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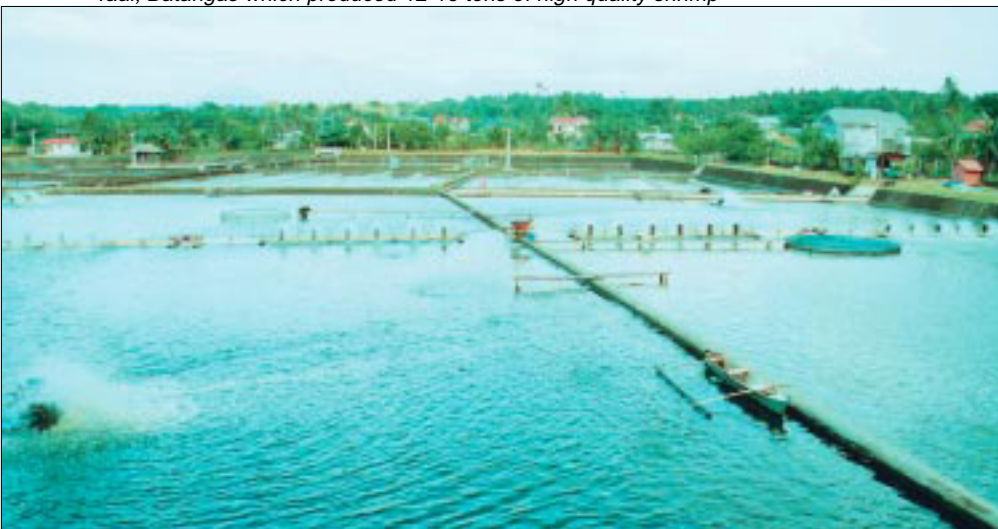
Shrimp: 1 problem, 4 solutions

A P30 billion industry, direct employment to some 180,000 Filipino voters. This is the tiger shrimp industry in the Philippines, which is feeling hopeful these days as it assembles its stakeholders again after four years. (The last shrimp congress was held in Iloilo City in 1998). There had been one problem, shrimp farmers had no way to combat the diseases, specifically the more virulent strain of luminous bacteria spawned by self-generated pollution and irresponsible use of chemicals and antibiotics, that devastated their farms.

"It was impossible to produce the tiger shrimp *Penaeus monodon* and come out a winner," says a farmer. "We had to downsize our family shrimp operations in 1996, and let go of the best trained people in shrimp production." That was then. Things have changed. R&D institutions have field-validated and packaged environment-friendly shrimp farming techniques. Progressive fishfarmers have similarly tried their own solutions.

In July's *Shrimp Congress 2002* in Bacolod City attended by nearly 500 industry players, workable solutions were presented and discussed. What were these?

The SEAFDEC/AQD environment-friendly shrimp techno-demonstration farm in Taal, Batangas which produced 12-15 tons of high quality shrimp



□ **ENLISTING TILAPIA TO COMBAT SHRIMP DISEASE** As shown by SEAFDEC/AQD, FYD International and the Negros Prawn Producers Cooperative (membership controls some 3,000 ha), tilapia is raised in a separate headwater reservoir and inside the shrimp pond but enclosed with a net. AQD has verified that water from tilapia ponds consistently has zero or very negligible luminous bacterial count.

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Disease diagnosis using molecular biology or DNA-based techniques at SEAFDEC/AQD biotechnology laboratory



Introducing SEAFDEC's regional fish disease project

The Regional Fish Disease program of SEAFDEC, which implements the three-year project for the *Development of fish disease inspection methodologies for artificially-bred seeds*, is implemented by SEAFDEC/AQD under the ASEAN-SEAFDEC Fisheries Consultative Group (FCG) collaborative mechanism, with funding from the Government of Japan-Trust Fund.

The program was developed considering that aquaculture production in Southeast Asia (SEA) has grown rapidly over the past 10 years. However, this rapid and generally uncontrolled progress spawned infectious diseases that frequently threaten the sustainability of aquaculture in the region. Moreover, the safety of consumers has become an important issue when some aquaculture products were found to contain chemical residues like pesticides. Thus, it becomes urgent and necessary to develop disease control and monitoring systems for chemical residues in aquaculture products.

The project aims to promote disease-free aquaculture in SEA and healthy and wholesome trading of aquaculture products including seeds for aquaculture;

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The shrimp congress was held back-to-back with the meetings of the Philippine Aquaculture Society and the Society of Aquaculture Engineers of the Philippines (pages 28-29 for highlights)



Curious participants take a look at SEAFDEC/AQD's environment-friendly shrimp farm model at the exhibit. See also pages 7 and 8 for a summary of product presentations at the congress



Yep, during the field trip hosted by FYD for the participants to their farm south of Bacolod City, some tilapias were cooked! Tilapia harvest can augment income from shrimp

SHRIMP: 1 PROBLEM ... FROM PAGE 1

Tilapias are omnivorous and filter-feed on the algae that bloom with organic enrichment, and when inside the shrimp pond consume the sludge which invariably form. In effect, they take out the excessive nutrients and maintain good water quality. Tilapia slime is also said to be bactericidal against luminous bacteria, Philippine shrimp's most recent killer, but the mechanism of how this is possible is not yet clear. However, AQD researchers have isolated bacterial, fungal and algal colonies from the "greenwater" of shrimp and tilapia, and found that some of the metabolites these isolates produce can inhibit *Vibrio harveyi*.

By whatever name this technology is known -- AQD calls it environment-friendly schemes or mangrove-friendly shrimp culture; FYD calls it TIPS or tilapia-water integration in prawn systems; and the Negros Prawn Producers Cooperative, "greenwater technology" -- it involves more than tilapia. Among others, there should be re-assignment of ponds (e.g. head and tail reservoirs); use of filter boxes; crop rotation (to break the life cycles of pathogenic organisms); biological pre- and post-treatment other than tilapia (use of probiotics, use of filamentous algae, oysters and mussels); long-arm paddlewheels, sludge collectors and substrates; and stocking of fry that have been checked for pathogens. The use of high quality feeds and the right feeding scheme are also important, and AQD, for the former, has successfully tested a less polluting formulation in tanks and ponds.

For queries on the technical aspect of this new technology, join *Aquafarmers' Corner* on the internet by visiting the AQD website at <http://www.seafdec.org.ph>.

□ DIAGNOSE SHRIMP DISEASE QUICKLY AND ACCURATELY DNA-based tests are much discussed, with the PCR used on fry to exclude from stocking those batches shown to have known viral killers like monodon baculovirus and most recently, the white spot syndrome virus (WSSV). One good news is that the Philippine government has put-up PCR-based laboratories all over the country: Cebu, Zamboanga, Cagayan de Oro, Davao, Iligan, Buenavista (Agusan del Norte), and Maribojoc (Bohol). This is in addition to existing units at AQD in Iloilo and in the BFAR central office in Quezon City. Eight more BFAR offices are programmed to receive PCR facilities. The government is also collaborating with UP Los Baños and the University of Hawaii to develop farm-based quick detection kit (dipstick method). Likewise, SEAFDEC has a regional fish disease project.

But a premium must be put on prevention. "Do not translocate shrimp from foreign countries," an expert emphatically advised. "Even if certified-free of known viruses, there could be other unknowns riding on it."

Lessons can also be learned from Japan's comprehensive prophylaxis strategy against viruses which include: (1) proper farm management (disinfect, maintain good water parameters, feed right), (2) use screened, virus-free stock, (3) use quick and accurate diagnosis (like PCR), and (4) administer immunostimulants, viral inhibitors, probiotics.

□ DEALING WITH MARKET REQUIREMENTS: FOLLOW THE CODE! The congress focused on antibiotic residues, noting, for example, that 300 parts per trillion (ppt) detection limit for chloramphenicol (CAP) has been settled by the European Com-

Principles and concepts of mangrove-friendly shrimp culture

By

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For a long time aquaculture development in general and shrimp culture in particular paid only scant attention to the environment. As a result, mangroves and other ecologically sensitive areas were destroyed in the process. Belatedly people realize that being friendly to the environment in general and to mangroves in particular is actually important to the industry because of the following reasons.

- The growth and survival of shrimps depend on a clean and healthy environment;
- The international market is getting more and more sensitive to wholesomeness;
- There is a tremendous pressure on developed countries to ban the import of shrimps from countries practicing environmentally damaging methods of shrimp farming;
- It is the only way to ensure sustainability of the industry.

Shrimp farming in particular is coming under attack by well-meaning environmental groups due to the following reasons.

- A large percentage of the mangrove forests in the world is alleged to have been cleared to make way to shrimp farming.
- Irresponsible practices by shrimp farmers such as improper use of chemicals and discharge during harvest have led to conflict with coastal communities.
- Shrimp farming has not helped the poor in the coastal communities and may even have made them poorer because the destruction of mangroves deprives them of a source of livelihood and reduces the aquatic resources that would have been available to the poor.

The efforts of various international NGOs to require importing countries to buy shrimps only from countries deemed to be mangrove or environmentally friendly cannot be ignored. One NGO in the United States has succeeded in requiring “turtle-friendly” certification for all shrimps coming from Asia even if these are raised in farms and not captured by trawlers. It is for this reason that the ASEAN countries have decided to take an active move to show that they are just as concerned about the mangroves and the environment.

Importance of mangrove forest to aquaculture

It used to be that mangroves are simply clear-cut to make way for fish or shrimp farms. Even if there are laws requiring buffer zones

where the mangrove trees should not be cut these laws are often ignored. Little do the developers realize that mangroves are important to aquaculture for the following reasons.

- Protect perimeter dikes of fishponds and shrimp farms from strong wind and wave action;
- Act as natural biological filter by absorbing excess nutrients from pond discharges;
- Condition water for shrimp farms and fishponds;
- Act as nursery grounds for various species.

The role of mangroves as natural biological filters becomes even more important with intensification. Traditionally aquaculture is always regarded as recipient or victim of industrial pollution. Untreated effluents from various industries always cause problems for nearby shrimp farms. Traditional aquaculture at extensive levels generates very little waste all of which can be consumed by other organisms or will eventually decompose to more basic nutrients. With intensification, the amount of waste generated exceeds what can be consumed and decomposed naturally and aquaculture becomes a source of pollution as well, shrimp culture in particular produces both solid and soluble wastes. Solid wastes from shrimp culture include the following:

- Uneaten feeds;
- Feces;
- Bodies of dead shrimps (or fish);
- Shell cast off during molting.

The soluble wastes on the other hand can consist of:

- Excess fertilizers, chlorine, BKC and other chemicals used during preparation;
- Antibiotic residues during culture;
- Metabolic wastes from culture animals;
- Phosphates leached out from feeds.

With intensification the following events happen:

- Nearshore water becomes enriched adding to the organic load and therefore bacteria;
- Solid wastes accumulate on bottom, become growing area of disease organisms; natural process of decomposition becomes overloaded and cannot cope up;
- Water supply system becomes contaminated and adjacent farms start to pollute each other.

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In many of the shrimp producing countries in Asia, intensification set off a series of events which eventually lead to the collapse of the industry in many countries as shown in Figure 1.

Sustainability

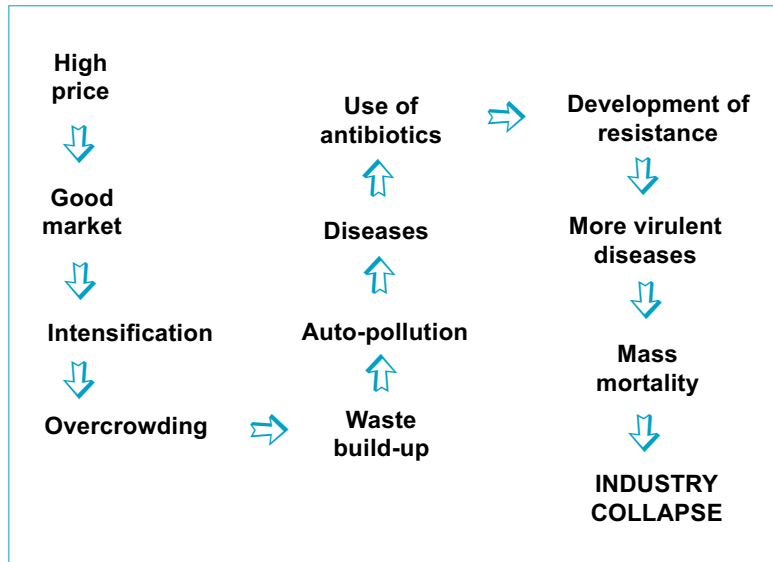
The single most compelling reason to farm shrimps without harming the environment is to ensure sustainability. Just what does sustainable aquaculture mean? To the fish farmer sustainable aquaculture would simply mean survival. Sustainable development in general has been broadly defined as development which meets the needs of the present without compromising the future (WCED, 1987). A more comprehensive definition of sustainable development comes from the Food and Agriculture Organization of the United Nations which goes as follows: "Sustainable development is the management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development (in agriculture, forestry and fisheries sectors) conserves land, water, plant and animal resources, is environmentally non-degrading, technically appropriate, economically viable and socially available."

In the welter of definitions one of the more comprehensive definition of sustainability comes from land management which appears to be just as appropriate for both aquaculture and agriculture. Sustainable land management according to Dumanski (1993 as cited by Greenland, 1997) is a condition where technologies, policies and activities are used together to integrate economic principles with environmental concerns so as to:

- Maintain or enhance production;
- Reduce the level of production risk;
- Protect the potential of natural resources and prevent degradation of water quality;
- Be economically viable;
- Be socially acceptable.

This definition embodies the various environmental, economic and socio-political issues which forms the matrix of sustainability. By going through the objectives of sustainability it becomes clear that aiming for sustainability not only has a practical value but also is

Figure 1
Events leading to diseases in shrimp farms and eventual collapse of the industry



essential towards attaining food security.

The various actions required to attain each of the five objectives are as follows:

- Maintaining and Enhancing Production*
 - Select sites which are suitable for the organism being cultured, in this case shrimps, has good soil characteristics and good supply of unpolluted water;
 - Employ proper design so that water in the shrimp farm can be maintained at the recommended depth, water can come in and out as required without delay, inputs can be

brought to all parts of the farm without much trouble, the stock can be easily harvested, the whole facilities is safe for workers especially when it comes to electrical connections.

- Make sure to select sites where it does not take too long to bring in fry from the hatchery.
 - Use good quality feeds and appropriate inputs.
- Reducing Production Risk Level*
 - Avoid areas that are subject to constant flooding and/or strong wind and wave action.
 - Employ sound engineering to maintain the integrity of the dikes, water conveyance and other structures.
 - Use healthy and uninfected seedstock.
 - Apply proper technical skills
 - Use appropriate prophylactic measures
 - Dispose of all wastes properly.

- Protecting Natural Resource Potentials*
 - Avoid ecologically sensitive areas such as mangroves.
 - Avoid use of harmful chemicals, antibiotics and other inputs which might affect the environment.
 - Treat water discharged from the shrimp farms.

- Economic Viability*
 - Find areas with favorable investment climate.
 - Find financing institutions that give favorable financing.
 - Make sure inputs will be easily available when required.
 - Employ efficient production methods.
 - Pay particular attention to product appearance and wholesomeness.

Environment-friendly schemes in shrimp farming

SEAFDEC/AQD's low discharge and closed-recirculating systems

By
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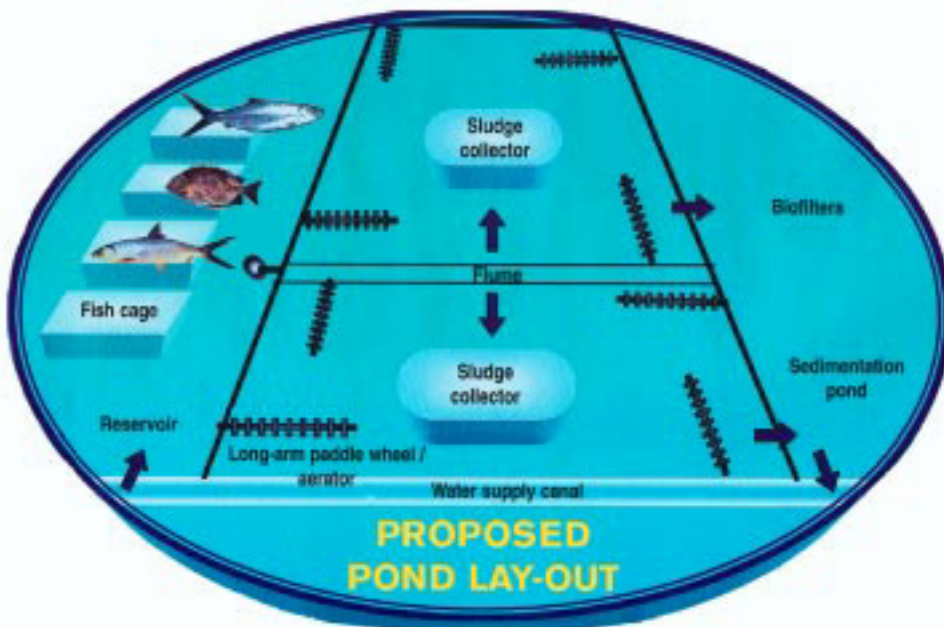
SEAFDEC/AQD through its Technology Verification Section has developed an intensive shrimp farming technology that is environment-friendly using the low-discharge and closed-recirculating systems. The protocol addresses the problem of, and provides solution to, our struggle against the dreaded luminous bacteria known to have caused the industry to decline sharply. It employs mitigation measures such as salinity reduction, physical and biological filtration of the culture medium, use of reservoir and settling ponds, biomanipulators, good quality shrimp fry, good quality feeds and an efficient feeding protocol, and long-arm paddlewheels for better aeration and water circulation. These physical and biological components are integrated in the pond design and layout in order to satisfy the requirements of the system.

To fast track the transfer of technology, field testing was conducted in various sites in the Philippines under the *Joint Mission for the Accelerated Nationwide Technology Transfer Program*, a collaborative project of SEAFDEC/AQD and the Bureau of Fisheries and Aquatic Resources (BFAR). Phase I started with field tests conducted in AQD ponds in Dumangas, Iloilo (western Visayas) and BFAR ponds in Batangas (southern Luzon), Bohol (central Visayas), and Lanao del Norte (northern Mindanao) which successfully demonstrated the technical

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Indicative cost and return data from AQD's techno-demonstration ponds in the Dumangas Brackishwater Station, Iloilo

Area	10,000 m ²
Total stock	400,000 pcs
Stocking density	40 pcs per m ²
Average body weight of shrimp at harvest	25 g
Biomass	7,500 kg
Survival rate	75%
Average price per kg	P270
Gross sales	P2,025,000
Expenses	
Fry	100,000
Feeds	535,000
Salaries/wages	180,000
Pond preparation	50,000
Lime	10,000
Biomanipulators	6,000
Probiotics	16,500
Power/lights/water	190,000
Fuel/lubricants	70,500
Sludge collectors/cages	14,850
Feeding bridge/tray	10,000
Laboratory analysis	10,000
Depreciation	67,000
Repairs of dikes/equip	60,000
Miscellaneous expenses	2,470
Total operating expenses	1,322,320
Equipment	300,000
Investment requirement	1,622,820
Net profit	702,680
Return on investment	43.3%



blue arrows note direction of water flow in low discharge system, red arrows for closed-recirculating

and commercial viability under different climatic conditions at stocking densities ranging from 25-60 shrimp fry per m² with production ranging from 6-15 tons per ha. [Very recently, AQD has demonstrated this technology overseas in coordination with the Department of Fisheries in Myanmar with a consistent production of about 10 tons per ha.]

Phase II engaged the participation of private tiger shrimp operators nationwide by using their farms, which is the ultimate goal to revive the shrimp industry. The JMANTTP also includes the conduct of nationwide skills development sessions that consist of lectures and practicals on the environment-friendly intensive shrimp farming technology. ###



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PRINCIPLES OF MANGROVE-FRIENDLY ... FROM PAGE 4

- Know the local market channels.
 - Market price of product
- e) *Social Acceptability*
- Farm should have minimal to zero impact on the environment.
 - Farm should have minimal to zero conflict with other users of common resource.
 - Use local human resource where advanced technical skills are not required.
 - Shrimp farm should have clear benefit to locality such as for instance payment of local taxes.
 - Pay farm workers just and fair wages.
 - Be a good neighbor

Conclusion

To sum up the above points a shrimp farm can be made mangrove-friendly by avoiding the selection of mangroves in the first place. Once operating, the farm should use only inputs that will not harm the environment. All types of wastes, whether solid or soluble, should be treated properly. Solid wastes which can be physically

gathered such as dead fish and shrimps should not simply be thrown into the water where it will find its way out and decompose. They should be properly disposed of by burying. Water coming out of the farm in the course of water change and during harvest should be allowed to settle in a treatment pond and not released directly to the water. Further treatment should be done with the use of filter feeders such as oysters and finally aquatic plants such as *Gracilaria* to reduce the nutrient load. It is not enough to just consider the immediate environment. The shrimp farm should maintain good relations with the farm workers and the community. Farm workers should be paid fair and just wages. Permits and licenses that may be required by all levels of government must be properly complied with. It is only by so doing that shrimp farming can be made sustainable.

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Probiotics: eliminating self-pollution in shrimp farms



Mr. William Kramer is the National Marketing and Sales Manager of HOC Po Feed Corporation, Philippines. Mr. Kramer is a graduate of Agri-business Management, major in Agronomy at the University of the Philippines at Los Baños College, Laguna. He's been involved in shrimp farming for 17 years. He made a presentation during the *Shrimp Congress 2002* in Bacolod City.

Its own wastes caused its decline - this is what happened to the shrimp industry.

The rapid development of the shrimp industry led to its own decline due to its self-generated pollution that reaches a level beyond the ecosystem's capacity to degrade. This polluted environment favored the emergence of various diseases such as luminescent vibriosis that caused significant drop in production, and eventually the sustainability of farm operations. The only way to maintain sustainability without drastically reducing stocking density and productivity is to reduce or even completely eliminate self-generated pollution. The use of probiotics is one of the means to achieve this.

Probiotics are viable monoculture or mixed culture of live microorganisms that when applied to culture systems benefit the farmed species by improving its indigenous microflora (microorganisms normally present) that compete with the pathogens (competitive exclusion) therefore, minimizing the effect of, if not eliminating, the pathogens directly.

HOC Po Feed Corporation developed the probiotic Maxima Microbial Inoculant (MMI). National Marketing and Sales Manager Mr. William Kramer presented at the *Shrimp Congress* the recorded production data of various tiger shrimp grow-out culture systems that used MMI as a control measure against luminous bacteria, *Vibrio harveyi*.

MMI is a mixture of generally recognized as safe (GRAS) cultures of fast-reacting photosynthetic sulfur and lactic acid bacteria, beneficial nitrogen-fixing bacteria, enzymes, yeast, fungi and actinomycetes combined with especially formulated microbial carriers (rice bran and fish meal) that provide and enrich the environment of microorganisms.

MMI is used in aquaculture as probiotic for disease prevention and control. "Beneficial microbes (microorganisms) prevent pathogen dominance," Mr. Kramer emphasized. MMI is also used as bioremediator (enhances soil microbial profile to efficiently oxidize the soil's organic load) and as bioaugmentor (introduces beneficial microorganisms to the culture system to improve its innate carrying capacity). It also hastens the elemental cycles of nitrogen, phosphorus and sulfur to eliminate unwanted products in the ponds. "MMI is a sustainable aquaculture technology," Mr. Kramer added.

To activate the microbial mixtures, MMI is soaked in water for 24 hours before it is evenly applied (broadcasted) into the pond. Application is done right after water change or while pumping water into the pond after draining. During application, paddle wheels should be in operation to hasten the mixing of the microbial culture with pond water. Water change is not advised for a couple of days (1 week) after inoculation to prevent the microbial culture from being flushed out and to promote further microbial multiplication. Re-application is advised after one week or every water change.

Mr. Kramer presented the collated data for the past six years of using MMI in semi-intensive culture of tiger shrimp *Penaeus monodon* in central Philippines. Productivity was compared in grow-out ponds utilizing direct pumping, and tilapia reservoir water systems. MMI application was at 10 kg per ha (1ppm), one to two times weekly for 120 to 150 days of culture in areas of both high (>20 ppt) and low (<20 ppt) water salinities.

Production results showed that average body weight is higher in all MMI treated ponds. In addition, survival rate is higher in shrimp grown with MMI whether they are in high or low salinities, or with direct pumping or tilapia reservoir water systems. However, survival rates on untreated ponds using tilapia reservoir water system is higher than untreated direct pumping because the luminous *Vibrio* from the sea water are reduced when allowed to stand for a few days before the water is supplied to the shrimp rearing ponds. "(The) multiplicative activity of luminous *Vibrio* is reduced (at) lower salinity levels," Mr. Kramer added.

While the MMI application seems promising, the consistent level count of the beneficial microbes in shrimp grow-out ponds is the most important in water management. Mr. Kramer reiterated that "the only way to minimize if not prevent the luminous *Vibrio*, is to keep the beneficial microbial count always higher than the luminous *Vibrio* in order to maintain the competitive exclusion process." This can be achieved through the use of probiotics.

MENTION OF TRADE NAMES IN THIS PUBLICATION DOES NOT CONSTITUTE ENDORSEMENT

Shrimp farmers' choices: feed, equipment, water treatment



Thailand's Dr. You Guanlin



Jojit Ronquillo



Ralph Lim

diseases, ensure superior water quality, offer most durable containment at the most economical cost, and speed harvest and turnaround.

Marc La Voie, Director of Biosolutions Co., Ltd presented their products Eco Marine, Pro Marine, and Bio Marine. He said it will not only enhance the "beneficial microorganisms" of the farmed species and prevent emergent diseases, but will also prevent soil and water degradation.

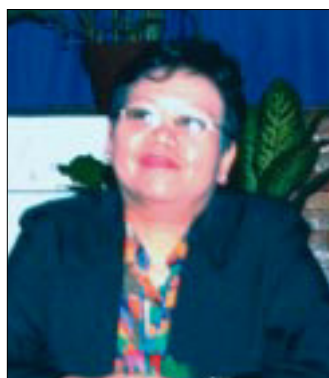
Presentations in *Shrimp Congress 2002* ranged from feeds to ozone technology. There were case studies, and technical and scientific sessions. Among the presentors are:

Karen Hogan, Executive Administrator and CEO of Aquatic Lifeline, Inc. (USA), presented their product **Lifeline Natural Artemia**, the natural food of shrimp larvae. *Artemia* was marketed in Japan in 1995, followed by Korea in 1996, Thailand in 1999, and the Philippines this year (distributed by Fi-Sh Pharma, Inc). See also our backcover article, this issue.

Dr. You Guanlin, Technical Manager of GSE Lining Co. Ltd., Thailand presented the high density polyethylene (HDPE) aquaculture **lining systems** for environmental containment applications or solutions that offer significant operation and cost benefits over soil, clay, concrete, and steel in aquaculture operations. GSE aquaculture lining systems, Dr. Guanlin said, can reduce operating costs and risks of



Peter Webster



Veronica Migo



Dr. Marc LaVoie

human health when taken in higher amount) analysis. RIDASCREEN has 50 ppt limit of detection and 150 ppt of limit of quantification.

Engr. Jojit Ronquillo of the Di-Catalyst International Corporation presented their product **Bio-Seb**, a microbial-based formula capable of releasing enzymes. He said that Bio-Seb cleans pond soil, conditions the water, prohibits the emissions of ammonia and sulfides, controls plankton growth at optimum level, and increases harvest. The company recommends farming protocol for proper application of their product, as well as proper monitoring during grow-out culture.

William Kramer, Marketing Director of HOCPO Feeds Co. presented their product Maximum Microbial Inoculant (MMI) that acts as **probiotic**, bioremediator, biostimulant, and bioaugmentator in shrimp culture. Thus, it can minimize, or even eliminate the self-generated pollution, which is critical in the sustainability of aquaculture. See preceding article, this issue.

Ralph Lim, representative of Pablo Altman of R-BIOPHRAM AG (Germany) presented the RIDASCREEN **chloramphenicol test** as a tool in detecting the presence of antibiotic residues in the processed shrimp in the market. The tool represents a rapid and cost effective alternative for the old techniques in chloramphenicol (antibiotic that threatens

Promising strategies against WSSV for kuruma shrimp in Japan

Kuruma shrimp, also called kuruma prawn or kurumaebi (*Marsupenaeus japonicus*) is one of Japan's most favored shrimps.

A native to the Indian Ocean and southwestern Pacific Ocean from Japan to Australia, these tasty creatures are chiefly consumed by the Japanese who utilize them in, among other things, a dish called dancing shrimp because the shrimps are served live. In Japan, live kuruma shrimp can fetch as much as \$100 per kilogram.

A hardy adaptable variety of shrimp, kuruma shrimp were among the first shrimp species to be cultured. However, outbreaks of the white spot syndrome (WSS) have been causing serious mortality to kuruma shrimp culture in Japan since 1993. Production declined from 3020 metric tons in 1988 to 1500 metric tons in 1994.

In 1993, about 80 % of kuruma shrimp production loss in Japan was due to white spot syndrome virus (WSSV) infection. The loss was estimated to be US\$ 20 million. Other losses recorded were due to *Vibrio*, *Fusarium*, and other unidentified infections.

WSSV can be easily detected by routine examination using 2-step PCR even in its early state of infection, but, it is highly pathogenic and can be easily transmitted either through vertical (spawners to eggs) or horizontal (from other crustaceans and shrimp to shrimp) mode of transmission.

On the contrary, the use of prophylaxis was recently proven to be effective against WSSV infection. Dr. Toshiaki Itami of the Department of Aquabiology, National Fisheries University, Japan recently developed a new prophylaxis strategy for WSSV: oral administration of peptidoglycan and fucoidan in shrimp diets.

Use of peptidoglycan (PG)

Peptidoglycan (PG) is an immunostimulant derived from *Bifidobacterium thermophilum*. Oral administration of PG was found to enhance the defense activity of kuruma shrimp against WSSV thus making it more resistant to infections.

In order to confirm the prophylactic efficacy of peptidoglycan to kuruma shrimp, Dr. Itami fed juvenile shrimp with PG at a concentration of 0.2mg/kg body weight/day with a 7-day intermittent schedule in which PG was fed for 4 days followed by 3 days of control diet (diet with no PG).



www.newmex.com/platinum/data/species/shrimpkuruma.html

This was administered for 30 days. Shrimp were challenged with WSSV in this period by being exposed to a WSSV-infected effluent seawater.

Survival rate of PG-fed shrimp was 97.6%, whereas survival for the control diet was only 19%. The latent virus was monitored using 2-step PCR. Examinations showed that the shrimps were still virus-positive but WSS did not become infectious.

Culture was continued after 1 month but the PG diet was replaced with the control diet. The shrimps started dying within 20 days after the replacement of feed. Final survival rate was 7.2% after 33 days of termination of PG feeding.

These results showed that PG enhanced the resistance of shrimp

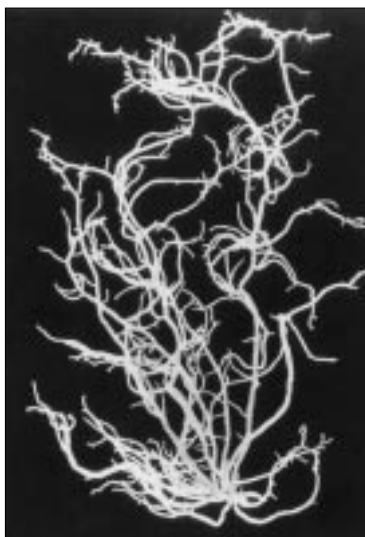
against WSSV because shrimp fed with PG exhibited a higher survival rate than did the control in the challenge trial, and because the shrimp started dying once the feeding of PG was stopped during the challenge.

In another experimental set-up, the quantity of WSSV in shrimp that survived infection after feeding with PG in the convalescent phase was studied using 2-step PCR in order to identify the exact period to clear the virus from shrimp.

Results showed that the virus was still detectable by PCR within 60 to 95 days of feeding with PG. On the other hand, no virus was detected after 95 to 125 days of feeding with PG. It was concluded that it would take time to clear the virus under the detection limit of 2-step PCR.

Shrimp fed with PG and survived after 30 days still carry a small number of virus parti-

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Dr. Toshiaki Itami presenting his paper at the National Shrimp Congress held at Bacolod City, Philippines from July 1 to 5, 2002

A brown algae is the source of fucoidan developed as prophylaxis for WSSV [cf. Ohno M, Critchley AT. 1993. Seaweed cultivation and marine ranching (1st ed). Japan International Cooperation Agency. p 51]

White spots on the carapace of kuruma shrimp
PHOTO COURTESY OF DR. ITAMI



cles and could be a source of infection. Hence, shrimp that survived WSSV infection by PG-administration should continue to receive PG for at least 2-3 months. This was to clear the virus by shrimp defense factors that have been strengthened by PG.

Use of Fucoïdan

Sulfated polysaccharides are previously shown to inhibit virus adsorption or attachment to the surface of animal cells. The sulfates in fucoïdan structure were shown to play an important role in the binding of WSSV. It also controls infection by inhibiting replication of enveloped viruses. Thus, it is a potent prophylactic agent against virus infections, WSSV included.

Fucoïdan is a kind of sulfated polysaccharide obtained from Okinawa-mozuko, *Cladosiphon okamuranus* a brown sea alga.

To test the efficacy of fucoïdan in inhibiting WSSV infection in kuruma shrimp, Dr. Itami prepared shrimp diets with different levels of fucoïdan and administered it to kuruma shrimp.

Fucoïdan were fed at concentrations of 10, 20, 60 and 100 mg/kg bodyweight/day for 15 days. The control group was fed with 0 mg fucoïdan. Four days after the first feeding, the shrimps were immersed in WSSV suspension. Mortality was monitored.

Results showed that survival rates of 100 mg fucoïdan-fed group was 82.3%, 60 mg group 78.9%, 20 mg group 46.1%, 10 mg group 38.4% and control group 12.4%. A dose of fucoïdan at 60 mg/kg bodyweight/day or higher is effective in preventing WSS in kuruma shrimp.

In another experiment, shrimps that were fed intermittently with fucoïdan showed a lower survival rate. Shrimps that were fed fucoïdan everyday exhibited the highest survival rate. It was concluded that fucoïdan should be fed everyday to prevent infection.

Conclusion

Peptidoglycan, an immunostimulant and fucoïdan, a viral inhibitor are both effective in prophylaxis against WSS as shown in experiments conducted by Dr. Itami. Significant effects on survival had been recorded when they were both incorporated in the diet of WSSV-infected shrimps.

Dr. Itami stressed the importance of being vigilant in translocating shrimp or crustaceans from other regions or countries into ponds to avoid entry of non-indigenous diseases. He said that some Japanese shrimp farmers committed this mistake by importing shrimp fry, which they think looked healthy and cheap. However, it was later found out that these shrimp fry were disease-carriers. Only a few farmers did this but it led to the collapse of the entire shrimp industry in Japan. This is the reason why Japanese farmers are now experiencing economic crisis in the shrimp farming business. "Learn from our failure!" Dr. Itami emphasized.

On its practical use, immunostimulants and viral inhibitors in shrimp farming is not a cure-all. Good health management, excellent pond management and stocking of genetically disease-resistant shrimp fry are still required to complement and increase the effectiveness of these prophylaxis strategies.

For details on use of PG and Fucoïdan as prophylaxis against WSS contact:

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PROBIOTICS ... FROM PAGE 7

Mr. Kramer thanked the support of his colleagues, Negros Prawn Producers Marketing Cooperative, Inc. (NPPMCI), and SEAFDEC/AQD. "Special thanks to Southeast Asian Fisheries Development Center for having to certify the use of MMI and their efforts to help resuscitate the much beleaguered shrimp farming," Mr. Kramer said.

The use of probiotics in aquaculture is hoped to boost shrimp production for sustainable aquaculture, not only in the Philippines, but also in the region. -CBL

The shrimp health papers

Presentations at the *Shrimp Congress 2002* organized by the Philippine Shrimp Industry Association Inc and BFAR in cooperation with SEAFDEC/AQD, Department of Trade / Board of Investments, and Industry and Negros Prawn Producers Marketing Cooperative Inc

Dr. Yasuo Inui, Fish Disease Expert of SEAFDEC/AQD, presented the activities of a regional fish disease control project with relevance to shrimp disease control. The Japanese Government Trust Fund is funding the project (see also our front cover story, this issue). The project consists of research and development, workshop, hands-on training, and development of a disease control network in the region. Dr. Inui discussed the establishment of effective control measures against infectious diseases and development of monitoring method for chemical use in aquaculture. The following studies are under the project: (1) control of viral diseases, (2) husbandry method for controlling luminescent vibriosis, and (3) development of evaluation methods for residual chemicals.

Dr. Toshiaki Itami of the Department of Aquabiology, National Fisheries University, Japan discussed the two new prophylaxis strategies he developed against white spot syndrome virus (WSSV) infection for kuruma shrimp in Japan (preceding article, this issue). He presented the results of his experiments on feeds supplemented with peptidoglycan and fucoidan. Mortality for WSSV-infected shrimps was found minimal.

Dr. Philip Loh, Professor and Head of the Virus Laboratory, Department of Microbiology, University of Hawaii presented the current detection or diagnostic technologies for shrimp viral pathogens, specifically for white spot virus, yellow-head virus, rhabdovirus and taura syndrome virus. He also discussed the advantages and limitations of these diagnostic technologies.

Akiko Sakai from the Institute of Applied Biochemistry, University of Tsubuka, Japan presented the development of a viral infection model using primary cell structure derived from penaeid shrimp through *in vitro* evaluation method. She also discussed the use of extracts of two species of mushroom, which were found to be effective against WSSV infection.

Dr. Nakao Nomura from the Institute of Applied Biochemistry, University of Tsubuka, Japan described the production of monoclonal antibodies against WSSV in shrimp. Dr. Nomura



Dr. Yasuo Inui



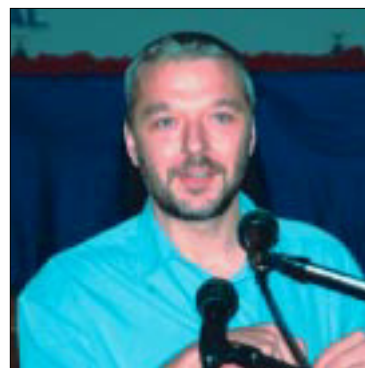
Dr. Philip Loh



Ms. Akiko Sakai



Dr. Nakao Nomura



Dr. Luc Grisez

explained that one of the most promising diagnostic tools to detect viral pathogens in shrimp farms employs immunological method by using monoclonal antibody. His work was focused on the development of a new method to purify WSS virion and obtaining monoclonal antibodies against the virion's envelope proteins. The antibodies obtained can be used for detection of WSSV in infected

shrimps. Moreover, some of these antibodies obtained have the ability to block viral infection.

Dr. Luc Grisez, Research Manager of INTERVET (AKZO NOBEL) Singapore, summarized available information on the luminous bacteria. One thing about the bacteria is that it doubles every 15 minutes if the conditions are right. He discussed vaccination as an excellent way to address the problem of luminous bacteria. Vaccination is most beneficial when used in shrimp hatchery operations. He said that vaccinated shrimps are healthier, more disease-resistant and grow better.

- SMW

Polymerase chain reaction (PCR) in disease diagnosis

By

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Various molecular biology techniques continue in becoming more important in fish and shrimp farming, particularly in detection and prevention of various diseases. One of the most prominent techniques is the Polymerase Chain Reaction (PCR). No technique has had a greater impact on the practice of molecular biology than the PCR. The PCR for amplification of specific nucleic acid sequences was introduced by Saiki et al. (1985) and has subsequently proved to be one of the most important scientific innovations of the past decade. With this technique, one can rapidly detect a virus or bacteria, few copies of mRNA (messenger ribonucleic acid), rapidly synthesize, clone and sequence virtually any segment of DNA (deoxyribonucleic acid). Despite the incredible power of the technique there has been one major limitation, that is, the DNA must be extracted from the sample. Thus one can not correlate PCR results with the pathological features of the material being tested.

The development of PCR means that small amount of DNA no longer limit molecular biology research or DNA-based diagnostic procedures. The technique is continuously improving and its full impact on molecular diagnostics is yet to come.

PCR currently has many applications, including analysis of ancient DNA from fossils, amplification of small DNA amounts for analysis by DNA fingerprinting, mapping the human genome and also those of other species, and detection of microorganisms present in low densities in water, food, soil or other organisms.

In aquaculture, PCR is a valuable tool for the prevention, control and management of various diseases. For fish and shrimp farmers, it permits fast, widespread, and sensitive screening of virus carriers, and also for early or light infections. The tests can be carried out non-destructively by using body fragments, blood or feces from fish and shrimp tested. PCR can be used to screen both broodstock animals and also larvae before stocking. PCR is rapidly becoming a critical instrument to detect fish and shrimp pathogens.

PCR assay has been widely used in the detection of fish viruses like stripe jack nervous necrosis virus (SJNNV) (Nishizawa et al. 1994), red sea bream iridovirus (RSIV) (Kurita et al. 1998), aquatic birnaviruses (Williams et al. 1999) and shrimp viruses like white spot syndrome virus (WSSV), monodon baculovirus (MBV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvo virus (HPV), baculovirus penaei (BP), Taura syndrome virus (TSV), yellow head virus (YHV) and

baculoviral midgut gland necrosis virus (BMN) (Lightner 1996; Wongteerasupaya et al. 1997; Lightner and Redman 1998; Tapay et al. 1999; Hsu et al. 2000; Magbanua et al. 2000). The assay has also been used in the detection of *Vibrio penaeicida* in shrimp (Genmoto et al. 1996; Nakai et al. 1997) *Aeromonas salmonicida* subspecies *salmonicida* (Miyata et al. 1996), *Pasteurella piscicida* (Aoki et al. 1997) and *Lactococcus garvieae* (Aoki et al. 2000) in fish.

PCR uses a thermo-stable polymerase to produce multiple copies of specific nucleic acid region quickly and exponentially. For example, starting with a single copy of a 1 kb DNA sequence, 10^{11} copies (or 100 ng) of the same sequence can be produced within a few hours. Once the reaction has occurred, a number of methods for identification and characterization of the amplification products are then applicable, of which the simplest is to identify the products according to their size following migration in the agarose gels. For many diagnostic applications, the simple visualization of a PCR product of characteristic size is a significant outcome since it indicates the presence of the target DNA sequence in the original sample.

Basic principle

Each PCR amplification is subdivided into three steps which are repeated in:

- (1) **melting or denaturation** (strand separation) of the double strand DNA (one to several minutes at 94-96°C). The PCR reaction requires a single-strand template. The first step denatures or melts the double-strand template DNA so that all the DNA is single-strand. This allows the oligonucleotide primers to anneal to the single-strand template DNA at specific locations. Temperature of 94°C for 30 seconds seems to work well, but shorter times have also been recommended. Remember, if the melting temperature is too low or time is too short the double-strand DNA may not denature thereby reducing the efficiency of the reaction. This is especially true for the first cycle in which the goal is to denature high molecular weight DNA. Some protocols suggest a long initial denaturation. On the other hand, the enzyme Taq polymerase will become less active after repeated denaturation cycle so, there is a need to balance between denaturation of the DNA and of the enzyme;

- (2) **annealing** of the two primers to opposite DNA strands (one to several minutes at 50-65°C). Once the template DNA has been denatured, the temperature must be lowered to a level that allows the primer to anneal. The trick is to lower the temperature to a level that allows the primer to anneal to the complementary sequence, if the temperature is too low the primer will sit down randomly (non-specifically) and if too high the primer will not sit down at all. Standard temperature seems to be about 55°C for 30-60 sec; and
- (3) **extension** of the primers by polymerase-mediated nucleotide additions to produce two copies of the original sequence (one to several minutes at 72°C). The Taq polymerase works best at temperatures between 72-75°C and so we raise the temperature from the relatively low annealing temperature to a temperature at which the Taq polymerase can function efficiently. The polymerase has to add nucleotides to the 3' end of the primer sequence annealed to the template DNA (please see figure below). The primers are necessary for the initiation of the reaction. The template DNA acts as a reference strand for the polymerase which adds the complementary nucleotide bases starting at the position just after the 3' end of the primer sequence (ADENOSINE pairs with THYMINE and GUANINE pairs with CYTOSINE). The primers are incorporated into all subsequently amplified DNA templates insuring perfect priming sites in subsequent PCR cycles.

PRIMER	5' TACAGCGCCACGTTA 3'
TEMPLATE	3' ATGTCGCGGTGCAATGATGCGTACGTAATGA 5'

During cellular DNA replication, enzymes first unwind and denature the DNA double helix into single strands. After the DNA is denatured, one more event must occur before DNA synthesis may be catalyzed by the DNA polymerase. It must find an area of transition from single stranded to double stranded DNA. At proper temperature typically around 55°C, sufficient primer-target DNA hybridization occurs which leads to the synthesis of complementary strands essential for the amplification step. During PCR, high temperature is used to separate the DNA molecules into single strands, and synthetic sequences of single-stranded DNA (20-30 nucleotides) serve as primers. Two different primer sequences are used to bracket the target region to be amplified. One primer is complementary to one DNA strand at the beginning of the target region; a second primer is complementary to a sequence on the opposite DNA strand at the end of the target region.

Components of a typical PCR.

Tris-HCl (pH 8.3)	20 mM	
MgCl ₂		2.5 mM
KCl		25 mM
dNTPs		50 μ M each
Primer 1	20 pmol	
Primer 2	20 pmol	
Taq polymerase	2.5 units	
Template DNA		10-100 ng
Mineral oil	optional	

As amplification proceeds, the DNA sequence between the primers doubles after each cycle. Following thirty cycles, a theoretical amplification factor of one billion is attained, assuming **100% efficiency** during each cycle. The final number of copies of the target sequence is expressed by the formula, $(2^n - 2n) x$, where:

- n - number of cycles;
- $2n$ - first product obtained after cycle 1 and second products obtained after cycle 2 with undefined length;
- x - number of copies of the original template.

There are factors that act against the process being 100% efficient at each cycle. Their effect is more pronounced in the later cycles of PCR. Normally, the **amount of enzyme becomes limiting** after 25-30 cycles, which corresponds to about 10⁶-fold amplification, due to **molar target excess**. The **enzyme activity also becomes limiting** due to **thermal denaturation** of the enzyme during the process. Another factor is the **reannealing of target strands** as their concentration increases. The reannealing of target strands then competes with primer annealing.

Two important innovations were responsible for automating PCR. First, a heat-stable DNA polymerase was isolated from the bacterium *Thermus aquaticus* that lives in hot springs. Hence, the term **Taq (DNA) polymerase** came to be. This enzyme remains active despite repeated heating during many cycles of amplification. Second, **thermal cyclers** were invented which automatically control the repetitive temperature changes required for PCR.

Following amplification, the PCR products are usually loaded into wells of an **agarose gel** and **electrophoresed**. Since PCR amplifications can generate microgram quantities of product, amplified fragments can be visualized easily following staining with a chemical stain such as ethidium bromide. The important point to remember is that the amplification is selective - only the DNA sequence located between the primers is amplified exponentially. The rest of the DNA in the genome is not amplified and remains invisible in the gel.

Primer design

Some simple rules aid in the design of efficient primers:

Typical primers are 18 to 28 nucleotides in length having 50 to 60% G+C composition.

The calculated melting temperatures for a given primer pair should be balanced. One can use the rule-of-thumb calculation of 2°C for A or T and 4°C for G or C. Depending on the application, melting temperatures between 55°C and 80°C are desired.

To prevent self-annealing, primers should not be complementary. This precaution is critical at the extreme 3' ends where any complementarity may lead to considerable primer-dimer formation and reduces the yield of the desired product.

Runs (three or more) of C's or G's at the 3' ends of primers may promote mispriming at G+C-rich sequences and should be avoided when possible.



Dr. Josette Biyo, leftmost / standing, with AQD staff and trainees in an aquasilviculture site in Aklan, west central Philippines

Science teacher thanks AQD

“I made special mention of SEAFDEC/AQD as a research institution in the Philippines which has been supporting my science research class,” science teacher Dr. Josette Biyo wrote AQD Chief Dr. Rolando Platon after she won Intel Foundation USA’s *International Excellence in Teaching Award* early this year.

“The judges particularly liked the mangrove studies conducted by my students under the guidance of (AQD scientist) Dr. Jurgenne Primavera,” Dr. Biyo explained. She showed slides of her students doing experimental work at AQD laboratories. This presentation was made to a board of judges and about 200 teachers from all over the world in Kentucky, USA during the *International Science and Engineering Fair*.

Dr. Biyo teaches at the Philippine Science High School – Western Visayas, Iloilo City. She is the first Asian teacher to receive the award since the competition started in 1997.

“Thank you for AQD’s support to our school, and for your contribution to my winning the international award. I hope (for your continued support) to our efforts to improve science education in the country,” Dr. Biyo concluded her letter.

To Dr. Biyo, thank you too for counting AQD in your success, and congratulations!

AQD celebrates its 29th year

SEAFDEC/AQD celebrated its 29th anniversary on July 9, honoring a new scientist, Dr. Edgar Amar, and 25 employees who have long served (~20 years) the Department. Dr. Amar was awarded a *Certificate of Appointment*, and the loyalty awardees were given certificates of service and loyalty rings in a short morning program.

In the afternoon, AQD scientist Dr. Felix Ayson delivered the annual Dean Domiciano K. Villaluz Memorial Lecture. He talked about facing the challenges of aquaculture through biotechnology. An extended abstract follows on page 17, this issue.

The lecture was followed by a booklaunching ceremony for AQD’s newest textbook titled *Nutrition in tropical aquaculture: essentials of fish nutrition, feeds and feeding of tropical aquatic species*. Ordering information can be found on page 33.

In addition to the day-long activity, AQD had a special week-long program for schoolchildren dubbed “Aqua Week 2002” which was organized by FishWorld, AQD’s environment education center, from July 8 to 19. The activities included kid’s day and film; contests on seafood dish, drawing and photojournalism, essay and poetry, write a fish story; and quizzes on nutrition and aquaculture, and ecology and aquaculture.

Aquaweek is a yearly activity celebrated to promote understanding of the importance of aquatic ecosystems and biodiversity and the research and development programs of SEAFDEC/AQD among school children and teachers.

Learning aquaculture technology via the internet

Twenty-five international learners completed on 19 August the first Internet based distance-learning course on “Principles of Health Management in Aquaculture” (AquaHealth Online). These learners came from ten countries, namely: Cambodia (2), Egypt (1), India (1), Indonesia (2), Malaysia (2), Myanmar (2), Singapore (3), Thailand (2), Vietnam (2) and the Philippines (7).

AquaHealth Online is a new and exciting learning experience conducted by SEAFDEC/AQD. Knowledge and skills needed in fish health management are actually transferred to learners via information technology. Learners from all over the world can participate without leaving their respective places of work.

Learners proceeded with the course as if they were in a classroom, except that they faced computer screens instead of instructors. Under guidance from specialists, learners performed learning-exercises on their own and submitted reports of their work through the Internet also. Learners took examinations administered by proctors near the places of their work. Of utmost importance was the unlimited interaction among learners, sharing insights and experiences, enhancing further the learning process. An enrollee introduced himself: “Hi everyone! I am Somporn of the National Institute of Coastal Aquaculture, southern Thailand. Currently I do research in marine fish diseases. I enrolled in this course to gain more knowledge and learn from my classmates’ experience. I want to make real friends with everyone as well.”

Health management in aquaculture is traditionally one of the most sought-after and well-attended international classroom type training courses at AQD. It is due to the realization that no aquaculture venture would ever succeed without due consideration to proper health management practices. What used to be taught in a student and teacher face-to-face setting is now taught in a distance-learning mode, AquaHealth Online.

After participating in the course, learners were expected to: (a) recognize shrimp and fish disease, (b) identify the cause(s) of the disease, (c) explain how a disease develops, (d) apply preventive and control measures to lessen the risks posed by the disease, and (e) use appropriate techniques for the preparation of samples for disease diagnosis.

How was AquaHealth Online conducted? Upon registration, learners were given a CD-ROM containing 12 learning modules and a course guide. The course guide provided the student with the course: introduction, description, goals and objectives, outline, requirements (skills and equipment), manner of assessment (grading system), and activities for each chapter.

How much computer skill was necessary to this course? An enrollee stated: *"I am chair of the research and extension department of our institution. This is my first time to experience an on-line course, much more navigate in a virtual environment. Anyway this is a very much welcome development in my profession for I will be able to refresh my clogged brain cells."* And another neophyte added: *"Sorry for the delayed introductions. I am new in computer operation. I beg your consideration."* And both of them managed.

Were there examinations? Yes. Online, the questions were handled much more easily. Self-assessment questions (SAQ) tested the learners' progress at each module. Answering these questions prepared the learners to take the proctored examinations.

What if the learner travelled or temporarily left his/her place? No problem, we considered it as the learner had access to the internet. Take this for instance: *"Hi everyone, this is "Zilong". Although this is quite late (due to my business trip), I'm glad that I still made it."*

The course is handled by a group of 11 fish health specialists at AQD. These scientists and researchers have a combined total of 210 years of experience in various fields of aquaculture health management -- in virology, bacteriology, mycology, parasitology, serology, immunology and molecular biology.

AquaHealth Online was developed with the technical assistance of the UP Open University, the country's premier institution in distance education and internet based learning systems.

Encouraged by the positive response of the AquaHealth Online learners, AQD launched another distance learning course "Basic Principles of Nutrition in Tropical Aquaculture" or AquaNutrition Online. It started 19 August. For more information contact: training@aqd.seafdec.org.ph.



Trainees with their instructors at SEAFDEC/AQD

"Aquaculture is the last frontier in seafood production owing to the decreasing catch in commercial and municipal waters. The aquaculture industry has huge export potential, and it is through diversification and sustainable management of aquaculture that the industry can help Southeast Asia attain its goal of food sufficiency and security. We at AQD are glad we are in the business of disseminating these technologies to help the region."

DR. ROLANDO PLATON
SEAFDEC / AQD CHIEF

AQD concludes two training courses this quarter

Two international training courses concluded in the third quarter of this year: the 4-week *Management of Aquafarming Systems*, and the 3-week *Mangrove-Friendly Shrimp Culture*.

The *Aquafarming* course was attended by 16 participants coming from Brunei Darussalam, Cambodia, China, Indonesia, Ma-

- S Pedrajas

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laysia, Thailand, Vietnam, Myanmar (one trainee each); and Philippines (8 trainees). The course discussed farming techniques for milkfish, grouper, shrimp, mudcrab, oyster, mussel, and seaweed.

The *Mangrove-Shrimp* course had 10 participants from Malaysia, Vietnam, Cambodia, Thailand, Indonesia, Brunei Darussalam, and Myanmar (one participant each); the other three came from the Philippines. The course was a component of an ASEAN-SEAFDEC program, and provided participants with basic understanding of the mangrove ecosystem and technical skills on shrimp culture so that they can grow shrimp in a sustainable and mangrove-friendly manner.

There have been four other training courses conducted in the first half of the year:

- *Freshwater Aquaculture*, attended by 11 participants coming from eight countries. The course included basic and advanced technologies in genetics, broodstock development, hatchery, nursery and grow-out operations of freshwater species like big-head carp, tilapia and catfish. The training session, for the first time, added a lecture topic on freshwater prawn *Macrobrachium rosenbergii*.
- *Health Management in Aquaculture*, attended by 10 participants from eight countries. AquaHealth is aimed at enabling trainees to apply the principles of health management into practice such that they can distinguish diseased from healthy fish/shrimp; identify disease agents and evaluate their effects on the host; and apply methods of disease diagnosis, prevention, and control. AquaHealth was conducted in Iloilo.
- *Marine Fish Hatchery*, the most attended, having 18 participants who came from ten countries. MarFish was developed as a course by AQD in an effort to transfer technologies on the spawning and larval rearing of commercially important aquaculture species. The course included milkfish, sea bass, grouper, snapper, rabbitfish and crabs.
- *Crab Seed Production*, with all-Filipino trainees (11 of them). The course provided technical skills so participants can establish or operate a crab hatchery. *Scylla serrata* was emphasized in the course.

AQD scientists in conferences

Four SEAFDEC/AQD scientists were in Rhodes, Greece from June 1 to 7 to present their papers (oral/poster) at the *International Symposium on Nutrition and Feeding in Fish*. They were:

- (1) Dr. Oseni Millamena – “Replacement of fishmeal by lupin meal in a protocol diet for grouper *Epinephelus coioides* juveniles”
- (2) Ms. Myrna Teruel, Ms. Ilda Borlongan and Dr. Corazon Santiago had papers on the utilization and/or potential of feed pea *Pisum sativum* as alternative protein source in practical diets for three cultured species — juvenile tiger shrimp *Penaeus monodon*, milkfish *Chanos chanos*, and tilapia *Oreochromis niloticus*, respectively

- (3) Ms. Borlongan and Dr. Arnil Emata (in absentia) – “Practical broodstock diet for the mangrove snapper *Lutjanus argentimaculatus*”

At the World Aquaculture Society-organized *World Aquaculture 2002*, six AQD senior staff presented various papers, as follows:

- (1) Dr. Gilda Lio-Po – “Experimental pathogenicity of the epizootic ulcerative syndrome (EUS)-associated rhabdovirus, *Aeromonas hydrophila*, and *Apanomyces* sp in snakehead *Channa striata*”
- (2) Dr. Josefa Tan-Fermin – “Some aspects of the reproductive biology, embryonic development and larval morphology in the blue tang *Paracanthurus hepatus*”
- (3) Mr. Armando Fermin – “Compensatory growth response of tropical abalone *Haliotis asinina* to feed cycling with seaweed and artificial diet”
- (4) Ms. Ilda Borlongan – “Efficacy of phosphorylated ascorbic acid as vitamin C source in diets for juvenile milkfish *Chanos chanos*”
- (5) Ms. Ofelia Reyes – “Nutritionally enriched free-living nematode *Panagrellus redivivus* as feed for grouper *Epinephelus coioides* larvae”
- (6) Mr. Wilfredo Yap – “Role of mariculture in securing food supply and reducing poverty in the Philippines”

In a related development, AQD scientist Ms. Neila Sumagaysay Chavoso won a Student Award (in absentia) during the WAS conference. The work cited for the award was her study “Water quality and holding capacity of semi-intensive and intensive milkfish ponds” which was submitted as an abstract to the Conference. The study is part of her dissertation for a PhD degree on Marine Science Institute at the University of the Philippines.

Who’s visiting at AQD?



Serge Raemaekers of the University of Ghent, Belgium, arrived on July 22 at SEAFDEC/AQD’s main station in Iloilo to conduct his Master of Science thesis, a study on the behavioral differences of mudcrab species (*Scylla serrata*, *S. tranquebarica*, and *S. olivacea*).

The study is under the European Commission-funded collaborative project on mud crab that involves four institutions -- AQD in the Philippines, the University of Wales in UK, the *Artemia* Reference Center in Belgium and Can Tho University in Vietnam. Serge’s thesis adviser at AQD is Dr. Emilia Quinitio. Serge will stay until October this year. Welcome!

The Dean Domiciano K. Villaluz Memorial Lecture Series is held every SEAFDEC/AQD anniversary, and is dedicated to the late Dean Villaluz, the first AQD Chief, in acknowledgment of his able leadership during AQD's organizational and formative years and for his contribution to the development of the aquaculture industry in the Philippines and the rest of Southeast Asia. Dean Villaluz was AQD Chief from 1973 until his retirement from active government service in 1979.

This year's lecturer is Dr. Felix Ayson on biotechnology (inset on right).

Facing the challenges in aquaculture through biotechnology

By

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Biotechnology, the science of manipulating genetic traits and substances, has been with us for centuries. The Chinese have created gold fish of different shapes, sizes and colors from a single species through centuries of breeding. New techniques in molecular biology, particularly genetic engineering, have made the field more exciting. Microorganisms act as the new drug factories. Harnessing them has made many medicines cheaper and more readily available. For example, insulin for diabetics is mostly produced by genetically altered bacteria through recombinant DNA technology.

Biotechnological tools fueled the Green Revolution through the development of higher yielding varieties of rice, wheat and maize. Biotech is being held as the engine of the second Green Revolution - one that would be less dependent on pesticides and fertilizers. The same tools have the potential to fuel another revolution, in another front - a Blue Revolution.

The rapid increase in population and the ensuing increase in the consumption of fishery products, as well as uncontrolled fishing, poor management, and the accumulation of chemical pollutants in the environments, have strained global fisheries production. Many countries have turned to aquaculture to increase fisheries production. At present, aquaculture is the world's growing food production sector.

The success of aquaculture depends on the control of reproduction and life cycle. The genetic background of the parent fish stock, efficient detection and effective prevention of diseases, thorough understanding of the optimal conditions for growth and development, sufficient supply of good quality water, and innovative management techniques. By improving some of these factors, the aquaculture industry has already made impressive progress over the last several years. Application of biotechnology can further speed up the expansion of the industry.

The cDNAs and the genomic sequences of growth hormone (GH) and other growth factors have been isolated and characterized for several fish species in recent years. Biologically active recombinant GH preparations have become available and exogenous

application of recombinant GH results in significant growth enhancement in fish. If new strains of fish produce elevated but optimal levels of GH, it would bypass many of the problems associated with exogenous GH treatment. Transgenic carp, salmon and rainbow trout have been produced to contain either GH gene promoter sequences or additional copies of the GH gene itself. Such fish has been shown to grow at increased rates compared to unmanipulated fish, resulting in increased production per unit time and a markedly shortened production cycle.

Occurrence or outbreaks of diseases during aquaculture operations substantially reduce profitability. Antibiotics are often used to prevent and control disease outbreaks. However, long-term use brings the risk of bacteria developing resistance and of residues in the cultured product. Apparently, the most effective method can only be the development of natural disease resistance in fish. Selections for specific pathogen resistant (SPR) strains are complementary approaches that are being addressed in current selective breeding programs in shrimps.

Rapid disease diagnosis and screening for pathogens is one area where molecular biology tools can be applied. In shrimp culture, kits have been developed and several more are being established for PCR-based detection of viral, fungal and bacterial pathogens before clinical symptoms of infection become evident.

Harnessing the hosts' specific and non-specific defense mechanisms for controlling diseases has considerable potential for health management in aquaculture. There are a large number of commercial immuno-stimulants and non-specific immune enhancers available in the market at present and these are incorporated to the diet to provide added protection to the animals. The use of probiotics and microbial food additives is also becoming widespread. Probiotics are living bacterial preparations that improve the balance of the intestinal microflora, such that digestive functions are enhanced or pathogenic microbes are inhibited or both. However, the effectiveness of many of these products still needs to be established.

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Technology caravan for Filipino fishfarmers

“SEAFDEC/AQD’s contribution to the government’s agriculture caravan in the (northern Philippine island of Luzon) was invaluable and well received by the fisherfolk,” Director Malcolm Sarmiento Jr. of the Bureau of Fisheries and Aquatic Resources told AQD Chief Dr. Rolando Platon. He asked AQD to join a fourth technology caravan.

AQD’s extension team was composed of Dr. Emilia Qunitio, Dr. Fe Estepa, Ms. Marietta Duray, Mr. Roger Edward Mamauag, and Mr. Isaac Abello. They gave lectures on the latest aquaculture technologies, including breeding and nursery of mud crab, tilapia and other fishes; grow-out management for fishes in ponds and shrimp farming techniques that have been demonstrated to be environment-friendly. The AQD lecturers also tackled mud crab culture in mangroves.

The caravan audience are mostly fisherfolk of overfished coastal areas in Region IV and V (southern Luzon). The caravan started off in Lucena City on July 22, continuing on to Calauag, Ragay, Mercedes, Calabanga, San Jose, and finally Sorsogon City on August 9.

AQD has prioritized its technology verification and extension program in pursuit of sustainable and responsible aquaculture in Southeast Asia.

Socio-economic monitoring guidelines crafted

Eight social scientists and marine resource managers participated in a mini-workshop to draft socio-economic monitoring guidelines for use by marine resource managers in Southeast Asia. The mini-workshop was held at the FishWorld audio-visual room from July 30 to August 1.

The participants were: (1) Susana Siar of SEAFDEC/AQD; (2) Leah Bunce of National Oceanic and Atmospheric Organization, also representing the Global Coral Reef Monitoring Network, USA; (3) Kuperan Viswanathan of International Center for Living Aquatic Resources Management–The World Fish Center, Malaysia; (4) Robert Pomeroy of International Marinelife Alliance, Washington DC; (5) Elmer Ferrer of the University of the Philippines’ College of Social Work, and Community-Based Coastal Resource Management Resource Center, Philippines; (6) Gregor Hodgson of The Reef Check Foundation, University of California at Los Angeles; (7) Johnnes Tulungen of Coastal Resource Management Project, Indonesia; and (8) Rebecca Pestaño-Smith of Coastal Resource Management Project, Philippines.

The socio-economic monitoring program was designed to help managers better understand the communities whose activities affect, and are affected by, marine resource management decisions. Thus, managers can minimize the socio-economic impacts of deci-



Concern for aquaculture technology’s bottomline: SEAFDEC/AQD scientist Dr. Emilia Qunitio gives a lecture during the techno-caravan in northern Philippines (TOP); while social scientists / resource managers met with AQD Chief Dr. Rolando Platon (leftmost in foreground, bottom photo) when they gathered to discuss how best to monitor the progress of communities taking part in resource management

sions, incorporate community concerns into decision-making and demonstrate the value of the marine resources.

The target audience of the guidelines includes local government units, non-government organizations, project managers, and the local community.

The socioeconomic guidelines will be pilot-tested in northern Antique and Cebu, central Philippines, towards the end of the year.

Training head is new aqua eng’g society president

SEAFDEC/AQD’s Head of Training and Information Division, Mr. Pastor L. Torres Jr., is the newly elected President of the Society of Aquaculture Engineers of the Philippines Inc. (SAEP) for 2002-2003. He was inducted into office July 1 in Bacolod City. SAEP is a non-stock, non profit technical service organization of engineers, architects and fisheries technologists involved in aquaculture engineering.

AQD’s Finance and Administration Division Head was also elected as Vice-President for the Visayas.

Hatchery-produced abalone juveniles released in Sagay Marine Reserve

SEAFDEC/AQD released 1,820 hatchery-produced abalone juveniles of 4 different sizes (1.5-2, 2.5-3, 3.5-4, 4.5-5 cm shell length) in Sagay Marine Reserve, Negros Occidental last June 17, 2002 as part of its stock enhancement program.

The experimental release was made to determine the optimum release size for stock enhancement of abalone *Haliotis asinina*. Sagay Marine Reserve was chosen as the pilot site based on a resource and ecological assessment of several potential sites conducted by AQD last year.

Prior to release, the abalones were 'diet-tagged' by feeding them a SEAFDEC-formulated diet to produce a bluish-green shell band, which serves as an identifying mark of hatchery-produced abalones. The abalones were packed and transported using a recently developed technique, which results to a 100% survival even after 8 hours of transport. A modified release module was used consisting of PVC pipes placed inside a net bag that allows the abalones to come out only when they are strong enough to look for food and natural shelter. In the hatchery, they were stocked in outdoor tanks with natural growth of algae as part of their conditioning and intermediate rearing.

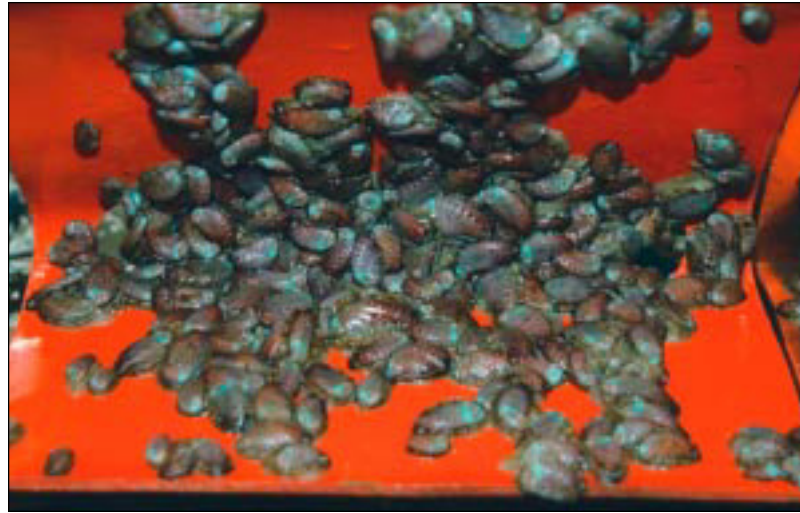
The abalones started to come out of the release modules within the first 2 hours after deployment. Only 2.3% remained in the release modules after the third day. On the first 3 days, dispersal from the release point was within a meter except for some large abalones which were found up to 4 meters away. During the 3-day monitoring, a total of 35 empty shells were found. Mortality was mostly due to crab predation. Majority of the released abalones was able to find good shelter among the dead corals encrusted with algae.

These preliminary results indicate that smaller-sized abalones have higher survival than the big ones because bigger abalones are more susceptible to crab predation due to its size and visibility. Of the 76 empty shells found during the first month, about 60% were from the large group (4.5-5 cm). The smaller-sized abalones are more cryptic and therefore more difficult to find by predators.

Recapture rates will be determined at least six months after the release when the abalone become adults and have the tendency to come out during the lowest tide.

The release of hatchery-produced abalones in a marine reserve is expected to increase the breeding stocks in the reserve, which in turn is expected to support abalone fishery in its surrounding areas.

Further monitoring and releases in Sagay Marine Reserve and other potential sites will be undertaken by the AQD research team composed of Dr. Wenresti Gallardo, Dr. Luis Ma. Garcia, Dr. Anicia Hurtado, and Dr. Clarissa Marte and their assistants Bernice Polohan, Shelah Mae Buen, Amadeo Biter, and Ephraim Doroteo.



Hatchery-reared abalone from SEAFDEC/AQD

Mariculture skills development session kicks off

The 4-month *Mariculture Livelihood Skills Development Session* opened on August 1 at SEAFDEC/AQD's Igang Marine Substation Mariculture Park.

The activity is a collaborative effort of AQD and the Bureau of Fisheries and Aquatic Resources under the Joint Mission for Accelerated Nationwide Technology Transfer Program (JMANTTP), and is co-organized with the Provincial Government of Guimaras.

Skills development session (SDS) is a unique process of learning wherein instruction is participative, primarily using hands-on skills and comprehensive on-site observation. SDS moves away from classroom type instruction.

The mariculture SDS will be participated in by one representative each from the five municipalities of Guimaras as selected by their respective municipal associations. Presently, 34 units of grouper cages have been installed in one municipality (Sibunag) through the assistance of AQD.

The trainees will be taught the technical aspects of operating fishcages for grouper, snapper and milkfish. Cage construction, stocking, feeding, management and marketing will be part of the curriculum.

The Mariculture Park facilities, where the SDS is ongoing, were constructed in 2001 with the following objectives: (1) demonstrate a well-regulated and environment-friendly approach to mariculture of economically important fishes as viable livelihood options for municipal fisherfolk and (2) enable fisherfolk and LGUs to learn a workable management set-up for sustainable cage culture livelihood activities.

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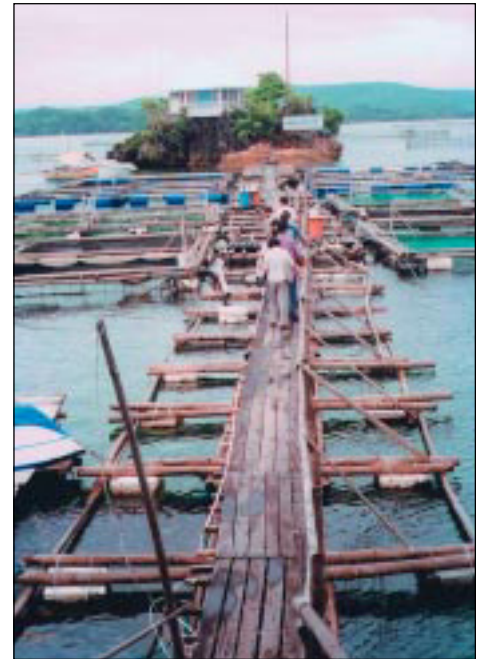
In their speeches during the opening program, local legislator Emilio Esmeralda and the governor's executive assistant Diosdado Gonzaga said that the mariculture livelihood project could reduce the pressure on existing Guimaras fishing grounds, which are now overexploited and depleted. Both expressed appreciation and full support to the collaborative undertaking among AQD, BFAR and the local government units of Guimaras.

At the end of the SDS, the participants and the LGU officials are expected to implement sustainable mariculture livelihood enterprises in their respective coastal areas. - J Genzola



The Guimaras governor's executive assistant, Diosdado Gonzaga, addresses the trainees at the opening of the skills development session

The trainees are oriented on the Igang Mariculture Park facilities during their first day



AQD welcomes DA Secretary Montemayor

Department of Agriculture (DA) Secretary Leonardo Montemayor visited AQD on August 4. He arrived with BFAR Director Malcolm Sarmiento Jr. and National Agriculture and Fisheries Council Executive Director Ricardo Villo Jr. Also present was BFAR Region VI Director Sonia Seville.

AQD staff members headed by AQD Chief Dr. Rolando Platon warmly greeted the guests at FishWorld, and toured them around the Biotech Wet Laboratory construction site and the renovated Biotech laboratory complex on the second floor of the Nutrition Building. They were briefed on the purpose of the laboratory, daily construction-related activities, and extent of work finished in the wet laboratory area and the Nutrition building. Dr. Platon briefly explained that the Governments of Japan and the Philippines provided the budget for the construction.

The guests were also briefed on the technologies developed by AQD. Some of the species presented were the seahorses, mudcrabs, molluscs (abalone, top shell), and finfishes (siganid, snapper). Their hatchery technologies were discussed.

Dr. Platon then accompanied the visitors to Tigbauan town proper for a turnover ceremony of materials for salt making and "payaw" project. In his speech, Secretary Montemayor expressed his confidence that AQD technologies will benefit the local fishers. "The culture technologies developed by AQD for seahorse and blue tang can benefit ordinary fisherfolk like you because these species are expensive (and potentially profitable)," he said. ###



FACING THE AQUACULTURE CHALLENGE ... FROM PAGE 17

Bioremediation, which involves the degradation of hazardous waste to environmentally safe levels by the use of selected microorganisms, bivalves, algae, etc., has been used to reduce organic loading and excess nutrients in shrimp ponds. Selection for and engineering of organisms to be more efficient in the removal of nitrogenous and other organic waste from the water and bottom sludge is another front that has to be explored.

Genetic modification may alter attributes of the organism or create new attributes that affect its interaction with the environment and other organisms.

One of the major concerns about releasing, accidentally or deliberately, GMOs into the wild is their effect to the ecosystem of the released site in particular, and on the genetic biodiversity in general. One of the more common measures taken to "reduce" these risks is sterilization. Aside from potential risks that GMOs pose to the environment, there are perceived risks to human health and safety.

A significant issue facing developed and developing countries alike pertains the ownership and patents of products and processes resulting from biotechnology research. Intellectual property rights (IPR) protect and ensure the exclusive rights of scientists to their inventions. Although IPR and patenting may encourage private sector investment in biotechnology research, there are fears that patenting may lead to monopolization of knowledge, restricted access to germplasm, controls over the research process, selectivity in research focus, and increasing marginalization of majority of the world's poor population.

Clearly, biotechnology has the potential to enhance aquaculture productivity. However, in order to fast track the use of biotechnology in aquaculture operations in developing countries, there is a need for a critical mass of trained manpower. The national governments will have to provide support for this undertaking. ###



AQD Research Publications

Reprints of papers listed here may be requested directly from SEAFDEC/AQD authors

[names of AQD researchers in boldface] or from the AQD Library

Compiled by the **AQD LIBRARY** <library@aqd.seafdec.org.ph>

Ayson FG, de Jesus EGT, Moriyama S, Hyodo S, Funkenstein B, Gertler A, Kawauchi H. 2002. Differential expression of insulin-like growth factor I and II mRNAs during embryogenesis and early larval development in rabbitfish, *Siganus guttatus*. *General & Comparative Endocrinology* 126 (2):165-174

Abstract. In rodents, the expression of insulin-like growth factor II (IGF-II) is higher than that of insulin-like growth factor I (IGF-I) during fetal life while the reverse is true after birth. We wanted to examine whether this is also true in fish and whether IGF-I and IGF-II are differentially regulated during different stages of embryogenesis and early larval development in rabbitfish. We first cloned the cDNAs of rabbitfish IGF-I and IGF-II from the liver. Rabbitfish IGF-I has an open reading frame of 558 by that codes for a signal peptide of 44 amino acids (aa), a mature protein of 68 aa, and a single form of E domain of 74 aa. Rabbitfish IGF-II, on the other hand, has an open reading frame of 645 by that codes for a signal peptide of 47 aa, a mature protein of 70 aa, and an E domain of 98 aa. On the amino acid level, rabbitfish IGF-I shares 68% similarity with IGF-II. We then examined the relative expression of the two IGFs in unfertilized eggs, during different stages of embryogenesis, and in early larval stages of rabbitfish by a semiquantitative reverse transcription-polymerase chain reaction. Primers that amplify the mature peptide region of both IGFs were used and PCR for both peptides was done simultaneously, with identical PCR conditions for both. The identity of the PCR products was confirmed by direct sequencing. Contrary to published reports for seabream and rainbow trout, IGF-I mRNA was not detected in rabbitfish unfertilized eggs; it was first expressed in larvae soon after hatching. IGF-II mRNA, however, was expressed in unfertilized eggs, albeit weakly, and was already strongly expressed during the cleavage stage. mRNAs for both peptides were strongly expressed in the larvae, although IGF-II mRNA expression was higher than IGF-I expression.

Bombero-Tuburan I, Coniza EB, Rodriguez E. 2002. Preliminary report on nursery and grow-out culture of hatchery-bred grouper (*Epinephelus coioides* Hamilton) in ponds. *Aquaculture Research* 33 (5): 379-381

Catacutan MR. 2002. Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratios. *Aquaculture* 208 (1-2):113-123

Abstract. The effect of different dietary protein and lipid levels, and protein to energy (P/E) ratios on growth and body composition of the mud crab, *Scylla serrata*, was evaluated. Six practical test diets were formulated to contain three protein levels (32%, 40% and 48%) at two lipid levels (6% and 12%), each with P/E ratios ranging from 20.5 to 31.1 mg protein/kJ. Individual crabs were stocked in 36 units of 60-l tanks and maintained on a 40% protein diet until each molted (M-0). Newly molted crabs were weighed and fed the test diets until termination at 30 days from the third molt (M3+30). Crabs were monitored daily

and body weight (BW) taken after each molt, at intermolt and at termination. Average initial BW (11.18 +/-0.66 g) was taken at 18 days after M-0. Carapace width (CW) at M3+30 and of the exuviae (at molt 1, 2, and 3 or M-1, M-2 and M-3), weight of exuviae (M-1 to M-3), feed conversion ratio or FCR, duration of intermolt, and total number of days of feeding test diets (M-0 to M3+30) were determined. At the end of the study, crabs were freeze-dried for analysis of nutrients in the flesh, exoskeleton and fat body. The FCR (3.21-4.21), intermolt duration and total number of days of feeding test diets (111.3-131.2 days) were not affected by dietary treatments ($P>0.05$). Analysis of covariance was used with CW at M, and BW at M0+18 as covariates. CW in the 40% protein with 6% lipid or 40/6 diet (P/E ratio, 27.5 mg protein/kJ) did not increase when lipid was increased to 12% (40/12), and it was significantly wider than crabs fed the 48/6 and 48/12 diets (P/E ratios, 31.1 and 27.2 mg protein/kJ). CW and BW did not differ in the 40% and 32% protein diets and were not affected by dietary lipid level at every level of protein. Ca in the exoskeleton was lowest in the 32/6 diet, while exuviae weight was about one-fourth of BW. Crude fat in the lipid deposit of crabs fed 48% protein diets were low. Results showed that the mud crab, *S. serrata*, grow well when fed diets containing 32-40% dietary protein with either 6% or 12% lipid at dietary energy ranging from 14.7-17.6 MJ/kg.

Cuvin-Aralar ML, Santiago AE, Gonzal AC, Santiago CB, Romana-Eguia MR, Baldia SF, Palisoc FJ. 2001. Incidence and causes of mass fish kill in a shallow tropical eutrophic lake (Laguna de Bay, Philippines), p 233-236. In: 9th International Conference on the Conservation and Management of Lakes Proceedings. Shiga, Japan

Abstract. Mass fish kills in Laguna de Bay, the largest lake in the Philippines, has been reported as early as the 1930s. With the introduction and development of aquaculture in this lake, considerable attention and concern was focused on the problem. Records of mass fish kill in the lake mainly from unpublished sources and reports from fisherfolk were reviewed and the causes categorized. The data covered the period 1972 to 1998. Among the commercially important fish species affected were milkfish (*Chanos chanos*), Nile tilapia (*Oreochromis niloticus*), bighead carp (*Aristichthys nobilis*), snakehead (*Channa striata*), catfish (*Clarias macrocephalus* and *C. batrachus*, *Arius manilensis*), silver perch *Terapon plumbeus* and goby (*Glossobius giurus*). The first three species are widely used in aquaculture and the rest are important in open water fishery. Sixty percent of mass fish kill incidents were due to low dissolved oxygen with more than half of these cases associated with blue-green phytoplankton blooms. Fish kills due to pollution from agriculture and industries, fish pathogens and other causes are also discussed. The incidence of mass fish kill reached its peak between 1977 to 1986. Record show that the most number of fish kills (80%) occurred between the months of May to September. The lakeshore towns in the central arm of the lake had the highest incidence of fish kill reported with 46% and followed by the west arm of the lake with 38% of all fish kills recorded.

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de Jesus EGT, Ayson FG, Amemiya Y, Moriyama S, Hyodo S, Hirano T, Kawauchi H. 2002. Milkfish (*Chanos chanos*) growth hormone cDNA cloning and mRNA expression in embryos and early larval stages. *Aquaculture* 208 (1-2):177-188

Abstract. In an attempt to understand growth regulation in milkfish, the milkfish growth hormone (GH) and its cDNA were characterized and the expression of GH mRNA in embryos and larvae was examined by RT-PCR. The milkfish GH was purified from an alkaline extract of the pituitary by reverse-phase high-performance liquid chromatography and detected as an immuno-positive protein with anti-salmon GH serum. The complete sequence of milkfish pre-GH was determined by cDNA cloning and nucleotide sequencing. On the basis of the N-terminal amino acid analysis of the native protein, the pre-GH was found to consist of a signal peptide of 22 amino acids and a mature protein of 188 amino acids. Milkfish GH shows higher amino acid sequence identity with GHs of carps (91-94%) and salmonids (70%) than with GHs of more advanced teleosts (<60%) in good accordance with its taxonomic position in teleosts. It has five half Cys residues, four of which are at positions homologous with those of other known GHs and the extra Cys with those of carp GHs. The molecular weight of milkfish GH was estimated to be 22 kDa, which is comparable to the theoretical value. This suggests that milkfish GH is a simple protein, although it has two potential N-glycosylation sites. Semiquantitative RT-PCR showed that GH mRNA expression was relatively weak in embryos and newly hatched larvae but was already strong in 2-day old and older larvae.

de la Pena MR. 2001. Use of juvenile instar *Diaphanosoma celebensis* (Stingelin) in hatchery rearing of Asian sea bass *Lates calcarifer* (Bloch). *Israeli Journal of Aquaculture-Bamidgeh* 53(3-4):128-138

Abstract. The effects of size, dry mass intake and nutritional value of the brackishwater cladoceran, *Diaphanosoma celebensis*, on the growth and survival of 15-30 day sea bass (*Lates calcarifer*) larvae reared in a static green water system were determined. The highest specific growth rate (29.4%/day) was attained in larvae fed a 1:1 combination of *Artemia* nauplii and adult *Diaphanosoma* but it was not significantly different ($p>0.05$) from fish fed only adult *Diaphanosoma* (28.8%/day) or only juvenile instar *Diaphanosoma* (28.6%/day). Survival rates of larvae (92.4-99.0%) fed the different live diets did not significantly differ ($p>0.05$). Larvae markedly preferred juvenile instar *Diaphanosoma* over *Artemia* nauplii and adult *Diaphanosoma*. The crude protein contents of juvenile *Diaphanosoma* (58.7%), adult *Diaphanosoma* (58.3%) and *Artemia* (56.7%) were substantially high and satisfied the dietary protein requirements of larvae. The fatty acid profile of the sea bass fry reflected the lipid composition of the live diet. Improved growth, survival and dry mass intake in larvae indicate the potential of juvenile *Diaphanosoma* in the hatchery rearing of sea bass larvae.

de la Pena L, Marte CL. 2001. The plight of older women in a fishing village: the women fish traders of Bugtong Bato, Aklan, Central Philippines, p 165-172. In: Williams MJ, Nandeesh MC, Corral VP, Tech E, Choo PS (eds). *International Symposium on Women in Asian Fisheries* (1998: Chiang Mai, Thailand). ICLARM - The World Fish Center

Abstract. The changing nature of the fisheries in Bugtong Bato, a small fishing community in Central Philippines has also changed the

role of women, particularly of older women, in the community. Until the 1980s, fishing used mainly traditional gears and methods and was highly seasonal. The livelihood activities of men were highly diversified, and fishermen undertook seasonal outmigration to the sugarcane plantations in Negros. Younger women and women of child-bearing age attended to domestic chores, helped their husbands prepare for the day's fishing activities, sought employment as domestic helpers in the capital town or Manila, or engaged in seasonal jobs such as rice harvesting or *sinamay* fiber knotting. Older women mainly attended to domestic chores. With the introduction of new fishing gears and methods, seasonal outmigration has virtually stopped. The men who participated in group fishing financed by local financiers earn better incomes from these new fishing techniques. However, as fishing now requires longer periods at sea, older fishermen are unable to join their younger counterparts, and rely only on traditional fishing methods for their livelihood. Due to physical limitations and poor health, older fishermen are unable to earn enough to support their families, and their wives seek supplementary means of livelihood. The introduction of new fishing gears and methods has increased considerably the volume of fish landed and spurred the development of a new economic activity in this community – that of fish trading. Most fish traders are older women whose husbands are unable to fish or whose income from fishing cannot meet their daily needs. As such, older women in this community have assumed the role of primary providers for their families.

Eusebio PS, Coloso RM. 2002. Proteolytic enzyme activity of juvenile Asian sea bass, *Lates calcarifer* (Bloch), is increased with protein intake. *Aquaculture Research* 33(8):569-574

Abstract. The effect of high dietary protein intake on proteolytic enzyme activity of feeding juvenile Asian sea bass, *Lates calcarifer* (Bloch) was studied. Ninety fish [mean body weight +/- standard error (SE) 304.62 +/- 34.84 g] were randomly assigned to two dietary treatments, each with three replicates. In treatment 1, fish were fed by-catch (*Thunnus albacares*) and in treatment 2, a formulated diet containing 50% protein. Proteolytic enzyme activity was determined in pyloric caecae and intestine at day 0, 7, 15, and 30. Initial proteolytic enzyme activity in sea bass ranged from 174 to 232 azocasein units (U-AC) per mg of protein. After 7 days there was no significant difference in proteolytic enzyme activity of fish fed the two diets. However, a marked increase was observed in fish fed the formulated diet at day 15. After 30 days, the proteolytic enzyme activity in fish fed the formulated diet was threefold higher than that in fish fed the by-catch diet. Fish fed the formulated diet had significantly higher total protein intake at day 7 than did fish fed by-catch. Thereafter, a twofold weekly increase in the total protein intake was observed in both fish fed the by-catch and formulated diets until day 30. These results suggest that a high dietary protein intake induces increased proteolytic enzyme activity in Asian sea bass.

Fermin AC. 2002. Effects of alternate starvation and refeeding cycles on food consumption and compensatory growth of abalone, *Haliotis asinina* (Linnaeus). *Aquaculture Research* 33 (3): 197-202

Abstract. The effects of alternate starvation and refeeding on food consumption and compensatory growth of hatchery-bred abalone, *Haliotis asinina* (Linnaeus), were determined. Two groups of abalone juveniles (mean shell length = 29 mm, body weight = 5 g) were alternately starved and refed a macro-alga, *Gracilariopsis bailinae* at equal

duration (5/5 or 10/10) over 140 days. A control group (FR) was fed the seaweed ad libitum throughout a 200-day experimental period. Starved and refed abalone showed slower growth rates (DGR, 63 and 70 mg/day in the 5/5 and 10/10 groups respectively), as a result of reduced food intake (DFI 15% and 16% day⁻¹ respectively), after repeated starvation and refeeding cycles. Percentage weight gains (5/5 = 196%, 10/10 = 177%) were significantly lower than that of the control (397%). When refed continuously over 60 days, the starved groups exhibited increased DFI and fed at the rate of 24% and 25% day⁻¹, which were not significantly different from that of the control at 26% day⁻¹. At the end of the experiment, no significant differences were observed among three treatments in terms of shell length (range: 4648 mm), body weight (range 25-28 g), % weight gain (392-465%) and per cent survival (range 87-98%). The results indicated that *H. asinina* had a complete compensatory growth following a return to full rations after a series of intermittent starvation and refeeding cycles.

Fermin AC. 2000. Illuminated cage nursery of the Asian sea bass, *Lates calcarifer* Bloch (Centropomidae): effects of initial body size and stocking density, p 267-271. In: Liao IC, Lin CK (eds). Cage Aquaculture in Asia: Proceedings of the 1st International Symposium. Asian Fisheries Society, Manila and World Aquaculture Society - Southeast Asian Chapter, Bangkok. 318 p

Abstract. This study was conducted to determine the appropriate initial body size and the corresponding stocking density of sea bass, *Lates calcarifer*, during nursery rearing in illuminated cages. Hatchery-produced sea bass fry of different initial sizes of 7.2 (day 15), 13.2 (day 22), and 15.2 mm (day 29) were stocked at densities between 300 and 1,500 m⁻³ in decreasing order with fish size. Nylon net cages (1x1x1 m) set in a protected sea cove area were individually lit at 300 lux using incandescent bulb placed at 1 m above water surface. Artificial lights attract wild zooplankton that served as prey to young sea bass. After 42 days of culture 22-day old sea bass fry with 13.2 mm TL initial size and stocked at 400 m⁻³ showed the highest growth (35.3 mm TL, 535.7 mg BW) and survival rates (64.4%). At a stocking density of 800 m⁻³, the survival rate was the second highest at 43%. Although day 15-fry at 7.2 mm TL initial size showed higher specific growth rates (11% day⁻¹) and size at harvest (29-31 mm TL, 346.2-374.4 mg BW), survival rates (11-15%) were lower than the day 22-and 29-fry (30-64%). Calanoid copepods of the genus *Calanus*, *Paracalanus* and *Acartia* dominated the diet (81-90%) of sea bass at different size groups. Percentage number of shooters ranged from 0.5-1.4% of total stocks and were not significantly different among treatments. The present results indicate that sea bass should spend 21 days in the hatchery prior to nursery rearing in illuminated sea cages. Sea cages are inexpensive and more cost-effective than ordinary cage or earthen pond for sea bass fingerling production.

Fermin AC, Buen SMA. 2001. Photoperiod effects on feeding, food conversion, growth, and survival of abalone (*Haliotis asinina* Linne) during nursery rearing. Phuket Marine Biological Center Special Publication 25 (1): 113-117

Abstract. Juveniles of 10 mm shell length were subjected to four photoperiodic regimes namely, 6L:18D, OL:24D, diffused 12:12D and ambient light (12L:12D) serving as control. Juveniles were fed fresh seaweed, *Gracilariopsis bailinae*, in excess amounts throughout the experiment. At the end of a 105-day experiment, juveniles held under ambient photoperiod were significantly bigger and had higher average daily growth rate than the rest of the treatments. Feed conversion efficiency was higher

at ambient light than at other photoperiodic regimes. Daily feeding rates at 65-day culture period were similar for all treatments; however, towards the end of culture period, feeding rate of abalone at ambient light was lowest compared to the rest of the treatments. Percent survival was significantly higher in animals at ambient light and 6L:18D with 99% respectively, than at other photoperiodic regimes.

Kautsky N, Folke C, Ronnback P, Troell M, Beveridge M, Primavera J. 2001. Aquaculture, p 185-198. In: Encyclopedia of Biodiversity. Academic Press

Laron M, Kamarudin MS, Yusoff FM, Saad CR. 2001. Evaluation of different live food organisms on growth and survival of river catfish, *Mystus nemurus* (C&V) larvae, p 299-302. In: Hendry CI, Van Stappen G, Wille M, Sorgeloos P (eds). Larvi '01-Fish and Shellfish Larvicultures Symposium. European Aquaculture Society, Spec Publ No 30, Belgium

Leano EM. 2002. *Haliphthoros* spp. from spawned eggs of captive mud crab, *Scylla serrata*, broodstocks. Fungal Diversity 9:93-103

Abstract. Monitoring of the fungal flora of spawned eggs of captive mud crab, *Scylla serrata*, was conducted in several hatchery runs at the Aquaculture Department of Southeast Asian Fisheries Development Center in Iloilo, Philippines. Quantification of the egg mycoflora revealed the dominance of oomycetes, particularly *Haliphthoros* spp. among spawners which aborted their eggs prior to hatching. Two species of *Haliphthoros* (*H. philippinensis* and *H. milfordensis*) were identified from the 24 isolates collected. *Haliphthoros milfordensis* was the dominant species. Physiological studies on vegetative growth and sporulation of the two species show that *H. philippinensis* have wider optimal range for salinity and temperature requirements than *H. milfordensis*, especially in sporulation. The pathogenicity study showed that only *H. milfordensis* was pathogenic to spawned eggs of *S. serrata*, while *H. philippinensis* was not. Infection of *S. serrata* eggs by *H. milfordensis* was observed starting at two days after inoculation of zoospores with 2-5% infection rate, reaching up to 10% at five days post-inoculation.

Lim C, Borlongan IG, Pascual F. 2002. Milkfish, *Chanos chanos*, p 172-183. In: Webster CD, Lim C (eds). Nutrient Requirements and Feeding of Finfish for Aquaculture. Oxon: CAB International Publishing. 418 p

Madrones-Ladja JA, dela Pena MR, Parami NP. 2002. The effect of micro algal diet and rearing condition on gonad maturity, fecundity and embryonic development of the window-pane shell, *Placuna placenta* Linnaeus. Aquaculture 206 (3-4): 313-321

Abstract. Immature *Placuna placenta* (Linnaeus) broodstock were given the micro algae *Isochrysis galbana* (T-ISO) Parke and *Tetraselmis tetrahele* (G.S. West) at different combinations and density levels and reared in tanks with or without mud substrate. The animals were also reared in the estuary and fed on available natural food. Monthly examination of gonad histology showed a significantly higher gonad index in broodstock reared in tanks with mud substrate than without ($P < 0.05$). In addition, those given a high-density algal diet (200,000 cells ml⁻¹, 3:1

☞ next page

mixture of *I. galbana* and *T. tetrahele*) had a significantly faster gonad development than the low-density diet (100,000 cells ml⁻¹, 1:1 mixture) fed broodstock reaching sexual maturity and gonad index of 330 +/-14 after 1 month ($P < 0.0001$), *P. placenta* given the low-density algal diet attained gonad maturity after 3 months and estuary-reared animals after 2 months.

Seawater irradiated by ultraviolet light (925-1395 mW h/1) induced spawning of all mature *P. placenta*. Estuary-reared animals had a higher fecundity and larger eggs and a higher percentage of fertilized eggs developed to straight-hinged larvae than those reared under other condition. Animals given a low-density algal diet released low number of small eggs which did not fertilize. Natural spawning also occurred in the estuary 2 months after stocking.

Madrones-Ladja JA, Polohan BB. 2001. The effect of stocking density, temperature and light on the early survival of the abalone *Haliotis asinina* Linne. Phuket Marine Biological Center Special Publication 25 (1): 207-210

Abstract. Newly hatched trocophore larvae of the abalone *Haliotis asinina* Linne were stocked at densities of 1,000, 3,000 and 5,000 larvae/l at low (20-25°C) and high (ambient, 28-30°C) water temperature levels in light (transparent) and dark (black cloth-covered) glass containers. Larvae were reared in UV light-irradiated sea water until pre-settlement stage. Aeration was not provided during the 20-h incubation period. A 3x3x2 factorial design with three replicated per treatment was followed. A three-way analysis of variance (ANOVA) showed a significant interaction among the factors tested. Analysis at each density level showed that at stocking density of 1,000 larvae/l, no significant difference between temperatures and between light or dark conditions was observed. However, at densities of 3,000 and 5,000, significantly higher survival was obtained at low, than high temperature ($P < 0.05$), but no difference was detected between the light and dark conditions. Analysis at each temperature showed that, at high temperature, better survival was obtained at stocking density of 1,000, than higher densities ($P < 0.001$), and at light than at dark condition ($P < 0.05$). However, at low temperature, no significant difference between densities or between light and dark conditions was detected. ANOVA at light or dark condition showed that at any of these conditions, larval survival was always higher at 1,000 stocking density than at other densities ($P < 0.05$). Survival was not significantly different between stocking densities of 3,000 and 5,000 larvae/l at any of these light conditions. Therefore, during incubation of newly hatched trocophore larvae of *H. asinina* to pre-settlement stage, the optimum stocking density at high temperature (28-30°C) was 1,000/l in a light-penetrable rearing container. When reared at high stocking densities of 3,000 or 5,000, a higher survival was obtained when temperature was lowered to 20-25°C in either rearing conditions tested.

Maeno Y, de la Pena LD, Cruz-Lacierda ER. 2002. Nodavirus infection in hatchery-reared orange-spotted grouper *Epinephelus coioides*: First record of viral nervous necrosis in the Philippines. Fish Pathology 37 (2):87-89

Abstract. Mass mortality occurred in 34-day old larval orange-spotted grouper *Epinephelus coioides* reared at a hatchery in the Philippines with clinical signs such as anorexia and abnormal swimming behavior. Histopathology of moribund fish demonstrated marked vacuolation of the brain, spinal cord and retina. Cytopathic effects were observed in SSN-1 cells inoculated with the tissue filtrate of affected grouper. Electron microscopy revealed non-enveloped virus particles measuring 20 to

25 nm in diameter in the cytoplasm of degenerated SSN-1 cells. Piscine nodavirus (betanodavirus), the causative agent of viral nervous necrosis (VNN), was detected in the affected tissues and SSN-1 cells inoculated with the tissue filtrate of affected fish by RT-PCR. This is the first record of VNN in the Philippines.

Marte CL, Cruz P, Flores EEC. 2000. Recent developments in freshwater and marine cage aquaculture in the Philippines, p 83-96. In: Liao IC, Lin CK (eds). Cage Aquaculture in Asia: Proceedings of the First International Symposium. Asian Fisheries Society, Manila, and World Aquaculture Society – Southeast Asian Chapter, Bangkok

Abstract. Fish production from freshwater cages and pens, and marine cages constitute 19% of the total foodfish produced from aquaculture in the Philippines. In 1998, production from freshwater cages and pens contributed about P2.5 billion or about 10% of the total revenues from aquaculture. Freshwater cage and pen culture is practiced in most of the major lakes and reservoirs in the country. The most important species cultured in freshwater cages are tilapia and bighead carp (*Aristichthys nobilis*) while milkfish (*Chanos chanos*) is farmed in freshwater pens at Laguna de Bay. Small water impoundments intended for the irrigation of upland farms are also being used to culture tilapia and other freshwater fish in cages. The unregulated expansion of cages and pens, use of high stocking densities, and excessive feeding has resulted in the deterioration of the water quality in many areas. This has prompted the more informed local government authorities to adopt measures limiting further expansion of cage and pen culture activities beyond the carrying capacity of freshwater bodies to prevent periodic occurrences of mass fish kills. While cage mariculture of groupers has been practiced in the Philippines since the 1980s, it was only in the early 1990s that much of the growth and expansion of the industry occurred with the popularization of milkfish mariculture. In the last five years, no less than 1,000 cages with an aggregate capacity in excess of 10,000 metric tons a year have been invested in milkfish sea farming. The species now accounts for about 90% of the production from marine cages. In recent years, a drop in milkfish prices has motivated the industry to focus its attention on other fishes, primarily grouper (*Epinephelus* spp.), snapper (*Lutjanus* spp.), sea bass (*Lateolabrax* spp.) and siganid (*Siganus* spp.). Currently, imported species such as red tilapia, yellow-wax pompano (*Trachinotus blochii*) and red drum (*Sciaenops ocellatus*) are also being tested by the private sector. A high production cost due to low feed conversion efficiency and high seed cost is presently the greatest concern of marine cage farmers. In some areas, unregulated expansion has already led to problems in water quality.

Primavera JH. 2000. Aquasilviculture trials in mangroves in Aklan Province, Panay Island, central Philippines, p 142-146. In: JIRCAS International Workshop on Brackishwater Mangrove Ecosystems: Productivity and Sustainable Utilization. Tsukuba: Japan International Research Center for Agricultural Sciences. 166 p

Abstract. To integrate production of crabs and shrimp with mangrove conservation, the SEAFDEC Aquaculture Department initiated studies on Mangrove-Friendly Aquaculture (MFA). Culture pens and ponds in old growth and newly regenerating mangrove sites in Aklan, central Philippines were stocked with mudcrab *Scylla olivacea*/S. *tranquebarica*. Investment costs, survival and production, and cost-return analysis for mudcrab culture in pens and ponds are reported in the paper. Aside from



FROM AROUND THE WORLD

Thailand: shrimp industry strives to reduce chemical use

Using fewer chemicals will be a key solution for the Thai shrimp industry, as it attempts to overcome more technical barriers to trade imposed by countries citing food safety reasons. Dhammarong Prakobboon, director-general of the Fisheries Department, said that shrimp farming under the Code of Conduct (CoC) practices would assure buyers that Thai products are free from chemical residues. The method requires the minimal use of chemicals and medicines and relies more on natural conditions.

The Department, in co-operation with the French government, has already trained farmers and hatchery operators on CoC practices and granted certificates to 23 farms and three hatcheries.

Mr. Dhammarong outlined potential solutions at a recent seminar on solving the residue problem in shrimps. The seminar was organized by the National Research Council, Chulalongkorn University and Kasetsart University. He said promoting the new farming method would not be easy. There are more than 30,000 families raising shrimps in areas totaling 450,00 to 500,000 rai, along with 2,500 hatcheries. [6.25 rai = 1 ha]

"I believe however, that more farmers will switch to the new method when they realize that their products will fetch higher prices. It will take a few years to convince all farmers," he said.

To deal with the tougher requirements and inspections, especially by the European Union, Mr. Dhammarong said the Department had asked shrimp-exporting countries supplying raw materials to Thailand to issue health certificates verifying that their shipments are free of chloramphenicol and nitrofurans.

Thailand imported about 6.8 billion baht worth of shrimps in 2001. Major suppliers are China, India, Indonesia, Bangladesh, Malaysia, and Burma.

As well, he said, the Department tried to help shrimp farmers reduce production costs by initiating saline irrigation systems in several provinces. It has already spent almost three billion on the infrastructure in eight coastal projects, far short of the previous 20-billion-baht plan involving 97 projects nationwide.

However, Chalor Limsuwan of

Kasetsart University said the Department should construct freshwater irrigation systems instead because shrimp farmers normally need freshwater to reduce salinity, thus lowering the risk of disease.

He said the best ways to prevent the residue problem were the use of microbes in farms, the reduction of shrimp density, as well as the cleanliness of pond floors.

Mr. Chalor said farmers sometimes adopted what they thought to be a preventive measure by applying antibiotics to shrimps without realizing the actual causes of diseases, resulting in excessive use of medicines and residues.

Janenuch Wongthawatchai of Chulalongkorn University said the residue problem stemmed from the application of incorrect or mixed drugs. She urged drug distributors to sell all quality products to farmers, who normally followed their instructions.

Jiraporn Kaesornchan of the Fisheries Department said several samples of medicines collected from shops were found to have been incorrectly labeled. He urged the government to crack down on substandard products.

Yuwadee Pattanawong of the Food and Drug Administration said it was difficult for the agency to control the use of 16 banned chemicals, which in fact covered 80 items when byproducts were included. She said the government had to consider establishing drug application standards and upgrading the food analysis system in preparation for possible, future bans of other chemicals.

Pot Aramwattananond, vice-president of the Black Tiger Shrimp Raisers and Exporters Association, said the incident was a blessing in disguise, as it had encouraged all government agencies to cooperate in solving the problem faced by an industry that generated 100 billion baht of revenue annually.

He said that as buying countries were paying more attention to food safety, exporters were concerned that the US would follow in the footsteps of the EU, after China and Vietnam, which could not sell their products to the EU, dumped the goods in America.

In January and February, the US imported 15% more shrimps. However, exports from China jumped by 250%, Vietnam by 186% and India 80%, while those from Thailand dropped by 10%. [For more info, contact: J Enright of the Mangrove Action Project. mapasia@loxinfo.co.th; <http://www.earthisland.org/map.html>]

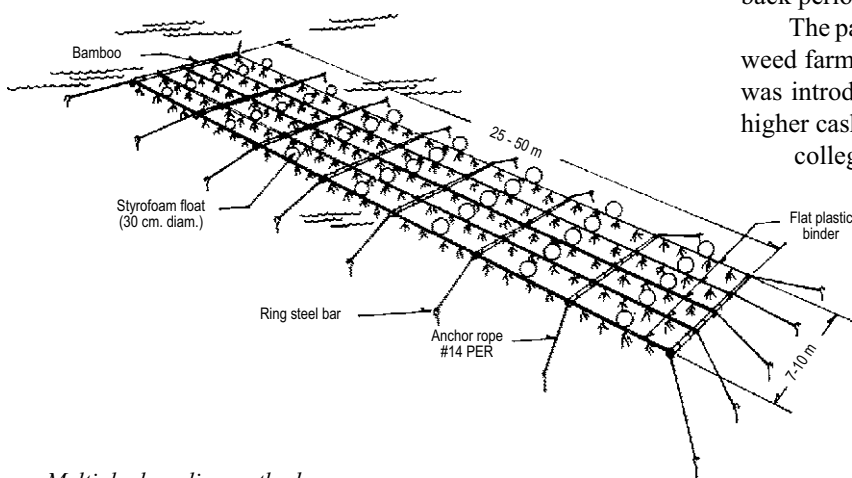


Philippines: national seaweed training promises to expand farming areas

Long been a producer and exporter of seaweeds, the Philippines significantly increased its production only just recently. In 2001, the Philippines was number three producer after China and Japan. It had employed 120,000 workers.

As indicated by activities in the seaweed farming sector, the Philippines is into an aggressive pursuit of increasing farm production through research and development. For example, the Bureau of Fisheries and Aquatic Resources recently finished a *National Trainer's Training on Seaweed R & D* (1-8 August 2002 at SEAFDEC/AQD in Iloilo) to strengthen national seaweed development. Attended by action officers from the various regional offices, the lecturers from AQD provided scientific and technical know-how. The training course covered topics such as assessment of sites and biological studies and production. Production covered development and improvement of farm technologies, establishment of demonstration farms and seaweed hatcheries, assessment of potential seaweed farms, health management, and other related topics. Training in management, manpower, and institutional development will be conducted soon.

Considering active government and private sector involvement, increase of seaweed farming in shallow waters can create culture problems associated with favorable growth such excessive grazing by herbivores like sea urchins, siganids, and starfish. Fortunately, a new research paper by seaweed researchers Dr. Anicia Hurtado and Renato Agbayani has shown that deep water (more than 10 meters deep) farming of the seaweeds *Kappaphycus* is possible and very profitable similar to the other methods in shallower waters. This method in deeper waters is commonly called *alul*. The paper assessed the seaweed planters' practices using the multiple raft, long-line method in three cultivation areas of Zamboanga del Sur, Mindanao. The result showed that though the



Multiple, long line method

method is expensive, production was greater and the revenue higher. Estimated production of *Kappaphycus* ranged from 38.5 to 67.5 t dry weight per year per family with 5-7 rafts. Average return on investment (ROI) based on 5 crops per year was 169.67 with a pay-back period of 0.62 years.

The paper further acknowledged the significant effects of seaweed farming on the seaweed farmers' lives since *Kappaphycus* was introduced. Food, clothing, and shelter have improved with higher cash income and schooling of children were upgraded to collegiate level. Consequently, the number of professionals in the community has increased and are now leaders.

Even semi-permanent to permanent homes have been built which were non-existent before seaweed farming method was introduced.

Even so, according to the researcher, production rates can still be improved such as the use of PVC pipe with polyurethane foam and plastic buoys with longer economic life could be used and mechanized harvesting could be introduced to reduce harvesting time and cost which degrade the quality of the harvested seaweed. - MBS

Marine information available via text

They “texted” and got the information they have been looking for. At the other end of the line to answer their queries were specialists of the Los Baños-based Department of Science and Technology’s Philippine Council for Aquatic and Marine Research and Development (DOST-PCAMRD).

The questions were on Philippine aquatic resources, fisheries and related fields. Called Aqua Info-Text Services (AITS), the unique information tool, which uses the text messaging capability of cellular phones, started in January 2001.

Under AITS, anyone with a cellphone can “text” dedicated cellphones of the council and receive information for free, said

PCAMRD executive director Rafael Guerrero III. The service is available from 8 am to 5 pm, Monday to Friday.

Last year, 404 people sent inquiries to PCAMRD through their cellphones. Most of the questions (255 or 63%) were about inland aquatic resources; 63 (16%) on marine aquatic resources; and 51 (15%) on post-harvest. The least number of questions was noted for socioeconomics and policy (27 or 7%).

Here are the numbers for AITS: 0917 353 8440 (queries on inland aquatic resources), 0917 609 5998 (marine), 0917 219 8204 (fish processing and postharvest), 0917-346 8543 (socioeconomics and policy). - R Fernandez

SHRIMP FARMERS’ CHOICES ... FROM PAGE 8

Peter Webster, Eco-Seafood Production Inc and **David Christmas**, Project Biotech Ltd, talked about a **land-based shrimp production system** that can produce 1,500 tons of shrimp per year. They explained that their company aims to produce a shrimp with an “eco-pedigree,” coming from a hi-health hatchery using natural algae (*Chlorella*) and a recirculating growout system that uses biological and computerized mechanical processes to maintain pristine water quality. Plans are underway to establish the fully integrated Eco-Seafood Project in a Cebu Economic Zone.

Veronica Migo of UP Los Baños described biotech institute’s study on **ozone technology** which is used for disinfection and for oxidation of toxic metabolites such as ammonia in shrimp grow-out ponds. She said alkalinity tends to decrease in ozonated ponds, and this condition can be helped by the use of lime (highly pure sodium bicarbonate for grow-out ponds and hydrated lime for reservoir ponds). In addition to ozone, algal bloom in ponds can be controlled by electrolysis.

Congress exhibitors

Argent Laboratories Inc. sells anesthetics, tranquilizers and water conditioners (for transport); hormones for sex reversal, breeding and spawning; pond and hatchery medicines; equipment; and hatchery larval feeds and other hatchery supplies.

Santeh Feeds Corporation manufactures shrimp, grouper, milkfish, tilapia, catfish, tropical fish, sea bass, snapper, “pompano,” and crab feeds. Feed types are floater, sinker pellets, crumbles, and mash.

Dakila Trading Corporation distributes equipment for monitoring water parameters such as dissolved oxygen, pH, salinity, total dissolved solids, temperature, chlorophyll, turbidity, among others.

B-MEG, Overseas Feeds Corp, Tolong Hatchery, Universal Scope Philippines, Inc, Guill-Bern Corp, Cybertech, Intaq Aqua, Res. Corp, Philippine Foremost Milling Corporation, Bio-Vintage Corp, Aquatic Ranch, Medtest, Floatech/FFX/Cruz Hatchery, Marine Technology Center Corp, and Infratex. - CBL



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Philippine Aquaculture Society

Society of Aquaculture Engineers in the Philippines

HIGHLIGHTS OF THE PAS-SAEP BIENNIUM MEETING 2002*

□ DAGUPAN CITY'S MILKFISH DEVELOPMENT PROGRAM

Incumbent Mayor Benjamin Lim of Dagupan City -- famed for its Bonuan *bangus* which is regarded as the best tasting in the country -- has taken steps to rationally manage the city's aquatic resources after pen culture of milkfish expanded to cover nearly all of the city's interlocking rivers. Fish kill was just the first problem, costing P10 million in the May incident alone.

Mayor Lim hired technical consultants who used aerial photography to map out the pens and ponds. Dagupan has 1,484 units of pens covering 95 hectares. Seven years ago, there were just 96 pens.

The *Dagupan Bangus Development Plan* centers on the concept of contract growing (fishpen lease agreements) by fisherfolk in designated or zoned areas. Taking into account traditional use of the river system, 10 zones have been designated, including pen and cage zones, pond and fish conservation zones, and fish traps zones. Aquaculture will be limited to no more than 10% of the river area.

A new fishery ordinance is being prepared to include these key elements: (1) a water quality monitoring system which will be used as basis to determine carrying capacity of each river zone and the number of permits that will be allowed annually; (2) a strict feed quality standards which will be tied to yearly feed quotas; and (3) the standardization of pen size, design, and construction.

The plan also includes a fish processing and marketing strategy, specifically, a publicly listed export-oriented milkfish processing and marketing corporation owned by Dagupenos and the city. Around 5,000 new jobs are expected to be created. To date, the city's fish pens employ 3,400 farmer-fisherfolk who earn about P2,500 a month (fishers earn less than P1,500).

Dagupan's *bangus* is priced P5 higher than other sources for the same size bracket, and the city intends for it to stay this way. The local government had organized a *Bangus Festival* in April, and attempted to have the "Longest Grill" on the Guinness Book of Records.

*1 July 2002, Bacolod City. PAS and SAEP are non-stock, non-profit organizations of aquaculturists/fisheries technologists, engineers/architects, and others involved in the aquaculture industry. (02) 372 3878 to 3886

□ MARICULTURE PARKS OF SAMAL AND GUIMARAS ISLANDS

Two mariculture parks recently opened in the country: one off Samal Island in Davao Gulf and the other in Igang in Guimaras province.

The *Samal Island Mariculture Project* covers 224 ha subdivided as follows: 50 ha for lease to small-scale cage farmers, 50 ha for medium-scale, 50 ha for big-scale, 50 ha for research and development (BFAR). The remaining 24 ha is kept open for navigation. In the BFAR area, a floating station, initial mooring for fish cages, a seaweed nursery, and motor boats are in place. Leasees would find convenient the nearby Davao Fish Port, with its ice plant and cold storage, freshwater supply and net mending yard.

The park is a joint undertaking of BFAR, the City of Samal and SEAFDEC/AQD and will be administered by an administrative council composed of the founding institutions and other representatives including the SAEP.

The *Guimaras Mariculture Park Demonstration and Training Project* is intended only for residents of Guimaras province through their respective LGUs. It is under AQD's close supervision and serves as a venue for skills development in fish cage operation and management. In place are two units of 10 x 10 x 6 m cages, eight units of 5 x 5 x 6 m, and ten of 5 x 5 x 3 m bamboo cages. The mariculture park is within AQD's Igang Marine Substation where skills development training on cage farming are conducted. The park is a collaborative project among AQD, BFAR and the provincial government of Guimaras.

The concept of a mariculture park is akin to the land-based industrial park where aquaculture spaces are available for lease, and the cost required for deep-sea cage farming is shared by tenants. Fees are based on a fair return of investment.

□ RESEARCH AND EXTENSION

Government funding is poor for research and extension in agriculture and fisheries: only 0.10% of the national gross domestic product. Of this money, about 20% goes to the fisheries sector's basic (20%) and applied research (80%).

If this expenditure pattern does not change, the country's research and extension agenda and programs (RDE) which were initially conceptualized and formulated for the period 1994-2004 may well extend into the next 20 years.

The Aquaculture RDE covers a wide spectrum of issues and concerns of the industry, from technology to information generation to policy formulation and extension. The general aim is to improve the productivity of aquaculture systems, expand aquaculture options, and manage environmental impacts of aquaculture operations.



SEAFDEC/AQD's **Dan Baliao** discussed the *Igang Mariculture Park* in Guimaras



UPV-CF's **Dr. Crispino Saclauso** on the country's R&D set-up



Technical consultant **Philip Cruz** presented Mayor Lim's *Dagupan concept*

DA Undersecretary Cesar Drilon swears in the officers of the *Philippine Aquaculture Society*



PAS officers, 2001-2002 Dr. Rolando Platon (President), Melchor Tayamen (VP Luzon), Philip Cruz (VP Visayas), Sani Macabalang (VP Mindanao), Wilfredo Yap (Secretary), Catalino de la Cruz (Treasurer), Marcelino Tumanda Jr (Auditor), Rolando Edra (Business Manager), Ruben Sevilleja (Press Relations)

New SAEP officers, 2002-2003: Pastor Torres Jr (President), Westly Rosario (Executive VP), Tereso Abella (VP Luzon), Dan Baliao (VP Visayas), Sani Macabalang (VP Mindanao), Nelson Lopez (Secretary), Catalino de la Cruz (Treasurer), Mario Santos Jr (Auditor), Virgilio Dureza (Press Relations). Rolando Edra is immediate past President

Standing committees: Valeriano Corre Jr (Aquaculture Management), Remus Landoy (Tanks and Tank-based Systems), Jose Llobrera (Mariculture), Virgilio Dureza (Pond Layout and Design), Dan Baliao (Pens and Cages), Philip Cruz (Equipment and Facilities)

Special committees: Herminio Rabanal (Publications and Editorial), Julita Abulon (Membership and Elections)

BOARD EXAM FOR FISHERIES PROFESSIONALS

Plans are underway to give licensure examinations to accredit fisheries professionals. In fact, the first examination is planned for October 2003.

Why the exam? To effectively supervise and regulate the practice of fisheries technologist profession as provided for in Republic Act No. 8550 also known as Fisheries Code of 1998.

Who can take it? A citizen of the Philippines with good moral character, and a graduate of BS Fisheries, Master's, PhD or doctorate degrees with majors in fisheries, aquaculture, fish processing technology, fisheries biology, fisheries management, oceanography/limnology, fishing boat engineering, and marine science biology from educational institutions duly recognized by the government.

Exemptions are allowed for those with fisheries and closely related degrees who have served in the government or private sector for not less than 5 years before the effectivity of RA 8550.

The practitioners of the fisheries profession include those in the consulting and management services, research and development, testing and evaluation of fisheries and related facilities, teaching and education, and other expertise.

The still-to-be-formed Board of Fisheries under the Professional Regulatory Commission (PRC) will later adopt and promulgate a *Code of Ethics for the Practice of Fisheries Technology* and a *Code of Professional Standards for the Practice of Fisheries Technology*.

SHRIMP: 1 PROBLEM ... FROM PAGE 2

mission based on current analytical methods. This is just one type of residue. What's a farmer to do?

Follow the government's "Code of Practice for Sustainable Shrimp Farming"! The Code guides and summarizes the primary environmental and social responsibilities of the aquaculture industry and recommends management practices. Good environments within and around shrimp farms mean decreased risk of diseases and non-use of therapeutic agents and other chemicals. And export-quality shrimp.

The Code is available from the Bureau of Fisheries and Aquatic Resources, and has been prepared in cooperation with SEAFDEC, Philippine Council for Aquatic and Marine Research and Development, and the Negros Prawn Producers and Marketing Coop, Inc. These institutions have signed a commitment to promote shrimp farming in the Philippines.

It should be noted that the UN's Food and Agriculture Organization wrote a global code for sustainable fisheries, SEAFDEC has regionalized it for Southeast Asia, and the Global Aquaculture Alliance has written one for shrimp farming. The latter is the blueprint of the Philippine code of practice.

The Code addresses the following issues: (1) guiding principles for responsible aquaculture; (2) mangroves; (3) site evaluation; (4) design and construction; (5) feeds and feed use; (6) shrimp health management; (7) therapeutic agents and other chemicals; (8) general pond operations; (9) effluents and solid wastes; and (10) community and employee relations.

□ PARTNERSHIP OF GOVERNMENT AND THE PRIVATE

SECTOR Filipino shrimp farmers want to emulate Thailand's and China's successes. Thai government support is crucial to their industry which is mostly family shrimp farms less than 2 ha in size. This support comes in the form of infrastructure, technology, diagnostic services and product promotion. China's recovery was mainly due to leaving their farms fallow for five years after disease devastation.

Not that the Philippine government and R&D institutes are not doing anything. They are. But shrimp farmers worry about financing and product promotion so much so that the Philippine National Shrimp Congress, registered as Philippine Shrimp Inc. (to cover all sectors), has discussed resolutions asking the government to:

- abolish the Power Purchase Adjustment because this increases the cost of production
- study the repackaging of Philippine shrimp as "green shrimp" and promote it in traditional and new markets like Europe
- venture into massive information dissemination on the principles of Global Aquaculture Alliance and environment sustainability, and give official acknowledgment to existing farms and production clusters that already observe the Code of Practice for Sustainable Shrimp Farming
- reevaluate the business viability of shrimp culture and to create new financial schemes for shrimp farming

- fully implement FAO Series 207, 2001 and its related laws and regulations which ban the importation of foreign shrimps (spawners, larvae, fry, juvenile or adult) as these could be carriers of pathogenic microorganisms and therefore pose a threat to the industry
 - establish a regular program on extension, training, technology demonstration, equipment upgrading, and policy formulation to support the industry's development; the research and development direction will be formulated by both private and government sectors and the country's research institutes
 - recognize PHILSHRIMP Inc. as the sole legitimate and official body to represent the industry in the country
- The shrimp congress was held back-to-back with the meetings of the Philippine Aquaculture Society and the Society of Aquaculture Engineers of the Philippines. Both societies also urge closer partnership between private and government sectors. ###

INTRODUCING FISH DISEASE PROJ ... FROM PAGE 1

and develop standardized diagnostic methods for important diseases of aquacultured organisms in Southeast Asia and establish a surveillance system.

The project has the following components:

- Research to develop standardized diagnostic methods for the region, disease control husbandry techniques, and monitoring methods for residual chemicals in aquaculture products
- International workshop for the regionalization of the standardized diagnostic methods, as well as disease control husbandry methods
- Hands-on training on diagnostic methods for important viral diseases in the region
- Development of a surveillance system for disease problems in the region

The implementation of the project started upon the dispatch to AQD of a fish disease expert, Dr. Yasuo Inui, by the Government of Japan in 2000. At the start of the project, AQD solely conducted the research and development activities. However, to make the project more efficient and more relevant to the region, the Department of Fisheries-Thailand was asked to collaborate in the implementation of the project starting in 2001. The three agencies in Thailand involved in the project are Aquatic Animal Health Research Institute (AAHRI), Marine Shrimp Research Development Center (MSRDC), and Samutsakhorn Coastal Aquaculture Development Center (SCADC). Other agencies will also be invited to collaborate during the later part of the project, especially in the establishment of the regional network.

To date, the ongoing research subjects are the establishment and standardization of diagnostic methods, biology and pathogenesis of disease agents, disease prevention and control, and establishment of evaluation methods for residual pesticides in aquaculture products. The project had convened with Office International des Epizooties (OIE) a seminar/workshop on "Disease control in fish and shrimp aquaculture in Southeast Asia: diagnosis and husbandry technique" late last year. - CBL

If all else fail, it usually helps to try a different primer pair. A less obvious reason for some primers failing to work is the presence of secondary structure in the template DNA. Software is also available from many commercial and academic sources to assist in the process. Most software packages for DNA sequence analysis now include menus for PCR primer design.

Nested PCR

Nested PCR primers are ones that are **internal** to the first primer pair. The larger fragment produced by the first round of PCR is used as the template for the second PCR. Nested PCR can also be performed with one of the first primer pair and a single nested primer. The sensitivity and specificity of both DNA and RNA amplification can be dramatically increased by using this method. The specificity is particularly enhanced because this technique almost always **eliminates any spurious nonspecific amplification products**. This is because after the first round of PCR, any non-specific products are unlikely to be sufficiently complementary to the nested primers to be able to serve as a template for further amplification, thus the desired target sequence is preferentially amplified. However, the increased risk of contamination is a drawback of this extreme sensitivity.

RNA (RT) PCR

PCR amplifies DNA sequences. In order to perform PCR on RNA sequences using *Taq* DNA polymerase, the **RNA must first be transcribed into a cDNA (complementary DNA) copy of the RNA sequence** because *Taq* has limited reverse transcriptase activity. This is called **reverse transcription (RT)**. Thus, RNA amplification is achieved by the reverse transcription-polymerase chain reaction (RT-PCR). There are several different kinds of primers that can be used to make cDNAs, like oligo-dT will prime cDNA synthesis on all polyadenylated RNAs, random-primed cDNA synthesis gives a broad range of cDNAs and is not limited to polyadenylated RNAs and lastly, oligo-nucleotide primers complementary to the RNA(s) of interest may be used to synthesize highly specific cDNAs.

One-tube RT-PCR incorporates both the reverse transcriptase enzyme and a thermostable DNA polymerase in a single tube for synthesis and amplification of the target RNA sequence. This is the preferred procedure for routine analysis. Commercial RT-PCR kits are available and alternatively, reagent mixes can be prepared also from separate component parts.

Problems with PCR

PCR is an extremely powerful technique, but its very power can also lead to considerable problems, particularly when detecting virus or bacterial genes for diagnostic purposes. It is important to remember that **nucleic acid from dead as well as viable microorganisms will give a positive reaction**. Since even a single molecule of DNA can be amplified by PCR, it is also vital to pre-

vent cross-contamination of DNA samples with amplified or foreign DNA. The slightest contamination of glassware, pipettes or reagents can result in the production of false-positive reactions. Such contamination problems impose a need for extreme cleanliness and rigorous controls. Amplification reactions should be performed in physical isolation (i.e., in a different room) from the parts of the laboratory where specimens are received and target nucleic acid is prepared. Various techniques for reducing extraneous DNA contamination of PCR products have been described, but it is vital that each set of PCR amplifications should include control reactions to verify the purity of reagents and the cleanliness of equipment.

PCR is vulnerable to contamination that will cause erroneous results. **False positives** will result from contamination of the reaction with target RNA or RT-PCR products. **False negatives** can be caused by the presence of inhibitors in the test samples or badly degraded target materials. For competent PCR, the sample must be either fresh and in good condition prior to nucleic acid extraction or preserved to maintain nucleic acid suitable for extraction. Proper consideration to the extraction procedure is also important. Maintenance of rigorously clean experimental techniques, use of standard reaction conditions and inclusion of internal standards as positive and negative controls are essential to gain accurate interpretation of the results.

Other problems may arise from the relatively high error rate of *Taq* polymerase. Base substitutions occur at about one in every 9,000 bp, and frameshifts at about one in every 40,000 bp. Although such error rates may seem to be insignificant, they may have profound effects on the homogeneity of the amplified products.

Although PCR can now be semi-automated because of the availability of the thermocycler, the technique still requires a certain amount of technical skill and some specialized equipments to prepare samples and perform amplification reactions successfully.

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the aquasilviculture trials in collaboration with local government units, other activities in the Aklan mangrove sites are the survey and mapping of the 75-ha area in Ibajay, construction of a treehouse, and the educational use as field site by Coastal Resource Management trainees (of SEAFDEC-AQD) and field biology students (of the University of the Philippines in the Visayas).

Santiago CB, Laron MA. 2002. Growth and fry production of Nile tilapia, *Oreochromis niloticus* (L.), on different feeding schedules. *Aquaculture Research* 33 (2): 129-136

Abstract. The effect of scheduled use of high-protein and low-protein diets on body weight and fry production of Nile tilapia, *Oreochromis niloticus* (L.), was determined. A preliminary feeding trial was first conducted on fingerlings. These were fed a high-protein diet (H, 25% protein) or a low-protein diet (L, 18% protein) daily, or diet H for 1-3 days followed by diet L for 1-4 days. Final body weight was significantly higher ($P < 0.05$) in fish fed diet H daily and in fish fed diet H for 2-3 days followed by diet L for 1 day (2H-1L and 3H-1L). Fingerlings on 1H-1L and 3H-2L had slightly lower growth. Based on the response of the fingerlings, five feeding schedules were tested with the broodstock. A high-protein diet (HP, 40%) and a low-protein diet (LP, 25%; same as H for fingerlings) were used. Feeding schedules significantly influenced body weight of female but not the male fish. Fry production was not significantly affected by the feeding schedule for broodstock. When growth, fry production and saving in feed cost were all considered, the broodstock on 1HP-1LP and 3HP-2LP feeding schedules both gave the highest overall performance. These findings give fish farmers an option in the management of feeding of tilapia broodstock.

Santiago CB, Focken U, Becker K. 2001. Voluntary feed intake and energy partitioning in tilapia (*Oreochromis niloticus*) fed diets with different protein/energy levels, p 181-184. In: Chwlibog A, Jakobsen K (eds). *Energy Metabolism in Animals: Proceedings of the 15th Symposium*. EAAP Publication No. 103, Snekersten, Denmark

Abstract. In order to investigate the effect of different protein/energy levels of diets (two commercial and one laboratory) on voluntary feed intake and energy partitioning in Tilapia (*Oreochromis niloticus*), 15 fish with an initial body mass of 33 g were reared individually in respirometric chambers for 42 days and offered 3 diets *ad libitum*. The protein contents of the diets were 36.1, 33.8 and 36.8% (dry matter base); the energy content 18.9, 18.4 and 19.2 kJ GE/g and 11.7, 10.5 and 15.4 kJ ME/g. The initial body composition and energy content was estimated from a control group. Feed consumption was recorded for each individual fish. Body mass development was monitored weekly. At the end of the experiment, the fish were sacrificed and their chemical composition (protein as N.6.25, lipid, ash) and gross energy content determined. To establish energy budgets, ingestion (I) was calculated from feed intake, retention (P) from accretion in the carcass, heat production (R) from oxygen consumption (indirect calorimetry) and apparently non-utilised energy (faecal and non-faecal losses, U) by difference from energy ingestion. In the beginning, food consumption amounted to approximately 5% body mass equivalent (BME) per day for all groups and gradually decreased to 2.5, 2.8 and 1.6% BME by the end of the experiment. While the food consumption was significantly different between the treatments, there were no significant differences in the body mass development. Average final body mass was 98.6, 93.8 and 103.7 g. Energy retention was 29.7, 29.2 and 44% of GE ingested; heat dissipation 32.1, 27.9 and 36.0%; faecal and non-faecal losses 38.2, 43.2 and 19.6%. For all energy budget parameters, values for the laboratory diet were significantly different from those of commercial feeds 1 and 2. Calculation of metabolisable energy from ingested feed revealed no significant differences in the energy uptake, suggesting that the voluntary feed uptake was controlled by the metabolisable energy. The fish were able to completely compensate for the lower ME content of the commercial feeds by increasing voluntary feed intake.

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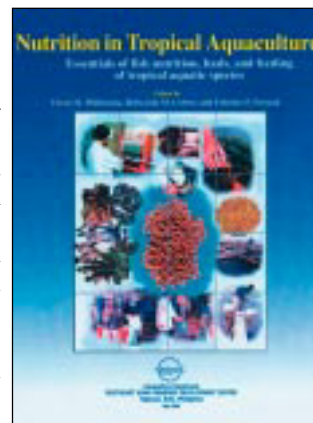
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that are best suited to temperate conditions.

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The nutrition textbook was launched on July 9, on the occasion of AQD's 29th anniversary; editor Dr. Felicitas Pascual (leftmost) signs a complimentary copy



New website

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Home of Fish Health Section of the Asian Fisheries Society whose Secretariat is presently hosted by AQD. The webpage is the official information clearing house for the forthcoming *5th Symposium on Diseases in Asian Aquaculture* to be held in Australia from November 25 to 28, 2002.

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The book was prepared in 2001 by SEAFDEC/AQD for the Asia-Pacific Economic Cooperation (APEC) Fisheries Working Group FWG 01/2000.

The Southeast Asian Fisheries Development Center (**SEAFDEC**) is a regional treaty organization established in December 1967 for the purpose of promoting fisheries development in Southeast Asia. Its Member Countries are Japan, Malaysia, the Philippines, Singapore, Thailand, Brunei Darussalam, the Socialist Republic of Viet Nam, Union of Myanmar, and Indonesia.

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Nota bene Mention of trade names in this publication is not an endorsement.

Karen Hogan Rides A Hog

For Karen Hogan, it has been a long way from Salt Lake City, Utah, USA to Bacolod City in western Visayas, Philippines, literally and figuratively.

Karen was one of the speakers at the technical session of the recently concluded *Shrimp Congress 2002* held in Bacolod City from July 1 to 4. Karen was representing her company Aquatic Lifeline, Inc (USA). Her topic was on larval nutrition. She emphasized quality nutrition in shrimp culture which she said is achieved by: less use of antibiotics, use of naturally healthy disease-resistant stocks and the best nutrition in the marketplace. And in case people forget, Karen reminded the participants that shrimp farming is about money – and therefore, the best feed that can improve the natural immuno-stimulation in shrimp should be used.

Karen is the Executive Administrator and CEO of Aquatic Lifeline Inc. based in Salt Lake City. Karen is married to Don Hogan, who runs the family hatchery business of African dwarf frogs for over 32 years. Her husband is an outdoorsman, nature-lover, trophy hunter, and a fisher.

According to Karen, their first foray into aquaculture was with the African dwarf frogs, *Hymenochirus boulengeri*. “They are very small, underwater frogs, living their entire life in water. They eat almost exclusively *Artemia* (a rotifer) from the Great Salt Lake. They grow to a size of one inch in three months, which is the marketable size. It takes them one and a half year to reach breeding maturity, and their size then reach to three inches long, stretched-out fully,” explains Karen.

They sell them to hobbyists and pet shops in New York for US \$5 apiece. They harvest about 60,000 dwarf frogs a month. These frogs consume a lot of *Artemia*, which has given way to another business. According to Karen, when an *Artemia* shortage happened in 1994, they were forced to go into the *Artemia* business in the Great Salt Lake. They used to buy from their competitor, The San Francisco Bay brand, but during the shortage, they packaged and marketed their own brand. Eventually, they expanded into larval diets supplements for the aquaculture industry. She marketed their *Artemia* product through the Internet. First, it was Japan in 1995,



followed by Korea in 1996, Thailand in 1999, and the Philippines this year.

Aquatic Lifeline, Inc. is distributed and marketed here in the Philippines by Fi-Sh Pharma, Inc., who specializes in aquatic medicines and pharmaceuticals. According to Mr. Leodegario Alba, DVM, General Manager, his outfit is relatively new in the aqua business. It is an addition to animal husbandry, poultry, swine and beef product lines. He welcomes the partnership with Karen Hogan’s Aquatic Lifeline, Inc., viewing the partnership with optimism. “There is a huge potential market,” says Dr. Alba.

One interesting aspect of Karen’s personal circumstances is her hobby: she drives a Harley-Davidson bike which they call a “hog.” It’s a 1990 Harley Sportster Sport 1200 cc engine with a Market Thunder Pipes (for enhanced window rattling sound).

“For the last 3 years, I’ve been riding the Hog on the scenic by ways all over the western United States; along western coast lines of the Pacific Ocean, through mountain passes and over deserts. Riding offers peace of mind, as well as stimulation and escape from daily life. I love it!” enthuses Karen.

Karen was born and raised near Phoenix, Arizona, then spent some time in Colorado near Denver, before settling in Salt Lake City with her husband. She attended Brigham Young University.

The three-day Shrimp Congress at Bacolod City was organized to bring all sub-sectors of the industry to get their act together. It was well attended by the movers and shakers of the shrimp industry including Karen’s Aquatic Lifeline, Inc., which was one of the participants in the Trade Exhibits.

The shrimp congress was organized by the Philippine Shrimp Industry Association, Inc. (PHILSHRIMP) and the Bureau of Fisheries and Aquatic Resources (BFAR), in cooperation with the Southeast Asian Fisheries Development Center - Aquaculture Department (SEAFDEC/AQD), Department of Trade and Industry (DTI), the Negros Prawn Producers Marketing Cooperative (NFFMC) and the Board of Investments (BOI). - APS

MORE STORIES ON THE SHRIMP CONGRESS INSIDE.