

# AQUA CULTURE

A s i a P a c i f i c

**D**iseases of the Barramundi

**V***ibrio* vaccine as a prophylactic tool

**A** business model for nursing the grouper in Aceh

**P**rocessing of microfeeds

**P**angasius seed quality in Vietnam

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# From the editor

## Science and industrialisation

Aquaculture, like agriculture, goes through the same evolution in its phases of growth and hence, we can learn from the poultry industry which serves as a role model. Aquaculture has expanded tremendously in recent history and is expected to carry the responsibility of providing seafood and freshwater fish and shrimp for the future as sea catches decline. However, some argue that this expansion has been seen as growth of backyard operations, significantly in Asia, where land availability and labour costs are advantageous. Following the poultry role model, we should be looking at a paradigm shift toward industrialisation. Industrialisation is the simple model of efficiency which allows for predictability and a standard cost of production but this model has to be supported by science. So where are we today?

In poultry, it was genetic selection that started a paradigm shift that changed the industry which can achieve a one kilogram bird in 42 days with excellent FCRs. The result is the cheapest protein source today. In aquaculture, the success of developing efficient and sustainable Atlantic salmon farming in Norway was attributed to selective breeding started in the late 1970s. The focus was on economically important traits such as growth, disease resistance and quality and this resulted in genetically improved salmon which grew twice as fast as wild salmon and required 25% less feed. The Norwegian salmon farming industry then reduced its feed costs by more than USD 230 million per year, according to Thodesen & Gjedrem (2006). This also spawned specialised breeding companies, similar to what we have with the tilapia and vannamei shrimp. In Australia, work at CSIRO showed that genetic selection resulted in a 10-fold higher nauplii production in 5th generation *P. monodon* as compared to that in the first generation. Thus, science has shown results which now require commercialisation.

In the marine fish sector, there is a tendency to indulge in new species rather than forge ahead with domestication and selective breeding in a selected few. It is said that there are some 67 species of marine fish being cultured in Asia. The question is how many species do we need to farm and surely a few is sufficient to meet the global fish demand. Again, look at how the poultry industry concentrates on a few species and is driven only by cost reduction, quality and yield.

In most Asian hatcheries, traditional methods still apply. Although biosecurity has been stepped up, production success is dependent on individuals and artisans working 24/7 as opposed to a standard operating procedure. The grow-out stages of both fish and shrimp show poor industrialisation where the most important performance indicator is predictability or rather, 'the lack of'. Current production performance is highly dependent on the weather and the capacity to control diseases. Without a paradigm shift, the only way forward is to test the limits of both the biology of the species and the environment by overstocking and putting the whole stock at the risk of disease.

The current practice also promotes undercutting prices and lack of attention to health management - an integral part of the practice and procedure. In the poultry industry, significant scientific work and investments have been made to identify the vectors of major diseases and production of vaccines to reduce disease occurrence. Although there have been developments, both scientific work and investments in aquaculture pale in comparison.

At the risk of sounding like a broken record, we are still unable to meet the nutritional requirements for most commercial aquaculture species in Asia today. Instead, there is significant overcompensation of nutrients in the feed to ensure that growth and performance are maintained. This gap will only increase at a faster rate as new genetic strains selected for faster growth emerge and nutrition is unable to unleash this growth potential.

With the recent food scandals, the market is demanding for traceability. This will push aquaculture towards integration that has a tendency to crowd out smaller farmers. However, we have seen farm associations working with large feedmillers and processing plants to form alliances to meet the requirements of traceability. Can this be a starting point for industrialisation?

Which comes first - science or industrialisation? One could argue that it does not matter as one will prompt the other. The problem still remains that neither party is moving fast enough.

### WRITE TO THE EDITOR

We want to hear from you. Write your comments on the industry to the editor.

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Zuridah Merican

# A NEW GENERATION OF ARTEMIA CYSTS

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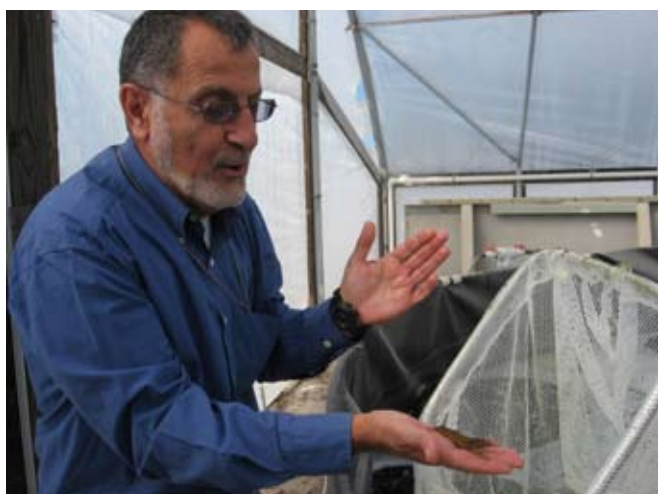


- Complete separation
- Clean biomass
- Undamaged nauplii
- Increased biomass output

# New production system with high yields

**Breakthrough could be a new world record in the production of the Pacific white shrimp *Litopenaeus vannamei* in an environmentally friendly system with no water exchange throughout the cycle.**

In Texas, Dr. Tzachi Samocha, a Regents Fellow and professor at the AgriLife Research Mariculture Laboratory in Corpus Christi and an expert in closed culture and bio floc systems, said that in the 'super-intensive raceway system', developed over five years, the production was 9.75 kg of shrimp/m<sup>3</sup> of water with very little use of pure oxygen and producing 22 g size shrimp. Ammonia nitrite in the culture water was very low throughout the production cycle. The production is about ten-fold higher than in pond production in the US and this new management could potentially revitalize the US shrimp industry.



Samocha said he was able to create the new system by improving on existing management practices. This management only requires top up water to compensate for losses from evaporation and management of particulate matter. The practice include adequate method for mixing and oxygenating the culture medium, careful feed-management and control of the bio-floc control while using a commercially available feed.

"The higher production translates into reduced cost per unit", said Samocha. "When considering fixed and variable costs, the economic analysis suggests that with an initial investment of USD 992,000, a successful commercial operation could show annual returns of about 38% and a payback period of about 2.8 years."

In the US and many parts of the world, the way to increase shrimp output has been through more intensive culture of existing pond systems or expansion of culture areas. The limited growing season and high labour cost are some of the obstacles of the shrimp farming industry in the US. This no discharge, high-density production system is a promising breakthrough that can also be constructed in colder climates using insulated buildings with water temperature control to produce shrimp year round, according to Samocha.

"Locally produced shrimp, means restaurants will be able to buy fresh, never frozen, or live shrimp from producers they know and trust. Buying live shrimp locally instead of importing frozen shrimp from other countries means food safety will increase, and our food dollars will stay in this country. Also of great significance is that shrimp raised in this system are never treated with antibiotics."

## Black tiger shrimp to make a comeback

### Better breeding stock is reviving export hopes

In 2004, 84% of Thailand's shrimp exports comprised 200,000 tonnes of the black tiger shrimp *Penaeus monodon*. In the years following, encouraged by high yields/ha and with the availability of specific pathogen free brood stock, farmers began to culture almost 99% of white shrimp *P. vannamei*. Exports also surged with 360,000 to 380,000 tonnes of vannamei shrimp in 2008 and 2009 respectively. Only a very small number of farms continued to culture the black tiger shrimp. Exports were 5,000 and 2,000 tonnes in 2008 and 2009, respectively. Now with availability of better breeding stock, Thailand's top shrimp and feed producer, Charoen Pokphand Public Foods Plc. (CPF) is looking at the black tiger shrimp making a comeback.

Sujint Thammasart, executive Vice-President, said in the Bangkok Post. "We have been successful in developing specific pathogen-free (SPF) black tiger breeding stock in greenhouses. The system keeps parent breeders free from diseases and produces healthy post larval shrimp. With bio-secure farming, these small shrimp will have strong disease-resistance".

CPF plans to export about 1,000 to 2,000 tonnes of black tiger -shrimp in 2010, from its total exports of 50,000 tonnes of shrimp. Sujint is confident that with this move by CPF, more farmers will begin to culture the black tiger shrimp. However, he suggested that farmers enter contracts with buyers (normally cold-storage operators and food processors), before farming to ensure that their output will find a market. The niche market will be larger shrimp of 20 to 25g and demand is strong for these sizes in Europe and Japan, which is currently supplied by India and Vietnam.

However, an industry source also advised farmers to study the market properly to avoid business risks and a price slump due to oversupply. Raising black tiger shrimp for export would not only balance Thai exports but also keep Thai products competitive in the world market.

"Vannamei shrimp are a perfect case study of mismanagement of demand and supply," said the source.

The average price of white shrimp (60-70 pcs/kg) has declined steadily over the past five years to THB 97.85/kg last year, from THB 122/kg in 2005.

"It's good for the country to have the second product line ie black tiger shrimp to maintain our export competitiveness," added Sujint.

The Office of Agricultural Economics expects Thailand's white shrimp to face tough competition with shrimp production in Vietnam and Indonesia. Vietnam has a goal of becoming the regional shrimp processing hub within a few years. Indonesia has also announced that it will support private and public ventures in the integrated white shrimp industry, starting with breeders, hatcheries, and farms and processing plants. Both countries aim for volume on par with that of Thailand in the near future.

India's production of the black tiger shrimp has been declining but of more concern are the small volumes of exports to the US markets, attributed to poor branding. The Marine Products Export Development Authority (MPEDA) expects a major boost when it signed a branding pact with American food service marketer and distributor Sysco, using its network to promote the merchandise as "Portico" shrimps. The campaign is estimated to cost USD2.3 million, of which 50% will be borne by Sysco.

Due to lack of funding, Samocha said, he had to release what he calls the “golden water” from his experimental shrimp production tanks but is confident he could have continued growing shrimp had he had the proper heating equipment. Exactly how long the golden water can be used can be determined only by further research, he said. “After 108 days, the system did not reach its maximum carrying capacity,” he said. “Nevertheless, we had to terminate the production trial as we had no way to heat the water. As the ambient temperature here in Corpus Christi began to drop, so did the water temperature.”

### Advanced shrimp nursery systems

For the past 20 years, Samocha and the team at AgriLife Research Mariculture Laboratory have been working on developing intensive nursery systems with emphasis on biosecurity and sustainability issues. Intensive nursery facilities in the US in greenhouses mean that biosecurity is easier to implement and losses to diseases minimised. When these systems are operated with minimal or no water exchange, the environmental impact of nutrient –rich effluents is minimised or eliminated. Samocha said that including a nursery facility in a shrimp production system provides for several advantages over direct stocking. Nursery facilities increase shrimp survival, improve facility usage and increase shrimp growth. Nursery facilities in areas susceptible to disease outbreaks can also serve as primary quarantine centres.

The evolution of this work and some commercial applications of this research were described in a presentation at World Aquaculture 2009, Veracruz, Mexico in September 2009. Commercial applications in Texas, Panama and Mexico used modified AgriLife Research prototypes. A comparison using juveniles from the nursery with those direct from the hatchery in a farm in Yucatan, Mexico, showed that survival of shrimp in grow-out ponds stocked with juveniles from the nursery raceways was 71% compared with 55% for direct stocked ponds. The



nursery was used to weed out weak post larvae before stocking in grow out ponds. The stocking of juveniles from raceways enabled a farm in Texas to harvest shrimp before other farms and secure premium prices. It has also enabled the farm to purchase post larvae at lower, pre peak demand prices and increase the number of cycles. The number of cycle/year increased from 2.3 to 3.2. In a trial in greenhouse raceways in Texas, the post larvae (1 mg) stocked at 40,000/m<sup>2</sup> grew to 0.1 g in 34 days with a survival of 99.5% and the food conversion ratio was 1:1. The total harvest was 5.61 kg/m<sup>2</sup>.

More information: Samocha, T., 2009. Advances in shrimp nursery technologies, *The Rising Tide*, edited by Drs. Craig L. Browdy and Darryl Jory, Special Session on Sustainable Shrimp Farming, World Aquaculture Society.

## Shrimp contract farming scheme in India

In a first of its kind in India, the Oceanaa group, a business conglomerate in India announced that it has signed a MOU with the Indian Overseas Bank and State Bank of India (SBI) for shrimp contract farming. Under this tripartite arrangement, farmers in Tamil Nadu and Puducherry with at least one ha of pond area and a license from the Coastal Aquaculture Authority will be identified by Oceanaa and recommended for loans. Oceanaa will supply the post larvae, chemicals, feed and other inputs against invoices. The company has its own hatchery with a capacity of 350 million post larvae/year. It will also provide free technical services and will buy back the shrimp at prevailing market prices. SBI will extend loans to the farmers. The shrimp will be insured against diseases such as WSSV-white spot syndrome virus and MBV-monodon baculovirus. Bunds will also be insured by the New India Insurance Co in Chennai.

Oceanaa will value add the harvest and export the shrimp products. Shrimp will also be marketed through the company's seafood retail setup. Sales proceeds will be routed to the bank for repayments of the loan. The loan facility for the farmers was calculated at INR 320,000/ha, repayable in six months and it is expected that the profit will be INR 30,000/ha. The farmers have to pay 15% of the cost of culture (Fishing Chimes, February, 2010).

## Black tiger shrimp to lead in 2010

According to preliminary statistics from Vietnam Customs, in the first 11 months of 2009, Vietnam exported 190,490 tonnes of shrimp, valued at more than USD1.518 billion. The share of black tiger shrimp dropped to 75% as compared to 95% previously. Exports also included more than 50,000 tonnes of white shrimp *P. vannamei*, valued at USD 300 million. It was forecasted that the export volume of white shrimp in 2010 will increase to 190,000 tonnes with a value of USD 500-600 million. The value of shrimp exports in 2010 will be USD 1.4 million. According to VASEP, the black tiger shrimp will lead in shrimp exports in 2010, even as the volume of white shrimp exports will increase because of low prices. The price of black tiger shrimp fluctuates from VND 90,000 to 100,000/kg while that for the white shrimp is just over VND 80,000/kg. According to the calculation of seafood experts, cost of production of the white shrimp is around VND 30,000/kg while farming a kg of black tiger shrimp is between VND 65,000 to 75,000/kg. (Vietfish News, January 2010).

# News in Brief

## Regulating imports of BT brood stock and post larvae

After lifting an eight year old ban on imports in December 2009, the Bureau of Fisheries and Aquatic Resources (BFAR) has now legislated that only shrimp hatcheries accredited by BFAR will be allowed to import specific pathogen free (SPF) or specific pathogen resistant (SPR) *Penaeus monodon* brood stock and post larvae. This is contained in Fisheries Order (FAO) No 230, adding that BFAR-accredited white shrimp *P. vannamei* hatcheries can also import and breed black tiger shrimp provided that separate facilities are used. BFAR will inspect the hatcheries regularly. Exporting facilities must show a minimum of two years of disease-free status, certified by a competent authority in the country of origin. The facility is also required to show the disease history from the time of establishment to present.

## Third aqua feed plant for Uni President in Vietnam

Officials of Uni-President Enterprises Co held a ground breaking ceremony in February for a USD140 million aqua feed plant in Dien Nam, Dien Ngoc Industrial Park in Quang Nam Province, Vietnam. The new plant, which will be the company's third aqua feed plant in the country, will cover an area of 13ha and have an annual production capacity of 100,000 tonnes of aqua feed and 400,000 tonnes of flour, instant noodles and animal feed per year. In Vietnam, Uni President began shrimp feed production in its first aqua feed mill in Binh Duong Province in 2000 and July 2006, it started operations at the second aqua feed plant in the Mekong Delta, producing 250,000 tonnes of aqua feed. The new plant is scheduled to be operational in March 2011.

## Better quality freshwater prawn from India

The EU's Rapid Alert System for Food and Feed Data (RASFF) showed only one rejection in January 2010, said the Seafood Exporters Association of India. In comparison, there were more than 50 rejections in 2009. Some of the rejections were due to the presence of nitrofurans metabolites in freshwater prawn. The association said that since September 2009, a substantial reduction in the number of alerts on Indian seafood was attributed to a two-pronged strategy of revising the testing module and also through better enforcement and compliance by authorities. Nitrofurans testing is conducted on the meat, instead of on the shell and following this, the number of notifications and rejections fell sharply. The decision of the EU Commission on September 30, 2009 required all crustacean imports from India to be tested for nitrofurans metabolites prior to shipment and to be accompanied by the test certificate. Those without certification would be detained and sampled for nitrofurans metabolites.

## Bangladesh resumes prawn exports to Europe

The Bangladesh Frozen Foods Exporters Association (BFFEA) said that exports of the freshwater prawn to the EU resumed in January because of the implementation of the regulation to control fish and animal feed production and prevent contamination with antibiotic residues. In May 2009, the government imposed a six month ban on exports after frequent reports on contamination with antibiotic residues in consignments to the EU. The EU imports crustaceans, mostly freshwater prawn worth USD250 million annually. Bangladesh exported 21,000 tonnes of shrimp in 2009,

comprising 25% of freshwater prawns. During the 8 month ban, exports were directed to Malaysia, Hong Kong, Singapore, the Middle-East and the United States at low prices. This export move is important as stocks of freshwater prawn must be cleared before end February, to be ready for the storage of a new harvest of black tiger shrimp.

## China's tilapia production down in 2009

A streptococcus outbreak between July and September in 2009 reduced tilapia production in China's four major tilapia producing provinces, Guangdong, Guangxi, Hainan and Fujian. These provinces represented 90% of the national tilapia output. This was reported in the annual outlook on China's seafood industry, released by USDA's Foreign Agriculture Service (FAS) in [seafoodsource.com](http://seafoodsource.com). However, the impact is short term as there is a vaccine available to control the bacteria. China's tilapia production was 1.11 million tonnes in 2008 and despite the bacterial outbreak, it is expected to increase to 1.15 million tonnes in 2009, said FAS, citing industry sources. China's major tilapia market is the US which imported 100,000 tonnes of frozen tilapia in 2009. Mexico is China's No. 2 tilapia export market, followed by Russia and Israel.

## Sustainable mussel aquaculture in Tasmania

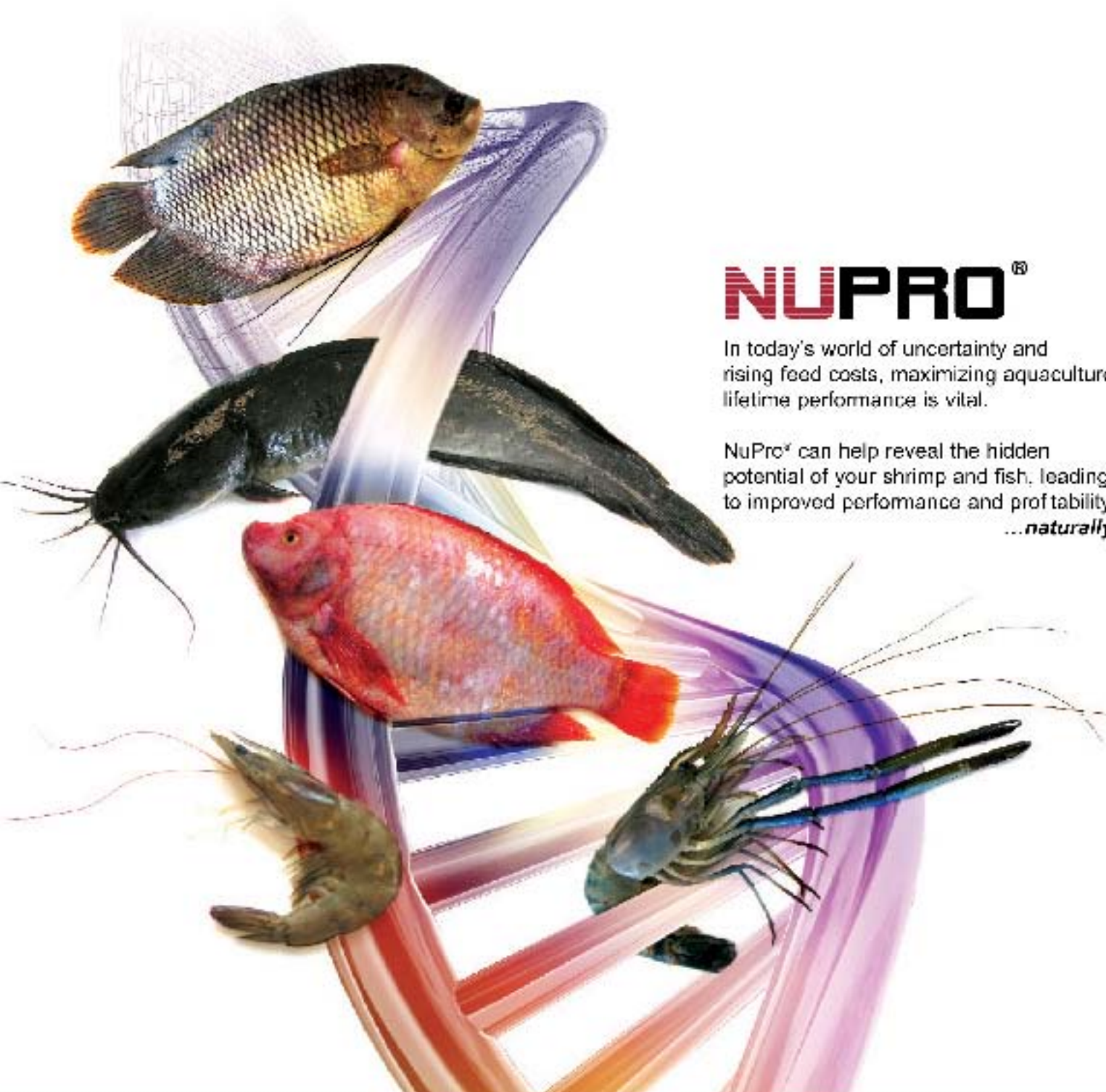
Friend of the Sea, an international organisation dedicated to marine habitat conservation, has certified Spring Bay Mussels on Tasmania's east coast as sustainable aquaculture. Spring Bay joins two other mussel producers, in Spain and Chile. The mussels are also certified organic by Australia's leading organic certifier, the National Association for Sustainable Agriculture, Australia. Spring Bay is the first commercial mussel hatchery in Australia and its sustainability accreditations rest on the hatchery production process. The company produces its own spat and does not deplete wild juvenile mussels. The secure spat supply allows farming all year and with the new process, Spring Bay produced 900 tonnes from 100 to 200 tonnes of mussels a year previously. The shellfish are exported to Singapore, Hong Kong, Malaysia and Thailand.

## Singapore's new seafood guide

WWF Singapore has introduced a seafood guide to help local consumers select seafood. The country is a large consumer of seafood, with a per capita consumption of 32.4kg, mainly supplied by imports from Asia. Local farms produce only 4% of the 105,163 tonnes of live, chilled or frozen seafood imported into Singapore in 2009. There are plans to increase this to 15%.

The guide is well intentioned as WWF wants to get citizens to play a larger role in the conservation and sustainability of marine resources. It has identified species into three categories; Green-recommended, Yellow-think twice and Red-avoid. In the 'think twice' category are several farmed species such as the four finger threadfin, milkfish and seabass, all cultured locally in Singapore's waters. To be avoided are several grouper species farmed in Indonesia and black tiger shrimp from Thailand. MSE certified Alaskan pollack, Asiatic hard clam from Ben Tre, Vietnam and wild caught Chilean sea bass and coral trout from Australia are in the recommended list. The impact of the list is important as in a recent poll, WWF said that 80% of respondents would like to either stop or reduce eating seafood if made aware that it was unsustainably produced or harvested.

# Reveal the Hidden Potential



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# A key segment in the value chain

By Soraphat Panakorn and Zuridah Merican

**Key to profitability in the business of rearing white shrimp nauplii to post larvae is survival rate.**

The role of Kunalab in Thailand's Chachoengsao Province is to nurse nauplii for 20 days to 10 day old post larvae (PL10) to supply to farms in the vicinity. The hatchery, some 50 km east of Bangkok, is located in one of the highest density areas for shrimp farming covering about 8,400 ha with an estimated 2,000-3,000 farms operating.

Thailand's hatchery sector is highly competitive. Ten years ago, there were around 4,000 hatcheries throughout Thailand and today, only 500 are in operation. Kunalab with a production capacity of 50 million PL/month is considered small in comparison to others in the area with capacities as large as 1 billion PL/ month. At present, there are about 60-80 hatcheries within a 20 km range from Kunalab.

"We started this business 6 years ago when we joined a feed company to supply post larvae to their aqua feed clients. Since then, the situation has changed as we are on our own and sell post larvae direct to independent farms. The connection with the feed company has helped us a lot", said Niramit Makmee, who with Kanokwan Pansawat are joint owners of the hatchery.

"Ten years ago we graduated, majoring in fisheries and decided to start work in a hatchery. Subsequently it was natural for us to start our own hatchery business, as we are so familiar with this industry. More importantly is that we have close relationships with farmers in the vicinity and this has helped us to run this business quite easily".



Kanokwan Pansawat is co owner of the hatchery



Niramit Makmee in the middle, left and right are his hatchery managers.

## Short process but affected by unpredictable weather

It takes a short time (20 days) to grow the nauplii. The first feeding is with various phytoplankton such as *Chaetoceros*, *Spirulina* etc and at the later mysis stage to post larva stages, live and encapsulated feeds, flakes, steam eggs etc are used. As a biosecurity measure, attention is focussed on water intake and on the production of phytoplankton. The cleaning process is simple, using surfactants such as dish washing liquid and exposure to sunlight for a week. Iodine and BKC (benzalkonium chloride) are also used to disinfect tanks and other equipment, when required. However, unlike other hatcheries, they do not use hydrochloric acid or chlorine.

"The rearing process is easy, provided all is well understood. Our main problems have not been diseases but the changing weather conditions have impacted our seed quality. We understand that weather fluctuations have also affected grow-out farmers. Other problems such as low post larvae prices, mortality and production costs can be controlled".

A stable temperature of 32-33°C is preferred for good growth and healthy post larvae, although the acceptable range is 28-35°C. The difference between day and night temperatures should not be over one degree as this will affect growth and a delay in metamorphosis to the next stage leading to mortality. High temperature fluctuations also affect the algal culture. A longer time to harvest increases production costs. One solution adopted is to warm the air flow into the tanks to stabilise water temperature.

## Traceability and quality

In nauplii grow-out, traceability of brood stock and determination of their quality are difficult, especially in hatcheries that are dependent on external supplies of nauplii. Despite the fact that nauplii suppliers are required to issue certificates on the condition of the nauplii, the actual quality of the nauplii is largely unknown. Kunalab buys nauplii from a hatchery which uses both local and imported brood stock in the spawning process.

"The hatchery supplying nauplii is very frank in revealing the brood stock used for a particular batch. We will choose the nauplii produced from imported or F1 brood stock which gives better performance. They have also said that the female spawners have been used 15-20 times and produce 150,000 to 250,000 nauplii each time. We also know that the spawners are fed with a local supply of polychaete worms which have been tested free from white spot syndrome virus (WSSV). Some local scientists have told us that these worms can be carriers of WSSV but so far we have not found any cases of infection".

Niramit added, "The post larvae that we produce are tested using PCR and certified free from WSSV. We have certificates to show this as this is an important demand from the farmers. Growth performance of our post larvae in farmer ponds has been above the current average daily growth (ADG) of 0.15g/day".

"We know that the growth performance of post larvae from local brood stock is lower. However, these post larvae have better tolerance to stress when compared to post larvae produced from imported brood stock, especially when conditions change rapidly, such as during the rainy and winter months and in locations where the culture conditions are not optimal".

## Business model

Post larvae are sold to farms within a 200 km range of the hatchery. Generally, grow-out farmers are pleased with the quality of post larvae sold by Kunalab. Prices are usually 30-50% higher than average prices and many farmers are willing to pay for higher quality fry. Niramit said that their close relationship with these farmers have helped them to secure higher prices for better quality post larvae.

"We have asked farmers to give us feedback on PL quality. For this reason, we maintain close connections with our customers. When there is a problem, we investigate and depending on the case, replace the batch. In Thailand, there is a general practice that hatcheries usually compensate with new seed stock and only in rare cases, do we return back money".

Competition is tough in the post larvae production business as there are hatchery operators selling lower quality post larvae at lower prices. Grow-out farmers then use this as a bargaining tool to bring down prices. In addition, when market size shrimp prices go down, farmers stop stocking ponds, reducing the demand and inevitably the price as well. In such cases, all hatcheries, including Kunalab have suffered as post larvae had to be fed and staff salary to be paid.



Rearing tanks

In general, the couple is happy to be in this hatchery business.

"We have the required skills and the business is profitable for most times of the year. The key factor determining profitability is the survival rate and on average we are able to achieve 40% survival rate. The cut-off point is 35% survival from nauplii to PL10. It will be good when we can reach 50% survival", said Niramit.

Currently, prices for post larvae range from THB 66 to 110 for 1,000 post larvae (USD 2 to 3.3/1000PL) and are very close to production costs. The reason for this range is that when determining their selling prices, most hatcheries, have only used fixed and variable costs in calculating production costs per batch. Niramit said that often, they have not costed in extras such as *ad hoc* tests for diseases and treatments, which may bring up costs by 10-20%.

However, to help these hatcheries, the Department of Fisheries of Thailand has set up laboratories to provide cost effective services. Hatcheries and farms can check for water quality, carry out general tests for diseases as well as use the PCR-polymerase chain reaction tests for viral diseases.

### Topping his wish list

"It would be better if we can see some standardisation on post larvae prices based on quality. If we have this, hatcheries will work at improving the genetic potential of the shrimp", said Niramit.

"We know that if hatcheries do this, growth performance will be better than what we have today. I believe that the ADG could be up to 0.25g/day with good management and using the current culture technology in earthen ponds. Survival rate in both hatcheries and grow-out ponds can be higher if we can get post larvae that can tolerate stress. However, because of the very low prices, it will be difficult for hatcheries to invest in better facilities, feed and rearing conditions".

Niramit added, "There are already some farmers who can produce post larvae which can achieve 0.25g/day growth but this has been inconsistent. A growth of 0.3g/day should be the target for the future if we work at reaching the genetic potential of the species".



**Soraphat Panakorn** is Technical Sales and Support Manager Aquaculture, Asia region Novozymes Biological, Thailand. Email: mamee\_d@hotmail.com

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# Micro aqua feeds By Zuridah Merican

At the recent aqua feed platform South East Asia in Vietnam, three feed processing experts shared their knowledge on production challenges.

In hatcheries, the leading feed is live feeds such as Artemia and rotifers and the reason why hatcheries use them is because they already have the infrastructure for live feed culture. However, as Artemia faces several issues; variable supplies, increasing costs (USD 30-60/kg), microbial and biosecurity risks, contamination with pesticides and heavy metals and enrichments to elevate DHA, EPA and vitamins, there is a need to look beyond using live feeds, said Süreyya Özkizilcik, Nutra Yem, Turkey. Micro feeds are relatively cheaper but Özkizilcik cautioned on the physical challenges posed by each type of micro feed. These aspects are further elaborated in his article on pages 14-16.

"Ideally it is possible to make good feeds with specifications on stability, floatation etc but the challenge is overcoming the fact that some micro feeds work in one hatchery but not in another. The delivery aspect is another challenge", said Özkizilcik.

Micro feeds can range in particle size from 50 microns to 1.0 mm. The desired feed particle sizes for larval feeds for feeding the zoea to early post larval stages of shrimp and post swim-up feeding of fish larvae are between 50-150 microns. Starter feeds for the later post larval stages of shrimp, fry stages of marine and freshwater fish and for small and medium size tropical ornamental fish have feed sizes of between 300 microns and 1.0 mm. Micro feeds are required to have specifications such as high water stability for shrimp post larvae and



Milkfish fry.

generally slow sinking for marine fish starter diets. Ingredients used to produce such feeds must undergo particle size reduction and the feed particle must be homogenous in its content.

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Paul Chen

Paul Chen, Wenger/Extru-Tech Taiwan said that “feed size does matter and that well formed pellets are preferred in feeding small fish and shrimp and that is the reason for the development of technologies to produce feeds in this range”. The three current processes are production of large pellets, either by pelleting or extrusion, followed by crumbling, Spherizer process (SAS) and direct extrusion.

Jacques Wijnoogst, Tema and Partners, Netherlands also outlined some technologies such as crumbling at the die of extruders and the new development called advance hydrolyzing technology. This new process for preparing a highly digestible feed with partially hydrolysed cell walls is yet to reach a commercial application stage.



Jacques Wijnoogst

### Particle size and the 90/90 rule

In the preparation of starter feeds for young fish, it is essential to have 99% of the ingredients in a feed particle and that there is homogeneity of the feed. Wijnoogst emphasised the 90/90 rule. It is important that in 90% of the daily amount consumed by shrimp and fish, 90% of the essential ingredients are present in the feed particle. By using sifting analysis and maths calculation, one can determine the best particle size distribution of the raw materials in the formulation. To get a homogenous feed particle, the smaller the quantity of raw material in a formulation, the smaller the particle size is required to get homogenous products.

Hammer mills can also make particles of less than 400 microns but with very fine screens installed (0.75-1 mm perforation), the capacity drops significantly. Pulverisers can grind finer in combination with an air classifier where particle sizes below 250 microns can be reached.

“Very fine grinding is required and hammermills are typically seen in the industry grinding for small starter feeds where 0.8 to 1.2 mm feed pellets can be achieved. Normally for the smaller direct extruded and micro feeds we see air swept pulverisers as the predominant grinding method. You can achieve very fine grinds which are quite acceptable for direct extrusion and SAS production methods which go down to pellet diameters to around 250 microns”, said Chen.

Special grinding equipment such as pulverisers with high rpm in combination with air classifier are already commonly used in shrimp feed production. These also have low heat to prevent nutrient degradation and can recycle oversize particles. There are also self cleaning screens, although these are not common in Asia. Some raw materials such as soybean meal with hulls and wheat bran should be avoided for crumbled starter feed or shrimp feed. The hulls are difficult to grind and will cause cracks in pellets and stay to large for small starter feed fractions.

“In crumbling, we know that there are aging effects of pellets which take place within at least 24 hours. Water stability is better and crumbling efficiency is higher if pellets are crumbled at least 2 days after pellet production. This means a separation of the crumbling line away from the main pellet line, but in many factories this is not done and the crumbling line is installed direct after the cooler and this is a design mistake”, said Wijnoogst.

### Extrusion

Both single and twin screw extrusion can be used to produce uniform micro feeds but with single screw extrusion the minimum is 600 microns pellets. Much smaller sizes are possible with double screw

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extrusion. In both cases, the raw material preparation is important such as deboning protein sources and sieving oil. Water must be filtered to prevent plugging of dies. Micro feeds cannot be oil coated and thus the capacity to add oil internally is an important criterion.

The advantage of direct extrusion is pasteurisation, excellent appearance, good durability and that both floating and sinking pellets can be produced. In addition, 95% yield is possible after post production sifting. The negative aspects are high cost and low production rate, size limitations and that there should be a dedicated line.

In the low temperature and low shear production using the SAS systems, yields are high at 95%. Advantages are that oil is added internally, smaller pellet sizes (300 microns) and homogenous mix in each particle whereas the disadvantage is that only sinking pellets can be produced. The low temperature process is also less destructive to nutrients and low temperature allows for the addition of medications and vitamins.

“Fish larval and fry feeds formulations usually specify relatively high fat levels and single screw extruders can accommodate 12% internal fat and twin screw extruders can have 17% internal fat. However, the oil has to be screened and sieved before addition. This is the advantage of extrusion”, said Chen.

“The latest development shows the critical requirement for phospholipids in early feeding. The addition of phospholipids showed better growth in the European sea bass larvae. Higher phospholipids increase survival rates and reduce malformations. Additionally, there are also benefits of additives such as betaglacans and phytogenics although it is too early to look at adding oral vaccines into these diets”, said Özkizilcik.

**Characteristics of some larval and starter feeds for fish and shrimp, marketed in Asia Pacific region**

Fish				
Name/type/species	Larval feeds (microns)			Fry feeds (mm)
NRD, Inve Aquaculture Microcapsules/ marine fish	100-200,150-300 (55%CP, 9% fat)	200-400, 300-500	400-600, 500-800	0.8-1.2, 1.2-2.0
Skretting, cold extruded/marine fish	<120 to 400 (55% CP, 15% fat)	500-800	900-1200	1.2-1.70 (52% CP, 20% fat)
Higashimaru Crumbles /minipellets /marine fish	<360 (59% CP, 10.3% fat)	410-650		1.0-2.5
Otohime marine fish	250	360-650	580-910	0.91-1.5 (52% CP, 20% fat)
Love larva, Extruded/marine fish	200 to 480 (48% CP, 10% fat)	480-1060 (52% CP, 12% fat)		1.1-2.1 (54% CP, 9.0% fat)
PT Global Indonesia, extruded/carp feeds				0.5 -1 (32% CP, 7% fat)
Aquaexcel, Cargill, extruded/ tilapia	0.4-2.2 (50-45% CP, 10-12% fat)			

**Marine shrimp –1. *P. vannamei* 2. *P. monodon* 3. *P. japonicus* 4. *P. indicus***

Name/type/species	Zoea to mysis (microns)	Early PL (PL1 –PL5) (microns)	Mid PL (PL6- PL10) (microns)	Late PL (PL11 to PL20) (microns)	Starter Feed Crumbles (mm)
Frippak, Inve Aquaculture Microcapsules	5-30, 30-90, 90-150 (54% CP, 16.5% fat)	100-200, (46%CP, 12% fat )	200-300	300-400, 400-600	
Higashimaru, crumbles (1,2,3,4)	125 microns (53% CP, 7-9% fat)	125-150	150-210	210-420	0.55- 1.7 (48% CP, 8% fat)
Encap/ micro-encapsulated Supreme/crumbles Gold Coin (1,2)	<77 (50% CP, 15% fat)	77-100	100-250	250-400	0.5-1.1, 1.2 -1.8 (42% CP, 6% fat)
EZ Larva, Zeigler /liquid, microencapsulated	10-50 /10-100	10-250/250-600			
Salt Creek, micro-encapsulated	5-20	50- 100/200-300	100-250	300-500	
Nicovita, powder/ extruded (1)		200-300 (40% CP)		300-800 (40% CP)	0.8 -2.0 (40% CP)
RICH Bioencapsulated	<2 to 50-100	100- 500/150-200	200-400		
UniPresident, crumbles (1,2)					0.4-1.0 (40% CP, 6-8% fat)
Prostar, Avanti, Crumbles (2)					0.2 -1.0, 0.6 to 1.0 (40% CP, 6% fat)
Waterbase, Crumbles (2)					0.1 -0.3, 0.6-0.8 (41% CP, 6% fat)
Bintang, CP Crumbles (2)				300 (42-41%, 5% fat)	0.425-0.71, 0.71-1.0 (42-41%, 5% fat)

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- Sound knowledge of fish nutrition and practical knowledge of raw material quality, extruded fish feed processing technology, cost formulation and fish farming techniques.
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## Aqua Feed Platform Asia 2010

The 9th practical short course on Aquaculture feed extrusion, nutrition and feed management was held in Ho Chi Minh City, Vietnam. Some 130 participants comprising mainly aquafeed producers, nutritionists and feed formulators attended the course. This year, the leading partner was Novus Aquaculture. The company also sponsored participants under its Aqua network program.

The series of courses which started six years ago has an extensive program covering a wide range of topics with specific practical aspects. Organisers are Filtration and Membrane World LLC. In this second course to be held in Asia, over two days, participants had the opportunity to meet experts in the field to discuss their current problems to enhance their plant operations. The course was divided into feed ingredients covering trends and raw material bottlenecks, utilisation animal by products, soybean concentrates, algae in aqua feeds and rapid analysis methods. In feed formulation, several presenters covered feed formulation and larval nutrition. In feed manufacturing technology, topics ranged from least cost formulation, feed preparation technology, pre and post conditioning, fundamentals of single and twin screw technology, new developments in extrusion technology, drying, plant design and quality assurance. Some of the current issues covered fat oxidation and stabilisation strategies, control of microbial contaminants in ingredients and feeds, energy savings and the reduction of carbon dioxide and increasing plant efficiency.

Novus Aqua supported the event with speakers. Dr. Farshad Shishehchian, Consultant for Novus International, Singapore said that the expectations for the aquaculture industry in Asia are increasing in his presentation on the 'Current and Future Market Trends in Aquaculture Feed'. Dr Jesus Venero, Aquaculture Nutrition Specialist, Thailand, covered bottlenecks in raw material formulation. In two presentations, Dr Anant Baradwaj, Aquaculture Nutritionist, discussed aqua feed quality control and mycotoxins management in aqua feeds.

## Process flow

In the production of micro feeds with direct extrusion, it was summarized that the requirement is a pulveriser with 250 microns particle grind, rotary sifter (300 microns screen) and extruder (with oil injection screened through 250 microns). A pneumatic conveying system is required to bring pellets to the fluid bed dryer or horizontal dryer with screen size of less than 250 microns. These are the dryers of choice for small feeds of 800 microns or smaller. These have adjustable temperature and residence time and efficient energy transfer into the product. They are also designed for easy cleaning and sanitation.

## References

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- Specific grinding and preparation for making extruded products with specific particle or pellet size- Jacques Wijnoogst, Tema-International, the Netherlands
- Micro aquatic feeds- Paul Chen, Extru-Tech/Wenger, Taiwan

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# Larval feeds for marine fish and shrimp larvae

By Süreyya Özkizilcik

**Production technologies face complex problems as feeds should be attractable to initiate ingestion, digestible to cross the intestinal barrier and complete to provide all the nutrients required by the larvae.**

What we know about the nutritional requirements of fish and shrimp larvae are mostly extrapolated information obtained from research on juveniles or adult forms. However, marine larvae are significantly different in physiology, anatomy and biochemistry from their juvenile forms. The digestive system consists of an undifferentiated tube without properly functioning liver, pancreas and stomach.

Indeed many larvae change their habitats after metamorphosis, migrating from pelagic to demersal. This sudden remarkable transformation occurs when larvae reach a specific size after obtaining essential nutrients and energy that sustain a steady growth. The larval feeds cover the life span from hatching until metamorphosis.

After hatching larvae obtain nutrients from two different sources. The energy and nutrients stored in the yolk sac provide the endogenous nutrition until active feeding initiates. Often these nutrients from the maternal broodstock supplement the exogenously ingested feeds in a mixed nutrition form. When the entire yolk sac is absorbed larvae depend solely on the intake of nutrients from the feeds.

Most marine larvae (both fish and crustaceans), cannot be raised solely on artificial diets commercially. Many attempts to grow marine larvae on artificial feeds have failed in providing satisfactory results for the aquaculture producers. Today, hatchery practices heavily depend on the use of live feeds such as *Artemia* and rotifers.

The production and maintenance of live feeds require separate biological systems that turn out to be more problematic than the intended juvenile production. Live feeds are costly, labourious, unpredictable and often nutritionally insufficient. It is very well documented that *Artemia* from many sources are deficient in highly unsaturated fatty acids (HUFA), mainly DHA and EPA. A common practice in hatcheries is to enrich *Artemia* and rotifers with essential nutrients and boost them with other vitamins, minerals and immunostimulants. The enrichment process is very costly and energy consuming.

## Larval feeds

From the technical point of view, larval feeds are classified under 500 micron feed particles containing essential nutrients that can support growth and well-being compared to those in the wild. They should be attractable to initiate ingestion, digestible to cross the intestinal barrier and complete to provide all the nutrients required by the larvae.

## Physical aspects

Most of the physical forces valid for larger grower feeds are not applicable to microscopic larval feeds. Surface charges, van der Waals forces and water movements significantly alter the behaviour of such a small particle in the water column. It is usually anticipated that a larval feed is neutrally buoyant with densities closer to that of the culture water. While this property appears to be an advantage, in extended periods of feeding, it makes uneaten feeds difficult to evacuate from the culture environment. In most hatchery practices, floatation is required in order to judge the amount to be delivered to the culture tank. However, it creates a rich matrix that is highly suitable for pathogenic organisms to thrive (Photo 1.)



*Photo 1. Uneaten floating feed particles clumping on the surface of larval tanks.*

The colour of the commercially produced feeds has been a long continuing discussion between nutritionists and commercial manufacturers. Most of the feeds available in the market today mimic the red-orange colour of live feed *Artemia*. However, it is very well documented that the contrast between the culture tanks and the colour of the feed is more critical than the presumed red/orange effect. Many studies showed that most marine fish larvae can capture feed particles (live feeds and artificial feeds) at less than 10 lux light intensities

The texture and general surface structure of microfeeds are not well understood. Marine larvae feed on a variety of zooplankton in the wild. While shape and texture of these organisms vary greatly, it is commonly accepted that soft, gel-like particles with high moisture contents (70-80%) are preferably ingested by larval fish and shrimps. Recent studies indicated that fish larvae prefer soft particles. When rapidly hydrating soft microencapsulated diets are fed to the Sea Bream larvae, the ingested size of the particles doubled.

## Nutritional aspects

Although it is more related to the physical structure, it appears more appropriate to mention the leaching of micro molecules from larval diets under the nutritional aspects. Most marine larvae are highly stimulated by some small molecular compounds to attract them to the feed particles. This phagostimulant activity of free amino acids and nucleotides are well studied in marine species. However, when these molecules leach out of micro feeds, they reduce the nutritional quality of the feed. Along with these stimulants many other water soluble nutrients are also released to the culture environment potentially reducing the water quality.

Presuming the ingestion of the larval feed, the digestibility of proteins and lipids seems to play a central role in sustaining growth and well being of the larvae. Some previous research indicated that live feeds contain enzymes to facilitate ingestion of nutrients in the primitive digestive tract of the larvae. However, recent research proved that most of the digestive enzymes (proteases and lipases)



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are present and live feeds contribute very little activity to the whole enzymatic processes. Furthermore, adding enzymes to larval feeds did not significantly enhance growth.

The extensive research strongly indicates that partially digested proteins (<10 Kdalton peptides and free amino acids) are important in providing larvae with amino acids required for growth. In terms of lipid nutrition, the essentiality of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are well established. Arachidonic acid, on the other hand, is proved to reduce stress and positively improve immune response. Furthermore, phospholipids appear to help reduce the skeletal malformations in fast developing marine larvae.

## Larval feed production technologies

The production technologies of larval feeds can be categorized in five main groups:

### *Semi-moist hydrocolloids*

These feeds are traditionally produced on-site from raw marine animals (fish, squid, shrimps etc.) as homogenized moist slurries and dispersed in the culture tanks for feeding marine larvae. As there is no standard production technology for these larval feeds, they will not be reviewed in this article. However, it is worth mentioning that these semi-moist colloids can be a venue of potential research if a standard production technology is developed.

### *Microbound feeds*

This technology relies heavily on the standard feed production methods for grower feeds of much bigger sizes. Typically, a mother pellet of 3-4 mm is produced with conventional pellet presses or extruders. After drying the mother pellet is coarsely crushed and sifted to the desired particle sizes. The binding matrix is usually starch, sometimes strengthened with the addition of gums.

### *Agglomerated feeds*

This production method is adopted from pharmaceutical production of some vitamins and medicines. Raw materials of desired formulation are finely ground and introduced to an agglomeration disc. A liquid

binding agent is sprayed on these finely ground particles causing them to form round-shaped larger particles. The resulting feed particles are usually well round agglomerates of different sizes.

### *Microextruded and spherized feeds*

A relatively new technology is employed for extruding strands (spaghetti-like) of raw material through small die holes under relatively low temperatures (<40°C). These semi moist strands (up to 10 cm) are broken and spherized on a spinning disc, forming round shaped small feed particles. Gelatin, carrageenan and other gums have to be used as binding agents since starch cooking does not take place at low temperatures.

### *Microencapsulated feeds*

Microencapsulation has a wide range of uses in many industries. This technology is also applied to marine larval feed production, mostly on experimental level. There are almost as many methods of microencapsulation as there are applications. In microencapsulated larval feeds, ingredients (proteins, lipids and/or solubles) are entrapped in a microshell or a matrix such as cross-linked proteins, lipids and various other coating materials. (Photo 2). These highly versatile microcapsules can be simple or complex which contain other incompatible microcapsules within a larger microcapsule (Photo 3).

## Conclusions

Larval feed production technologies deal with more complex problems in comparison to conventional grower feeds. Each technology offers many advantages as well as inherent problems (Table 1). Microbound crumbles are relatively cheap and easy to produce with existing machinery in a conventional feed plant. Although they are widely used water soluble nutrients leach out of these micro crumbles almost immediately after contact with culture water. Agglomerated microfeeds suffer from the same physical conditions. Microextruded feeds have lower leaching rates but have difficulties in density adjustments therefore sink to the bottom quite rapidly. Microencapsulated feeds offer solutions to most of the above problems. Nevertheless, they are relatively new, unknown and costly.

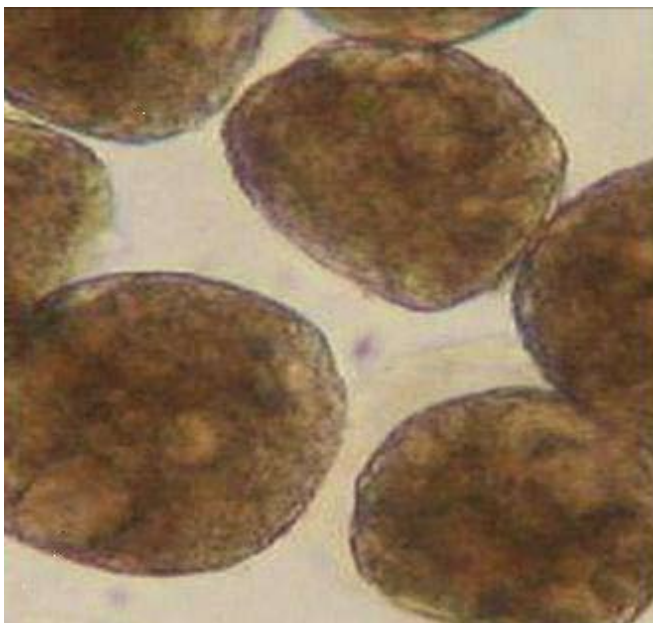


Photo 2. Simple alginate microcapsules containing the feed ingredients.

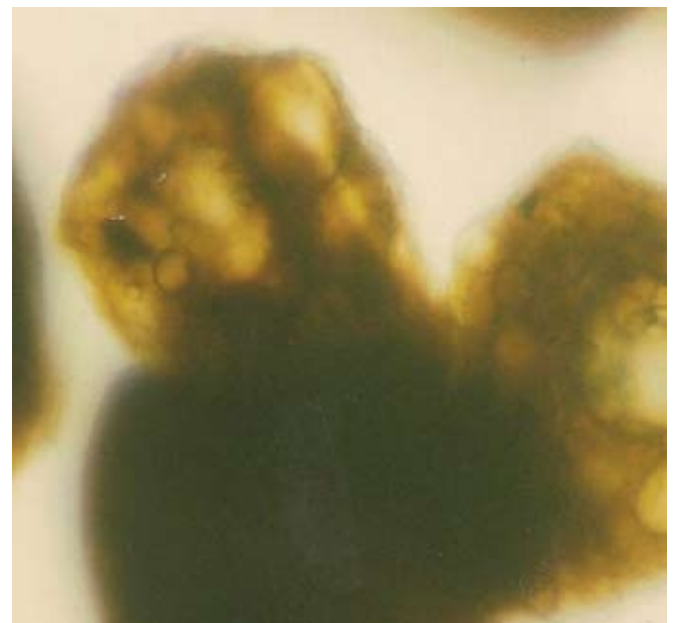


Photo 3. Complex cross-linked protein wall capsules containing lipid-wall capsules with other feed ingredients.

Figure 1. Physical properties of an ideal feed particle for marine fish larvae.

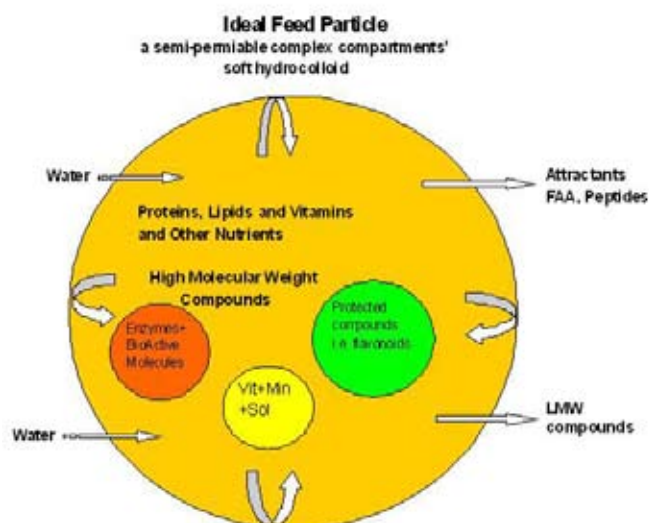


Table 1. Advantages and disadvantages of various microfeeds for marine larvae.

	Physical properties	Leaching	Ingestibility	Digestibility	Production costs
Semi-moist hydrocolloids	dispersed liquids	very high	high	high	cheap
Microbound crumbles	swell and disintegrate fast	very high	average	average	addendum to existing production line
Agglomerated particles	high leaching	high	high	average	dedicated equipment
Microextruded feeds	fast sinking dense particles	average	average	average	dedicated equipment
Microencapsulated feeds	well formed, dust free	low	high	low/average	dedicated facility


An ideal microfeed for marine larva production must overcome all the obstacles characterized for other technologies (Figure 1). It should be made to the desired size range with neutral buoyancy of the culture water, without any dust or clumps. This particle should hydrate rapidly allowing water molecules to penetrate the microfeed while leaching the small molecular weight compounds and retaining the larger molecules to stimulate the larvae for ingestion. From a nutritional standpoint, practically all the entrapped nutrients should be easily digestible/absorbable in the primitive digestive system of the larvae. In order to achieve this, a microfeed should be complex with other compartments that selectively protect the sensitive nutrients and release on the specific targeted site.

References available upon request.



Süreyya Özkizilcik, the owner of NutraFeeds Ltd. Turkey, holds a Ph.D. in microfeeds for larval fish and lipid nutrition and patents in microencapsulated feeds and enrichments.  
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# Using feed with reduced levels of fish meal for commercial production of the Pacific white shrimp in Asia

By Herbert E. Quintero, Michael C. Cremer, Lukas Manomaitis and D. Allen Davis

**Evaluation of diets with soybean meal as the main plant protein source and poultry by-product meal as the second animal protein source showed that shrimp growth performance was not affected.**

The commercial culture of shrimp is one of the most successful sectors of the aquaculture industry. Currently, the Asia-Pacific region accounts for 88% of shrimp and prawn production, with the top five producers China, Thailand, Viet Nam, Indonesia, and India accounting for 81% (FAO 2009a). Production in this region has shifted from indigenous species to mainly the Pacific white shrimp or white leg shrimp *Litopenaeus vannamei*.

This shift has resulted in increased world production of this white shrimp from 145,386 tonnes in 2000 to 2,296,630 tonnes in 2007. The value has increased correspondingly from USD 792,883 to USD 8,815,854 (FAO 2009b). In 2000, Ecuador, Mexico and Brazil accounted for 75% of the world production of the Pacific white shrimp. In 2007, these Latin American countries represented only 14% of world production whereas China, Thailand, Indonesia and Viet Nam accounted for 82% (Table 1). In 2007, 1,065,644 tonnes of Pacific white shrimp were harvested in China, representing 84% of production. Thailand, the second largest producer of *L. vannamei*, produced 490,000 tonnes in 2007 and this represented 98% of their shrimp production. In 2007, Indonesia reported 164,466 tonnes of *L. vannamei* production and 133,113 tonnes of *P. monodon* production, while Viet Nam's production reached 153,000 tonnes of *L. vannamei* and 170,000 tonnes of *P. monodon* (FAO 2009b).

## Fish meal as a cost-effective feed ingredient

Despite the continued expansion of commercial shrimp farming, the industry faces a number of environmental, social and economic challenges. For instance, the rapid increase in production has exceeded demand, resulting in declines in value. In addition, the worldwide economic crisis has reduced consumption in the main markets, which has reduced industry profitability. There are also other constraints such as the use of fish meal as a cost-effective source of high quality animal protein and essential dietary lipids

It is estimated that in 2003, 22.8% of the fish meal available for aqua feeds was used in shrimp culture, followed by marine fish and salmon with 20.1% and 19.5%, respectively. For a sustainable



*A semi-intensive shrimp farm in Latin America. Latin American farmers typically use significantly lower shrimp stocking density than farmers in the Asia-Pacific region.*

industry these figures need to be reversed, since fish meal prices are increasingly affecting the cost of feed production. Also, there is substantial pressure from environmental groups to reduce the use of fish meal in aqua feeds because of the negative relationship between what it is consumed and what it is produced. Another concern is that fish meal production is also affecting global fish stocks. This forces producers to look for alternatives to reduce feed cost and ensure a sustainable shrimp culture industry.

Therefore, shrimp nutrition and feed management is emerging as a tool for producers to achieve cost reductions, leading to better profitability and sustainability of operations. Typically, shrimp feeds represents 40% to 70% of variable costs of most farming operations ranging from semi-intensive to intensive systems, either in Asia or Latin America. Thus, replacement of fish meal using other animal protein sources and/or plant proteins, with balanced formulations, may reduce substantially the feed cost of shrimp production.

**Table 1. World production of Pacific white shrimp in 2000-2007 in tonnes (Source: FAO, 2009 Fish Stat Plus).**

Country	2000	2001	2002	2003	2004	2005	2006	2007
Ecuador	50,110	45,269	63,600	77,400	89,600	118,500	149,200	150,000
Mexico	33,480	48,014	45,853	45,857	62,361	90,008	112,495	113,540
Brazil	25,388	40,000	60,253	90,190	75,904	63,134	65,000	65,000
China	0	87,839	175,286	526,446	638,490	702,484	887,838	1,065,644
Thailand	0	0	60,000	132,365	251,698	374,487	490,000	490,000
Indonesia	0	0	0	0	53,217	103,874	141,649	164,466
Vietnam	0	0	10,000	31,717	40,000	100,000	150,000	153,000
World Production	145,386	267,953	473,449	982,663	1,297,935	1,647,405	2,090,935	2,296,630

## Reducing fish meal in shrimp diets

In a recent study, commercial shrimp feeds with different levels of fish meal inclusion and formulated to contain 35% crude protein and 8% lipids were evaluated. Fish meal was reduced gradually from 9% to 0%, using poultry by-product meal as a second animal protein source with a constant inclusion level of 16%, and soybean meal as the main plant protein source (Table 2). The inclusion rate of soybean meal ranged from 32.48% up to 39.52%. Nutritional composition for all the diets was similar.

**Table 2. Ingredient composition of practical diets for *L. vannamei* (values expressed on as fed basis, g/100 g) (Amaya et al. 2007).**

Ingredient	0% FM	3% FM	6% FM	9% FM
Soybean meal	39.52	37.17	34.82	32.48
Fish meal	0.00	3.00	6.00	9.00
Poultry by-product meal	16.00	16.00	16.00	16.00
Milo (commercial sorghum)	30.68	32.33	33.82	35.47
Corn gluten	4.84	3.17	1.67	0.00
Fish oil	4.72	4.47	4.22	3.96
Di-calcium phosphate	2.65	2.27	1.88	1.50
Bentonite	1.00	1.00	1.00	1.00
Mold inhibitor	0.15	0.15	0.15	0.15
Vitamin premix	0.34	0.34	0.34	0.34
Mineral premix	0.08	0.08	0.08	0.08
Stay-C 35%	0.02	0.02	0.02	0.02

Diets were evaluated under experimental pond conditions in 0.1 ha ponds for 18 weeks, and the production parameters from those diets did not exhibit any significant effect from totally replacing fish



*Sampling shrimp at an intensive shrimp farm in China.*

meal. Key production parameters had the following average values: final weight 19.6 g, production 6,026 kg/ha, growth rate 1.12 g/week, FCR 1.22, and survival 88.15%. However, total feed input cost was significantly reduced when more plant proteins were included in the diets (Amaya et al., 2007). Another step in this research consisted of replacing the poultry by-product meal with an all plant protein diet. Soybean meal reached 58% in the feed formulation without affecting shrimp growth performance or pond production.

Currently, the American Soybean Association International Marketing program is disseminating these results globally. Methodology for this effort includes a series of regional seminars where results of

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research are presented. During these seminars, producers and feed manufacturers to replicate this technology were identified. In such field trials, shrimp farmers are asked to compare their traditional feed against a nutritionally balanced feed with higher inclusion rates of soybean meal.

Although controlled conditions on commercial farms can be difficult to maintain, farmers are asked to follow a protocol that includes the use of replicate ponds (three replicates per feed treatment) and shrimp post larvae from the same origin and that will be stocked under similar conditions. Farmers are also encouraged to use their traditional feeding and culture procedures, so there is only one variable to evaluate. Transfer of this technology also includes improving manufacturing practices at the feed mill to achieve better feed quality and lower the cost of feed.

Commercial demonstration feed trials have been performed under semi-intensive conditions in shrimp farms in Ecuador, Mexico and Columbia, with substitution of 50 to 75% of the fish meal inclusion. Considering that culture conditions in the Asia-Pacific region are intensive to super-intensive, while in the Americas, culture is extensive to semi-intensive, validation of the technology under Asia-Pacific conditions is important. Preliminary trials under intensive conditions have started in China and the initial results encourage the use of diets with high inclusion rates of soybean meal.

*The increasing use of soybean meal and other plant proteins as protein sources in shrimp feeds will allow for some reduction in feed cost. However, we would like to highlight that feeds should not only be nutritionally complete and cost effective but they must also be properly applied to prevent both overfeeding and waste.*

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# Grouper nursing in Aceh, Indonesia

By Ujang Komarudin, Michael A. Rimmer, Islahuttaman, Zaifuddin and Samsul Bahrawi

**A technical and economic evaluation shows this as a profitable business which is providing coastal farmers in Indonesia with an alternative to shrimp culture.**

Similar to small-scale farmers in many other parts of Asia, farmers in Nanggroe Aceh Darussalam province of Indonesia (Aceh) are struggling to profitably produce shrimp. Shrimp farming is an important livelihood in Aceh with an estimated 73,000 ha of coastal ponds (*tambak*) and perhaps as many as 200,000 people are directly employed in coastal aquaculture. However, even before the devastating earthquake and tsunami of 2004, shrimp farming in Aceh was affected by a range of problems, such as: disorganised supply chain; crop losses due to disease outbreaks; lack of technical and market information to farmers; poor control of farm inputs (in particular seed quality); food safety issues arising from the use of restricted chemicals; decreasing price due to global market changes; and adverse environmental impacts including mangrove clearing (Padiyar et al. 2005).

Since 2005, many aid agencies and NGOs have supported rehabilitation of coastal aquaculture in Aceh. Most projects have focussed on the restoration of damaged ponds and associated infrastructure (inlet canals, etc.). Several agencies have supported implementation of better management practices (BMPs) for shrimp farming but because of the large numbers of farmers in Aceh, the geographic impact of these schemes has been limited.



*Grouper nursing cages (kelambu) in a pond in Samalanga district, Aceh.*



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Juvenile tiger grouper (*E. fuscoguttatus*) from nursing ponds.



Grouper are initially nursed in green (1 mm mesh) kelambu and then transferred to black (4 mm mesh) kelambu.

In response, many Aceh farmers are seeking alternatives to traditional shrimp farming. One alternative that has proven popular with farmers is grouper nursing.

### Grouper aquaculture in Indonesia

In 2006, Indonesia produced about 8,200 tonnes of farmed grouper (K. Sugama, pers. comm.). A substantial proportion of grouper fingerlings are produced in hatcheries in Bali (Sugama 2003). These hatcheries range from small-scale (without brood stock facilities) to large-scale facilities (with integrated brood stock facilities). In Buleleng Regency, northern Bali, 40 backyard and 3 large-scale hatcheries are producing grouper fingerlings (S. Ismi, pers. comm.).

The species most commonly produced is tiger grouper (*Epinephelus fuscoguttatus*). In 2006 and 2007, the annual production from Balinese hatcheries was around 6 million tiger grouper fingerlings (S. Ismi, pers. comm.). Balinese hatcheries also produce the mouse grouper (*Cromileptes altivelis*) and coral trout (*Plectropomus leopardus*) fingerlings, but in much lower numbers. Based on a survey done by S. Ismi (pers. comm.), generally, less than 100,000 fingerlings are produced per annum of each of these species. The Bali hatcheries usually do not incorporate nursery facilities and are keen to move fingerlings out of their tanks as soon as possible, generally when the fish are around 20 mm total length (TL), to make space for the next production cycle.

However, most grow-out farms are located elsewhere in the archipelago: Sumbawa in the east, Thousand Islands (*Kepulauan Seribu*) near Jakarta and Batam and Lampung in the west. The grow-out farms generally do not have the facilities for very small (< 70 mm TL) fingerlings and so a specialised nursery culture sub-sector has developed in Aceh and in East Java. These nurse grouper fingerlings from about 20 mm to 70–100 mm TL. Although there are no data available on the number of such nursing operations in Aceh, anecdotal information suggests that there may be 5,000 such operations established in Aceh and this sub-sector is expanding steadily.

### Grouper nursing in Aceh

To better understand the nature of this developing sub-sector, we undertook a survey of grouper nursing operations in two districts, Bireuen and Aceh Utara, where grouper nursing is concentrated. A total of 11 operations (7 in Bireuen and 4 in Aceh Utara) were surveyed by questionnaire in September 2008. The survey was undertaken through the Aceh Aquaculture Rehabilitation Project, which is funded by the Australian Agency for International Development (AusAID) and

the Australian Centre for International Agricultural Research (ACIAR) under the Australia Indonesia Partnership for Reconstruction and Development (AIPRD). The information below is derived from this survey.

### Culture methods

Two grouper species are cultured. The tiger grouper (*E. fuscoguttatus*) is sourced from hatcheries in Bali, and the green grouper (*E. coioides*), usually wild caught. Tiger grouper are transported from Bali to Medan (North Sumatra), then to the nursery sites in Aceh. Green grouper are caught locally using fixed nets. The nets are set facing towards the oncoming current, and are reversed when the tidal flow changes. Generally, green grouper is a lower-value species than the tiger grouper (Table 1).

Table 1. Names and price data for grouper species commonly cultured in Aceh.

Scientific name	<i>Epinephelus fuscoguttatus</i>	<i>Epinephelus coioides</i>
English name	Tiger grouper	Green grouper
Indonesian name	Kerapu macan	Kerapu lumpur
Local (Aceh) name	Kerapu kodok	Kerapu bulat
Seed price (15–20 mm TL)	Range: IDR 1,650 – 2,200 Mean: IDR 1,900	Range: IDR 300 – 1,700 Mean: IDR 1,200
Sale price (75–100 mm TL)	Range: IDR 2,500 – 4,000 Mean: IDR 3,500	Range: IDR 2,500 – 4,000 Mean: IDR 3,000
Conversion rate: one USD = IDR 9,365.00 (12 Feb 2010)		

The grouper are cultured in coastal ponds originally constructed for shrimp culture. Ponds used range from 500 to 8,000 m<sup>2</sup> in area. The fish are farmed in small cages (known locally as *kelambu*) fixed to the substrate with wooden poles. Two types of *kelambu* are used: 'green' (1 mm mesh) which range in size from 1.8 × 1.0 × 0.6m to 2.5 × 1.25 × 0.8m; and 'black' (4 mm mesh) which range in size from 1.5 × 1.0 × 0.5 m to 2.5 × 1.25 × 0.8m.

The rearing process is divided into two phases: the initial phase utilises 'green' *kelambu* and takes 10–15 days. Grouper are stocked at 500–2,000 fish per cage (depending on cage size), and fed mainly on small wild shrimp and fish captured from the ponds. After 10–15 days, the fish are moved to the larger mesh 'black' cages and the stocking density reduced to 300–1,000 fish per cage. During the second phase chopped 'trash' fish is used as feed. During both phases the grouper is fed 2–3 times daily.



Juveniles are graded by hand every 3 to 4 days to reduce cannibalism.



Preparing trash fish to feed juvenile groupers.

The fish are initially stocked at 15–25 mm TL, and are harvested when they reach 75–100 mm TL which generally takes 30–50 days. To reduce mortalities due to cannibalism, the fish are graded every 3 days. Farms undertake 7–8 production cycles per annum. Pond management is relatively simple. Water in the ponds is flushed twice each month during the highest tides.

Overall, quoted survival figures are high (Table 2). Survival is generally around 75%, but can be as low as 15% and as high as 98%. Low survival is reportedly associated with the rainy season, when

rapid changes in water quality can induce stress, leading to disease outbreaks.

**Table 2. Reported survival rates of grouper nursed in cages in coastal ponds in Aceh.**

	Minimum	Maximum
Low survival	15%	80%
Average survival	60%	85%
High survival	70%	98%

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Farmers learning to make farm-made feeds to reduce the use of trash fish in feeds for juvenile groupers.

Production costs (mainly feed costs) are generally low, estimated at IDR 150–250 per fish. At a high stocking density of 1,000 fish per cage, with 75% survival, a cage will provide a profit of about IDR 900,000 (USD 96.10). This does not include payment to farm workers, which is usually on a production basis.

### Business models

In general, there are three types of business system used by the grouper nursing sub-sector: middleman's investment, individual farmer's investment, and farmer group investment.

A single middleman (*toke*) may be responsible for grouper nursing by between 30 and 105 farmers. In this model, a farmer provides his own pond or rents a pond then uses it to raise an amount of grouper fry. Any operational expenses for fish seed, feed, and nets are supplied by the middleman. The profit is split usually 50:50 between the middleman and the farmer. The middleman investment model was the most common model observed in this survey (6 out of the 11 operations surveyed).

Individual farmers may use 1 to 3 ponds and employ 2 to 7 staff to assist with farming operations. Three farmer groups (*kelompok*) comprising 7 to 10 farmers, were surveyed. The larger group (10 farmers) employed an additional 10 employees, operating a total of 200 cages in 4 ponds. For two of these groups, profit is split 50:50 between the farmers and the employees. For the larger group, profit is split 70% to the farmer, with 30% going to the farmer group for future investment.

### Discussion

Grouper nursing is attractive to small-scale farmers in Aceh because of the short culture period (30–50 days) which reduces the risk of losses due to diseases. Many farms will stop production during the short rainy season (November – December) to reduce the risk of disease outbreaks caused by rapid water quality fluctuations.



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Grouper nursing is profitable. Five thousand grouper will generate an income of about IDR 2,375,000 to IDR 5,000,000 (USD 253.61 to USD 533.90) per cycle. Poverty levels in rural areas of Aceh are high by Indonesian standards with over 30% of rural households living below the poverty line (USD1.55 /day) (World Bank 2008). Hence grouper nursing is an opportunity for poor farmers in rural areas of Aceh to earn income.

Grouper nursing offers flexible investment models, including individual investment, farmer group investment, or middleman investment. This provides farmers with a range of investment options. Many small-scale farmers have limited capital for investment, even the modest investment required for grouper nursing. Banks are reluctant to loan money for aquaculture because of the high risk associated with shrimp farming. This is reflected in the popularity of the middleman investment model as found in this survey.

The farmer groups surveyed provide an interesting investment model. In areas where there are existing farmer groups, or where conditions of social cohesion support the establishment of farmer groups, formation of farmer groups provides an opportunity for farmers to co-invest and share risk. Based on our survey data, one hectare of coastal pond could allow up to 15 farmers to work together.

### Sustainability issues

Grouper nursing in Aceh offers a combination of sustainable and less sustainable practices. The practice of feeding wild shrimp and fish (usually gobies) sourced from the ponds reduces production costs and minimises impacts on local fish populations. However, there is still substantial demand for 'trash' fish which is provided by local fishermen. In response, we are planning to train grouper farmers in the use of farm-made feeds, which will reduce the use of 'trash' fish.

Another potentially unsustainable practice is the collection of juvenile green grouper from the wild using nets and traps. Much has been written on larval and juvenile grouper collection, and this topic is well covered in Ottolenghi et al. (2004). Although hatchery production technology for green grouper exists, the relatively low price of this species means that demand for higher-value species, such as tiger grouper, is greater. Hatcheries respond to this demand by producing tiger grouper rather than green grouper. The popularity of green grouper for nursing in Aceh seems to be based on their availability from the local juvenile capture fishery, as well as this species' general hardiness in culture.

Another species for which there is substantial potential in Aceh is the giant grouper (*E. lanceolatus*). The Brackishwater Aquaculture Development Centre at Ujung Batee, near Banda Aceh, is planning to produce fingerlings of this species following reconstruction of hatchery facilities destroyed by the 2004 tsunami. This species has potential, not only for nursing, but also for grow-out in coastal ponds and in sea cages in Aceh.

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From left: Samsul Bahrawi, Michael Rimmer, Ujang Komarudi.

Ujang Komarudin, Samsul Bahrawi, Islahuttaman and Zaifuddin are staff of the Brackishwater Aquaculture Development Centre Ujung Batee, Banda Aceh, Nangroe Aceh Darussalam, Indonesia

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# Important diseases of farmed barramundi in Asia

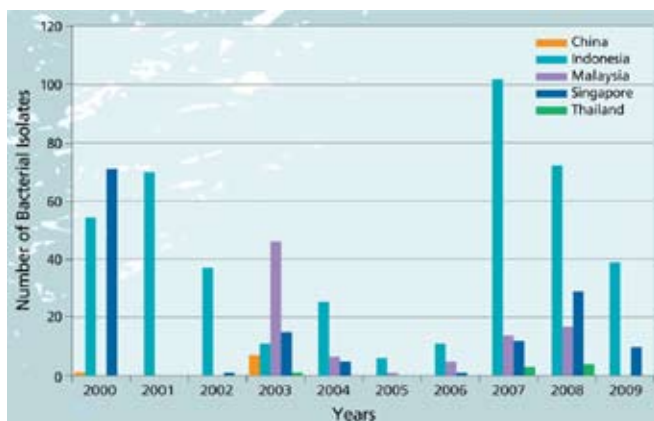
By Neil Wendover

**With more barramundi being cultured intensively in Asia, extensive epidemiology surveys conducted provide insights into the major diseases. With better understanding of these diseases, industry can look forward to vaccination and other disease management protocols.**

The barramundi (also known as the Asian seabass *Lates calcarifer*) is native to the Indo-Pacific region and is well suited to aquaculture. Although most of the current production is consumed domestically, due to its tasty, firm white flesh, barramundi is rising in popularity as a seafood item in the US and Europe since the late 1990's. Recently, several large international aquaculture operations have been set up in South East Asia to produce barramundi to take advantage of the suitable growing conditions and lower labour costs. The species is however not without its problems. Diseases are a major concern for the future sustainability of this and indeed any aquaculture industry.

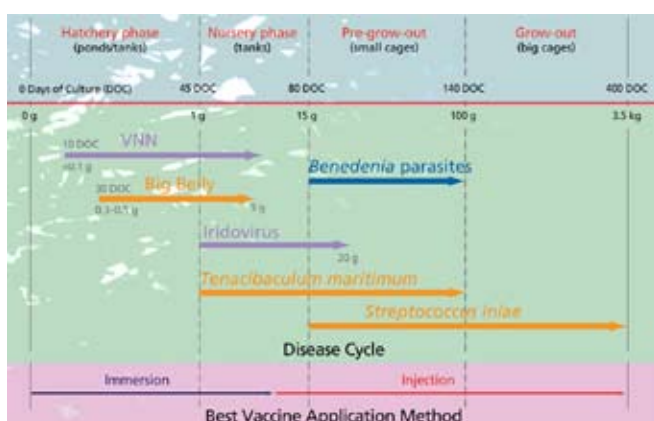
Building upon extensive experience in salmonids, Intervet/Schering-Plough Animal Health established a research centre in Singapore in 2000 dedicated to the development of novel vaccines and other products for commercially important farmed warm water species.

**Graph 1. Strain collection data base\*; barramundi bacterial isolates since 2000.** (NB. not true epidemiology as some countries were visited more frequently than others)



\* Intervet/Schering-Plough Animal Health

**Chart 1. An overview of the major diseases of farmed barramundi in South East Asia.**

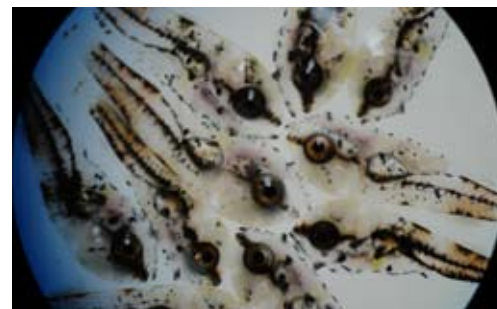


The company identified barramundi as a major farmed species for the region and therefore initiated extensive epidemiology surveys. Over the last 8 years, this routine surveying has resulted in a stored bank of barramundi-specific bacterial isolates from Indonesia, China, Malaysia, Singapore and Thailand (Graph 1) and has allowed the team in Singapore to identify the most important diseases in this species (summarised in Chart 1).

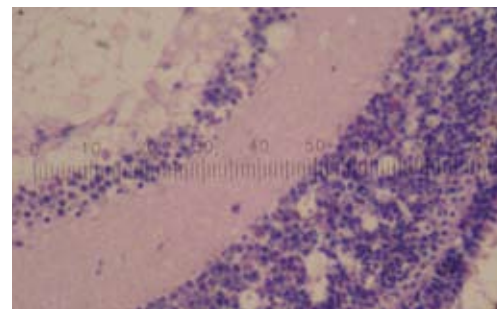
This article will highlight each of the major diseases in barramundi and their specific characteristics.

## Viral Nervous Necrosis

Viral Nervous Necrosis (VNN) is acute in larvae that are ten days old and often results in 100% mortality. The transparent larvae (due to the contraction of chromatophores) will display corkscrew or whirling swimming patterns and have hyper-inflated swim bladders.



Picture 1: VNN infected barramundi larvae, transparent with contraction of chromatophores



Picture 2: H&E stain (\*200) Characteristic Viral Nervous Necrosis vacuoles present in retina of an infected fish

The clinical signs are very characteristic of the disease. Histopathological sections clearly illustrate pathognomonic cell vacuolisation and necrosis of the central nervous system with lesions also occurring in the retina and spinal cord. A combination of histopathology and nested Polymerase Chain Reaction (PCR) will confirm the outbreak.

The disease can be passed either horizontally through the broodstock with evidence of the virus detected in brood-fish gonads, fertilised eggs, and larvae or vertically through the water with VNN outbreaks in fish farms occurring after introduction of infected juveniles. There is also possible transmission between wild and farmed fish.

There is no specific treatment; however, ozone treatment of all incoming eggs and larvae has been shown to reduce transmission potential. Development of VNN free broodstock with broodstock screening and isolation will be an important strategy in managing this disease.

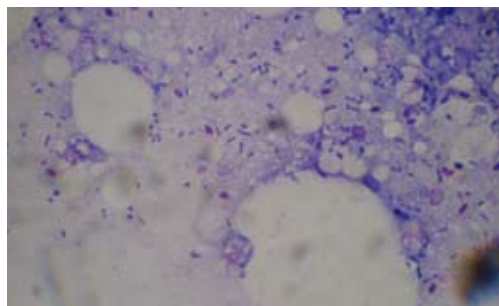
## Big Belly

Big Belly Disease is an intracellular, bi-polar large coco bacillus bacterium characterised by the company in 2004. It is present in several South East Asian countries including Indonesia, Singapore and Malaysia. To date, the disease has only been isolated from barramundi.

Big Belly is typically severe in 25-day-old fry in the nursery causing severe clumping of internal organs, abdominal distension and muscular atrophy. Often, infected individuals will take on a darker colouration, become lethargic, separate from the 'school' and lose equilibrium with either surface swimming or resting on the bottom of tanks. Considered to be a slow systemic infection, onset of mortality is gradual but consistent and without treatment has the potential to kill 90-95% of the population.



Picture 3: Typical 'knife edge' tail and abdominal distension characteristic of Big Belly in juvenile barramundi



Picture 4: Impression print of large bi-polar coco-bacillus bacterium.

Diagnosis in the field is based on the occurrence of clinical signs with a typical 'knife edge' to the tail (Picture 3) and a glutinous mass of intestines. Also, impression prints of visceral fat and other intestinal material will reveal the characteristic form of the bacterium (Picture 4).

Isolation of the bacterium is difficult due to its intracellular nature and the need for a specific agar medium to inhibit most of the environmental contamination. The R&D group in Singapore has developed Big Belly specific primers for PCR amplification.

In the field Aquaflor®, a premix formulation containing 50% active florfenicol, has shown promising results in reducing mortality due to the disease.

## Iridovirus

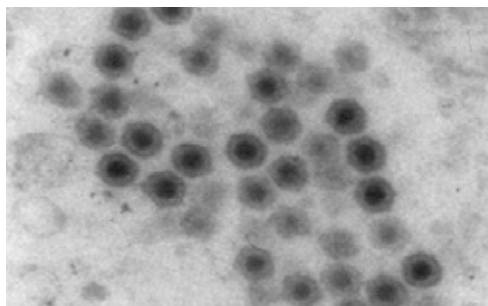
Iridovirus is one of the most severe diseases of tropical marine species such as barramundi and grouper (*Epinephelus spp*) but also affects many other fish species including some in freshwater. In barramundi the disease mainly occurs in fish of 10 to 50g and causes acute peaks of mortality of up to 80 – 90% (Picture 5). The fish will turn black and lose appetite. On closer clinical examination the gills will appear very



Picture 5: Acute mortality peaks typical in barramundi leave few survivors



Picture 6: 'Red eye', an often-typical clinical sign of Iridovirus in barramundi juveniles because of massive internal haemorrhaging



Picture 7: Electron microscopy of grouper Iridovirus illustrating typical icosahedral viral particles

pale and may bleed when handled and blood may also leak into the iris giving a 'red eye' appearance (Picture 6). Internally, a pale spleen is characteristic of this disease.

Mortality peaks and indicative clinical symptoms are generally good indicators of this disease in the field. Electron microscopy of spleen and kidney tissue reveals icosahedral viral particles typical of iridoviruses (Picture 7) and may be confirmed by PCR analysis using primers against the major capsid protein. Conventional treatment strategies have so far not proved effective.

## Parasites

It is almost impossible to avoid parasites because usually they are present as part of the aquatic ecosystem. Health monitoring and early diagnosis is key for control of parasitic diseases. Protozoa (particularly *Cryptocaryon irritans* and *Trichodina spp.*) and capsalid monogeneans on the body surface (particularly *Neobenedinia spp.*) most commonly affect newly stocked barramundi in open ocean cages. Current observations and reports from South East Asia indicate that capsalid monogenean infections are the most serious and pathogenic amongst all of the parasitic diseases. If left untreated barramundi quickly develop skin and tail rot and mortality rates of 30-40% are common.

*Neobenedinia* most commonly affects younger fish but if a population has been compromised in anyway (even from the nursery stage) then they will always be susceptible. Typically, a proportion of the fish will be 'off feed' and lethargic. The parasite also irritates the eyes causing opacity and exophthalmia ('pop eye') and gradually the caudal and pectoral fins will become frayed (white appearance in the water) and hemorrhagic when handled.

*Neobenedinia* is particularly easy to spot at the farm because after the whole fish is immersed in freshwater for some minutes, the parasites will turn opaque (Picture 8). A specific parasite prevention program with



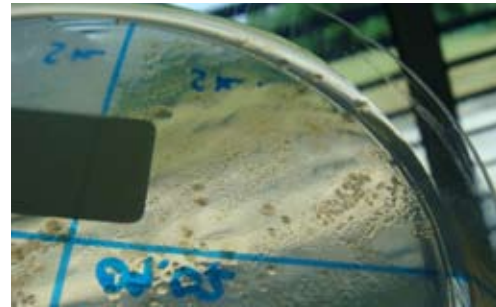
Picture 8: Dorsal fin of a barramundi illustrating opaque *Neobenedinia* parasites after immersion in freshwater



Picture 9: Indonesian farmers performing a bath treatment in response to a *Neobenedinia* outbreak in barramundi



Picture 10: Late stage progressive 'saddleback' skin lesions due to *T.maritimum*



Picture 11: Isolation of the rod shaped bacterium on selective media illustrating the rusty colouration

routine freshwater immersion, skin scrapes and gill clips should be considered an integral part of the health management protocols.

## Bacteria

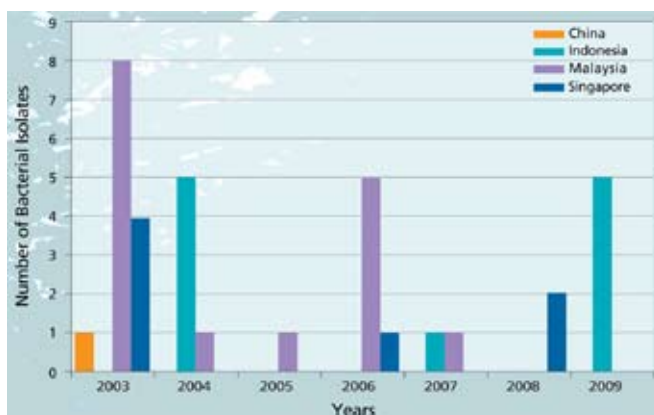
### *Tenacibaculum maritimum* (*T. mar*)

*Tenacibaculum maritimum*, also described as *T. mar*, is a rod shaped gram negative, filamentous bacterium. In Singapore, the team has been working with this pathogen since 2003 (Graph 2). There are 95 isolates from China, Indonesia, Malaysia, Singapore, Korea, Japan and Greece stored in the laboratory. The majority of isolates come from tropical saline species including barramundi, snapper (*Lutjanus* spp), grouper, cobia (*Rachycentron canadum*) and pompano (*Trachinotus blochii*).

*T. mar* can be especially severe when combined with skin parasites and outbreaks of the disease often occur after a stressful event. Typically, a *T. mar* outbreak will start as small lesions on gills and the ventral side of the fish, resulting in chronic scale loss and spreading to other cartilaginous body parts including the face and jaw.

Associated mortality in barramundi may be gradual or acute depending on the nature of the outbreak but often results in cumulative losses of 50-60%. Recent reports indicate that this disease is becoming more severe with devastating losses of nursery fish after stocking in pre-grow out cages (Note that in Graph 2, *T. mar* is still a concern with isolates collected in 2009).

Graph 2. Strain collection data base\*; Barramundi specific *T. maritimum* bacterial isolates since 2000.



\*Intervet/Schering-Plough Animal Health.

Presumptive diagnosis on the farm is possible using a gram stained wet mount of a skin scrape from the edge of an infected lesion. However, before confirming *T. mar* as the primary reason for the lesions it is important to first rule out other possible causes such as parasites or any possible trauma history. *T. maritimum* is a difficult bacterium to isolate due to the requirement for a selective media and the need to sample fish at a very early stage in the progression of the disease before other more common environmental bacteria mask the isolation of the initial pathogen. Isolated colonies on selective agar media display a rusty coloured branching morphology illustrated in Picture 11.

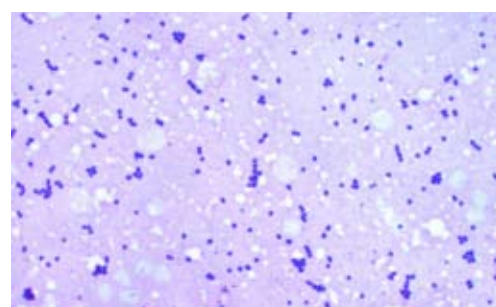
The characteristics of *T. maritimum* with infections starting on the fish surface (seen clearly in Picture 10) coupled with rapid onset and transmission make therapeutic control extremely difficult. Prevention of the disease through vaccination prior to exposure is the strategy for the near future.

### *Streptococcus iniae*

To date, the most important bacterial species affecting the culture of barramundi throughout the tropics is *Streptococcus iniae*. Indeed, *S. iniae* is considered to be one of the most serious bacterial diseases



Picture 12: Typical clinical signs associated with *S. iniae* of exophthalmia and internal septicaemia



Picture 13: A gram stained, impression print of *S. iniae* infected brain tissue illustrating the gram-positive chains of cocci

of all warm water fish. The R&D team has isolated *S. iniae* from barramundi, groupers, pomfret (*Pampus argenteus*), red sea bream (*Pagrus major*), snappers, croakers (*Pseudosciaena* sp.) and threadfin (*Polynemidae* sp.).

*S. iniae* is a gram-positive bacterium, the cocci of which group together in pairs or chains and may be clearly seen from impression prints of infected brain tissue as in picture 13. In barramundi, the disease is systemic and characterised by massive acute mortality peaks with cumulative mortality of 70% being very common. Infections can occur throughout the entire growth cycle but are more often seen in large and harvest size fish. *S. iniae* is therefore considered to be an 'expensive' disease for farmers who will experience a loss of production efficiency and marketable product but more importantly a reduction in overall feed conversion rates.

The disease is associated with typical clinical signs of uni or bilateral exophthalmia ('pop eye'), swirling swimming behaviour and scale lesions. Commonly, internal clinical signs may include cerebral meningitis (brain necrosis), septicaemia, ascites (accumulation of fluid) in the body cavity and splenomegaly (enlarged spleen) as shown in Picture 12.

Since 2005, Intervet/Schering-Plough Animal Health has Norvax® Strep Si, a water-based inactivated vaccine, registered for use against *S. iniae* infections in farmed fish. The vaccine was designed with two applications in mind; the first of which is as an immersion vaccination in which fish are immersed for 30 seconds at approximately 2g. In the laboratory, protection from immersion vaccination has been confirmed for 5 weeks. The second application method is by intraperitoneal injection (IP) and is conducted when fish reach 15g. Laboratory studies indicate that when 15g barramundi are vaccinated using a single intraperitoneal (IP) injection with 0.1ml of Norvax® Strep Si, maximum protection is achieved as soon as one week after vaccination. Studies conducted over 12 weeks in sterile laboratory conditions have demonstrated protection remains throughout this period. Studies under field conditions after IP vaccination at 15g have demonstrated protection up to 18 months post vaccination when fish are >2kg.

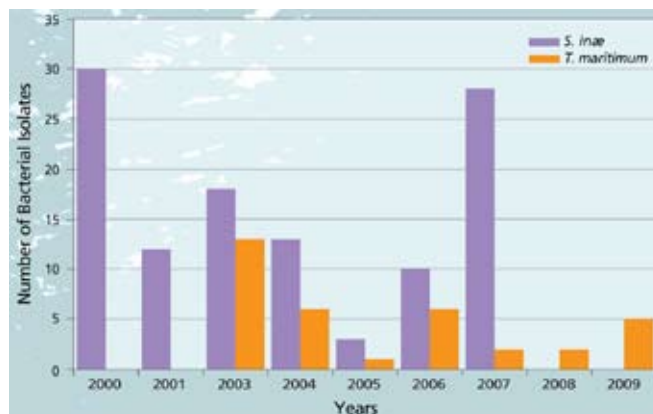
When a farming operation confirms *S. iniae* as a problem this should initiate a large-scale commercial vaccination program, consequently farmer profitability improves through an almost complete eradication of *S. iniae* outbreaks. A vaccination program of this nature typically involves the implementation of strict sanitary measures in the hatchery/nursery to ensure 100% vaccination of healthy juveniles.

Immersion vaccination at 2g in the nursery will afford protection against *S. iniae* until IP injection vaccination at 15g (Picture 14); this then protects the fish for the final grow out period until harvest. Recently however, some farmers have phased out the early immersion application and reports have emerged of occasional *S. iniae* outbreaks in unvaccinated nursery size fish.



Picture 14: IP vaccination of barramundi.

Graph 3: Regional isolate bank of *S. iniae* and *T. maritimum* in barramundi\*



\*Intervet/Schering-Plough Animal Health

This means the disease is still very much present in the farming environment and that farmers should remain vigilant and continue with the original strategy of immersion vaccination followed by an injection booster.

## Conclusions

Extensive mortality investigations in South East Asia by Intervet/Schering-Plough Animal Health and other research groups have resulted in a clear impression of the most important diseases of commercially farmed barramundi. Those diseases, briefly summarised in this article, include a range of parasitic, viral and bacterial pathogens. Understanding the problem is always the first step in dealing with it and unfortunately, for most farms in the region, veterinary and on farm diagnostic support is still lacking. Even so, vaccination with Norvax® Strep Si is a commercially viable preventative strategy for the most economically devastating bacterial disease *S. iniae*.

When an operation decides to embark on a vaccination program it is important to consider that this is just one tool to be used in combination with a series of good husbandry and stress reduction practices. If the farming environment is poor, the vaccine might still be effective but other diseases will undoubtedly occur. Vaccines are also very specific tools and cross protection is rare, a vaccine against *S. iniae* for example will protect against *S. iniae* infections only and not against *S. agalactiae*.

Iridovirus and *Tenacibaculum maritimum* are two important pathogens of barramundi that still require effective health management strategies.

**Author's notes:** This article contains information on veterinary, pharmaceutical and biological animal health products based on international registration dossiers. Brand names may differ between countries and the safety and efficacy data and the withholding periods for a specific product are subjected to local regulations. Norvax® and Aquaflor® are the property of Intervet International B.V. or affiliated companies or licensors and is protected by copyrights, trademark and other intellectual property laws.



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# Establishing safety and efficacy of an injectable form of a *Vibrio* vaccine for the orange-spotted grouper

By John S Clark, Jirasak Tangtrongpiros and Nantarika Chansue

**The European model of using vaccination as a primary prophylactic tool was applied successfully to orange-spotted grouper juveniles. This demonstrates a new direction for health management in grouper culture in Asia.**

Asian aquaculture is characterised by an enormous diversity of species, with several dozen marine fish species being farmed. Consequently, more resources are needed to understand the basic epidemiology of diseases in these species. In Asia, some disease-causing agents have been described but comparative studies between isolates from different geographical locations and fish species are generally not available (Tan et al., 2006). Epidemiological data is scarce, in the same way as is basic data on the immune systems of Asian fish species. This hampers the development of effective preventative strategies for disease control. Furthermore, most farming is operated on a small scale and technical support, including disease diagnosis and training, is often lacking at the farm level.

## Learning from the salmon industry

Salmon has been considered as the model species of modern aquaculture, especially for cage farming. In the last 20 years, this industry has developed dramatically and now produces nearly 1.5 million tonnes annually (FAO, 2006). Produced largely by two countries (Norway and Chile), in marine cage culture, the focus is on only one family of cultured fish, namely salmonids. Therefore, most resources available are used for the optimisation (including disease control) of its culture. This is in stark contrast to the above-mentioned situation in Asia. A survival rate lower than 95% in salmon farming is a sign of disease outbreak, whereas, a survival rate of 50% is often acceptable in Asia. The intensification of salmon production has led to the separation of fry production (hatcheries) and on-growing sites, optimised feed and feeding strategies, good quality fingerlings (that are virtually disease free) and good farm management.

## The Asian challenge

In Asia, aside from producing different species of fish at the same site, there is no segregation in year classes. In contrast this is obligatory for salmon in Europe. In Asian farms, trash fish is widely used as feed, fry are often wild caught or derived from wild-caught brood stock and the culture techniques per species are as yet undefined. Furthermore, legislation and implementation regarding farm licenses and zoning policies are non-existent in most Asian countries.

This often results in too many fish and too many farms in a concentrated area, a situation that promotes the spread of diseases. The combination of all these factors, together with the diversity of organisms in tropical waters, leads to a truly challenging disease situation with a variety of entry points for pathogens.

Disease is undoubtedly recognised as one of the biggest constraints to the production, development and sustainable expansion of marine fish aquaculture in the Asian region. As most farms operate on a small scale and with limited technical support, disease diagnosis

and training is usually lacking at the farm level. Even if fish suffer from disease and overall survival is low, epidemiological data is rarely collected, reported and analysed. This situation is in the process of being corrected.

In past few years, more and more attention has been given to the identification of aetiological agents involved in fish disease epidemics. Pathogens can be classified into bacterial, viral, parasitic and fungal groups and the following studies give a solid indication of the diversity of major pathogens affecting the fish farming industry in Asia (Bondad-Reantaso et al., 2005; Komar et al., 2005; Labrie et al., 2005; Leong et al., 2005, 2006 and Tan et al., 2003).

In Thailand, the diversification of culture is evident in recent years (Rimmer et al., 2004) and includes the orange-spotted grouper *Epinephelus coioides* and other coral trout or grouper species of high economic value (de Silva and Phillips, 2007). Diseases of bacterial origin are the most significant causes for economic losses affecting fish culture (Austin and Austin 1999) and in cultured marine fish, *Vibrio* spp. are the pathogenic microorganisms most frequently isolated during epizootics. In particular, many epizootics have been attributed to *Vibrio cholerae*. The economic losses caused by these microorganisms indicate the need to develop prophylactic strategies based on the European model for salmonids (Smith et al., 1999), such as vaccination, in order to prevent disease.

## A *Vibrio* vaccine

Vibriosis is the most common disease affecting marine finfish grow-out culture in the Asian region. It is responsible for catastrophic economic losses, in particular in the culture of the snapper, pompano and most recently in higher value species such as grouper. Conventional treatments using antibiotics have failed due to a lack of technical expertise in their administration and use which has led directly to the spread of bacterial resistance and hence to lack of efficacy. The aim of this study was to assess the effectiveness of an injectable bivalent *Vibrio* vaccine against *Vibrio* species in Thailand affecting farmed orange-spotted grouper and to assess its possible impact on grouper farming practices in Thailand.

## Fish samples

In this study, 840 juvenile of the orange-spotted grouper weighing approximately 5.75g and of approximately 4.31 cm length were used. These fish were purchased from a private farm. Fish were acclimated in 60 litre plastic aquaria for 14 days prior to initiation of the experiment. Three replicate tanks with 20 fish each were used in all immunisation and infection experiments. A light and dark period of 12:12h was maintained and aeration was supplied using an air stone. Water exchange was 50% every alternate day. The fish were fed daily to

satiation with frozen Artemia. To verify the *Vibrio* sp. free status of the fish, two fish were sacrificed and samples were obtained for bacterial culture by passing an inoculation loop into spleen, kidney and liver. The samples were streaked directly on TCBS and blood agar and were subsequently incubated at 30°C for 24-48 hours.

Water quality parameters were monitored. Temperature was measured daily. The pH, hardness, ammonia, nitrite and alkalinity were determined daily using a commercial test kit (AQUA-VBC, Thailand). The salinity was measured weekly with a refractometer (Table 1). Water quality parameters appeared to be normal although ammonia did give cause for concern.

**Table 1. Means of water quality parameters.**

Parameters	Mean level and S.D.	Parameters	Mean level and S.D.
Temperature	27.5±0.5°C	Alkalinity	60±19.27 ppm
pH	7.66±0.23	Ammonia	0.88±0.77 ppm
Salinity	34ppt	Nitrite	1.56±1.55 ppm
Hardness	>1000 ppm		

## Bacterial preparation

*V. cholerae* isolated from moribund sea bass (*Lates calcarifer*) were provided by the Veterinary Medical Aquatic Animal Research Centre (VMAARC), Faculty of Veterinary Science, Chulalongkorn University, Thailand. *V. cholerae* was plated onto Tryptic Soy Agar supplemented with 1% NaCl and harvested after 24 hours growth. Bacteria were identified using API® 20E (BioMerieux, Madrid, Spain) and the API profiles compared with the API database (Apilab Plus, version 3.3.3.; BioMerieux). The pure isolate was kept in stock media at room temperature.

## Virulence assays/Dose response curve

Virulence assays were performed with 540 juvenile orange-spotted grouper via intra-peritoneal injection. Bacterial doses ranging from  $10^3$  to  $10^{10}$  CFU/fish in a volume of 0.1 ml, injected intra peritoneally (IP) were used to establish pathogen doses that would kill 50 and 100% of injected fish within 3 days. Each dose had 3 replicates with 20 fish in each replicate. Control groups were challenged with sterile 0.9 % (v/v) saline.

After injection of the pathogen, fish would be observed daily. Mortalities were recorded on a daily basis, and symptoms associated with the ensuing pathogen attack were recorded and photographed. Samples of liver, spleen, heart, kidney, digestive tract and brain were preserved for histological examination and a photographic record was maintained. The mean lethal dose (LD) was calculated by the Reed and Muench (1938) procedure for establish optimal pathogen dose to be used during the efficacy stage of the trial.

## Methods of vaccination

### Vaccine manufacture

The vaccine was a bivalent *Vibrio* vaccine composed of 2 virulent strains of *V. harveyi* and *V. parahaemolyticus* isolated from diseased, moribund fish located in a farm near Phuket, Thailand. Cell concentration was  $1 \times 10^{11}$  CFU/ml for each strain, which were then formalin killed. A small amount of adjuvant was added to enhance vaccine potency. The sterile vaccine was contained in 500ml polyethylene bottles with a rubber stopper with an aluminium foil cap.

### Injection vaccination

Fish were not sedated. The vaccine (NovaQsol Holdings Inc., Philippines), was administered intraperitoneally to each fish. Injection volume was

0.05 and 0.10ml/fish of 5g average body weight. Fish are then returned to their respective tanks.

## Vaccination protocol and establishing vaccine safety

In this part, 240 juvenile orange-spotted grouper were separated into 4 groups of 60 fish/group. Each group was further sub-divided into 3 groups of 20 according to the following schedule;

- Negative control (NC), no injection.
- Positive control (PC), 0.1 ml saline injection.
- Test (T1), Injection vaccinated 0.05 ml injection.
- Test (T2), Injection vaccinated 0.10 ml injection.

Immunised and control fish were held for 21 days before the challenge. All groups were monitored for mortality on a daily basis. In this manner, vaccine safety could be clearly established.

## Efficacy test of vaccination

At 21 days post-vaccination, the positive control and all test groups received a 0.1 ml aliquot of a 100% lethal dose of pathogen administered intraperitoneally. The positive controls were administered an injection of 0.1 ml sterile saline. The fish were monitored for mortality for 3 days post-challenge.

Fish mortalities were observed and recorded on a daily basis and symptoms of the ensuing pathogen attack were recorded and photographed. Tissue samples of liver, spleen, heart, kidney, digestive tract and brain were preserved for histological examination, again with an accompanying photographic record. The percentage mortality rates and relative percentage survivals (RPS) of each vaccine dose were calculated against the relevant controls. In this way, the efficacy of both vaccine doses was clearly established.

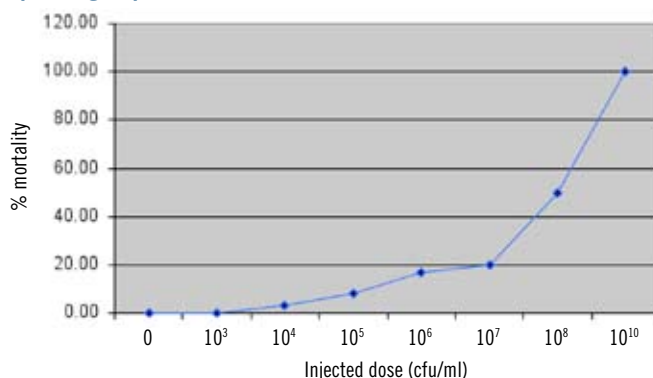
Dead fish were removed twice daily and upon post-mortem examination, specimens were obtained aseptically from spleen, kidney and liver tissues for examination of *V. cholerae* infection. Specimens were culture directly onto TCBS at 30°C for 24 hours and biochemically identified by API 20E.

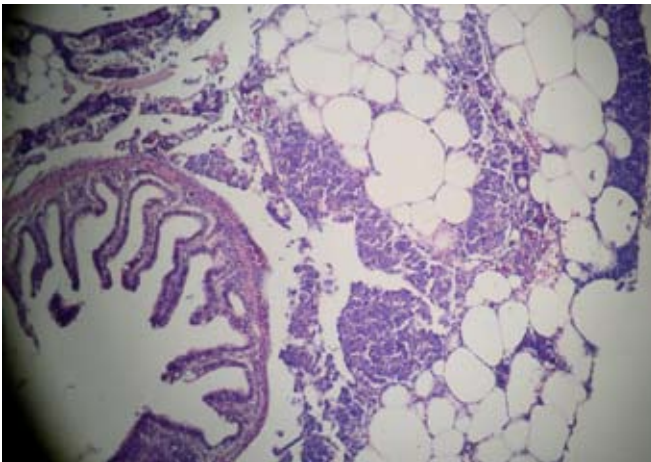
*Vibrio* sp. was not isolated from the two randomly selected fish, indicating that fish held at the start of the experiment were clear of actual and potential *Vibrio* infection.

The virulence assays/dose response curve was very clear for this particular isolate of *V. cholerae*. Figure 1 clearly indicated both LD50 and LD100 of *V. cholerae* were  $1 \times 10^8$  CFU/ml and LD100 and  $1 \times 10^{10}$  CFU/ml respectively. The LD100 dose was used to challenge 21 days vaccinated fish. Results are shown in Table 2.

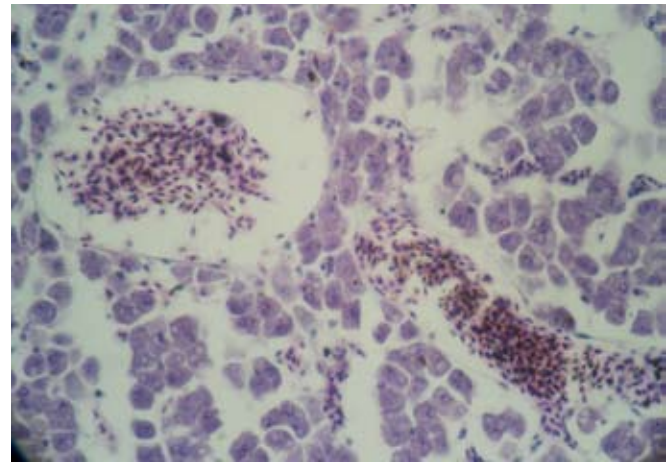
In this efficacy test of vaccination, the highest rate of protection against *V. cholerae* was observed in 0.10 ml vaccinated fish (T2), with RPS values up to 77.59% and significantly lower percentage mortality

**Figure 1. Determination of LD50 and LD100 for *V. cholerae* in juvenile spotted grouper.**





Acute peritonitis.



Septicaemia of hepatic tissue.

**Table 2. Percentage mortality of juvenile orange-spotted grouper, non-vaccinated and vaccinated by two intraperitoneally injected doses of a *Vibrio* vaccine and relative percentage survival (RPS) after challenge with *Vibrio cholerae*.**

Group		% Mortality*	RPS
NC	Control, no infection	2.63±3.72c	97.44
PC	Control, normal saline IP	100.00±0.00a	0.00
T1	0.05 ml. vaccine IP	54.04±31.73b	45.76
T2	0.10 ml. vaccine IP	23.15±18.61bc	77.59

\* Data was subjected to two way analysis of variance using an SPSS program. Significant differences were determined at P<0.05.

rates than in all infected groups (P<0.05). In this group (T2) there was no significant difference in the percentage mortality rate when compared to the control group (P>0.05). The 0.05 ml vaccinated fish (T1) displayed a 45.76 RPS value, which was a lower RPS value than in 0.10 ml vaccinated groups but was not significantly different from the percentage mortality rate at a (P>0.05) confidence limit. The positive control group displayed a 100% mortality rate.

The dead fish from this challenge experiment were confirmed positive for *V. cholerae* infection by re-culture and re-identification using API 20E. Histopathological studies of the dead fish showed atypical symptoms such as acute peritonitis and hepatic septicaemia.

## Discussion

It is a well known fact that there are many problems associated with the use of antibiotics. In addition to developing antibiotic resistance, many antibiotics are water soluble leading to leaching from feed, and some, like oxytetracycline, will complex with calcium and magnesium ions in seawater or hard water, rendering their efficacy highly reduced. Sick fish often do not eat and the efficiency of delivering antibiotics

orally is therefore questionable. Two key technical comments should be made regarding antibiotics:

- by their very nature they are active mainly against bacterial pathogens and therefore have no direct effect against viral and other pathogens and
- antibiotics work only as long as they can be delivered at the appropriate concentration to the target organ or tissue.

Application of antibiotics is a curative measure used to treat an existing infection. In contrast, vaccination is a preventative measure, dependent on the immune system of the animal. Vaccines can act against bacterial, viral and, at least experimentally, parasitic infections and they will usually act only against specific targeted pathogens. The duration of protection obtained with vaccines normally largely exceeds that of antibiotics. Historically, there are clear indications that the introduction of vaccines has greatly reduced the use of antibiotics in Norwegian salmon production from a high of over 50 tonnes in 1984 to less than 2 tonnes in 2004; with salmonid production in the same period rising from 25,000 tonnes to just over 500,000 tonnes.

In the past, fish vaccines were only available for salmonid species. This situation is changing with new vaccines being registered in Asia for Asian species (Grisez and Tan, 2005). Significant progress has been made in the field of vaccine research and development (Grisez and Tan, 2005). Besides yellowtail in Japan and grass carp in China, a commercial vaccine has recently been launched for use in Asian sea bass, tilapia and other species in some Southeast Asian countries (Komar et al., 2005).

It must be remembered, however, that vaccination is only one of the tools employed in good health management strategies and it is not sufficient on its own to guarantee high survival and profitability (see box).

In addition to the above, various studies have been carried out on the efficacy of various *Vibrio* vaccine types on survival of different grouper species. In the present study, results indicated that the 0.10 ml

Measures to sustain a successful aquaculture (Tan et al., 2006).	
Recommended	NOT recommended
<ol style="list-style-type: none"> <li>1. Use healthy (not necessarily cheap) fry</li> <li>2. Quarantine incoming animals</li> <li>3. Use formulated pelleted feed</li> <li>4. Grade fish periodically</li> <li>5. Monitor water quality</li> <li>6. Record diseases and feeding</li> <li>7. Observe withdrawal period of drugs</li> <li>8. Remove dead fish at least once a day</li> <li>9. Clean and disinfect equipment</li> <li>10. Vaccination if available</li> </ol>	<ol style="list-style-type: none"> <li>1. Place your farm too close to others</li> <li>2. Have several species in one farm</li> <li>3. Use fingerlings from unknown sources</li> <li>4. Overstock (to overcome low survival)</li> <li>5. Use trash fish</li> <li>6. Overfeed</li> <li>7. Use drugs without diagnosis</li> <li>8. Leave or throw dead fish in the water</li> <li>9. Restock fish without cleaning the cages</li> <li>10. Ignore diseases until heavy mortality occurs</li> </ol>

vaccinated fish group had a clearly higher RPS and lowest % mortality rate than the 0.05ml group. However, this method of administration is difficult to apply in routine farm management. In conclusion, the high protection afforded by the *Vibrio* vaccine injection in cultured orange-spotted grouper strongly suggests it should be a good prophylactic measure against mortalities caused by both pathogens.

## Bottomline

As Asian aquaculture continues to grow, disease problems will inevitably become worse unless key steps are taken. Under the threat of disease epidemics and the vigilance of governments and consumers regarding food safety, the industry must undergo changes. Therefore, disease research and the implementation of new disease control concepts are inevitable. Collectively, this includes the use of healthy fry, quarantine measures, optimised feeding, good husbandry techniques, disease monitoring (surveillance and reporting), sanitation, vaccination, and the responsible use of chemicals and antibiotics when diseases occur. Overall, the emphasis must be on prevention rather than cure (treatment). This is the only way to sustain a responsible yet profitable Asian aquaculture industry.

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*References are available on request.*



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# Partnerships to advance hatchery best practices and improve production

by Jan Koesling

In terms of volume, China dwarfs Thailand but the latter leads with quality white shrimp post larvae production, advanced hatchery management technologies and innovations. A series of seminars by Thai experts is helping the hatchery sector in China with best practices and emphasis on quality.



Some 100 end users, retailers and distributors attended the international white shrimp workshop in Haikou, Hainan organised by Bayer Sichuan. Thai experts Gunnawit Ruchirawat and Thitiwat Leepaisomboon are in the centre in light blue shirts.

Asia leads the world in shrimp production, and in the region, Thailand is recognised for its technology and management practices in hatchery operations leading to superior results and quality post larvae. China, on the other hand, is the world's largest producer of post larvae with a production of almost 500 billion annually.

Similar to all industries, all eyes are on China. As the largest shrimp producer, its ability to produce large quantities influences the market elsewhere. Helping China's producers to maintain quality aside from just large volume is in the interest of all in the industry.

China, in turn, has its eye on Thailand. The country has for decades taken the lead in developing best practices and in particular the application of technology in hatchery production. The paradise island

of Phuket has become the centre where advanced technology is being developed by industry leaders and hatchery operators committed to implementing revolutionary hatchery management practices in large commercial operations.

## Back to Basics

The emphasis is on the importance of larval technology. Successful Thai hatchery operations such as Biotech Farm Consult, work with an associated farm, Burapa Farm which produces 4,000 to 6,000 tonnes of premium quality shrimp annually. Both parties recognise that larvae quality is a key success factor for shrimp performance at the grow-out stage.

Biotech Farm Consult, produces 3,000 to 4,000 top quality domesticated broodstock pairs per month together with 200,000 million nauplii. The broodstock and nauplii are distributed locally and worldwide. The hatchery has in place, a biosecurity program that includes:

- a three to four week quarantine period for the pre broodstock prior to maturation
- health checks for major viruses such as White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV)/ Gill-Associated Virus (GAV), Taura Syndrome Virus (TSV), Hematopoietic Necrosis Virus (IHNV), and Infectious Myonecrosis Virus (IMNV) and
- an emphasis on broodstock which have growth performing characteristics, rather than resistance or tolerance to pathogens. The post larvae from such specific environmental resistant (SER) broodstock have been proven to be more productive in the grow-out ponds.



Teams from Biotech Farm Consult, Guangdong Yuehai Feed, Haimao and Nanjiang companies.

Such systems are generally absent from China's hatcheries. However, while Thailand's hatchery operations are impressive in its management and technology, it is dwarfed by China in terms of volume.

Guangdong province alone has 500 hatcheries, with another 400 facilities operating in Fujian province and more than 300 shrimp hatcheries in Zhanjiang province. In Hainan, 340 shrimp hatcheries have been granted national licenses and many more are operating without licenses. Despite the staggering number of hatcheries, China's industry is in its infancy to some extent. Some may describe most of these hatcheries as backyard operations, with little application of advanced technology.

Bayer sees itself as partner to China's hatchery operators and realises that the backyard technology is hindering the local industry. To address this problem and to promote greater productivity, the company hosted a series of training seminars to allow customers from the different regions of China to learn from Thai shrimp breeding, hatchery and farming experts.

## Coaching China

In early December 2009, Bayer Sichuan conducted an international white shrimp workshop in Haikou, Hainan. This was attended by more than a hundred end-users, retailers and distributors from across China. Invited experts from the Biotech Farm Consult partnering with Bayer Animal Health provided hatchery and grow-out training. The expert group was led by the Thai experts Gunnawit Ruchirawat and Thitiwat Leepaisomboon. The experts sat down with hatchery operators in a discussion group where practical experiences and solutions were shared. Participants agreed that shrimp hatchery operations in Thailand are more advanced than in China, with sophisticated hatchery management processes and biosecurity protocols.

Seeing the need for more expertise-sharing and dialogue, Bayer Animal Health then arranged a field visit and training at Biotech/Burapa Farm Group located in Phuket Island for three of China's top aquaculture groups: Haid group, Evergreen group, and Wenqiang hatchery. The Haid group of Guangdong is the largest shrimp feed producer in China, and in 2009 it purchased two billion post larvae. It has plans to double this to four billion in 2010. The Evergreen group, also of Guangdong, is the second largest shrimp feed producer in China and one of the largest in the world. Wenqiang, on the other hand, has a new hatchery which has 1,500 pairs of broodstock.

Teams from the visiting companies learnt best practices on water treatment and water preparation, water quality management and water exchange during larval culture. In the Artemia hatching tank, stocking procedure for nauplii, larval feeding management, as well as post larvae harvesting and handling were taught.

Following the positive feed-back from this successful training session in Hainan and the farm visit to Phuket, the company decided to



give more farmers and hatchery tank operators in China the opportunity to obtain first-hand knowledge on applied hatchery technology from Thailand before the start of the season.

## Leading the way with innovation

Bayer Animal Health will continue to introduce innovations to the shrimp industry with the objective of producing a technology in Asia's aquaculture industry that is cost efficient and sustainable, and adheres to food safety standards. Last year, Bayer Sichuan opened the first exclusive aquaculture store just outside Haikou city. The shop provides proven technical support for the application of Bayer's products in hatcheries and grow-out farms.

Beyond that, Bayer Animal Health has proven itself to be a strong partner to China's shrimp hatchery and grow-out operations. It strives to service the industry with training and seminars designed to elevate the shrimp industry to one where sound technology and management practices are in place.



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# Part 1: User and producer perceptions on broodstock and hatchery production *Pangasius* catfish seed quality in Vietnam

By Ben Belton, David C Little and Le Xuan Sinh

**Variability in seed quality was linked as much to user perception as to technical or environmental conditions.**

This article details some of the findings of a British Council funded research project implemented in 2008–2010 by Can Tho University, Vietnam and the University of Stirling, UK, titled “Promoting sustainable high quality river catfish seed production in Vietnam”. The project’s goals were to investigate the status of catfish seed quality in the Mekong Delta and to identify any emerging problems with seed quality which might impact the industry’s development in future.

To this end a survey of hatcheries, nurseries and growout farms in the three main catfish producing provinces was conducted in order to determine user perceptions of seed quality and to identify practices which might affect them (Table 1).

The first part of the article deals with practices in the hatchery sector, whilst the second part in the next issue will address perceptions in nurseries and grow-out farms.

**Table 1. Fieldwork locations with types of operation.**

Province	Type of Operation		
	Hatchery	Nursery	Grow-out Farm
Dong Thap	17	11	4
An Giang	10	11	13
Can Tho	2	3	16
Total	29	25	33

## The hatchery sector

The Vietnamese *Pangasius* hatchery sector became established at the end of the 1990s, following successful research effort. Following that, farmers were no longer so dependent on the limited stocks of wild juveniles from the Mekong River. Prior to this, several communes on the upper Hau and Tien branches of the Mekong, close to the Cambodian



*Stripping eggs.*



border, have a long history of nursing wild seed. These experienced nursery operators became the first hatchery owners and most *Pangasius* hatcheries are still to be found in these same few communes today.

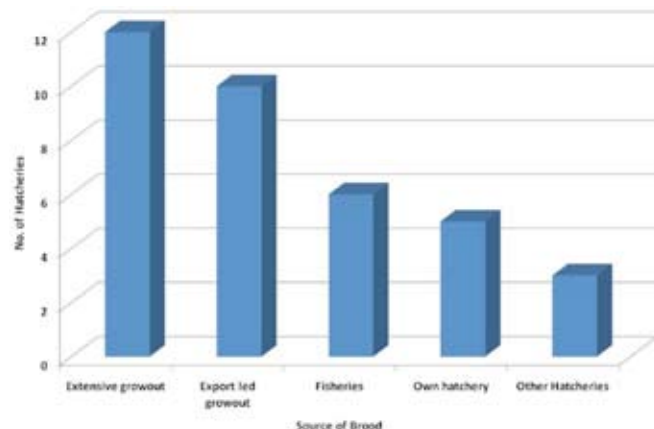
## Sourcing broodstock

Hatchery operators practice a very diverse set of strategies for sourcing broodstock, based on a variety of beliefs, opportunities, and constraints. These include:

- Perceptions of the relative productivity of wild and artificially propagated broodstock
- Attempts to maintain genetic diversity by sourcing broodstock from a number of locations
- Local availability of broodstock which varies from place to place

Figure 1 shows all the sources of broodstock reported by interviewees. Around half of those interviewed obtained broodstock from more than one source. However, the relative amounts of broodstock from each

**Figure 1. Sources of broodstock used by hatcheries.**



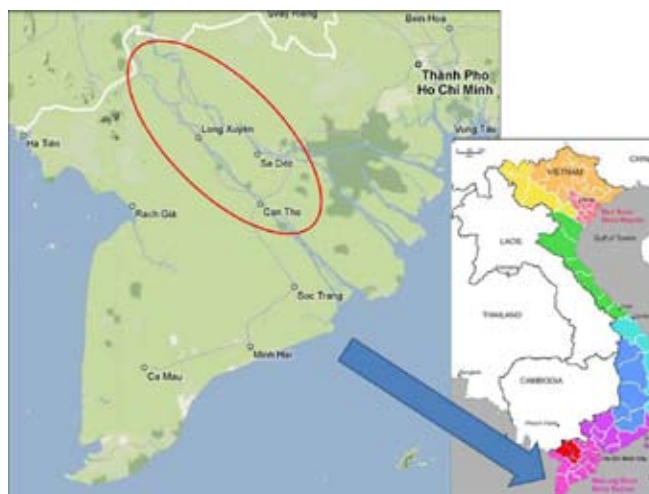
source used varied from hatchery to hatchery. The most common source of broodstock was extensive grow-out ponds culturing marketable fish for local markets and for subsistence purposes. Interviewees claimed that in most cases these systems are stocked with wild caught seed and some hatchery owners choosing broodstock from this source saw this as an important advantage.

Equally or more important however, fish stocked in these systems receive minimal supplementary feed, leading to slow growth. All of those interviewed who used broodstock from extensive ponds purchased sexually mature fish of three years old or more and weighing only 1.5-2kg.

During conditioning when they were fed a high protein diet, these fish grow very rapidly, become ready for spawning in just a few months and are therefore a very cost effective option.

Broodstock from intensive, export-led growout ponds are much more readily available. The market size is around 1kg and are rarely more than 12 months old. This means that they require time to mature and feed costs to condition fish prior to spawning is high. In general, a significant portion of broodstock continues to come direct from wild capture fisheries. Many hatcheries seek to obtain wild broodstock in order to diversify the genetic makeup of their stocks. Finally, fry produced in hatcheries can be kept and grown for use as broodstock. This approach has the advantage of allowing hatchery operators to select for favourable characteristics such as rapid growth from a very large pool of individuals by keeping only those which perform best, but requires long term planning and considerable investment.

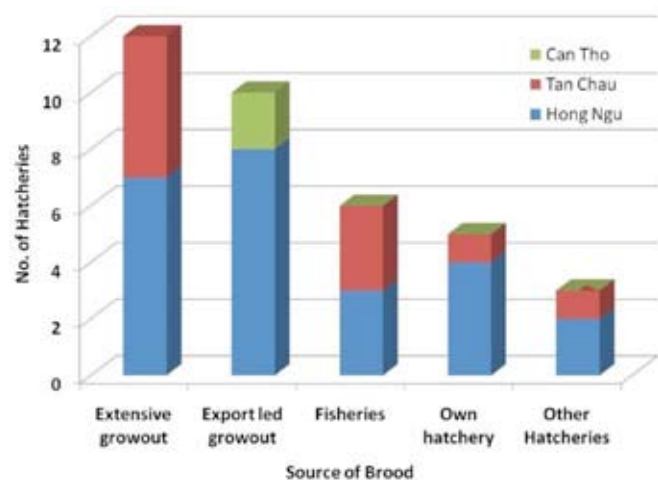
Map showing the main areas of catfish production in the Mekong Delta. The provinces of Dong Thap, An Giang, and Can Tho where the research took place are highlighted in red on the bottom right hand map.



broodstock used and hatcheries strongly favoured broodstock fish of artificially propagated origin on the basis that they found artificially propagated broodstock easier to spawn than those of wild origin. Hatchery owners also said that broodstock of farm origin produce up to 30% more eggs than wild broodstock, the eggs have higher hatching rates and the seed produced grow faster.

A number of historical factors may help to explain these differences. The hatcheries visited in Can Tho were recently established with the purpose of providing fry for the large growout farms to which they are linked. These farms provide an immediate source of broodstock. In Hong Ngu district, there are a very large number of hatcheries located very close together. This competition means that it is necessary to select broodstock from a large variety of locations and sources, and also explains the large diversity in the sources of broodstock used. Hatchery operators are also aware of the potential for inbreeding when using artificially propagated seed so most actively look for broodstock from a variety of locations and sources.

Figure 2. Sources of broodstock used in hatcheries by location.



## Location

If sources of broodstock are broken down by location, some interesting patterns in broodstock use preferences emerged (Figure 2). Only two hatcheries were visited in Can Tho (reflecting the small numbers of hatcheries located in the province) and both of these were part of large integrated commercial growout farms. These hatcheries therefore, had a large pool of fish close at hand to use as broodstock. None of the hatcheries visited in Tan Chau district of An Giang used broodstock from export oriented growout ponds. Every interviewee there claimed to use at least some broodstock of wild origin. Most of these hatchery operators obtained broodstock from local extensive growout ponds, said to be stocked with fry harvested from the Tien branch of the Mekong River.

Hatchery operators in Tan Chau district displayed a strong preference for broodstock of wild origin because they felt that they had a better shape and produced more eggs during spawning and that the survival of the seed was better. In sharp contrast, on the island district of Hong Ngu in Dong Thap province, which has the largest concentration of hatcheries in the Delta, there was far greater variety in sources of



Hatching fertilised eggs



Mixing eggs and milt

In Tan Chau district in An Giang province there are far less hatcheries than in Hong Ngu district and the traditional practice of harvesting and stocking wild seed in extensive grow-out ponds has continued despite the government ban in 2000 on using wild seed. Interviewees were quite open about this and the practice is still common, although on a small scale.

Interestingly, the state-run AnGiang Afiex Co (AFIEX) hatchery in Long Xuyen uses both fish of wild origin and their offspring as broodstock and reports that there is no difference in their performance because the environmental conditions in which they are raised and the nutrition they receive is exactly the same. Many interviewees also recognised the importance of feed in determining broodstock and egg quality and felt that feed quality and broodstock management had improved over the years since they started their operations.

This seems to suggest that there may actually be little substantive difference in the performance of wild and artificially propagated broodstock, provided good broodstock management practices are put in place. Improvements in egg productivity such as those reported in Hong Ngu district may therefore reflect general improvements

in management rather than the results of moving from use of wild broodstock to use of artificially propagated broodstock. However, while the inhabitants of Hong Ngu district strongly identify with artificial propagation because of its importance to the community, in Tan Chau district, where there are far fewer hatcheries, the traditional capture of small quantities of wild fish for stocking and on-growing continues. The perceptions expressed by hatchery owners in these two locations may therefore relate as much to localised cultural preferences as to any substantive differences in performance between wild and artificially propagated broodstock.

### Broodstock management

Other hatchery management practices vary considerably too. One hatchery (owned by AFIEX) maintained male and female brood at a ratio of 1:1, while some private hatcheries condition only one male fish for every nine females. Most hatcheries fall somewhere in the middle. These choices reflect a tradeoff between trying to maximise genetic variation and minimise feed costs. The number of times each fish is spawned in a year and the total period of time over which each fish is used for spawning is also variable.

Most hatcheries say they usually use broodstock weighing between 3 and 7kg, and spawn each female 2-3 times a year over a 3-year period. If certain fish are particularly fecund they will be retained for much longer, whilst poorer fish will be discarded more quickly. Many hatcheries give broodstock individual identification marks and maintain detailed records to facilitate management decisions. Several hatcheries reported that they spawn fish up to six or more times in a year if demand for larvae is high.

Some hatcheries reported that hatching rates vary by around 20% seasonally, with lowest rates during the late rainy season and cold season. Others reported no variation in hatching rates throughout the year. Similarly, some hatchery operators reported getting big improvements in hatching rates since starting their operations because their techniques had improved, whilst others reported no change at all. Egg productivity is lowest during the months from October to December, falling to 5% of body weight or less. This causes many smaller hatcheries to cease production at this time and causes a large jump in the price of larvae.

The market value of table sized catfish is the most important overall factor affecting hatchery output. A good farm gate price allows grow-out farmers to sell quickly and farmers then quickly restock with nursery seed. It follows that nurseries also require new larvae for restocking. Hatcheries adjust their production accordingly in line with this demand.



Measuring larvae for sale



Ben Belton



David C Little



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## Effect of *Bacillus* S11 on black tiger shrimp larvae

By Wannipa Phianphak, Pissamai Powedchagun and Sirirat Rengpipat

**The view that heterogeneous bacterial flora gives better growth and survival of shrimp larvae, is supported by this work in a commercial hatchery. Preliminary results indicated that the presence of probiotics in culture water increased survival rate of nauplii to 12 day old post larvae by 28% and profits were 22% higher.**

In 2005, the yield per crop in most shrimp farms in Thailand began to decline because of low quality brood stock and outbreaks of viral and bacterial infections. This resulted in stunted growth and high mortality rates. Farmers began to use antibiotics, which aside from increasing production costs also increased incidences of antibiotic residues in shrimp. The usage also increased bacterial resistance to certain antibiotics. The Thai Department of Fisheries then introduced the “zero tolerance” detection of antibiotics to produce quality and hygienic Thai shrimp. The farming is under the Code of Conduct (CoC) guidelines and included farm inspections in accordance with Good Aquaculture Practices (GAPs) for antibiotic residues (Prompoj, 2002).

### Probiotics

Shrimp farming using probiotics should be considered as a way to reduce the use of antibiotics and produce shrimp safe for consumption.

However, a suitable probiotic bacteria should be non-pathogenic strains of microorganisms and very specific to the intended host (e.g. *Penaeus monodon*). It is also required that the probiotic bacteria provide a balance of indigenous microorganisms within the intestines of the host and are easily and economically prepared at a commercial scale. It needs to have significant probiotic properties, as previously defined (Fuller, 1989, 1992, 1997; Gatesoupe, 1999; Tannock, 1999). Such selected probiotic strains should be well evaluated. This includes information on the mechanism of probiotic action on their host(s) and from a food safety aspect (Gatesoupe, 1999; Verschuere et al., 2000).

Recently, probiotics have been developed specifically for aquaculture, but they are still not widely used. There are, however, substantial potential advantages on the use of probiotics in shrimp and fish aquaculture (Austin et al., 1995; Gildberg et al., 1997; Rengpipat et al., 1998). *Bacillus* S11 (BS11) originally isolated from the intestines

of the black tiger shrimp, showed positive probiotic properties with shrimp growth and health in laboratory studies, including their ability to adhere onto the intestinal surface of the shrimp (Rengpipat et al., 2003; 2005; 2009).

However, the benefits of this probiotic bacteria during commercial scale production remains unknown. Therefore, this work looked at the effect of the supplementation of BS11 in culture water on growth and mortality rates of *Penaeus monodon* larvae during the rearing stages in a commercial hatchery.

## Bacterial strain

*Bacillus* S11 (BS11) was grown in Trypticase Soy Broth (TSB; Difco, Sparks, MD, USA) and maintained on Trypticase Soy agar at 30°C. Bacterial pellets were washed twice with sterile 0.15 M Phosphate buffered saline pH 7.2 (PBS), re-suspended in PBS and the absorbance of the final bacterial suspensions were adjusted to 1.0 at 660 nm using PBS. Bacterial suspensions were kept in glycerol (20% (v/v)) at -70°C until further use.

Live cells of 24-h old BS11 cultures were washed three times in sterile normal saline solution (SNSS) by centrifugation at 9820 x g at 4°C for 10 minutes and were then kept in ice before adding to the shrimp culture tank water.

## Larval culture

The trials were conducted in a private commercial shrimp hatchery, located near Chachoengsao Province, Thailand. The farm uses wild broodstock from the Andaman sea. Due to commercial and logistical reasons, only four circular cement tanks were available for use in these trials. The tanks were accordingly divided into two treatments (probiotic treated and control) with only two replicates.

Two such trials, conducted at different times, were performed. In both trials, 300,000 nauplii were added to each tank on day 1. The frequency of entering the hatchery area, including the time per visit were strictly controlled for biosecurity reasons and in order not to interfere with the daily commercial activity of the hatchery. As such only a small number of samples (n=100) were collected during each visit. This also prevented statistical analysis and limited the strength of the data. Therefore, these results can only be considered as preliminary indication and a stimulus for future work. The advantages, as well as the limitations, in this study are discussed later.

Live weights, survival rates and bacteria levels in post larvae (PL) shrimp were monitored. In each trial, each tank contained approximately 2 tonnes of seawater. Shrimp in the probiotic treated and control tanks were fed *Chaetoceros* sp. and a feed supplement.



The level of *Chaetoceros* sp. was checked four times a day to maintain a stable amount.

A third of the tank water volume was replaced with new seawater daily. Water temperature was 30 °C. The salinity at day 1 was 30-31.6 ‰ and this was gradually decreased to 18 -19.6 ‰ over the first 20 days of the culture period. In each trial, approximately 2g wet weight of live BS11 cells were added to the treatment (probiotic) tanks. Tanks were supplemented with the cells to maintain ~10<sup>4</sup> CFU/ml in the culture water, on day 3 (zoea-1), day 6 (mysis-1), day 10 (PL2), day 13 (PL5), day 17 (PL9) and day 20 (PL12).

The survival of PL15 shrimp on day 23 was reported by the hatchery owner after the shrimp harvest. Water samples were collected, along with live shrimp from each treatment, for determination of salinity, pH, temperature, ammonium, nitrite, nitrate, phosphate and total alkalinity.

## Bacterial counts

In each trial, on day 20, the live weights of 100 PL12 shrimp from each tank (20 shrimp collected randomly from five parts of the tank) were determined. Shrimp were immediately rinsed three times with SNSS and aseptically transferred into a new tube each and filled with SNSS up to 10 ml. The tube was sonicated for a few seconds and a serial dilution of the suspension was performed into SNSS. From each dilution, a 0.1 ml aliquot was transferred onto each of two plates of plate count agar (PCA), Trypticase soy agar (TSA), Chromocult® Coliform Agar (CCA, Merck KGaA, Darmstadt, Germany) and Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS). Water samples (100 ml) were randomly collected from five areas in each tank and kept in sterile bottles before plating 0.1 ml aliquots of serial dilutions. Colonies were counted.

The plate count agar method for total aerobic bacteria count followed the procedure specified in the FDA (1998). A 0.1 ml aliquot of the appropriate serial dilution was aseptically spread over the surface of each Petri dish (100 mm diameter) containing PCA (pH 7.0) and cultured in duplicate per dilution for 48 h at 35 °C. Plates with total colonies in the range of 30 – 300 within 48 h at 35 °C were counted. Aliquots (0.1 ml) of each serial dilution were plated and cultured on Chromocult® Coliform Agar (CCA). Plates with 15 – 150 colonies were selected for counting on a colony counter (Model 3328, Scientific Instrument, USA) and were identified as coliform (red) and *E. coli* (blue) colonies.

The growth and identification of *E. coli* from all methods was reconfirmed by re-isolation on EMB agar and IMViC testing. *E. coli* ATCC 25922, *Enterobacter aerogenes* ATCC 1415 and *Klebsiella pneumoniae* ATCC 27736 were examined at the same time as the controls.

Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS, Difco, Sparks, MD, USA) was used to plate 0.1 ml aliquots of each serial dilutions and incubated at 35°C for 24 h. Plates with 30- 300 colonies were selected for counting on a colony counter as above. Yellow (sucrose fermenters) and green (non-sucrose fermenters) *Vibrio* colonies were identified.

## Results and discussion

### Culture conditions

Water temperatures and other water quality parameters were similar in the four circular cement tanks of the two trials. There was no clear effect of BS-11 after addition to larval tanks during the experiment as shown in Table 1.

### Growth performance

Post larvae cultured in the probiotic treated tanks (with BS11 added) showed higher average weights (~2 times) and a higher survival rate

**Table 1. Changes in water quality parameters during the 20 day culture period.**

Parameters	Probiotic treated tank	Control tank
Water temperature (°C)	~29.1 to ~31.6	~29.1 to ~31.6
Salinity (‰)	~30-31.6 to ~19.6	~30-31.6 to ~18.1
pH	~7.5 to ~8.0	~7.5 to ~8.0
Dissolved oxygen (mg/L)	~4.2	~4.2
Ammonium (mg/L)	~0.5 to ~3	≤ 0.15 to ~1.0
Nitrite (mg/L)	≤0.1 to ~1.0	≤0.05 to ~1.0
Phosphorus (mg/L)	~0.1 to ~2.0	~0.1 to ~1.5
Total alkalinity	~119 to~156	~102 to ~153

**Table 2. Live weights and survival of post larvae shrimp fed with feed supplemented with live BS11 (Probiotic) or without (Control) after culture for 20 (weight) or 23 (survival) days.**

Parameters	Average live weight (g) <sup>a</sup>	Survival (%) <sup>b</sup>
Trial 1	~5.6	~90
Probiotic	~2.9	~70
Control		
Trial 2	~5.1	-
Probiotic	~2.6	-
Control		

*Note:*  
<sup>a</sup> Average weight of PL12 (n = 100) after random sampling 100 PLs per tank, from two tanks for each treatment and trial, after 20 days of culture.  
<sup>b</sup> The total number of PL15 shrimp that were harvested for sale from the two tanks per treatment in the first trial as the % of the original number of nauplii that were added into the tanks at the first day of culture.  
 All PL15 were harvested after 23 days of culture.  
 No results (-) are available for the second trial.

when compared with those in the control shrimp (Table 2). The projected PL15 shrimp yields (for two tanks) were 540,000 and 420,000 for the probiotic treated and control tanks, respectively. There was about 25% extra yield in the probiotic rearing tanks. According to the farm owner, the net profit was 22% higher for the probiotic treated shrimp as compared to the control shrimp.

This was in spite of the limited sampling (only two tanks per trial, two trials, and the addition of antibiotics and termination of BS11 supplementation within one of these trials) and which did not allow for statistical analysis. The data does support the possibility of using BS11 as a probiotic with significant benefits in larval shrimp culture water, although there is clearly a need for some confirmation studies. Similar results have been reported previously for other probiotics in shrimp (Boyd & Gross, 1998; Chintanapunt, 2003; David et al.,1998; Duangbansao, 2001; Moriarty, 1998) and shrimp larvae (Zhou et al., 2009).

**Luminescent bacteria**

In the second trial, on day 10 of the culture period, visible luminescence of the water in the control tanks during the night was observed with the naked eye. The antibiotic rhodomycin was added (15g/tank/day). This is a standard commercial practice. However, the luminescence continued after a week and post larvae shrimp began to die. On day 17 of the trial, luminescence was also observed in the probiotic treated tanks, where again the antibiotic was added and BS11 supplementation of the water was stopped.

Nevertheless, random samples of PL12 shrimp (n = 100 /tank) after 20 days of culture from all four tanks were weighed. Three days later, luminescence was again observed and the remaining post larvae shrimp were harvested a week afterwards. Therefore, no data for the survival rate was recorded.

However, the average weight of the probiotic PL12 shrimp (n = 100; ~5.1 g) was more pronounced at ~2 times that of the control shrimp (~2.6 g). Thus, despite the clear potential differences in *Vibrio* infection which may have had an adverse effect on shrimp development, the data from the two trials were within the general trend in terms of a larger weight gain in the probiotic treated shrimp relative to the control shrimp. The addition of BS11 directly into the culture water (this study) gave the same beneficial results on shrimp as that reported for probiotics added to feed for shrimp cultured in earthen ponds (Rengpipat et al., 2003).

**Bacterial counts in shrimp**

After 20 days of culture, the bacterial flora of shrimp was determined (Table 3). The total viable bacteria count (TBC) of shrimp larvae from the probiotic treatment tank was some 2.6 and 4.1 times higher than in the shrimp larvae in the control tank in trial 1 and trial 2, respectively. As expected, BS11 was detected in the probiotic treated shrimp but not in the control PL12 shrimp in both trials. It was likely that the lower level of BS11 found in probiotic treated shrimp in the second trial reflected the effects of the termination of the BS11 supplementation and addition of the antibiotic following the luminescent *Vibrio* infection. Thus, BS11 accounted for 62.5% of the TBC in probiotic treated shrimp from the first trial, but only 1.4% in the second trial.

Coliforms were only detected in trial 1, and not trial 2 (perhaps due to the antibiotic effects). In contrast, *E. coli* was only found in trial 1 but only in the probiotic treated PL12 shrimp and at low level.

Green *Vibrio* spp. were found in both trials and were ~45 and ~104-fold higher in the control than the probiotic treated PL12 shrimp in trials 1 and 2, respectively.

More yellow *Vibrio* spp. were also found in the control ~1.6 and ~9.6 fold higher than probiotic treated shrimp in trials 1 and 2, respectively and comprised a larger proportion of the TBC in the second trial (~5.7% and ~23.6% versus ~9.2 and ~36.1% for probiotic treated and control PL12 shrimp, respectively, in trial 1 versus 2, respectively). Generally, *Vibrio* spp. are widely distributed in normal flora in sea water. It is likely that this is the main reason why they were detected in healthy shrimp, shellfish and fish (Nakai et al.,1997; Vandenberghe et al.,1998). However, they can cause secondary infections as opportunistic pathogens in immunologically compromised shrimp (Lavilla-Pitogo et al.,1990; Lightner, 1992; Moriarty, 1998; Saulnier et al., 2000). Our results indicated a heterogeneity of bacteria, including *Vibrio* spp., coliforms and *E. coli*, in shrimp of trial 1 which may reflect a good balance of flora in shrimp. Moreover, it is possible that BS11 as a probiotic could exist in shrimp and may reduce green *Vibrio* spp. level by competitive exclusion.

**Table 3. Bacterial counts of PL12 shrimp larvae after the 20 day culture period.**

Experiment	Bacterial count (CFU/g)					
	TBC	BS11	Coliforms	<i>E. coli</i>	<i>Vibrio</i> spp.	
					yellow	green
Trial 1						
Probiotic	~7.76x10 <sup>4</sup>	~4.85x10 <sup>4</sup>	~7.50x10 <sup>2</sup>	~9.97x10 <sup>1</sup>	~4.40x10 <sup>3</sup>	~9.90x10 <sup>1</sup>
Control	~2.98x10 <sup>4</sup>	nd	~1.00x10 <sup>2</sup>	nd	~7.05x10 <sup>3</sup>	~4.50x10 <sup>3</sup>
Trial 2						
Probiotic	~6.70x10 <sup>4</sup>	~9.05x10 <sup>2</sup>	nd	nd	~6.13x10 <sup>3</sup>	nd
Control	~1.63x10 <sup>5</sup>	nd	nd	nd	~5.88x10 <sup>4</sup>	~1.86x10 <sup>4</sup>

*Note:* Data represents the average value from duplicate tanks per trial  
 nd = not detected

**Table 4. Bacterial counts of culture tank water after culture of shrimp for 20 days.**

Experiment	Bacterial count (CFU/g)					
	TBC	BS11	Coliforms	<i>E. coli</i>	<i>Vibrio</i> spp.	
					yellow	green
Trial 1						
Probiotic	~1.19x10 <sup>5</sup>	~4.24x10 <sup>4</sup>	~1.82x10 <sup>2</sup>	~1.32x10 <sup>2</sup>	~2.69x10 <sup>3</sup>	~3.33x10 <sup>2</sup>
Control	~1.42x10 <sup>5</sup>	nd	~3.39x10 <sup>2</sup>	~2.27x10 <sup>2</sup>	~2.02x10 <sup>3</sup>	~6.42x10 <sup>2</sup>
Trial 2						
Probiotic	~1.99x10 <sup>5</sup>	~4.41x10 <sup>2</sup>	nd	nd	~2.80x10 <sup>3</sup>	~2.24x10 <sup>4</sup>
Control	~2.77x10 <sup>5</sup>	nd	nd	nd	~1.57x10 <sup>5</sup>	~2.51x10 <sup>5</sup>

Note: Data represents the average value from duplicate tanks per trial  
nd = not detected

### Bacterial counts in tank water

These were summarized Table 4. After 20 days of culture, the TBC was at the same level of ~10<sup>5</sup> CFU/ml in both trials. As expected, BS11 was detected in probiotic treated water but not in the control rearing water in both trials. The lower level in the second trial presumably reflected that the supplementation of the water with BS11 was stopped after the luminescent *Vibrio* infection was observed.

Neither coliforms nor *E. coli* were detected in the water of probiotic treated and control tanks in trial 2 (Table 4), consistent with their absence in shrimp (Table 3) but were present in both probiotic and control tanks in the first trial, and were at a higher (1.9- and 1.7 fold for coliforms and *E. coli*, respectively) level in the control culture water.

In the first trial, green *Vibrio* spp. outnumbered yellow *Vibrio* spp. in control tanks ~1.93-fold, as compared to probiotic tanks in accord with that seen in the shrimp tissues. Whilst in the second trial green *Vibrio* spp. outnumbered yellow in control ~11-fold as compared to probiotic shrimp which is consistent with the luminescent bloom seen in the water and in the shrimp.

The green and luminescent colonies on TCBS were randomly selected and restreaked on TCBS for identification. They were Gram-negative rods, showed no oxidase production and gave biochemical tests which could be confirmed as *Vibrio harveyi* (data not shown). LD50 of *V. harveyi* of 10<sup>2</sup>-10<sup>3</sup> CFU/ml on post larvae shrimp (Lavilla-Pitogo et al.,1990) and of < 10<sup>3</sup> CFU/ml on protozoa or >10<sup>3</sup> CFU/ml on the mysis of *P. monodon* by bathing (Prayitno & Latchford, 1995), were all lower than the results indicated here, namely that the presence of green luminescent *Vibrio* spp. at higher levels > 10<sup>4</sup> CFU/ml in culture water should be considered as an unsuitable condition for shrimp larvae culture.

The role of BS11, one of the heterogeneous shrimp associated bacteria, may have been to contribute to the ecological microbial

balancing of microorganisms in post larvae shrimp and the culture water (Tables 3 and 4) by interfering or competitively excluding the existence of other bacteria. The results from this preliminary study indicated this possible trend and evidence of the number of luminescent *Vibrio* spp. that could cause mortality in shrimp.

Green luminescent *Vibrio* spp. should be kept at <10<sup>3</sup> CFU/g in shrimp to maintain healthy shrimp with good production (Table 3) or <10<sup>4</sup> CFU/ml in culture water (Table 4). The ability of BS11 to produce extracellular caseinase (protease), amylase and lipase enzymes may function beyond probiotics in the gastrointestinal tract of shrimp, as it may also help in degrading all the left over nutrients and facilitate a better and healthy environment for the relatively high densities that post larvae shrimp are commercially reared in.

### Conclusion

BS11 can be transmitted to shrimp either via culture water or as a feed additive, depending on the developmental stage of the shrimp. The ecological balancing of microorganism in water for shrimp culture would be an important factor to be considered and should be further evaluated. It is apparent that heterogeneous bacterial flora in shrimp culture water tends to indicate a healthy habitat for post larvae shrimp culture. After these BS11-fed post larvae shrimp are transferred from the hatchery to earthen ponds, the outcome of shrimp production should be monitored in order to evaluate the benefit of using probiotics.

### Acknowledgments

We greatly appreciate the contribution by PP farm, Chachoengsao, Thailand with the commercial shrimp rearing facility for this research. This research was supported by the Chulalongkorn University Graduate School Thesis grant, the Thailand Research Fund via Chulalongkorn University and the NEDO fund from Japan.

References are available on request.



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# Vaccine to control VNN in groupers and sea bass

This has been developed by scientists at the Iloilo-based Aquaculture Department (AQD) of the Southeast Asian Fisheries Development Center (SEAFDEC).

Viral Nervous Necrosis (VNN), also known as Viral Encephalopathy and Retinopathy (VER), has in the last two decades inflicted serious economic losses in the culture of high value marine fish species, not only at the larval and juvenile but importantly at the grow-out stages. An urgent need for preventive measures that include the selection of disease-free broodstock, disinfection of eggs to control the vertical transmission of the virus from broodstock and vaccination to enhance fish immunity is required, said Leobert D. de la Peña and Rolando Pakingking, Jr, who worked on vaccine development at the hatchery and grow out stages respectively.

Leobert D. de la Peña artificially infected eggs with the virus and were either washed with ozone-treated seawater or immersed in iodine and Virkon. The eggs were checked for the virus before and after infection as well as after disinfection using the polymerase chain reaction (PCR) test and cell culture. The experiments showed that the effective time exposure and concentrations using ozone-treated seawater, iodine and Virkon were 2.5 minutes, 25 parts per million (ppm) and 1.5 ppm, respectively.

The study also determined that the groupers (*Epinephelus fuscoguttatus* and *E. coioides*) were susceptible to the virus. The milkfish (*Chanos chanos*) and siganid (*Siganus guttatus*) were not. Grouper larvae weighing 8g and below were highly susceptible with a mortality rate of 80%-100% in four to eight days. Fish weighing 90g and above are no longer susceptible to the virus.

The most susceptible size of the grouper was then vaccinated. The vaccine, which was prepared from DNA plasmid encoding the capsid protein of the virus, was intramuscularly injected to the fish at 5, 10 and 100 nanograms (ng). Challenge tests using intramuscular



injection of the virus were conducted after 15, 30 and 45 days from the vaccination. Results showed that fish vaccinated with 100ng had the highest survival rate.

Pakingking and colleagues developed a formalin-inactivated vaccine to control the disease at the grow-out stage. Three studies pertaining to the efficacy of the vaccine were conducted from June 2007 to November 2009 under the project "Fish Disease Surveillance System" funded by the Government of Japan Trust Fund 4. The vaccines were tested on sea bass (*Lates calcarifer*) and grouper (*E. fuscoguttatus*).

The first study, published in the Journal of Fish Diseases 2009, focused on the immune responses of the Asian sea bass to a formalin-inactivated betanodavirus vaccine. The fish produced neutralizing antibodies at high titer levels from Day 10 to Day 116, with the highest titer at Day 60 post-vaccination. No mortalities were reported after the vaccinated fish were challenged with the red-spotted grouper nervous necrosis virus (RGNNV, a betanodavirus). The result showed that the potential of the formalin-inactivated RGNNV vaccine against viral nervous necrosis of Asian sea bass.

The second study dealt with the vaccination of the brown-marbled grouper (*E. fuscoguttatus*) while the third study was conducted to determine the susceptibility of hatchery-reared pompano (*Trachinotus blochii*) to betanodavirus and its immune responses to a formalin-inactivated betanodavirus vaccine.



Tiger grouper and seabass, pictures courtesy of Christopher Co, Overseas Feed Co, Philippines



Dr. Leobert D. de la Peña and Dr. Rolando R. Pakingking, Jr.

## AQUATECH 2010



In the Philippines, AQUATECH 2010 will be held from April 6 to 8, 2010 at Bohol Beach Club, Panglao, Bohol, Philippines. Events Quality and Interactive Promotions, Inc. (Equip, Inc.) is organising this as an annual event to continuously update the aquaculture industry on new trends and technology. It is organised in collaboration with the Republic of the Philippines through the Municipality of Calape, Bohol and Bureau of Fisheries and Aquatic Resources (BFAR).

The theme of the event will be "Aquaculture Technology Philippines" which will be towards improving efficiency and profitability. It will showcase new frontiers in aquaculture activities, address issues affecting the sector, create awareness on best management practices among farmers and create new investment opportunities. The major component of the upcoming event will be an exhibition of stakeholders, farmers, hatcheries and other input suppliers, machinery manufacturers and seafood processors highlighting the achievements made by their respective sectors.

More information: Web: [www.equipinc.webs.com](http://www.equipinc.webs.com) Email: [info.aquatechcon@gmail.com](mailto:info.aquatechcon@gmail.com), Tel: +63 2 748-0847/482-4046/703-7938.

# Join us in Porto



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[www.aesonline.org](http://www.aesonline.org)

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# A firm commitment to aquaculture at Aquaculture 2010



This also confirms Biomin's keen focus on this dynamic growth sector. For the first time, Biomin will partner the World Aquaculture Society (WAS) with the sponsorship of the Aquafeeds session. This will be held in San Diego from 1 to 5 March. Aquaculture 2010 is the largest event for the global aquaculture industry. The event is expected to draw some 4,000 participants from over 80 countries.

Held on the 4th of March, the session on 'Aquafeeds' will see expert speakers from commercial practice and academia share their insights on major issues in aquafeeds formulation and alternatives to fishmeal and protein replacement strategies for a variety of aquatic

species. Dr. Pedro Encarnação, Biomin's Aquaculture Specialist, said, "We are a research-focused and -driven company. We have translated our longstanding competence in animal nutrition for terrestrial species into aquaculture and aquafeed research. Our Aquatic Centre for Applied Animals (AQUACAN) in Thailand actively conducts feeding trials for many aquatic species, from which new findings have been translated into innovative and practical solutions in aquafeeds."

Biomin will also participate as a sponsor at the Australasian Aquaculture 2010, to be held in Hobart, Australia in May, and at the Aquaculture Europe 2010 that will take place in Porto, Portugal in October, both of which are key events for aquaculture in 2010.

## New corporate slogan "Naturally ahead"

For over 25 years, going 'the natural way' has been Biomin's mission. With its focus on natural growth promotion in animal nutrition, the company is anchoring its firm commitment towards sustainable animal production with a new slogan 'Naturally ahead'.

From February 2010, 'Naturally ahead' replaces 'The Natural Way' as the new corporate slogan that strongly encapsulates its vision to be at the forefront of advances in animal nutrition and science, while effectively communicating its steadfast dedication towards improving animal health and performance 'the natural way'.

Erich Erber, Founder, said, "We at Biomin are dedicated to finding innovative trend-setting solutions that empower our customers to master existing and future challenges in animal nutrition. 'Naturally ahead' conveys this commitment to enable our customers to stay naturally ahead in their business."

The company was the first to promote the use of natural feed additives in animal production during the early-80s, during which interest in such products was negligible. This foresight and commitment to its vision for natural alternatives have paid off. Today, the company is widely recognised in mycotoxin control and risk management, with equally strong portfolios in acidifiers, probiotics and phyto-genic feed additives.

A successful concept for over 25 years now, 'The Natural Way', far from losing relevance, has paved the way for the company's new slogan 'Naturally ahead'. With that, Biomin continues to affirm its long-standing commitment towards groundbreaking research and cutting-edge technologies in animal nutrition.

More information: Web: [www.biomin.net](http://www.biomin.net)

## Real-time PCR shrimp virus detection

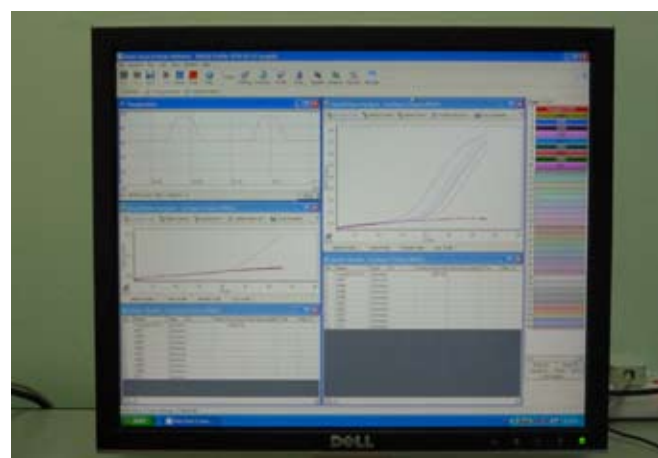


Malaysian based Lab-Ind Resource (LIR Biotech), a leader in shrimp and fish PCR (polymerase chain reaction) viral detection has invested and upgraded its laboratory to offer real-time PCR services to the aquaculture industry. It is the only private

laboratory in Malaysia and in the region, offering shrimp viral detection using a real-time platform that allows qualitative and viral load (for research purposes) determinations. The laboratory is in the process of working towards ISO 17025.

In a press release, the company said that it now offers viral screening and diagnostics for shrimp the detection of OIE listed diseases; WSSV-white spot syndrome virus, TSV-Taura syndrome virus, IHNV-infectious hypodermal and haematopoietic necrosis virus, YHV-Yellow head virus, IMNV-infectious myonecrosis, MBV-Penaeus monodon-type baculovirus, HPV-hepatopancreatic parvovirus disease. GAV- Gill-associated virus, MrNV-Macrobrachium rosenbergii nodavirus. Currently, it offers the detection of WSSV, TSV, IHNV, YHV and IMNV using real-time PCR. The real-time PCR is much faster and result is specific and sensitive. In fish, diagnostics are for detection of KHV-koi herpes virus, VNN-Viral nervous necrosis and Iridovirus.

The company provides support services all along the whole aquaculture production and supply chain from hatchery to processing



Tests running on Rotor-Gene Q System

plants. It carries out residue screening where it has the capability to help evaluate screening tests of antibiotics according to EC/657/2006 Guidelines) and food safety and hygiene. Aside from Malaysia, the professional team has worked extensively with government agencies and aquaculture industry in Indonesia, Thailand, Brunei and Bangladesh.

More information: [www.myladind.com.my](http://www.myladind.com.my); Email: [labind@myjaring.net](mailto:labind@myjaring.net)

# Strategic cooperation agreement on recirculation technology for white shrimp

Charoen Pokphand Foods Ltd., leading producer and supplier of farmed white shrimp, and AKVA group ASA, leading supplier of technology for the aquaculture industry, have signed a strategic cooperation agreement for developing a complete new production system for the production of white shrimp utilizing AKVA's recirculation technology and CPF's broad knowledge in shrimp farming.

CPF is working actively to improve their production methods and technology. A natural next step for the shrimp farming industry is to utilise new technological opportunities to address many of the challenges of the industry and society today. The first step of the strategic cooperation includes developing a full scale prototype of a 200 tonnes/year intensive facility for white shrimp (*Litopenaeus vannamei*), as an integrated solution.

"This strategic cooperation agreement is an important step for us to strive towards a transformation of the shrimp farming industry. By this project we are addressing important issues for the industry such as the level of which marine resource and land areas are utilized; the escaping of shrimp from farms to interfere with wild stocks; the control of diseases and efficient battling of parasites; the elimination of, and risks of pollution from shrimp farms and other possible effects on the habitats where the farming is done. Further to this we believe that the technology that this strategic cooperation agreement will produce has the potential also to limit the carbon footprint of the produced shrimp significantly", said Dr. Chingchai Lohawatanakul, Chairman of the executive Board of CPF.

"For our group this is an important step forward in the development in the Asian market and the furthering of our recirculation technology in the global market. We appreciate the initiatives and eagerness that CPF is showing in this project and are looking forward to the results from this cooperation for both parties", said Knut Molaug, CEO of AKVA group.

More information: Email: [kmolaug@akvagroup.com](mailto:kmolaug@akvagroup.com) (Knut Molaug).



Back from left: Robins P. McIntosh (Senior Vice President, CPF), Jan Erik Svensson (Regional Director, AKVA group SEA Co., Ltd.), Dr. Chingchai Lohawatanakul (Chairman of the Executive Board, CPF). Front from left: Knut Molaug (CEO, AKVA group), Dr. Sujint Thammasart (Executive Vice President, CPF)

## European investment in land based aquaculture

Cell Aquaculture has secured an USD2.4 million investment from Dutch based private equity fund, Linnaeus Capital Partners. The equity fund targets high growth potential companies and now plans to develop a global presence in aquaculture.

Anita Hamilton, Linnaeus Director, said, "We see tremendous opportunity in the aquaculture industry particularly in the land-based recirculating sector. Following thorough due diligence and having searched the world for a suitable aquaculture investment, we believe that Cell Aquaculture is exceptionally well positioned to capitalise on the booming aquaculture industry".

Cell Aquaculture said the investment was the first stage of a planned long term funding and investment strategy. The principals of Linnaeus will be meeting with Cell Aquaculture representatives in both Malaysia and Australia to review the company's growth plans and

develop a further funding and investment strategy. Discussions will also include European expansion opportunities.

Cell Aquaculture Limited (Cell) with its headquarters in Western Australia, is ASX listed. Cell supplies a full range of environmentally sustainable, vertically integrated seafood production services, encompassing everything from 'Hatch to Dispatch'. This Cell™ propriety system is a complete land based environmentally responsible aquaculture system developed for the production and supply of premium quality fin fish. Cell has established hatchery operations for Australian Barramundi at James Cook University, Queensland and has plans to construct a multi-species hatchery in Malaysia. It has developed large scale land-based recirculating seafood production facilities in Terengganu, Malaysia and signed MOUs for a large scale production in South Africa and Malaysia as well as for a land based grouper production facility in Singapore.

# New feed additive improves fish survival and performance



In January, Zamira Life Sciences launched their new microbial feed additive, Marine LABS, in Malaysia.

Other Asian markets will follow in

2010. Dale Harris, Zamira Sales and Marketing Director has just been travelling in Malaysia explaining to fish farmers on this new product and how it can help the Malaysian industry.

"The Malaysian fish farming industry is facing a range of challenges at present, with bacterial disease being a major issue with freshwater and marine fish farms. It has been great to see that the commercial farms currently using this feed additive in Malaysia are seeing excellent results with increased survival and fish performance", Harris said. "We expect more and more farmers will need to use disease prevention strategies, with the increasing pressure on the use of antibiotics and high levels of mortality from Streptococcal and other bacterial diseases that we are seeing in Malaysia at present".

Marine LABS contains a specially selected strain of bacteria called *Bacillus polyfermenticus* (KUS2 strain), which has proven efficacy in protecting fish from pathogenic infection. It is designed to be used throughout the growing cycle, from hatch to harvest, to ensure maximum protection from a wide range of diseases. Disease challenge experiments and commercial field trials with this feed additive in Korea and Malaysia have shown significant improvements in survival rate of marine and freshwater fish species. Growth rate and feed conversion



improvement has also been demonstrated with its inclusion in feeds through the grow-out cycle.

In-vitro testing at Australia and Korean universities on a wide range of pathogenic bacteria has shown excellent antibacterial activity against major aquaculture disease species such as *Streptococcus spp.*, *Vibrio spp.*, *Flavobacterium columnare*, *Flexibacter tractuosus*, *Edwardsiella tarda* and *Photobacterium damsela*. The product is included with the feed at a rate of 1 kg per tonne of feed and can play an important role in fish health management at all commercial fish farms.

More information: Email: [genpharma@pahangpharmacy.com.my](mailto:genpharma@pahangpharmacy.com.my) (Genpharma) for Malaysia; Web: [www.zamira.com.au](http://www.zamira.com.au).

## BAP standards for Pangasius farms

# GAA welcomes public comments on BAP pangasius standards

Drafts of the new Best Aquaculture Practices standards for Pangasius farms, developed under the guidance of the Standards Oversight Committee of the Global Aquaculture Alliance, are now posted for public comment at [www.gaalliance.org/bap/comments.php](http://www.gaalliance.org/bap/comments.php).

The new BAP standards apply only to facilities that raise Pangasius in ponds. The Best Aquaculture Practices certification program has separate standards for channel catfish farms. Two draft documents, the standards/guidelines and accompanying audit form, are provided online in PDF format. The first file outlines the standards, as well as the reasons for their requirements and how best to comply with the standards. The draft audit form indicates how auditors will review Pangasius farms for compliance with the BAP standards.

In addition to social and environmental responsibility, the Pangasius farm standards encompass food safety and traceability. Audit questions address biodiversity protection and quantify water quality in pond effluents. They also require controlled use of chemical

treatments and monitoring of feed ingredients from marine sources. Another standard specifies ongoing management and proper disposal of pond sludge.

The Standards Oversight Committee (SOC) recently approved the release of the new standards for public comment after reviewing an initial draft submitted by the Pangasius Farm Technical Committee. After evaluating comments received, the technical committee will consider changes to the standards and forward a revised document to the SOC for further approval. All properly submitted comments will be acknowledged.

Comments can also be sent by e-mail or fax to BAP Standards Coordinator Daniel Lee ([dangaelle@aol.com](mailto:dangaelle@aol.com), +44-1248-716729) or Technical Committee Chairman Philippe Serene ([sereneconco@vnn.vn](mailto:sereneconco@vnn.vn), +84-8-5121-259). Interested parties must submit comments by March 31. For additional information on the comments process and Best Aquaculture Practices certification, contact Daniel Lee at +44-1248-712906, e-mail [dangaelle@aol.com](mailto:dangaelle@aol.com).

## New appointment

# Regional Marketing Manager, Aqua

Animal health and nutrition company, Novus International Inc., has appointed Ramakanta Nayak as Regional Marketing Manager, Aqua for Asia-Pacific region. Ramakanta is a post graduate of the Central Institute of Fisheries Educations, Mumbai, He also has an MBA from Indian Institute of Management, Calcutta. He has been in the industry for almost 12 years, previously with Sankyo, Kanoria Chemicals and Novozymes. This new appointment reflects the commitment of Novus to expand its business in the aquaculture industry with sustainable solutions in the region. Rarnakanta will be based in Bangkok, Thailand.



# Opens new plant in Singapore to expand global reach

**A milestone for this industry leader in animal health and nutrition, Novus International's new manufacturing facility in Singapore brings more flexibility in delivery of solutions to customers in Asia. For the opening on 16 January 2010, Novus hosted customers from Singapore, Thailand, Malaysia, Vietnam and India as well as regional press.**

"With increasing demand, we did a logistical study and decided that Singapore's vast distribution network would help. Raw materials for Novus products come from Asia, Europe and USA. The common factor is that all our products follow the Novus quality standard", said Thad Simons, President & CEO. "The plant will be producing animal feed supplements for the poultry, pig, pet, ruminant and aquaculture industries in the Asia Pacific region, aside from China where there is already a plant".

The new plant will produce its first product, a trace mineral blend, followed by ACTIVATE® DA, nutritional feed acid and Cibenza™ DP100, feed additive. The raw material addition, liquid addition and blending processes are all PLC controlled to ensure precise product formulation control. The site is also fully compliant with the Singapore regulatory authority (Agri-Food & Veterinary Authority of Singapore).

The new plant is part of the company's focus on a 'knowledge driven product expansion' in six core areas: nutrition and health, amino acids, minerals, energy metabolism, specialty and feed quality for the poultry, pork, pet, ruminant and aquaculture industries.

The plant will offer several solutions specific to the aquaculture industry. MERA™ Met Ca, a small particle calcium salt of HMTBa, and MERA™ Cid, a proprietary blend of organic acids. Both of these offerings are beneficial to aquaculture producers as they support favorable bacteria by acidifying the gut environment. These products also help to support antibiotic free production. MINTREX® chelated trace minerals feature fully-defined structures which are stable at various pH ranges found in the gastrointestinal tract. This results in enhanced mineral absorption by fish and shrimp.



Guests from Thailand, from left; Bunluesak Sorajjakit, Thai Union Feed Mill, Thanomvong Taepaisitphongse, Betagro Group, Ladda Siriprasertsilp, Cargill Siam Ltd and Chamlong Termglinchan, Sun Feed Co Ltd.

"We understand that a customer's needs vary based on their geographic location," said Giovanni Gasperoni, Executive Vice President, Marketing and Sales. "Manufacturing facilities like this one empower our employees to support our local and regional customers in a way that brings indisputable value to their businesses."

The new plant covers an area of 4,000 square meters. Areas of the facility are dedicated to a raw material warehouse, a two-level integrated manufacturing area and a one stop distribution warehouse for the Asia Pacific region.

Yiannis Christodoulou, Regional Director, said, "I believe that by having a plant here, we are offering flexibility to our clients, in terms of the delivery of specific solutions to achieve their goals. We have experienced growth in the Asia Pacific and China regions and as a result, have set up offices and added local staff to service our clients. We are also expanding our research programs to other countries. Currently, we are connected with individuals in India, China and Vietnam."

The plant has targeted ISO 22000 in quality management, ISO 9001 in food safety management and HACCP (food hygiene) to be completed by the first quarter in 2010. It is also targeting FAMI-QS, the European Feed Additive and Pre Mix Quality systems which will open doors for clients to export finished products to European Union customers.

"We believe that to give the best to the customer is via these internationally recognized quality systems, said Leow Tong Hoe, Plant Manager. "The overall capacity of the blending plant is 60 tonnes/per day. To ensure precise product formulation, the raw material, liquid addition and blending processes take place in an air conditioned environment of 25°C and 45% humidity. The process is fully automated with very little human involvement. Product traceability is ensured through a supplier approved program and quality control is currently conducted by a Novus approved laboratory in Singapore", added Leow.



A traditional lion dance to open the new plant. From left: Jeffery B Klopfenstein, Senior VP operations & IT, Giovanni Gasperoni, Thanomvong Taepaisitphongse, Betagro group, Thad Simons, Shah Habibul Haque, Aftab Bahumukhi Farms Limited, François Fraudeau, VP, Europe, Asia & Africa Sales, Novus Europe SA/NV, Yiannis Christodoulou and Leow Tong Hoe.

# Freshwater Prawns: Biology and Farming

By Michael Bernard New, Wagner Cotroni Valenti, James H. Tidwell, Louis R. D'Abramo and Methil Narayanan Kutty (Editors)

ISBN: 978-1-4051-4861-0; Hardcover; 560 pages

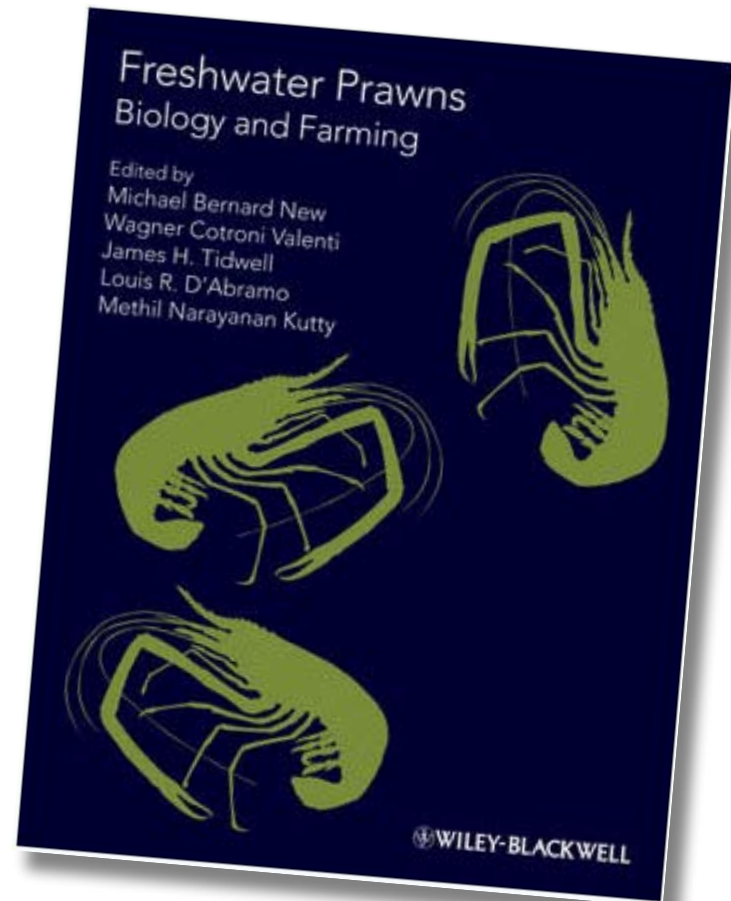
The rapid expansion in the farming of the freshwater prawn culture since 2000 has continued into 2007 in Bangladesh, China, Thailand and Vietnam but has declined in Brazil, India, Indonesia, Malaysia and Taiwan. Reasons cited for the decline in production range from poor brood stock, low quality post larvae and diseases. This comprehensive compilation of information and reviews by 44 internationally well known experts is timely as the industry is looking at addressing these problems. With 24 chapters, the book covers almost all of what is known today on the biology of the species and technical and commercial aspects of the industry.

In the first chapter which gives an overview of global production of the species up to 2007, the prognosis of the author, Michael B New is that the future is bright for the industry but transfer of more improved rearing and processing techniques are fundamental for its expansion. Subsequent chapters in the book are designed to impart as much of the available information on each aspect of its farming. Overall, the information targets the global culture of the main species *Macrobrachium rosenbergii*, even though most of the information would be relevant to the culture of other species. Nevertheless, a chapter is dedicated to the culture of several other *Macrobrachium* species of importance.

The book is well planned as the chapters are arranged to provide essential information as one starts farming to marketing the prawn. It starts with the biology of the species, followed by brood stock management, hatchery systems and management, larval feeds and feeding and nursery systems and management. The chapter on hatchery systems taps on previous practical information presented in manuals, but is coupled with personal experiences of the authors. It also details recent changes in management systems and tank requirements for the desired post larvae production and live feed production. The chapter on larval feeds also discusses the supply and processing of artemia, including its nutritional enhancement.

There are six chapters covering grow-out systems, starting with site selection to grow out in monoculture, polyculture and integrated systems and in temperate zones. This is completed with the chapter on water quality and soil management. The state-of-the-art information required for successful culture is given. Specific chapters on nutrition and feeding strategies, health management and genetics complement the chapters dealing with technical challenges. A chapter on biology and management of size variation and references to sex reversal and monosex culture demonstrated the awareness that this is current challenge in the industry. However, a more practical approach to the topic would be preferable to help commercial farmers address this problem.

The reviews on culture systems describe results of stocking strategies and operations in several polyculture systems in combination with several species of fish, hybrids monoculture and in integrated operations with rice etc. The editors have digressed from the norm with chapter 17, where developments on commercial prawn farming in several countries are based on information derived from informal



surveys with industry. Although the results of this survey do not comply with normal scientific enquiry, it has added value to the book as it shows the diversity in farming practices and brings practical information to the attention of scientists working on freshwater prawn farming and the entrepreneur considering the species as an aquaculture species.

In the last part, there are two chapters which make this book unique. The editors have decided on a chapter discussing the sustainability of the culture of the prawn and one on marketing and processing of the end product. Unusual for a scientific text book, there are some recipes for the cuisine of the prawn. Lastly, economics and business management completes the book with information on costs, profitability and investments required for hatchery and grow-out production.

In general, it is a well conceived book on freshwater prawn farming, encompassing information presented in previous editions and is updated with more recent information. This should fulfil the needs of anyone seeking information on its farming and serve well as a valuable reference book and teaching aid for those in academia. It is a useful book to have for those planning or already involved in commercial production. - Zuridah Merican (More information: [www.wiley.com](http://www.wiley.com); email: [cs-books@wiley.co.uk](mailto:cs-books@wiley.co.uk))



# The New Dimension for Aquaculture in Vietnam

This is the theme for the one day ILDEX Vietnam Aquaculture Conference 2010 to be held on March 26, 2010 at the New Saigon Exhibition and Convention Center, Ho Chi Minh City, Vietnam. It will be held in conjunction with ILDEX Vietnam, to be held from 25 to 27 March. The program is designed to reach various stakeholders in the industry from pond/hatchery operators to technical level personnel to managers, feed manufacturers and government officers. It will cover issues faced by the industry. At press time, the program is as follows:

## Program

**Keynote:** General trends of aquaculture in Vietnam.

**Keynote:** Challenges for sustainable shrimp and catfish farming in SEA

### Sustainability & Certification

- Global gap: exporting to the EU-Jos Exters, Anova Seafood BV
- WWF's result from the Pangasius Dialogues and what is happening to the shrimp dialogues-Dr. Flavio Corsin, WWF Vietnam
- Certification: the costs and benefits -Xavier Bocquillet, ISTOM, Vietnam

### Innovations in culture technology

- Biosecurity and controlling shrimp diseases – Dr. Sri\_Nhonhang Supornchai, Dupont, Thailand
- Probiotics in aquaculture: do they work – Dr. Pedro Encarnacao, Biomin, Singapore
- Diseases in catfish farming: the essence of monitoring – Cedric Komar, Intervet/ Schering Plough Animal Health, Vietnam

### Feed Management Strategies

- Nutrition approach to fish & shrimp health – Dr. Jacques Gabaudan, DSM Nutritional Products – Aquaculture Centre Asia Pacific, Thailand
- Phytase in extruded feeds: looking into the future – Dr. Dirk Lorenz-Meyer, Behn Meyer, Animal Nutrition, Malaysia
- Extrusion: new developments for ingredient usage

Online registration is available at <http://www.sptmrk.com/index.php/conference/ildex-vietnam-aquaculture-conference-2010>. All presentations will be in English with translation to Vietnamese. More information: ILDEX Vietnam Aquaculture Conference 2010, Email: [info@sptmrk.com](mailto:info@sptmrk.com); Web: [www.sptmrk.com](http://www.sptmrk.com). ILDEX Vietnam 2010, Email: [info@ildex.com](mailto:info@ildex.com) (Lertwat Chanthatharath); Web: [www.ildex.com](http://www.ildex.com)

## The speakers

**Dr. Flavio Corsin** is Senior Aquaculture Advisor, Aquaculture Program, WWF, based in Vietnam. Flavio is also the coordinator for the WWF's Pangasius Aquaculture Dialogue. In his presentation, he will talk on WWF's involvement in aquaculture, the Aquaculture Dialogues in developing multi stakeholder performance-based standards for the aquaculture industry, updates on the Pangasius Aquaculture Dialogue (PAD) and the Shrimp Aquaculture Dialogue (ShAD), the development of the Aquaculture Stewardship Council (ASC) and challenges of small scale farmers in obtaining certification.

As head of Biomin's aquaculture department, **Dr. Pedro Encarnação** is responsible for product development and application. He is also the director of the Applied Center of Aquaculture Nutrition (ACAN), Biomin's aquaculture research centre based in Bangkok. In his talk on 'Probiotics in aquaculture: do they work', Pedro will discuss the use of probiotics to control pathogens by competitive exclusion. Although, probiotics play an important role as biological control agents in pond culture, particularly with respect to performance of fish and shrimp, disease control and water quality of the pond, the selection of specific beneficial bacteria and their ability to thrive in the gut and aquatic environment is key to its success.

**Cedric Komar** is Business Manager Asia, Intervet/Schering Plough Animal Health and has recently relocated to Vietnam. His presentation is on 'Diseases in catfish farming: the essence of monitoring'. Cedric said, "We are at a turning point in the Pangasius farming industry where globally recognised production standards have become critical success factors. Naturally, aquatic health management is also drawing more attention from industry stakeholders since the new game rules include a full traceability of the production phase where the veterinary follow-up has a key role".

## What to expect in AQUA CULTURE Asia Pacific Magazine in 2010

Volume 6 2010						
Number	1	2	3	4	5	6
	January/February	March/April	May/June	July/August	September/October	November/December
Issue focus <i>Current trends and challenges</i>	Aqua feed Production	Hatchery	Sustainable & Responsible Aquaculture	Health & Biosecurity	Cage Culture	Food Safety & Traceability
Industry review with <i>profiles and outlook</i>	Marine Shrimp	Marine Fish	Catfish	Freshwater Fish	Tilapia	Marine Fish
Feed Technology	Animal & plant meals	Larval feeds & processing Feed additives	Feed standards Feed enzymes	Pre and Probiotics Immunostimulants	Processing Technology Feed additives	Nutrition Novel meals & oils
Production Technology	Aeration & Disinfection Technology	Brood stock & genetic Improvement	BMP, Standards and Certification	Recirculation aquaculture technology	Feed management	Health management
Shrimp/Fish culture developments	Coverage on experiences from industry, including role models, benchmarking and opinion articles.					
Markets	Contributed reports on market trends, product development, issues and challenges.					
Show Preview/Issue	Victam 2010, Bangkok, Thailand 3- 5 March World Aquaculture 2010, San Diego, USA March 1-5	Bonus distribution: Ildex Vietnam 2010, Ho Chi Minh City, Vietnam, March 25-27 2010 ASAIM Aquaculture Conference, Bangkok, Thailand, March 21-25	Australasian Aquaculture 2010 Hobart, Tasmania 23-26 May Vietfish 2010, Ho Chi Minh City, Vietnam 12-14 June		Aquaculture Europe 2010, Porto, Portugal 6-8 October Aquaculture China 2010/China Fisheries and Seafood Expo, 2010, November 2-4 Dalian, China PRC	



# RECIRCULATING AQUACULTURE TECHNOLOGY WORKSHOP

May 22 & 23, 2010, Hobart, Tasmania

Recirculating aquaculture technology continue to attract attention and are the subject of considerable capital investment worldwide. This workshop is designed for a broad audience and seeks to provide participants with nonbiased, research-based information about the design and management of recirculating aquaculture fish production systems.

The information presented at this workshop comes from the first-hand research results, findings from the global research community and the experiences of the presenters with commercial scale producers. Species covered are tilapia, yellow perch and southern flounder, summer flounder, hybrid striped bass, black sea bass, and three species of sturgeon.

This workshop will be presented by Professor Thomas Losordo, and Todd Guerdat, Department of Biological & Agricultural Engineering at North Carolina State University, USA. Thomas is involved in aquaculture for 35 years and currently heads a program of applied research and

extension (public service) in the area of recirculating aquaculture production systems design, development and management. Todd Guerdat is a Graduate Research Assistant and PhD candidate. Todd has worked as a research assistant operating the NC Fish Barn and has extensive experience in the construction, operation and management of both research scale and commercial scale recirculating aquaculture systems. His research is on physical, biological, chemical means of treating marine aquaculture effluents.

Reservations are essential and Australasian Aquaculture conference delegates can receive a discount of AUD100 on registration for this workshop. Workshop registration includes a technical workbook, a compact disc containing useful publications and spreadsheets and lunch. For information: Web: [www.australian-aquacultureportal.com](http://www.australian-aquacultureportal.com) Email: [sarah-jane.day@aquaculture.org.au](mailto:sarah-jane.day@aquaculture.org.au) (Sarah-Jane Day, Conference Coordinator), Tel: +61 437 152 234 Fax: +61 2 4919 1044.

**March 17-19**

## 7th Philippines Shrimp Congress

Bacolod City, Philippines

Email: [jalbaladejo@bfar.da.gov.ph](mailto:jalbaladejo@bfar.da.gov.ph)

[jalbaladejo99@yahoo.com](mailto:jalbaladejo99@yahoo.com)

Web: [www.philshrimp.org/](http://www.philshrimp.org/) [www.da.bfar.gov.ph](http://www.da.bfar.gov.ph)

**March 21-25**

## 2010 ASAIM Aquaculture Conference

Bangkok, Thailand

Email: [organizer@soyevents.com](mailto:organizer@soyevents.com)

Web: [www.soyevents.com](http://www.soyevents.com)

**March 25-27**

## Ildex Vietnam 2010

Ho Chi Minh City, Vietnam

Email: [info@ildex.com](mailto:info@ildex.com)

Web: [www.ildex.com](http://www.ildex.com)

**March 26**

## ILDEX Vietnam Aquaculture Conference 2010

Ho Chi Minh City, Vietnam

Email: [info@sptmrk.com](mailto:info@sptmrk.com)

Web: [www.sptmrk.com](http://www.sptmrk.com)

**April 6-8**

## Aquaculture Convention 2010

Bohol, Philippines

Email: [info.aquatechcon@gmail.com](mailto:info.aquatechcon@gmail.com)

Web: [www.equipinc.webs.com](http://www.equipinc.webs.com)

**May 5-7**

## International Conference and Exhibition on Shrimp Aquaculture 2010

Jakarta, Indonesia

Email: [donedwin@bimatama-inka.co.id](mailto:donedwin@bimatama-inka.co.id)

**May 19-21**

## Offshore Mariculture 2010

Dubronik, Croatia

Email: [iroberts@mercatormedia.com](mailto:iroberts@mercatormedia.com)

Web: [www.offshoremariculture.com](http://www.offshoremariculture.com)

**May 22-23**

## Recirculating Aquaculture Technology Workshop

Hobart, Australia

Email: [sarah-jane.day@aquaculture.org.au](mailto:sarah-jane.day@aquaculture.org.au)

Web: [www.australian-aquacultureportal.com](http://www.australian-aquacultureportal.com)

**May 23-26**

## Australasian Aquaculture 2010

Hobart, Australia

Email: [sarah-jane.day@aquaculture.org.au](mailto:sarah-jane.day@aquaculture.org.au)

Web: [www.australian-aquacultureportal.com](http://www.australian-aquacultureportal.com)

**May 31-June 4**

## 14 International symposium on fish nutrition & feeding

Qingdao, China

Web: [www.isfnf2010.com](http://www.isfnf2010.com)

**June 9-12**

## Global Conference on Aquaculture 2010

Bangkok, Thailand

Email: [aqua-conference2010@fao.org](mailto:aqua-conference2010@fao.org)

Web: [www.aqua-conference2010.org](http://www.aqua-conference2010.org)

**June 12-14**

## Vietfish 2010 – Vietnam Fisheries International Exhibition

Ho Chi Minh City

Email: [quocthanh@vasep.com.vn](mailto:quocthanh@vasep.com.vn)

Web: [www.vietfish.com.vn](http://www.vietfish.com.vn)

**September 26-October 1**

## 17th Annual Practical Short Course on Aquaculture Feed Extrusion, Nutrition, & Feed Management

Email: [mnriaz@tamu.edu](mailto:mnriaz@tamu.edu) (Mian Riaz)

Web: [www.tamu.edu/extrusion](http://www.tamu.edu/extrusion)

**October 5-8**

## Aquaculture Europe 2010

Porto, Portugal

Web: [www.easonline.org](http://www.easonline.org)

Web for exhibition: [www.marevent.com](http://www.marevent.com)

**October 17-20**

## GOAL 2010

Kuala Lumpur, Malaysia

Web: [www.gaalliance.org](http://www.gaalliance.org)

**October 21-23**

## Tilapia 2010

Kuala Lumpur, Malaysia

Email: [infish@po.jaring.my/](mailto:infish@po.jaring.my/) [infish@tm.net.my](mailto:infish@tm.net.my)

Web: [www.infofish.org](http://www.infofish.org)

**November 2-4**

## Aquaculture China 2010

15th China Seafood and Fisheries Expo 2010

Dalian, China PRC

Website: [www.Seafare.com](http://www.Seafare.com)



# australasian aquaculture

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