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Letter from the Director

Aloha,

This month marks the official beginning of our next development cycle with the release of our "Request for Pre-Proposals." This request was compiled based on information provided by regional stakeholders in the recent CTSA "Priority Areas" and "Species Issues" surveys. We greatly appreciate those who took the time to participate and share with us the specific challenges and opportunities for aquaculture development within our region.

In addition to the FY2012 Request for Pre-Proposals, this month's newsletter features a CTSA project update by the "Tuna Roe in Shrimp Maturation Diet" project out of the University of Guam. There is also an announcement for a finfish hatchery workshop at the Oceanic Institute, open to technicians and farmers from the U.S. Affiliated Pacific Islands (excluding Hawaii). Last but not least, this month's AquaClip.

As always, please let us know if you have any questions, comments, or suggestions!

Mahalo,

Cheng-Sheng Lee
Executive Director, CTSA

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CTSA FY 2012 Request for Pre-Proposals: Due June 29, 2012

The Center for Tropical and Subtropical Aquaculture (CTSA) requests pre-proposals for applied research that addresses problems and opportunities in the regional aquaculture industry. In a recent region-wide survey, CTSA stakeholders identified the below strategic areas and species as the top aquaculture development priorities. Pre-proposals that target these 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. CTSA strongly encourages collaboration between institutions and agencies in the region, as well as shared funding of large priority projects.

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CTSA's mission is to support aquaculture research, development, demonstration, and extension education in order to enhance viable and profitable aquaculture in the United States. CTSA is funded by an annual grant from the U.S. Department of Agriculture's National Institute of Food and Agriculture (NIFA). The CTSA region includes the following areas: American Samoa, Guam, Hawaii, the Commonwealth of the Northern Mariana Islands, the Republic of the Marshall Islands, the Federated States of Micronesia, and Palau.

Please note: Desired outcomes and/or deliverables are included where applicable. They represent industry-identified requests and it is strongly recommended that they be addressed in your pre-proposal.

FY 2012 Strategic Areas & Priority Species

Cost Effective Locally-Made Aquatic Feed
Nearly all survey participants identified availability of affordable feed as one of the major constraints in the regional development of aquaculture. CTSA would like to solicit a proposal that will develop a cost-effective aquaculture feed using locally available ingredients. The major goal should be to create a local feed that costs less than imported feed for species currently being farmed and/or species identified in the CTSA survey as desired species for regional farming. These include but are not limited to tilapia, marine shrimp, moi, Kahala, rabbitfish, freshwater prawn and groupers.

Tilapia Farming Development
In our recent survey, tilapia was identified as the most desired species for aquaculture farming throughout the CTSA region. Although most farming technology is available elsewhere, the development and expansion of tilapia farming still faces regional challenges, including but not limited to sources of fingerlings and farming and importation restrictions of some desired species. CTSA encourages studies to develop desired strains from those that are already locally available, and cannot provide funding to introduce non-native species. We also encourage proposals to develop locally made feed for tilapia.

Rabbitfish farming development
Rabbitfish is a highly desired species for farming on almost all Pacific Islands. Currently, the rabbitfish supply for local markets is dependent on wild harvest. Although farming technology is established in several Asian countries, it is still not widely available in the Pacific Islands. CTSA is seeking a project to adapt, demonstrate and transfer rabbitfish farming technology to different Pacific Islands.

Sea Cucumber farming technology
With an increased demand for sea cucumber in Asian markets, natural stocks of the species have been over harvested in some Pacific Islands. To mitigate this problem, CTSA recently funded projects to transfer sea cucumber hatchery technology to Pohnpei and Yap. It is important to ensure that any technology transferred throughout the Pacific region is adapted to local conditions, and is being operated wholly by local technicians. Currently, the major issues facing the development of the sea cucumber industry are the lack of farming technology and sources of seedstock within different communities. CTSA is calling for a proposal to continually transfer and demonstrate sea cucumber farming technology in waters around the islands, using locally desired species.

Marine Shrimp farming
The CTSA region is known as the main source of SPF white shrimp broodstock for many shrimp farming countries around the world. However, the local shrimp farming industry is still struggling to achieve profitable and sustainable operations, mainly due to the high costs of feed, energy, labor, and transportation on most islands. CTSA is therefore soliciting a proposal for a collaborative effort between researchers and industry members to improve production efficiency and sustainability of marine shrimp technology.

Marine Finfish Farming Technology
Farming of marine finfish such as moi, Kahala and groupers has been identified as a commercial aquaculture practice with potential for growth in the region. CTSA has previously supported the development of farming technology for moi and Kahala. However, stakeholders have indicated that existing operations are still struggling with impeding issues, including the lack of a reliable source of fingerlings, expensive feed, and the high cost of energy. Stakeholders have also indicated that groupers are another desirable marine finfish for farming in the region. Grouper farming technology that is currently available in several Asian countries can likely be adapted to regional conditions. CTSA will accept proposals to adapt and/or develop technology that improves the practices, profitability, and sustainability of marine finfish farming in the region.

Freshwater Prawn industry
CTSA stakeholders also identified freshwater prawns as a desirable species for regional farming. Freshwater prawn culture was developed in Hawaii over three decades ago. Although the number of prawn farms has decreased in recent years, data from the CTSA survey indicated that freshwater prawns remain a species with potential for profitable farming in the region. However, revitalizing the prawn industry will require solutions for key issues, including seedstock source and cost of feed,

energy, and labor, etc. The Center will consider proposals that will improve freshwater prawn farming industry in the region.

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Click here to view the complete announcement, which contains important details on the Process and Instructions to submit a pre-proposal. If you have any questions, please contact Meredith Brooks via email at mbrooks@oceanicinstitute.org, or by telephone at (808) 259- 3176.

A Value Added Approach for Tuna Roe on Guam: Can It Be Used as a Local Ingredient in Shrimp Maturation Diets?

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Figure 1. A tuna fish processing plant on Guam

Figure 2. Making semi-moist tuna roe based diet

Introduction

Shrimp reproductive performance parameters in captivity including the rate of gonad development during the sexual maturation process, the frequency of induced spawning, and initial larval development of post-spawning (which relies exclusively on the nutritional reserves from egg yolk) are greatly affected by the quality of the maturation diet. Although the nutrition requirements related to fertility and fecundity are not fully understood, the commonly used maturation diets such as bloodworms, squids and bivalves are good sources for promoting shrimp maturation and reproduction with bloodworms being considered as the gold standard from a nutritional perspective.

However, there are two primary concerns with the "fresh-frozen" maturation feeding regimes. One is the biosecurity concern. The fresh-frozen ingredients are sometimes collected from coastal areas, where shrimp pathogens may be present from local shrimp aquaculture activities. These feed organisms may be potential shrimp pathogen carriers, and shrimp could be infected by pathogens through food ingestion, which is ranked as the second highest risk for disease transmission in shrimp. Once the disease spreads via either horizontal or vertical transmission or both, the catastrophic shrimp disease outbreaks would occur.

The second concern is the high cost. The costs of certified specific-pathogen-free (SPF) shrimp maturation supplements, such as bloodworms from the United Kingdom or squids from California, USA are very high. The importation of those frozen products is both time consuming and extremely expensive, especially to isolated areas such as Guam. It was estimated that the landed price on Guam for the certified SPF bloodworm was over $90/kg, which was too expensive for it to be used as shrimp maturation diet. There was a clear need to seek a local alternative supplemental feed for shrimp broodstock to sustain the industry's development.

Migratory tuna species are the most important fish resource in Pacific region, with a harvest worth about $3 billion US dollars annually. Guam is the largest tuna transshipment center in the Western Pacific. The tuna average 45 to 65 pounds. There are several loining operations on Guam that process the fish before the loins are air-lifted to Japan. This produces a volume of tuna scraps which is estimated at 3-4 tons per week. The scraps are generally viewed as wastes and sent to the landfills at a significant cost. Tuna roe comprises 16% or so of the total scraps, and the local availability of good quality tuna fish roe exceeds 30,000 kg annually.

This project was to develop new knowledge to increase the value of the underutilized tuna roe by exploring its potential use as an aquaculture feed ingredient in a shrimp maturation diet and to do so in a sustainable manner. In this study, we assessed the nutritional and health status of fish roe and developed a semi-moist maturation diet for Penaeus vannamei using the tuna roe as the major

ingredient. The diet was evaluated against other feeding regimes to evaluate their impacts in boosting the reproductive performance of shrimp broodstock.

Baseline information of tuna roe

Samples from 23 batches of Yellow Fin tuna roe were randomly collected and pooled for health and nutritional baseline analyses.

Health status

DNA and RNA were extracted from six pooled raw roe samples, and checked for the C-1 viruses of the US Marine Shrimp Farming Program SPF list, including WSSV, IHHNV using PCR, and TSV, YHV, IMNV using RT-PCR, following the published PCR diagnostic protocols provided by the Shrimp Pathology Lab at University of Arizona. The diagnostic results confirmed the absence of the listed shrimp viral pathogens in the tuna roe samples (Table 1).

Table 1. PCR results of the six randomly selected tuna roe samples.

Sample #	WSSV	IHHNV	YHV	TSV	IMNV
UOG#1	Not detected	Not detected	Not detected	Not detected	Not detected
UOG#2	Not detected	Not detected	Not detected	Not detected	Not detected
UOG#3	Not detected	Not detected	Not detected	Not detected	Not detected
UOG#4	Not detected	Not detected	Not detected	Not detected	Not detected
UOG#5	Not detected	Not detected	Not detected	Not detected	Not detected
UOG#6	Not detected	Not detected	Not detected	Not detected	Not detected

Protocols:
WSSV: Two-step nested PCR modified method from Lo, et al (DAO, 1996, 25:133-141)
IHHNV: PCR described by Nunan, et al. (Marine Biotechnology, 2000, 2: 319-328)
YHV: RT- PCR described by Tang and Lightner (DAO, 1999, 35:165-173)
TSV: RT-PCR described by Nunan, et al. (DAO, 1998, 34:87-91)
IMNV: RT-PCR nested protocol described by Poulos and Lightner (DAO,2006,73:69-72)

Nutritional status

Prior to sending samples for proximate analysis, a series of experiments on drying procedure were conducted to check for the appropriate freeze drying conditions for processing the tuna roe and to compare the results between conventional oven drying and freeze drying procedures. With the standardized freeze drying procedure (30 gram each sample freeze dry for 48 hours), the dry matter reached 18.6% on average.

Following the same drying protocol, six samples were shipped to two labs in University of Hawaii for biochemical analysis for protein, total lipids, mineral, ash, as well as fatty acids profiles. Baseline information of tuna roe nutritional values indicated that the fish roe is abundant in protein (74.5% of dry matter) and lipids (10%) (Table 2).

Table 2. Proximate analysis of tuna roe samples

Sample #	Ash (%)	Protein (%)	Lipid (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	B (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
UOG#1	5.79	72.95	12.94	1.19	0.78	0.05	0.16	1.19	1	6	63	2	376
UOG#2	7.46	76.99	7.95	1.42	1.27	0.05	0.14	0.88	1	5	80	2	422
UOG#3	5.69	73.03	9.28	1.16	0.88	0.04	0.14	1.18	2	4	55	1	315
UOG#4	7.32	73.44	16.27	1.22	0.84	0.05	0.12	0.96	1	5	64	2	344
UOG#5	7.17	74.32	7.54	1.22	0.9	0.04	0.13	0.98	1	5	55	2	348
UOG#6	7.28	76.09	5.97	1.28	0.94	0.08	0.13	0.98	1	5	48	1	357
Mean	6.79	74.47	9.99	1.25	0.94	0.05	0.14	1.03	1.17	5.00	60.83	1.67	360.33
Std.dev.	0.82	1.70	3.87	0.09	0.17	0.01	0.01	0.13	0.41	0.63	11.09	0.52	36.15

The total fatty acid accounts for 3.91% ± 0.71% of tuna roe in dry matter. Previous studies on PUFA and HUFA profiles of natural foods for P. vannamei maturation suggested that three HUFA are needed for dietary inclusion, which are arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA). Bloodworms are generally considered as the gold standard of shrimp maturation feeds, therefore the fatty acid profile of tuna roe was compared with bloodworm's in order to assess the suitability of fish roe for shrimp maturation diets (Table 3). ARA was suggested to be the precursor of prostaglandins, which play a role in controlling vitellogenesis. The fact that

shrimp oocyte EPA level was found to be positively related to fecundity and DHA highly correlated with hatching rate support their dietary inclusion beneficiary for shrimp maturation. Among the total fatty acid fractions, ARA comprises 5% of total fatty acid fraction in tuna ovaries, which is slightly higher than that in bloodworms (4%). On the other hand, significantly lower EPA content was present in tuna roe as compared with that in bloodworm, 6% vs. 29.3%, while DHA in tuna ovaries triples the amounts in bloodworm. As a result, the EPA/DHA ratio is far lower in tuna roe (0.16) than that is bloodworm (2.27), the latter of which was similar to that from shrimp ovary tissue. However, the relationship between the dietary EPA/DHA ratio and shrimp maturation remains unknown.

Table 3. Fatty acid levels (% of total fatty acid fraction) of the six tuna roe samples compared with the published bloodworm fatty acid profile.

Fatty Acid	Blood Worm	Tuna Roe (Mean ± Std.dev)	
Myristate	14:00	0.89	2.11 ± 0.23
Palmitate	16:00	7.5	22.57 ± 1.09
Palmitoleate	16:1n7	3.58	3.62 ± 0.34
Stearate	18:00	6.51	5.53 ± 0.23
Oleate	18:1n9	7.09	14.03 ±1.98
Linoleate	18:2n6	1.03	1.00 ± 0.11
Linolenate	18:3n3	0.28	0.33 ± 0.06
Octadecatetraenoate	18:4n3	???	0.39 ± 0.14
Eicosenoate	20:1n11	14.9	1.02 ± 0.25
Arachidonate	20:4n6	4.1	5.02 ± 1.09
EPA	20:5n3	29.3	6.18 ±0.79
Erucate	22:1n13	0.41	0.39 ± 0.08
DHA	22:6n3	12.9	38.10 ± 2.52
EPA/DHA		2.27	0.16

Dietary evaluation of tuna roe based semi-moist diet on shrimp reproductive performance

Based on the nutritional content, we developed a semi-moist diet (Table 4) containing 55% protein and 15% lipid on a dry weight basis, which possessed the suitable texture and water stability. It was well accepted by shrimp broodstock. The ingredient cost for the semi-moist diet is about $11/kg.

Table 4. Dietary composition (%) of the tuna roe based semi-moist diet and cost.

Ingredient	Price	Cost/g	Composition	Cost ($)
Fish Roe	$2.50/lb	$0.01	55%	3.029
Soybean meal	$2.75/lb	$0.01	12%	0.727
Glutinous rice flour	$2.00/lb	$0.00	5%	0.220
Spirulina	$25.66/lb	$0.06	5%	2.826
Black algae powder	$26.15/lb	$0.06	2%	1.152
Mineral mix	$25.53/kg	$0.03	1%	0.255
Vitamin mix	$130/kg	$0.13	1%	1.300
Lecithin	$50/kg	$0.05	3%	1.500
Distill Water	$0.25/gal	$0.00	15%	0.008
Soybean oil	$5.00/kg	$0.01	1%	0.050
Total	---	---	100%	11.07/kg

The effect of the diet on the reproductive performance of shrimp broodstock was also evaluated and details are as follows. SPF P. vannamei adults (30.5g average) used for the stage I experiment were produced from the same cross and ~10 shrimp/m2 were stocked in each 30 ton fiberglass tank (bottom area 23.6m2) with male to female ratio of 1.4:1. One month prior to the experiment, shrimp had been conditioned with feeding the two regimes (tuna roe diet and control regime A (50% proprietary commercial shrimp maturation diet, 50% Squid). We chose this specific commercial shrimp diet as the control because it had yielded the best results in boosting shrimp's maturity among all the available dietary regimes at UOG hatchery, including the frozen squid. During the one-month stage I experimental comparison, a natural mating scheme was applied to evaluate the dietary effect of tuna roe on shrimp broodstock reproduction indexes, such as ovary maturity level, number of mating per day, eggs per spawning, hatching rate, etc. The semi-moist tuna roe diet yielded an inferior reproductive performance as compared to regime A.

A new batch of shrimp broodstock was used for the stage II and III comparisons. In both

experiments, we compared the semi-moist diet with the same commercial diet alone (regime B, 55.3% Protein, 9.83% lipid). During the stage II comparison, broodstock from both sexes were fed the diet, and natural mating was adopted. Results showed that similar effects were achieved on most reproductive traits, except that significantly fewer females reached maturity level of stage IV ($p<0.0001$) and consequently, a lower number of female spawners ($p = 0.077$) were found in the tuna roe dietary group. The latter may be resulting from the lower sperm quality of male broodstock from the fish roe dietary group.

Therefore, the stage III comparison consisted of the same feeding regimes as stage II (semi-moist diet vs. regime B), but only female broodstock were used after the unilateral eye ablation was performed. As soon as the female brooder reached stage IV, artificial insemination was applied by utilizing the best spermatophore available from the males that were fed regime A. Semi-moist diet seemed to be able to produce similar eggs per spawning, fertilization rate and hatching rate in shrimp brooders as the regime B, though the maturity of female to stage IV was still a little behind ($p<0.05$).

Summary

This project explored the possibility of utilizing the local available tuna roe for shrimp maturation diet and provided baseline information in terms of the nutritional and health aspects of such a diet. The information generated from the project could serve as a useful tool towards developing a cost effective, nutritionally complete and biosecure maturation diet. Utilized as a shrimp maturation diet, tuna fish roe was found to be:

Figure 3. Female shrimp fed with the semi-moist diet.

- High in HUFA content. Tuna roe contained similar level of arachidonate (ARA), less EPA and higher DHA than bloodworm.
- Free of shrimp viral pathogens. This is a good attribute for SPF shrimp.
- Suitable for developing semi-moist diet with proper texture and palatability.
- More favorable to female reproduction than male reproduction.
- Value-added as both nutritional values and environmental benefits are realized.

The primary findings of this project could serve as a good starting point for further development of a nutritionally complete maturation diet to benefit a larger region than Guam and CNMI, as carotenoids, vitamins, stimulating compounds, certain HUFA ratios could be further identified or incorporated to the diets in promoting shrimp maturation and reproductive performance.

Acknowledgements

The authors wish to thank Mr. Steve Stenson for his generous provision of tuna roe, Ms. Jarupan Channarong and Mr. Adrian Rojas for their valuable contribution in the semi-moist diet preparation and evaluation of shrimp reproductive performance, Ms. Liza Blas for tuna roe collection, Mr. Yanhao Yang on experimenting the various drying procedures, Dr. Yongde Zhang for assisting in shrimp disease diagnosis. Our thanks to the Dr. Harry Ako and Ms. Page Iida from University of Hawaii at Manoa, CTAHR for providing the fatty acid analysis of tuna roe samples, as well as Agricultural Diagnostic Service Center, University of Hawaii for their service in proximate analysis.

Pacific Island Spotlight: Marine Finfish Hatchery Training Workshop at OI for U.S. Affiliated Pacific Island Technicians & Farmers

On August 6-18, 2012, the Oceanic Institute Finfish Department will conduct a workshop for aquaculture technicians and farmers from the U.S. Affiliated Pacific Islands. The workshop is part of a CTSA-sponsored project to establish marine finfish farming in the CNMI and surrounding region. Only applicants from the CNMI, Guam, Palau, FSM, and American Samoa are eligible to participate.

Hands-on, intensive training sessions will include:
- Broodstock husbandry
- Feeding, sex determination, tagging, spawning, egg quality criteria, egg collection & health maintenance
- Live feeds production
- Production systems for algae, rotifers & Artemia
- Larval rearing
- System design, operation, stocking, feeding, harvest
- Water quality, tank management & maintenance
- Nursery/Grow-out Systems
- System configuration, operation, feeding & management

Participants will be provided with a new manual, outlining all of the techniques and procedures covered in the training. Sessions will run 0800-1700 daily.***The workshop is free, however participants are responsible for their own transportation to/from the location, as well their accommodations while on Oahu.*** To register for the workshop, contact Dr. Chad Callan at (808) 259-3149 or ccallan@oceanicinstitute.org. There is very limited space available, so please register as soon as possible!

AquaClip: Increase Expected in Farmed Bluefin Tuna in 2015

By Chris Loew, SeafoodSource contributing editor. May 10, 2012.

Kinki University, which pioneered captive tuna breeding, has teamed up with Toyota Tsusho Corp. for large-scale commercial farming for export to world markets.

Kinki University is known for the Kindai brand of captive-bred bluefin, which has limited distribution in New York and San Francisco. "Kindai" is short for Kinki Daigaku, the Japanese name of Kinki University.

Goto Tuna Dream, which operates holding pens in the ocean off Fukue Island, in Nagasaki Prefecture's Goto Island Chain, is a subsidiary of Nagoya-based Toyota Tsusho, a Toyota Group trading company.

A first batch of 14,000 juveniles, hatched at the university's facility six months earlier, was shipped to Tuna Dream's pens in November 2011. After two months, they were sold to aquaculture operations in Nagasaki and Kagoshima Prefectures to be fed for another three years, when they should reach a marketable length of 1 meter. This means a jump in sustainable bluefin can be expected from early 2015.

Click here to read the 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 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.