumping (B, injected with* C. irritans*).*

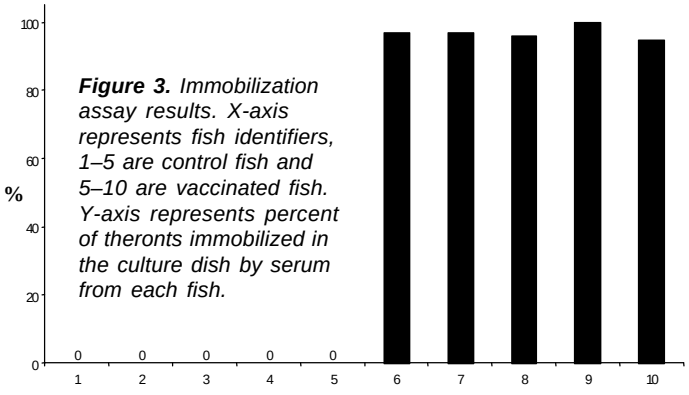


Figure 3. *Immobilization assay results. X-axis represents fish identifiers, 1–5 are control fish and 5–10 are vaccinated fish. Y-axis represents percent of theronts immobilized in the culture dish by serum from each fish.*

antibodies in the skin also cause the parasite to exit the host prematurely, providing additional protection to the fish (Xu and Klesius 2003). Antibodies with immobilizing properties caused theronts to stop swimming, settle, and form aggregates (Xu and Klesius 2002).

We originally proposed to use expression library immunization (ELI) to identify immunogenic parasite proteins to develop a vaccine effective against *Cryptocaryon.* ELI is an elegant molecular method that has been successfully employed in mammalian models to generate resistance and immunity to *Mycoplasma pulmonis* (Barry et al. 1995), *Taenia solium* (Manoutcharian et al. 1998), and *Leishmania major* (Piedrafita et al. 1999). Preliminary reports of efficacy of ELI against *Piscirickettsia salmonis* in Coho salmon (Miquel et al. 2003) also supported our efforts to develop a vaccine against *C. irritans* using this approach. However, to date, our attempts to construct expression libraries from *C. irritans* have failed. We are now following methods that have been used by Dickerson and others (cited above) to identify candidate proteins for a vaccine.

Methods

Vaccination procedure. Relatively large trophonts (> 200 µm) were harvested from a *C. irritans* infected Mozambique tilapia (*Oreochromis mossambicus*) using a pipette. Trophonts were washed and resuspended in filtered seawater (FSW) and placed in a 24-well culture plate. After confirmation of encystment of the trophonts, cultures were incubated for at least four days in ambient air conditions. Newly hatched theronts in the culture were counted using a microscope. Five tilapia (average size: 150 g) reared in freshwater and then acclimated to seawater for the purpose of this experiment were vaccinated with *C. irritans* by intraperitoneal injection of theronts in sterile FSW (~17 theronts per gram fish) or sterile FSW without theronts as a control. Six weeks after vaccination, fish were anesthetized using tricaine methane sulfonate (MS-222) and blood was collected from each fish from the caudal vein. Serum was isolated from the blood by centrifugation and stored frozen in aliquots at -20 ºC until used for immobilization assays and enzyme-linked immunosorbent assay (ELISA).

Immobilization assay. Assays were done as described by Clark et al. (1987) with modifications. Ten µl of fish serum collected from vaccinated or control fish was diluted in 490 µl of FSW and heat inactivated at 56 °C for 30 minutes. Twenty µl of this diluted sample was added into each well of a 96-well plate containing 100 µl of theront suspension with FSW (~30 theronts per well). After 30 minutes incubation at room temperature, the number of immobilized theronts was counted using a microscope to find the percentage that were no longer motile.

C. irritans *protein preparation.* Theronts (harvested as described above) were placed on ice in 1.5 mL microfuge tubes for 15 minutes and centrifuged at 16,000 x *g* for 40 minutes at 4 °C. After discarding the supernatant, the pellet was resuspended in 100 µl of FSW and sonicated to disrupt the cells. The protein suspension was diluted with 1 mL of phosphate buffered saline (PBS), and centrifuged using Centricon® (Millipore) units (10,000 kD molecular weight cutoff) to remove low molecular weight proteins from the preparation.

ELISA. A standard protocol for ELISA was used to test for antibodies against *C. irritans* (Harlow and Lane 1988). A 96-well ELISA plate (Corning Costar, Corning, NY) was coated overnight with a protein suspension of *C. irritans* in carbonate coating buffer (1.2 µg in 100 µl per well). The plate was blocked with PBS containing 1% bovine serum albumin or BSA (PBS-BSA) for 2 h, and, then, at sequential 1 h intervals, the ELISA plate was incubated with serum from vaccinated or control fish (diluted 1:1000), rabbit anti-tilapia IgM IgG (diluted 1:5000), and 1:2500 goat anti-rabbit IgG (diluted 1:2500). Between the addition of each new reagent, the ELISA plate was washed 5x with PBS containing

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CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

CTSA currently assists aquaculture development in the following areas:

Hawaii
American Samoa
Northern Mariana Islands
Federated States of Micronesia
Guam
Palau
Marshall Islands

REQUEST FOR PRE-PROPOSALS

Due February 28, 2006

The Industry Advisory Council (IAC) and the Technical Committee (TC) of the Center for Tropical and Subtropical Aquaculture (CTSA) met on September 27, 2005 to advise CTSA's Board of Directors on the areas of research CTSA should fund in order to achieve its mission. **CTSA is requesting pre-proposals that address these priority areas for its Year 20 funding cycle**. (Projects approved for Year 20 will likely begin in the Fall of 2007.) Please submit your pre-proposals to the CTSA administrative office by **Tuesday, February 28, 2006**.

CTSA's mission is to support aquaculture research, development, demonstration, and extension education to enhance viable and profitable aquaculture in the American Insular Pacific (see list at left for specific islands). Funded by an annual grant from the USDA's Cooperative State Research, Education, and Extension Service, **CTSA would like to fund projects that will enhance economic opportunities for aquaculture producers in our region. The Center strongly encourages interagency cooperation and shared funding of priority projects.**

- **Applicant Eligibility:** Universities, community colleges, or nonprofit research institutions and organizations must lead project execution. Private individuals or commercial companies may not directly initiate projects.
- **Pre-proposal Guidelines:** Pre-proposals should be no more than two pages (single-spaced, 10- to 12-pt. font) and include the following sections:

  Proposed title or main idea

  Problem statement
  Briefly explain the need for your project, which should address priority species and constraints as identified by the IAC and TC (see next page). Other areas of potential development, however, may be addressed if properly justified as to their importance to aquaculture in the Pacific.

  Proposed objectives
  Define and number objectives that are achievable and measurable. Please visit the CTSA Web site at www.ctsa.org to view previously funded projects so that your pre-proposal does not duplicate work in completed or current projects.

  Approach
  Describe the procedures and facilities that the project work group will use to accomplish the objectives defined in your pre-proposal.

  Duration
  If a project's duration is more than one year, then your pre-proposal must include the objectives and approach for each year.

  Estimated budget
  Estimate the funding needed to accomplish the objectives. Provide the estimates in dollars per year. CTSA typically does not fund projects for more than $100,000 per year. The Center gives preference to projects that will deliver the most benefits at the lowest cost. Due to its limited project budget (~$560,000), CTSA will distribute funding to the highest-ranked proposals until it exhausts all of its available funds.

CTSA Request for Pre-proposals (continued)

Project work group members
List individuals, by name and affiliation, who would be able to carry out your objectives. Project work groups should involve participation from individuals with different areas of expertise. When possible, these individuals should hail from different political entities or institutions within the CTSA region.

Please e-mail pre-proposals to kedennis@hawaii.edu. If you have any questions, please contact Kathryn Dennis via e-mail or by telephone at (808) 956-3529. If necessary, pre-proposals may be faxed to (808) 956-5966 or mailed to the following address:

The Center for Tropical and Subtropical Aquaculture
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3050 Maile Way, Gilmore Hall 104
Honolulu, HI 96822-2231

• Top Ten Species and their Constraints by Region as Identified by the IAC and TC

Hawaii

1. Yellow tang
   broodstock maturation, fertility
   first feeding
2. Shrimp (cleaner and snapping)
   reproduction
3. Kahala
   broodstock conditioning
   ectoparasites
4. Corydoras
   mass production
5. Moi
   operculum deformity
   selective breeding
6. Tuna
   scale of work
   government regulations
7. Sturgeon
   seedstock transportation
   spawning synchronization
8. Live rock
   lack of information
   biology and culture of coralline algae
9. Swordtail
   scale-up, quality of control
   lyretail production, gene marker
10. Grouper
    niche market
    lack of local technology

Hawaii's general constraints

high costs of land, labor, and energy
biological constraints
(seedstock limitations)
biosecurity/biocontainment

U.S.-affiliated Pacific Islands (USAPI)

1. Pearl oysters
   nursery survival and predation
   spat transport
   grafting technology
   economics—data, modeling
2. Tilapia
   value adding
   optimal stocking density
3. Grouper
   first feeding and feeds
4. Mangrove crabs
5. Corals (soft and hard)
   reproduction and fragmentation techniques
   land-based parameters, techniques
6. Moi
7. Sponges*
   pharmaceutically active, alternative species
   transport, introductions
8. Shrimp*
   disease
9. Milkfish*
10. Giant clams*

USAPI constraints applicable to all species

extension	seedstock availability	capacity
biosecurity	policy/regulation	biocontainment
marketing	economic feasibility	energy efficiency

* IAC and TC members did not necessarily think CTSA needs to fund work on sponges, shrimp, milkfish, or giant clams right now but selected them because they are important to the region.

Kona Blue continued from Page 1

then, is that customers—especially health and environmentally conscious consumers—will readily pay a premium for the benefits of the fish.

Recent lab analysis shows that Kona Kampachi has an average fat content of 30 percent or nearly 3 percent Omega-3 fatty acids per 100 g weight—a level higher than almost any other fish on the market, Sims claims. To further this healthy image, Kona Blue in early November switched to an organic feed.

As for sustainable aquaculture, Sims points to hatchery technology that eliminates the need to deplete wild stocks and to cage locations that minimize environmental impact. Kona Blue is the first company to integrate offshore cage farming with a hatchery, a setup that allows the company to control its product from "hatch to harvest," he touts.

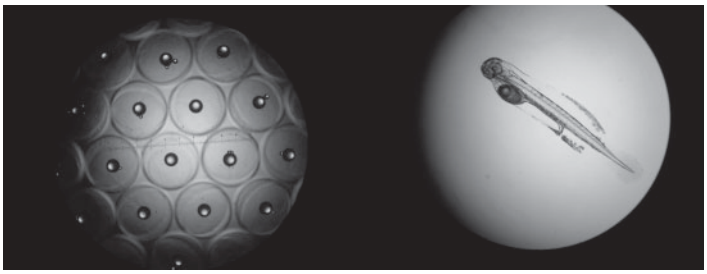
After six weeks in the company's hatchery and land-based nursery at the Natural Energy Laboratory of Hawaii Authority (NELHA) in Kailua-Kona, the fish go for another six weeks in a nursery of two surface cages located at Kona Blue's deep ocean ranch a half mile off the coast of Keahole Point. Then, also at the ranch, the fish spend seven to nine months for growout to three to five pounds in four open-ocean cages—each 50 feet high and 80 feet wide—that sit 30 feet below the ocean surface in waters more than 200 feet deep. Kona Blue hopes to add two more open-ocean cages in 2006.

This complex operation, not surprisingly, required a lot of time and money to put in place. On research and development alone, the company has spent four years and more than $2 million, says Sims. He and cofounder Dale Sarver spent three years on technology development and a fourth year of research to scale it up. Meanwhile, finding a site and obtaining the required permits took three years. Kona Blue is only the second company to obtain an open-ocean aquaculture lease from the state of Hawaii. Modeled after Hawaii's groundbreaking statutes and introduced in the United States Senate last June, the National Offshore Aquaculture Act of 2005 would create a framework for providing federal permits.

Building and maintaining a company like Kona Blue demands substantial capital. Fortunately, Sims and Sarver so far have been able to tap funds beyond their own pockets. Back in April 2002, the company won a three-year, $1.5 million federal grant through the Advanced Technology Program of the U.S. Department of Commerce.

Further assistance came in part from Hawaii's high-tech tax-credit law known as Act 221 and passed in 2001. The tax-credit helped attract $4 million in funding for Kona Blue in 2004 from Aspen, Colo.-based Cornerstone Holdings, which also connected the company with its chief executive officer Michael Wink. This feat earned the company the "2005 Venture Capital Deal of the Year" award from the Hawaii Venture Capital Association.

Despite all that financing, the company's bright potential still comes with a host of challenges. "I hope they make it, but it's going to be tough," says Randy Cates, president of Cates International Inc., the first open-ocean fish farm in the U.S. and the first in 2001 to obtain an offshore aquaculture lease from Hawaii. He temporarily has halted production of Pacific threadfin or moi in his four cages located two miles south of Ewa Beach, Hawaii, to focus efforts on construction of the company's commercial hatchery, which will support the offshore system with sufficient fingerling supplies.



Photos courtesy of Kona Blue

Kona Blue spent four years and more than $2 million on the research and development of its "hatch-to-harvest" system. Above: *Kona Kampachi eggs (left) and hatched larva (right) seen under a microscope.* Left: *Fish swim in one of the company's offshore cages, each 2,600 cubic yards in volume.*

Neil Sims expects difficulty. He positions open-ocean cage culture as the future of aquaculture in Hawaii yet admits that offshore fish farming operations in general face "real challenges." The "spectacular new industry" must tackle obstacles found in selecting sites, developing technology, engineering, and building hatchery capacity, he says.

Reluctant to point to any one worry for Kona Blue, Sims speaks with enthusiasm: "With aquaculture, no single issue [sticks out]. We're growing a species that's never been grown before ... It's exciting, and it keeps me up at night."

His dreams are big for Kona Blue Water Farms. In the short term, he aspires to ramp up production of Kona Kampachi from the current 3,000 pounds per week to 30,000 pounds per week by the end of 2006. Eventually, he hopes to expand the company's offshore cage culture to additional marine species. *—KD*

Allen C. Riggs steps in as ADP's new aquaculture vet

Allen C. Riggs, DVM, joined the Hawaii Department of Agriculture's Aquaculture Development Program (ADP) in June. Previously, he ran a private practice in Florida as an aquatic animal veterinarian. From 1999 to 2003, Dr. Riggs provided extension services through the University of Florida to the Florida aquaculture industry, conducting research on koi, carp, and sturgeon. As staff veterinarian at UF's College of Veterinary Medicine, he taught courses in water chemistry, histological interpretation, and general fish and shellfish health and preventive medicine. Also, from 1995 to 1999, he served as a veterinary consultant and aquarist at the Key West Aquarium in Florida, acquiring knowledge of diseases of marine tropical species. Editor Kathryn Dennis interviewed him in early December for this double issue.

Regional Notes*: What is your role at ADP?*
Allen C. Riggs: My primary role as the aquatic animal veterinarian with the Aquaculture Development Program's Disease Prevention unit (ADP-DP) is to summarize the findings of our diagnostic team and to formulate management and/or treatment protocols.

Why is this role important to Hawaii's aquaculture industry?
Disease can be defined as any condition other than normal or healthy. Basically, disease costs aquaculture operations money. Even if animals do not die but are negatively affected, it costs operators money. Compromised animals often have slower growth rates than healthy animals, increasing time to market and in turn increasing costs. An active disease prevention program can help pinpoint subclinical problems before they turn into major losses.

Why did you take this position?
The opportunity to work with both established and emerging aquaculture species/operations. And to do it in Hawaii.

"An active disease prevention program can help pinpoint subclinical problems before they turn into major losses."

—Allen C. Riggs, DVM

What have you accomplished so far?
Over the last six months, we have refined and standardized some of the programs/protocols already in place. The SPF Shrimp Farm Certification protocol is a good example. ADP-DP currently is working with Hawaii's shrimp producers and research community to review this protocol and schedule a summit to discuss it and other issues. We also have helped several producers and hobbyists by making site visits to their operations. Site visits allow us to review and evaluate culture systems and husbandry methods in combination with specific diagnostic tests on the animals to determine if a disease process involves multiple factors.

What are your main goals?
ADP-DP would like to communicate the following two goals to producers in Hawaii so that we can better assist them. First, please contact us early in the course of a disease event, so we can discuss and schedule an appropriate plan of action. Second, if possible, let us help you make a diagnosis before starting treatment protocols. Often times, medications applied to water and/or animals prior to our examining them can adversely affect our routine diagnostic tests.

What obstacles might stand in your way of achieving those goals?
The most likely obstacles are funding shortfalls from the state or from federal sources. Private sector aquaculture operations would have to meet any shortfall themselves. Or we'd have to reduce services in some capacity.

How do you like Hawaii so far?
Yes, Hawaii is a great place to be. Scuba diving and sushi are favorites of mine. Also, my cat Sammy and I have become quite fond of poke.

ANNOUNCEMENTS

New faces. The CTSA administrative center had some personnel changes at the end of 2005. After more than a decade of service to CTSA and the aquaculture community, Alcian Clegg left her position as administrative assistant in October to move to the United States mainland. Earlier, publications specialist Debra Sasaki departed in May to pursue her interest in history as an editor at the University of Hawaii's Center for World History. As Executive Director, I appreciate their dedication to and effort in meeting the CTSA mission, and I wish them both a bright future.

As for the future of CTSA, I am pleased to have two new staff members. Kathryn Dennis joined as publications specialist in late September and in December Erin Kochi became our new administrative assistant. Please help me welcome them. *—CSL*

2005 Census of Aquaculture. The National Agricultural Statistics Service encourages all producers to return their census forms, mailed on Dec. 15. Haven't received a form? Contact Steve Gunn at (808) 973-9588, (800) 804-9514 or nass-hi@nass.usda.gov.

Reef video. Big Island filmmaker Ziggy Livnat created a seven-minute video about how to treat coral reefs. To borrow a copy, contact K. Dennis. To help distribute, go to www.ForTheSea.com.

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0.01 % Tween 20 (PBS-T). ABTS [2,2'-azino-bis (3-ethylbenzioline-6-sulfonic acid)] peroxidase substrate was added to each well to allow for chromogenic (color) development. After 10 minutes, the absorbance at 405 nm was read using a microplate reader.

Gel electrophoresis and Western blot. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was run per protocols described in Harlow and Lane (1988) to identify the molecular weights of any *C. irritans* theront proteins immunoreactive with antibodies from tilapia. Six µg of homogenized *C. irritans* total protein was loaded into each lane of a 10 % acrylamide gel with a reducing or non-reducing sample buffer. Following SDS-PAGE, proteins in the gel were transferred to a polyvinylidene difluoride (PVDF) membrane, using a mini-Protean III Transblot apparatus (BioRad). Transfer was done in a refrigerated cold room, 100V for 1h. The membrane was blocked in PBS-T containing 1 % BSA (PBS-T-BSA) overnight at 4 ºC, and, then, immunoblotting of the membrane was done following a standard Western blot protocol (Harlow and Lane 1988), using fish serum collected from a fish that had been heavily infected in a cohabitation experiment, treated to remove parasites, and allowed to recover.

Results

Immobilization assay. Microscopic examination of the assay plate revealed clear evidence of parasite immobilization. Theronts co-incubated with serum from fish injected with FSW had active, normally functioning cilia (Figure 2A). Ciliary function was impeded in theronts co-incubated with serum from theront-vaccinated fish, and we saw clumping of cilia (Figure 2B). We performed easily the enumeration of the percentage of immobilized theronts, as none of the theronts co-incubated with serum from FSW-injected fish were immobilized. In contrast, an average of 97 % of theronts co-incubated with serum from the vaccinated fish were immobilized (Figure 3).

ELISA. An average absorbance of 0.36 ± 0.04 OD_{405} (n=5) was detected in the fish injected with FSW only, while fish injected with FSW containing theronts had a value of 0.90 ± 0.16 OD_{405} (n=5) against sonicated theront proteins. We observed a significant difference between the two groups based on t-test analysis ($p<0.01$). The assay requires refinement to become quantitative, but clearly the vaccinated fish are producing antibodies specific to one or more *C. irritans* protein(s).

SDS-PAGE and Western blot. The protein profile for sonicated theronts visualized by colloidal gold stain revealed a heterogeneous mixture of molecular weight proteins with a dominant protein band of 30 kD. The Western blot revealed an immunoreactive band at this same molecular weight (Figure 4) when the samples were run in a non-reducing sample buffer. No band was detected in reducing sample buffer conditions, a result also reported for *I. multifiliis* (Wang and Dickerson 2002). Under reducing conditions, the antigenic epitopes of this protein apparently changed form and lost immunoreactivity. The results of this experiment confirm that fish produce specific antibodies against *C. irritans*. The non-reducing Western blot, using serum from FSW-injected fish, showed no immunoreactive bands were present, although the colloidal gold stained membrane confirmed this dominant protein was in the sample transferred to the membrane (data not shown).

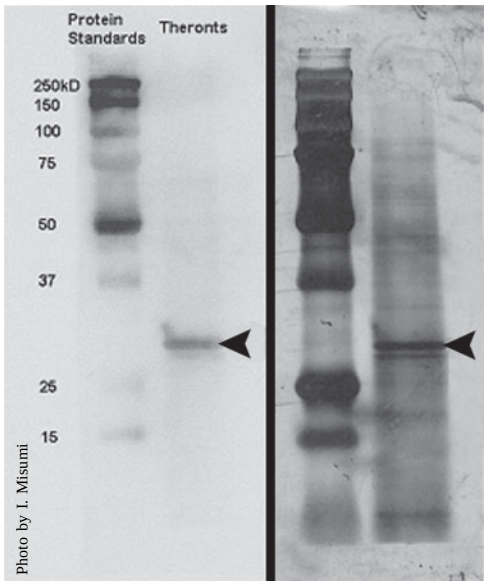

Figure 4. *Western blot results illustrating* C. irritans *protein reactive with antibodies from a heavily infected fish that had been treated and allowed to recover (arrows).* Left: *blot using chemiluminescence detection (Pierce);* Right: *total protein on the membrane using colloidal gold stain (BioRad).*

Discussion

The immobilization we observed suggests that theronts do induce a fish immune response and immunological memory against *C. irritans*. Once we ruled out ELI and decided to follow a more classical vaccine design, we selected theronts for the first vaccination trial instead of the tomont stage because they are the infective stage and possess cilia. I-antigens are a component of cilia proteins and are required to stimulate the production of i-antibodies. SDS-PAGE and Western blot have revealed since that in fish that produce i-antibodies, the antibodies recognize the 30 kD protein whether it is processed from theronts or tomonts. Encysted tomonts almost ready to hatch have ciliated tomites within the cyst, so this result is not unexpected. The protein profile for sonicated tomonts, however, is much more complex than the profile for theronts. The use of tomonts to identify suitable candidate proteins for vaccine development would be more involved and costly than the use of theronts.

Currently, we are vaccinating moi with theronts to confirm whether they have the same response against theronts as described for tilapia and have purified moi IgM. We will use this to produce a rabbit anti-moi IgM reagent, so we can perform ELISA and Western analysis for moi samples. We plan to purify the 30 kD protein by electro-elution in non-reducing conditions and to use it to inject fish in order to assess its effectiveness as a vaccine. In tandem, we will also conduct molecular characterization of the protein in the hopes we can produce a recombinant vaccine that does not have to be produced from live parasites. This work, along with additional vaccine and challenge experiments in moi using an increased sample size, comprises the next phase of our research.

Acknowledgments

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References

Barry, M.A., W.C. Lai, and S.A. Johnston. 1995. Nature 377:632-635.

Brown, E.M. 1963. Progress in Protozoology, Proceedings of the International Congress of Protozoology. 1:284-287.

Bryant, M.S., R.P. Lee, R.J.G. Lester, and R.J. Whittington. 1999. Diseases of Aquatic Organisms 36:21-28.
Burkart, M.A., T.G. Clark, and H.W. Dickerson. 1990. Journal of Fish Disease 13:401-410.
Burgess, P.J. and R.A. Matthews. 1995. Fish & Shellfish Immunology 5:459-468.
Clark, T.G., H.W. Dickerson, J.B. Gratzek, and R.C. Findly. 1987. Journal of Fish Biology 31:203-208.
Colorni, A. 1987. Aquaculture 67:236-237.
Colorni, A. and A. Diamant. 1993. European Journal of Protistology 29:425-434.
Dickerson, H.W., T.G. Clark, and R.C. Findly. 1989. Journal of Protozoology 36:159-164.
Harlow, E. and D. Lane. 1988. Antibodies: a laboratory manual. Cold Spring Harbor Press, pp. 412, 651.
Hines, R.S.A. and D. T. Thira. 1974. Journal of Fish Biology 6:373-378.
Lin, T.L. and H.W. Dickerson. 1992. Journal of Protozoology 39, 457-463.
Manoutcharian, K., L.I. Terrazas, G. Gevorkian, and T. Govensky. 1998. Immunology Letters 62:131-136.
Miquel, A., I. Muller, P. Ferrer, P.D. Valenzuela, and L.O. Burzio. 2003. Biological Research 36:313-323.
Piedrafita, D., D. Xu, D. Hunter, R.A. Harrison, and F.Y. Liew. 1999. Journal of Immunology 163:1467-1472.
Sigh, J. and K. Buchmann. 2001. Journal of Fish Disease 24:49-51.
Wang, X. and H.W. Dickerson. 2002. Clinical & Diagnostic Laboratory Immunology 9:176-181.
Xu, D.H., P.H. Klesius, and R.A. Shelby. 2002. Journal of Fish Disease 25:45-52.
Xu, D.H. and P.H. Klesius. 2003. Journal of Fish Disease 26:287-291.
Xu, D.H., P.H. Klesius, and R.A. Shelby. 2004. Journal of Fish Disease 27:135-141.
Yoshinaga, T. and J-I Nakazoe. 1997. Fish Pathology 32:229-230.

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many people as possible from Hawaii's aquaculture community was all part of a "buy-in process," says Tamaru. "We wanted everyone there to recognize their contribution," he says. For a meeting "memoir" or summary of responses and potential next steps, go to http://www.hawaiiaquaculture.org/docs/summMem.doc.

The process begun at the summit is not over. To turn vision into action, some stakeholders are working on follow-up tasks, Tamaru says: "We looked down the road ... The real nitty-gritty is 'How do we get there?' " Stay tuned. —*KD*

Around the Pacific

This column marks the first of a new regular feature in *Regional Notes*. In each issue, as the new editor, I'll provide updates on industry and research activity throughout the CTSA region, with an emphasis on the U.S.-affiliated Pacific Islands.

Aquaculture in the Commonwealth of the Northern Mariana Islands (CNMI) leapt forward with the launch of Marianas Sweet Shrimp on Nov. 26 in China Town, Saipan. The company plans to raise and sell specific-pathogen-free (SPF) shrimp broodstock and postlarvae of Pacific white shrimp (*Litopenaeus vannamei*).

An enterprise of a caliber not seen before in CNMI, the company, with funds from its sole stockholder, Saipan Ice & Water Co., already has put more than $200,000 into the development and construction of facilities for the farm.

Located on almost four acres of leased property in China Town, facilities include 22 tanks that vary from 10 spawning tanks in an indoor hatchery system to two 30-by-70 raceway tanks. The company also has a packaging and shipping building and water storage and disposal systems. Aeration, filtration, and power supply all have backups.

The company's priority markets are Guam and local farms; other targets include South Asian and Pacific Rim countries and Western Pacific states. In addition to broodstock and postlarvae, the company plans to raise shrimp for local consumption.

Nearly 1,800 nautical miles southeast of Saipan, meanwhile, in the Republic of the Marshall Islands, six men from five atolls last fall completed two months of training in the farming of black-lip pearl oysters. They were honored at a ceremony (picture at left), which attracted the attendance of the mayors and council members of the five atolls: Rongelap, Maloelap, Likiep, Ebon, and Bikini/Kili.

Photo courtesy of CMI

CMI ceremony. Back (l to r): *Diane Myazoe-deBrum (CMI), Joanis Reim (CMI), Anton Eknilang (Rongelap), Rand Dybdahl (CMI), Ranny Ohlwiler (Rongelap), Biel Jibas (Ebon), Manoj Nair (CMI), Moses Leon (Bikini/Kili/Ejit) and Tabwi Aine (CMI).* Front: *Georgeton Jerbal (Maloelap) and Aiji Aias (Likiep).*

With spat produced during their training at a hatchery at the Arrak campus of the College of the Marshall Islands (CMI), trainees are establishing pearl farms. "The idea is for the communities to grow out the spat and sell the product to commercial companies, ultimately creating a source of income beyond Copra (or dried coconut meat) production," says Manoj Nair, one of two trainers and an aquaculture research scientist with CMI's USDA-CSREES Land Grant Program. The other trainer was Rand Dybdahl, also of CMI Land Grant.

Pearl oyster farms started up in November on the Ebon, Likiep, and Rongelap Atolls. The Maloelap Atoll plans to set up a farm on Jan. 10, and the Bikini/Kili Atoll hopes to launch a farm early in 2006. Nair says, "For the people, the farms bring hope for the future and a fact of pride." —*KD*

CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

INSIDE

The Center for Tropical and Subtropical Aquaculture (CTSA) is one of five regional aquaculture centers in the United States established by Congress in 1986 to support research, development, demonstration, and extension education to enhance viable and profitable U.S. aquaculture. Funded by an annual grant from the U.S. Department of Agriculture's Cooperative State Research, Education, and Extension Service (USDA/CSREES), the centers integrate individual and institutional expertise and resources in support of commercial aquaculture development.

CTSA currently assists aquaculture development in the region that includes Hawaii and the U.S.-affiliated Pacific Islands (American Samoa, Commonwealth of the Northern Mariana Islands, Federated States of Micronesia, Guam, Republic of Palau, and Republic of the Marshall Islands).

In its 18 years of operation, CTSA has distributed more than $9 million to fund more than 185 projects addressing a variety of national aquaculture priorities.

Each year, the Center works closely with industry representatives to identify priorities that reflect the needs of the aquaculture industry in its region. After consultation with appropriate technical experts, CTSA responds with a program of directed research that has these pre-determined priorities as the focus of project objectives. The Board of Directors is responsible for overseeing CTSA's programmatic functions. The Center disseminates project results through its print publications, hands-on training workshops, and Web site.

CTSA is jointly administered by the Oceanic Institute and the University of Hawaii. The main office is located at the Oceanic Institute's Makapuu Point site on the island of Oahu in Hawaii.

For more information, contact Cheng-Sheng Lee, Ph.D., Executive Director, by telephone (808) 259-3107, fax (808) 259-8395 or e-mail (cslee@oceanicinstitute.org).

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