

July/August 2016

# Hatcheryfeed

Advances in feeding early life stage and broodstock aquatic species

*The magazine of Hatcheryfeed.com*



BROODSTOCK FEEDS ▪ PELLETED POLYCHAETES ▪ PROBIOTIC BENEFITS ▪ ALGAE  
CONCENTRATES ▪ NOVEL PROTEIN FEEDS ▪ NEW PLAYER IN MICRO-ALGAE



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\*Cover story  
Sea bass fry sorting

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### From the Publisher



Hatchery feeds are following the same trends that we see in the rest of the aquaculture feed sector: they have become ever more sophisticated and specific for purpose. In this issue we look at feeds for RAS salmon systems, a first feed for post-vaccinated Lumpfish and results of a feed that reduces deformities in European sea bass larvae. Another common development comes in the latest understanding of the role that beneficial bacteria play in promoting health: probiotics are at once ancient knowledge and a new and exciting science. Feeding probiotics, once dismissed as “snake oil” by many in the aquaculture industry, is now an accepted strategy and gaining prominence in early life stages. Broodstock feeds are not forgotten: we feature feeds from mussels, polychaetes and harnessing the potential of plants, as well as a fishmeal replacer from agricultural inputs. None of these exciting developments minimize the essential requirement for algae, a demand that two companies featured here are doing their best to meet.

*Suzi Dominy*, Editor & Publisher

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# Hatchery News Review

*News briefs from Aquafeed.com and Hatcheryfeed.com*

**UK** - Parent company of Inve Aquaculture, Benchmark Holdings plc, has acquired the breeding and genetics programs of Colombian firm, Centro de Investigación de la Acuicultura de Colombia Ceniagua (Ceniagua), Acquired under a technology transfer agreement, the move expands Benchmark's aquaculture breeding capabilities from tilapia and salmon to include *L. Vannamei*, cobia and grouper.

**SPAIN**—In a world first, the Spanish Institute of Oceanography (IEO) closed the life cycle of Atlantic bluefin tuna (*Thunnus thynnus*) in captivity. The captive-bred tuna were raised from eggs collected in cages in 2011, 2012 and 2013. They were grown to juveniles, weighing 3-5 grams, transported to cages and fattened until the end of 2014. 50,000 fertile eggs were successfully collected from the cage, in 2015.

**USA**—Rising Tide Conservation and the team at the University of Florida Tropical Aquaculture Laboratory have succeeded in raising the first ever captive-bred Pacific Blue Tangs. The collaborative success was over six years in the making. In previous

attempts, the team at UF Tropical Aquaculture Laboratory consistently had a survival rate of Blue Tangs to four days post hatch before the larvae would die. At most, the Blue Tang larvae would survive to 21 days post hatch. Success came after studying successful breeding techniques of the Yellow Tang at Hawaii Pacific University's Oceanic Institute, Hawaii.

**USA** – The University of Miami's Rosenstiel School of Marine and Atmospheric Science Experimental Fish Hatchery (UMEH) made an international mark as the first educational and research institution in the world to obtain the GLOBALG.A.P. Integrated Farm Assurance for Aquaculture producing Cobia eggs and fingerlings commercially.

**AUSTRALIA**— Western Australia's emerging aquaculture industry will receive a boost from the 2016-17 State Budget with a \$2.3 million investment in a new multi-species shellfish hatchery in Albany. The industry-run mussel, oyster and pearl production hatchery is expected to be fully self-funded within 10 years, once the industry has reached sufficient scale.

The State Budget also included \$1.3 million to expand investor-ready aquaculture zones across the State.

Western Australia's commercial fisheries and aquaculture are worth about A\$490 million per year .

**INDIA**— Shrimp feed manufacturer, The Waterbase Limited, has invested almost US\$3million to build a hatchery in Nellore, Andhra Pradesh.

The hatchery, which is scheduled to be operational by the end of March, 2017, will produce 500 million post-larvae a year for local farmers, with ambitions to increase over time.

Shrimp accounts for more than 60 per cent, of India's seafood exports, generating revenue of \$2.7 billion during FY15.

**CHINA**—VNU Exhibitions Asia Ltd has acquired Asia's most important ornamental fish trade show, Aquarama, from UBM Exhibition Singapore; VNU will operate these events beginning with the 2016 edition. The show now becomes an annual event and is relocated from Singapore to Guangzhou, China.

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# An innovative approach to RAS feed development

By Louise Buttle, Principal Scientist, Cargill Aqua Nutrition

The numbers of Recirculation Aquaculture Systems (RAS) are on the rise for salmonids in Canada, Chile, Norway and Scotland. For any new production facility being built for freshwater it is very likely that RAS will be system of choice. This developing technology for holding fish needs to be met with the right knowledge in terms of the feed input.

This is where Cargill Aquaculture Nutrition (CQN) EWOS has developed “EWOS Clear,” a targeted feed to meet the needs of the RAS salmon farmer



Photo: Robert Paterson. Scottish Salmon Producers' Organisation

This new concept feed uses a holistic approach to feed design for RAS salmon husbandry in freshwater, taking into account the many factors that impact the optimum running of these systems. This article describes the development of this feed at the Cargill Innovation Centre (CIC) and finishes with the key elements of EWOS Clear for RAS.

CIC is where most of the nutrition R & D for Cargill Aqua Nutrition is conducted. CIC does not have any recirculation R & D facilities in Norway however, this was not a hurdle for our team of innovative scientists. This challenge was turned into a novel approach, hence

the “CIC model for RAS feeds” was developed.

This mass balance and bioenergetics model, adapted from Losordo & Hobbs (2000) and Timmons & Ebling (2010), Brafield AE & Llewellyn MJ (1982), is based on nutrient mass balance, bioenergetics budget and RAS engineering principles. This model was used in the R & D to evaluate how feed characteristics affect the key RAS operating parameters. An overall picture of the model is given in Figure 1.

This illustrates the inter-relation between the feed, the fish and the RAS system parameters and how a holistic

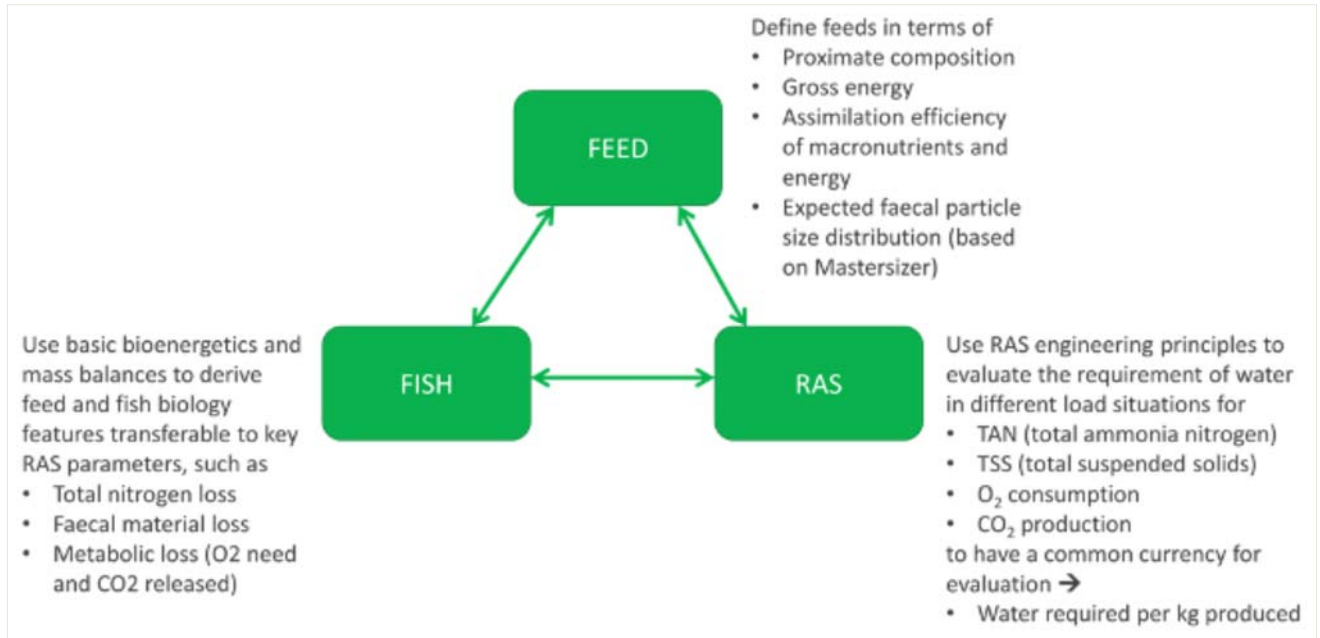


Figure 1. The Holistic Approach to RAS Diets

approach to RAS feeds is the only way forward.

The use of this model enabled scientists to test many feeds, without needing a full scale RAS facility with replicate systems. However without doubt, testing in a commercial RAS system was vital for full product development and final documentation was completed in one of Norway's largest smolt facilities.

Throughout the trials conducted in CIC for RAS development, fish weight gain and conversion factors were measured. In addition, many samples of feed, fish and faeces were sent for analysis in the CIC laboratory, Dirdal, Norway. CIC has the capability to analyze many parameters "in house" which makes for a very efficient turnaround of trial data. In addition to standard analyses, we also wanted to focus on the digestibility of the feeds,

and on the histological appearance of the gastro intestinal tract in these small fish.

Of course, there has been a focus on formulation, raw material selection with the highest digestibility as well as macro nutrient balance – output from the model findings demonstrated that changing the macro nutrient balance can impact the operating parameters in the RAS system without compromising fish performance. For example, some feeds led to a higher ammonia excretion. These types of findings guided the recommendation from CIC. Also, it is important to ensure that as much as possible of the feed proteins are used for growth by the fish, instead of being burnt as energy. That way we can minimize the nitrogen excretion over the gills, and into the water, and thereby reduce the load on the bio filter.

Another important consideration is the faeces particle size – a smaller faeces particle will lead to a potential clogging of the mechanical filters, and a higher possible demand on the bio filters. Therefore feed formulations that resulted in a larger particle size of the faeces were targeted, and CIC developed an ongoing routine testing procedure for this parameter. An example of some of the faeces particle testing is given in Figure 2. This shows how different feeds will result in different faeces particle size, and according to some of the results we have seen that these faeces particle sizes will have different rate of degradation.

Essential also for a RAS feed is optimized feed quality – leading to high water stability in the feed and homogeneous particle size, minimum dust and breakages. Throughout the R & D

AN INNOVATIVE APPROACH TO RAS FEED DEVELOPMENT

process, the feeds were tested for quality parameters to ensure it would meet the RAS Farmers expectations. Compared to a standard flow through system for holding freshwater salmon, the use of RAS is more complex and so there are many more parameters to consider when formulating and delivering the best feed. Accordingly, there may be a higher requirement on the digestibility of the raw materials used and the physical quality of the feed, and therefore, a different requirement in terms of the macro-nutrient specifications (digestible energy, digestible protein for example).

**Key elements of EWOS Clear**

Key Elements of EWOS Clear include

- Excellent technical quality

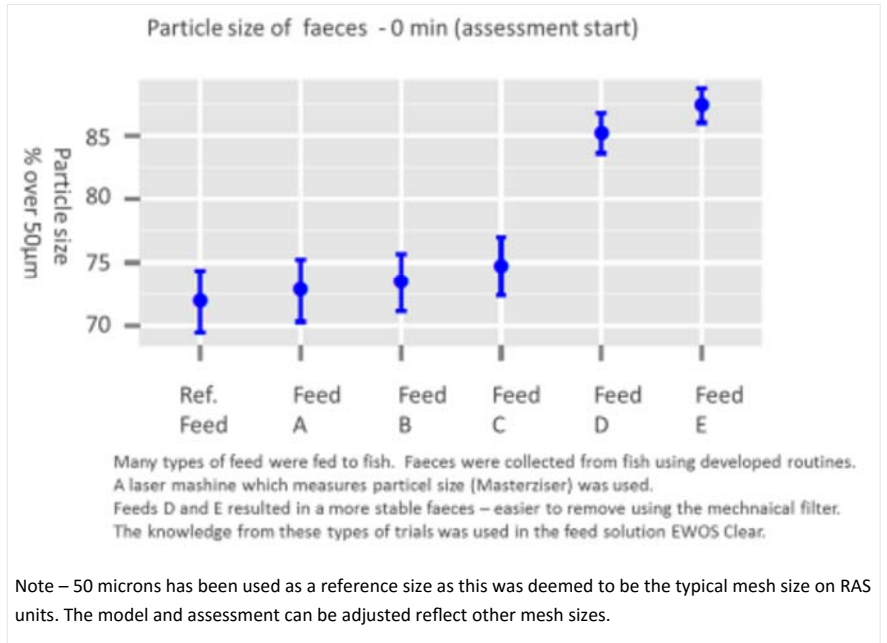


Figure 2. Faecal Particle size measurements with different RAS trial diets

- Highly digestible raw materials
- Optimized macronutrient balance and energy content
- Faeces stability to avoid disintegration before the mechanical filter
- Good availability of minerals
- High performance in fish growth, low feed conversion ratio
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It is well recognized that each RAS system has particulars to its design and therefore the system features of each RAS, will affect the outcome of the feed evaluation. This enforces a complication that feed development is best done with multiple RAS designs in mind. The parameters in the CIC model can easily be updated, allowing us to rapidly and continuously develop EWOS Clear to align it with new RAS technology in the future. The same feature in the model opens the possibility to do selection of optimal feed for a specific RAS on customer to customer basis. Therefore in the future it may be possible that CQN EWOS offers a bespoke customer service, particular to each RAS design – but that's for another day!

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Losordo T.M., Hobbs A.O. (2000) Using computer spreadsheets for water flow and bio filter sizing in recirculating aquaculture production systems. *Aquacultural Engineering* 23, 95-102.

Timmons M.B., Ebeling J.M. (2010) *Recirculating aquaculture*. 2nd edition. NRAC Publication No. 401-2010. Cayuga Aqua Ventures: Ithaca. 948 pp.

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# First feed for post-vaccinated Lumpfish

Turning conventional wisdom on its head, PT Aquaculture has developed a new 'low octane' feed that will slow down fish growth while boosting health and growth of cleaner fish.

By Suzi Dominy

From Canada to Norway, sea lice (*Lepeoptheirus salmonis*) impose a significant financial burden on farmers of Atlantic salmon (*Salmo salar* L.) – and with rising sea temperatures, it is a problem that is likely to intensify. In British Columbia, Canada's Queen Charlotte Strait, for example, 2015 saw an epidemic of sea lice throughout salmon farms. And according to a University of Toronto-led study<sup>1</sup>, high ocean temperatures during the winter months of 2014 likely accelerated sea-lice development, enabling populations to grow quickly and reach higher numbers than they would under normal ocean temperatures.

In recent years the use of chemicals has been heavily relied on to control infestations, but today, eco-friendly solutions are taking their place; cleaner fish are proving a highly successful and effective biological control. Ballan Wrasse (*Labrus bergylta*) were initially used but are now starting to give way to lumpfish (or lumpsuckers) (*Cyclopterus lumpus* L.) The lumpfish is easier to raise than the wrasse, be-



Photo: Emily Whalen NOAA Teacher at Sea.

cause it grows quickly and is less fussy about what it eats. It continues to eat even when it is cold, which means it can be used as a cleaner-fish all year round.

## Health issues

Farmed cleaner fish are produced in about 30 facilities in Norway and beyond. Along with this rapid increase, disease problems have emerged, such as *Vibrio* sp., atypical *Aeromonas*

*salmonicida* and *Pasteurella* sp., driving major research and development programs into disease control in Canada and Europe. While vaccination of lumpfish is now becoming standard, the research continues as a priority.

Earlier this year Researcher Gyri Teien Haugland and her colleagues at the University of Bergen's (UiB) Department of Biology were awarded NOK 10 million (approximately 1.2 million Euros) from the Research Council of

FIRST FEED FOR  
POST-  
VACCINATED  
LUMPFISH



A researcher live-lousing a juvenile pink salmon that will be released to continue its seaward migration . Photo Stephanie Peacock.

“Good health is absolutely essential for the lumpfish to be able to do its job as a cleaner-fish,” said Gyri Teien Haugland, who heads the research project, which is coordinated by UiB. Following some successful small-scale aquaculture trials, both the volume and the number of lumpfish users grew in 2013. More than two million farmed lumpfish were put into the salmon cages, but many of them died of diseases. In 2014, the researchers began vaccine trials with promising results, and about 5 million farmed lumpfish were introduced to the salmon cages. If the vaccination is successful, production can be further increased this year. This is good news

Norway for a four-year study into the health of the lumpfish and to develop a vaccine against the most common lumpfish diseases.

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FIRST FEED FOR POST-VACCINATED LUMPFISH

for salmon farmers: in 2016 it is expected that Norway alone will need some 50,000,000 cleaner fish.

Lumpfish are vaccinated at about 8g and must then stay in tanks on land for 500 degree days (between 40 and 50 days) before going to sea. However, during this critical time, they are quite susceptible to bacteria and disease and they also grow at such a fast rate, they can become too big for purpose and therefore, to some degree, ineffective in the cages. For the producers, this creates another problem: since lumpfish can double in weight every 15 days (at 12°C), the total biomass can overwhelm the grow out land-based facilities before the fish

can be sent to the cages, and ultimately that creates a bottleneck in production.

**A new specialty feed**

Pacific Trading Aquaculture, based in Dublin, Ireland, has worked with Marubeni Nisshin Feed Co., Ltd. (MNF) of Japan, to become the first company to develop and offer a diet specifically targeted at post vaccination lumpfish: a new formula Otohime EP. The product is offered in 1.3mm and 1.7mm pellet sizes that is very high in the essential vitamins and minerals (including B-Glucan) so fish health is very high, immune system is boosted and with a reduced level of protein and fat it will control or slow down

the rapid growth rate post vaccination.

“Initial trials of our reduced fat and protein 'low octane' EP diets in Norway are showing the fish on the EP diets are at least 30% smaller after 500 degree/days than those fed on our conventional diets”, Paul Coyne, Director, PT Aquaculture said.

A 30% reduction in fish size also means the farmer can accommodate 30% more fish in each tank, which in turn gives him much better return on his infrastructure. Cleaner fish are sold on a per fish price, not a per weight price, so the farmer doesn't get a higher price for bigger fish. Also

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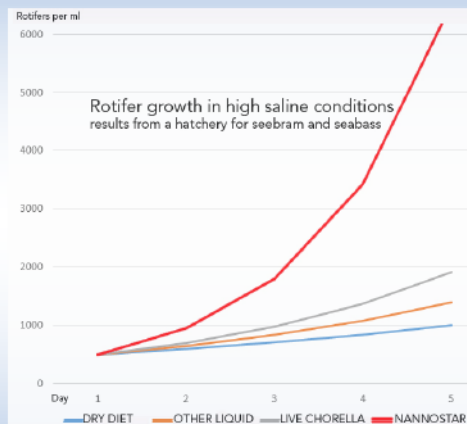
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smaller fish eat less which in turn leads to savings on feed costs.

“There was no change in mortality rates between our regular diets and the EP diets in trial. Both were below 1%” Coyne said. “We take from that we are succeeding in terms of producing a highly healthy diet essential in all nutrients and vitamins and boosting the fish immune system, but without the high fat/protein levels usually associated with fish feeds”.

“It is somewhat of an anomaly to be producing a feed to get fish to grow more slowly but in this instance that is what the market needs .... slow growing but healthy fish”, he added.

“This is a market we are working with constantly, always striving to make sure our feeds deliver the best performance across the full production cycle, from hatching to cages”, Coyne said. “It is imperative that the fish grow at a sustainable and controlled rate, but importantly they must be very healthy and robust, with increased survival, and therefore be able to do a better job in the salmon cages when they get there at between 16 and 40g”.

This EP range is at the larger size end of the Otohime spectrum and specifically targets the lumpfish. The new formula includes the essential Beta-Glucans required to boost the immune system around vaccination time and increase disease resistance in lumpfish.

The specific targets of this “high quality - low octane” diet are:

- To improve fish health
- To boost immune system
- To slow down and control growth post vaccination
- To increase survival rates
- To produce strong, healthy & efficient cleaner fish

“Importantly,” Coyne stressed “we have included a suggested feed table for lumpfish so producers can use the Otohime throughout the entire production cycle and produce a healthier, more robust and more efficient fish going to the cages”.

#### References

<sup>1</sup> Andrew W. Bateman, Stephanie J. Peacock, Brendan Connors, Zephyr Polk, Dana Berg, Martin Krkošek, Alexandra Morton. Recent failure to control sea louse outbreaks on salmon in the Broughton Archipelago, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences, 2016; 1  
DOI: [10.1139/cjfas-2016-0122](https://doi.org/10.1139/cjfas-2016-0122)

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*This EP range is at the larger size end of the Otohime spectrum and specifically targets the lumpfish. The new formula includes the essential Beta-Glucans required to boost the immune system around vaccination time and increase disease resistance in lumpfish.*

#### More information

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Product details and datasheet.



# Broodstock diets

## *an overview*

By Judith Kolkovski, Nutrakol Pty Ltd.

The broodstock condition, inclusive of physiological, nutritional and stress aspects, is one of the key issues for the success of marine fish and shrimp hatcheries.

It is well established that high quality larvae and therefore optimal growth and high survival is dependent on the broodstock nutrition and their health. The effect of nutrients on the gonadal development, maturation and fecundity of different aquatic species, including fish and crustaceans, has been the subject of research efforts in past decades. This research includes protein and lipid levels and fractions (i.e. phospholipids), essential fatty acids especially HUFA's such as DHA, EPA and ARA levels and ratios, vitamins such as ascorbic acid (C) and  $\alpha$ -tocopherol (E), in addition to many others. Specific nutrients such as carotenoids, yeast extracts and microalgae (*Spirulina*, *chlorella*, *Dunaliella salina*) have been



found to have a significant effect on fecundity and are currently included as a standard in broodstock diets.

Although there is a significant quantity of scientific literature on broodstock nutrition of marine fish and crustaceans, there are still many factors which are unknown. Due to the large

number of species grown in aquaculture which, in many cases are varied in habitat, behavior, feeding habits, activity etc., there is no one (or even several) optimal formulae that can suit all species. For example, nutritional requirements for flat fish such as *Solea solea* are very different from pelagic

**BROODSTOCK  
DIETS—  
AN OVERVIEW**

species such as *Seriola*. As Cobia is a tropical species, it has significantly different requirements from Halibut, which is a cold water species. While in recent years, more species-specific maturation diets have become increasingly available, the nutrition of broodstock is still far from optimal.

The use of fresh and frozen seafood as a partial or complete diet for broodstock is still the standard with many marine species, including established species such as gilthead sea bream and European sea bass in the Mediterranean. While there are commercial broodstock diets that can be used solely, marine organisms such as sardines, mackerels, squid, mussels are considered to be essential for marine fish broodstock. In shrimp aquacul-

ture, significant effort is made to grow polychaetes, which together with squid and *Artemia* is considered being essential for broodstock. Large hatcheries and broodstock centres usually have their own polychaete farms. Although this practice has a potential risk for introducing pathogen vectors which may affect broodstock quality, all shrimp industry worldwide is still reliant on live polychaetes and other fresh and frozen feeds.

**Lack of standard feeding protocols**

In general, there are no standard feeding protocols for marine fish broodstock and the nutrition and feeding protocols can vary significantly between hatcheries and species. A

mix of seafood such as sardines, mackerels and squid, in addition to commercial boosters, which contain vitamins, minerals, fatty acids and other nutrients are usually the norm. Moist diets (developed during the 80s) which are based on a mix of fishmeal and other marine organism meals (mainly krill and squid), oils and nutritional additives, as well as, fresh ingredients (squid, fish) are commonly used. In many hatcheries, these moist diets are produce in-house.

While the nutritional requirements of fish and shrimp broodstock may not be fully described, especially for 'new'-cultured species such as groupers (*Epinephalus sp.*), *Seriola sp.*, Cobia, and many others, they are partially or fully fulfilled with the supplement-

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tation of fresh feeds and nutritional boosters.

Current commercial broodstock diets and supplements focus on the nutritional requirements of the fish and shrimps. However, additives or supplements that support the hormonal cycle, and thus will lead to better gonadal development and higher fecundity, are scarce. This is especially true for out-of-season broodstock groups of well-established and new species in aquaculture. Moreover, other factors affecting the broodstock fecundity and maturation such as stress and immune system and digestive system are usually not directly addressed.

#### **Promise in herbs**

While herbal medicines and herbal therapy for women with reproductive hormonal problems have been recognized for centuries, there is very little adaptation of it in aquaculture. Medicinal plants are known to have hormonal boosting effects. Some herbs are utilized in herbal medicine as natural boosters for the hormonal cycle in both men and women and in hormone replacement therapy for menopausal women. The effect of herbal extracts on the development and performances of fish and shrimp broodstock have not been intensively investigated. However, scientific evidence suggests that aside from the known properties of plant extracts as antibacterial, antiviral and antifungal, these compounds

can significantly improve the broodstock condition and therefore the quality of their eggs and larvae. In black tiger shrimp, *P. monodon*, significant increases in fecundity, gonadal weight and reduced inter-molt period were observed when fed a maturation diet containing extracts of *W. somnifera*, *Mucuna pruita*, *Ferula asafoetida* and *Piper longum*. Similar results (increase in fecundity and gonadal weight and reduced inter-molt period) were found when a mix of *W. somnifera*, *Mucuna rurita*, *Ferula asafoetida* and *Piper longum* was fed to the spawner through bio encapsulated *Artemia*.

Many plant-derived compounds have been found to have non-specific immune-stimulating effects in animals, of which more than a dozen have been evaluated in fish and shrimp (Table 1).

Herbal compounds have the ability to inhibit the generation of oxygen ani-

ons and scavenge free radicals, therefore, reducing stress effects. Herbal anti-oxidant effect was demonstrated when *P. kurroa (picrorhiza)* extract was used as an anti-stress compound for black tiger shrimp. Other herbs such as, *Astragalus membranaceus*, *Portulaca oleracea*, *Flavescent ophora* and *A. paniculata* and many other are known to have specific and non-specific anti-stress effects.

Currently, several hatcheries both for fish and shrimps around the world are using commercially available herbal extract mixes, specifically designed to boost and modulate the hormonal system in aquatic animals. The herbal extracts are used with out-of-season broodstock and/or species with fertilisation and gonadal development problems such as groupers (low sperm motility and volume) and many other species (Table 2).

Using a commercial mix of herbal extract as a standard additive to brood-





Table 1. Examples of herbal extracts used as immune stimulants and growth promoters in aquaculture

Botanical name	Family	Distribution	Useful parts	Biological effect in aquaculture
<i>Hygrophila spinosa</i>	Acanthaceae	India, Sri Lanka	Whole plant	Growth Promoter
<i>Ipomea digitata</i>	Convolvulaceae	India	Root	Growth Promoter, Immunostimulant
<i>Solanum nigrum</i>	Solanaceae	India	Berries	Growth promoter
<i>Terminalia arjuna</i>	Combretaceae	India, Burma, Sri Lanka	Bark	Growth promoter
<i>Boerhaavia diffusa</i>	Nyctagineae	India, Tibet	Leaf and Root	Growth promoter, appetizer
<i>Carica papaya</i>	Caricaceae	India	Fruit	Growth promoter, appetizer
<i>Eclipta erecta</i>	Compositae	India	Whole plant	Hepato tonic, Immunostimulant, Anti-stress
<i>Eclipta alba</i>	Compositae	India	Whole plant	Hepato tonic, Immunostimulant, Antiviral, Anti-stress
<i>Cynodon dactylon</i>	Gramineae	India	Leaf and Root stalk	Immunostimulant, Antibacterial
<i>Emblica officinalis</i>	Euphorbiaceae	India	Whole plant	Immunostimulant, Antibacterial
<i>Urtica dioica</i>	Urticaceae	Europe, Turkey, India	Whole plant	Immunostimulant
<i>Vernonia cinerea</i>	Compositae	India	Whole plant	Immunostimulant
<i>Viscum album</i>	Loranthaceae	India, Himalayas, Turkey	Berries and Leaves	Immunostimulant
<i>Zingiber officinale</i>	Scitamineae	India, China, Bengal	Rhizome	Immunostimulant
<i>Picrorrhiza kurrooa</i>	Scrophulariaceae	India	Rhizome	Immunostimulant, Anti-stress
<i>Withania somnifera</i>	Solanaceae	India	Root	Immunostimulant, Growth promoter

stock diet, similar spawning performances are currently achieved with

*Seriola lalandi* broodstock groups during spawning season and off-season

(Kolkovski and La Camera, 2015).

Table 2: Examples of herbal extracts in broodstock diets

Botanical Name	Family	Distribution	Useful parts
<i>Cinnamomum Zeylanicum</i>	Lauraceae	India, Sri Lanka	Bark
<i>Elettaria cardomomum</i>	Scitamineaceae	India, Burma, Sri Lanka	Dried ripe seeds
<i>Eugenia caryophyllata</i>	Myrtaceae	India, Sri Lanka	Fruits and dried flower buds
<i>Mesua ferrea</i>	Guttiferae	Andaman, Nicobar Islands	Flowers buds, seeds and bark
<i>Asparagus racemosus</i>	Liliaceae	India	Leaves and Root
<i>Mucuna pruriens</i>	Papilionaceae	Tropics	Seeds, roots and legumes
<i>Witania Somnifera</i>	Solanaceae	India	Root and leaves



*Herbal extracts and phytotherapy compounds can contribute significantly to the broodstock condition through direct and indirect effects and more research is needed to introduce these compounds into modern aquaculture.*

### Conclusion

While a significant quantity of information is available on the marine fish and shrimp broodstock nutritional requirements, optimal nutrition and feeding requirements are still far from optimal. This is especially true for new and 'developed' species.

More attention is needed to reduce the reliance on live, fresh and frozen seafood as major ingredients in

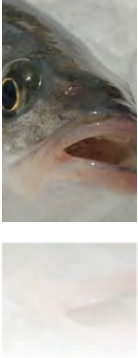
broodstock diet. Herbal extracts and phytotherapy compounds can contribute significantly to the broodstock condition through direct and indirect effects and more research is needed to introduce these compounds into modern aquaculture.

### About the author

Judith Kolkovski, ND is a nutritionist and herbalist and the general manager of Nutrakol Pty Ltd. Judith has 15 years of experience in natural therapy in humans. In recent years, Judith focused on the development of natural health solutions for aquatic animals.

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# Reducing deformity rates of European sea bass larvae

By Michel Autin, Technical Director, EMEA Division, BioMar Group.

New findings demonstrate that weaning European sea bass larvae (*Dicentrarchus labrax*) on BioMar's LARVIVA ProStart diet with an early weaning protocol is possible and delivers good results on deformity rate, survival, *Artemia* replacement rate and growth. By this better hatchery economics can be achieved.

One of the major problems in marine hatcheries in the Mediterranean is deformities in larvae. Hatcheries typically aim for fry batches that have deformity rates less than three percent in order to avoid costly manually sorting of the fish before shipping them to the farm.



The European market for sea bass is mainly a whole fish market and deformed fish look unappetizing to the end customer. Above A shows a sea bass with severe deformities, whereas B is an example of a well-shaped sea bass.

The problem is two sided: when deformed fry are stocked in cages they will start consuming feed and then either die, causing the overall feed conversion ratio (FCR) to go up, or they don't die and then the farmer ends up with deformed fish he can't sell. The European market is mainly a whole fish market and deformed fish look unappetizing to the end consumer.

Deformities are caused by many different factors such as genetics, tank man-

agement and temperature, but nutrition also has an impact. In the classical weaning set-up, the longer you feed live feed, especially *Artemia*, the less deformities you tend to have.

In the production cost of the larvae, live feed production, feed costs and survival play a prominent role and because of this there is also a quest for early weaning in order to reduce the *Artemia* cost.

According to Jef Peeters, product developer for BioMar's marine hatchery

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BASS LARVAE

feed line LARVIVA, the challenge is to find formulated larval diets and feeding regimes that allow for early weaning but that also maintain the deformity rates within reasonable limits, while also having a focus on overall survival and hatchery economics.

#### Testing early weaning of sea bass

“When BioMar’s top agglomerated weaning feed for marine fish, LARVIVA ProStart, was tested in sea bream hatcheries in 2012 the outcome was very successful and our sea bream customers have welcomed this,” Jef Peeters said.

“In order to possibly help our sea bass customers we started to investigate at which level would it be possible to perform similar early weaning results on sea bass larvae fed on LARVIVA ProStart. “

A 54 day trial was carried out on sea bass larvae at IFREMER, Palavas (France), to assess the effects on the performances, with a special focus on



The repeated process of controlling fry for deformities is labour intensive. Based on a daily sorting capacity of 15-20.000 fish per person and a total Mediterranean fry production of 900 million units the cost of hand sorting reaches easily 4-5 million EUR per year.

deformities and survival, with four different weaning modalities and with weaning starting at DPH 40, DPH 35 and DPH 30. Densities were in the same order of what is practiced in commercial hatcheries ( $\approx 100$  larvae/liter).

#### Start weaning at DPH 35 for best performance results

“One could have expected a decreasing gradient of performances with larvae fed on LARVIVA ProStart, with weaning starting at DPH 40, DPH 35 and DPH 30. However, weaning at

Table 1: Based on the trail results the table shows a calculation on the comparative cost for *Artemia* and feed that are needed to produce one million sea bass fry. By utilizing LARVIVA ProStart and by weaning at DPH 35 savings are equaling more than 2,500 EURO per million fry, compared to the control diet.

BioMar diet	Unit cost (€/kg)	Quantity (kg)	Total (€)	Commerical control diet	Unit cost (€/kg)	Quantity (kg)	Total (€)
Artemia cysts	101	10	962	Artemia cysts	101	7	709
LARVIVA ProStart 100	38	2	84	Control DPH 10-16	120	1	60
LARVIVA ProStart 200	38	10	374	Control DPH 14-34	100	10	980
LARVIVA ProStart 300	32	34	1108	Control DPH 32-53	100	34	3440
LARVIVA ProStart 400	18	11	198	Control DPH 49-53	9	11	99
		<b>Sum (€/million larvae)</b>	<b>2726</b>			<b>Sum (€/million larvae)</b>	<b>5288</b>

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DPH 35 exhibited an overall better performance,” Jef Peeters said and continued: “One explanation of this is that *Artemia* is relatively poor in nutrients compared to compound dry feed, but it is more attractive for the larvae than dry feed. Stopping feeding of *Artemia* at DPH 35 forces the larvae to feed more on dry feed compared to weaning at DPH 40, resulting in more energy and nutrient intake, which in turn results in better performance. On the other hand, stopping feeding *Artemia* at DPH 30 also results in lower performance, indicating that at that stage, sea bass larvae are still more attracted to *Artemia* than to compound dry feed.”

#### Six times less vertebral deformities

Vertebral deformities are by far the most important parameter in the economics of European marine hatcheries and Jef Peeters looked very much forward to analyzing the trial results: “We can now say that to start weaning sea bass with LARVIVA ProStart at DPH 35 gives six times lower vertebral deformities. And these are indeed optimal results in regards to deformities. But it seems that weaning at DPH 30 is not optimal for European sea bass larvae as the total vertebral deformities are high in the two treatments that started weaning at DPH 30.”

In the trial, prognatism was the most common deformity but the percent-

ages and the severity of this deformation was acceptable in all treatments.

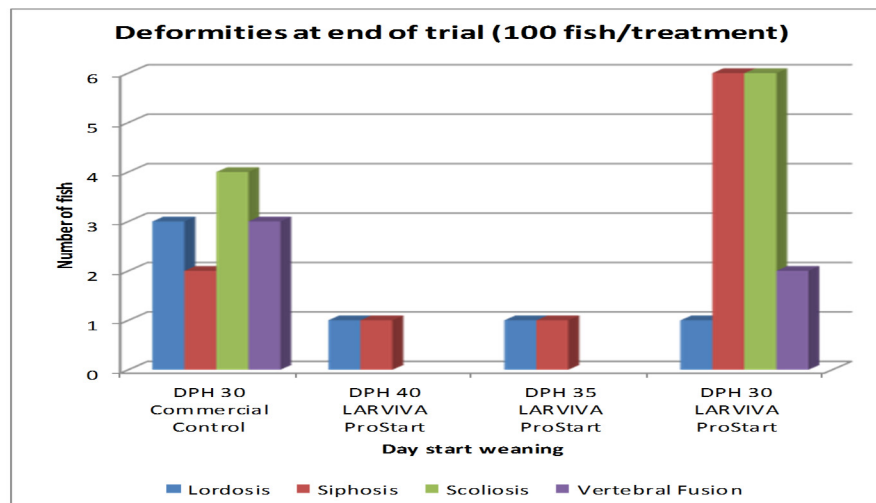
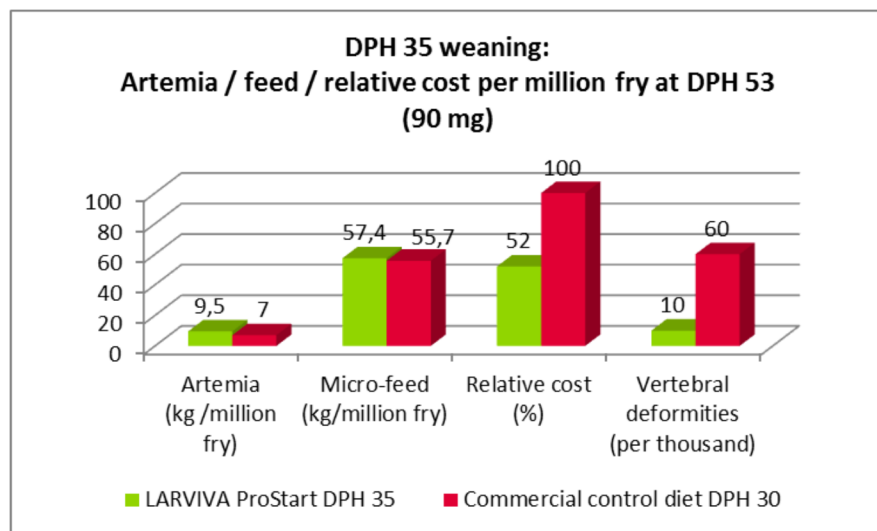


Table 3: Comparison between feeds: kg of *Artemia* and feeds consumed, relative cost (control = 100 %) and deformity rate of sea bass larvae at DPH 53 with weaning at DPH35.



er, even for the poorest result, and even though differences amongst treatments are statistically significant.”

#### Excellent survival values

According to Jef Peeters the absolute survival values of the trial are also excellent: “Survival was up to industry standards in Western Europe or high-

#### Good growth and less need for *Artemia*

“Weaning European sea bass larvae on LARVIVA ProStart with an early

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weaning protocol is possible and delivers good results,” said Jef Peeters. “Weaning the fish at DPH 35, LARVIVA ProStart delivers growth performances that are similar to the commercial control feed but with six times lower vertebral deformities. The *Artemia* quantity can be considerably lowered from the average of 35 to 50 kg per million fry produced in performant commercial hatcheries to only 9.5 kg as shown in the trial,” he said.

**Substantial savings in production costs**

Balancing the importance of deformity rate, survival, *Artemia* replacement

rate and growth with the results obtained in the trial, Jef Peeters concluded: “Using the LARVIVA ProStart diets with weaning starting at DPH 35 is our recommended protocol for sea bass larvae. By this it is possible to obtain very low deformity rates and a better total economic performance. Based on the outcome of the trial the savings in

mere productions costs are equaling more than 2,500 EURO per million sea bass fry.”

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# Overcoming the bivalve hatchery algae bottleneck with algae concentrates

By Eric Henry PhD, Research Scientist, Reed Mariculture Inc., Campbell, California, USA

The need for large quantities of quality microalgae is one of the most significant impediments restricting expansion of hatchery capacity for the bivalve industry. Ample feeding with microalgae is essential for hatchery productivity, but natural phytoplankton from ambient waters cannot provide a reliable supply of microalgae. Moreover, untreated seawater risks exposure of hatchery animals to parasites, pathogens, and toxic phytoplankton blooms.



*Crassostrea virginica*-veliger larva

Adequate feeding with high-quality algae is the key to both high growth rates and survival. But production of sufficient quantities of algae to satisfy the needs of a hatchery can be a daunting challenge, especially for smaller growers who are now faced with the need to establish their own hatcheries due to shortages of seed. Fortunately, there is a solution to the problem of ensuring reliable supplies of microalgae for hatcheries: the use of liquid algae concentrates. Reed

Mariculture Inc. produces algae concentrate feeds designed specifically for bivalves. Reed Mariculture's Shellfish Diet® 1800 provides a nutritionally balanced combination of six specially-selected strains of *Chaetoceros*, *Pavlova*, *Tisochrysis* ("T-Iso"), *Tetraselmis*, and *Thalassiosira*, providing a range of cell sizes from 4 – 12 microns. These marine microalgae strains have demonstrated success as feeds for a variety of bivalves including oysters, clams, mussels, and scallops. This mixed diet provides excellent nutrition

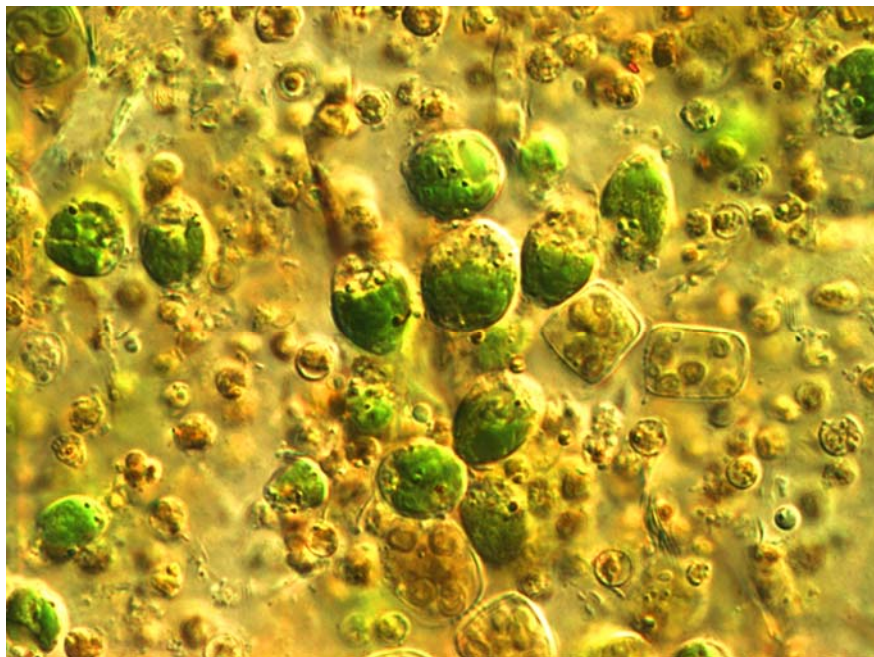
OVERCOMING  
THE BIVALVE  
HATCHERY ALGAE  
BOTTLENECK  
WITH ALGAE  
CONCENTRATES

for all life stages, from larviculture and spat setting in the hatchery, or for remote setting, all the way through to broodstock conditioning. Reed Mariculture also offers the algae species used in Shellfish Diet as single-species Instant Algae® products, so hatchery operators have the option to create their own custom mix of species for particular applications.

The FAO bivalve hatchery manual estimates that “The culture of algae accounts for about 40% of the costs of rearing bivalve seed to a shell length of about 5 mm in a hatchery.” Many bivalve hatcheries are now finding it more economical as well as more reli-

*For established hatcheries, use of Instant Algae to supplement in-house algae cultures can avert critical shortages when peak demand outstrips production capacity.*

able to feed only with Instant Algae. With no need to grow their own microalgae, the cost and the complexity of both constructing and running a hatchery are significantly reduced, making it possible for even small-scale bivalve growers to set up their own hatcheries. For established hatcheries, use of Instant Algae to supplement in-house algae cultures can avert critical shortages when peak demand outstrips production capacity.



Micrograph of Shellfish Diet

In the USA, Scott Rikard manages the Auburn University oyster (*Crassostrea virginica*) hatchery on Dauphin Island, Alabama. He says, “We use Shellfish Diet almost exclusively for rearing our oyster larvae. Since we opened the hatchery in 2003, production has increased from a

few million larvae and less than 100,000 oyster spat each year, to 188 million oyster larvae and 12.8 million oyster seed in 2014. All made possible by Shellfish Diet!” (see [www.nsgl.gso.uri.edu/masgc/masgcg12008.pdf](http://www.nsgl.gso.uri.edu/masgc/masgcg12008.pdf))

John “Barley” Dunn, Director of the East Hampton Shellfish Hatchery on Long Island, in the state of New York, says “We use Reed’s algae concentrates to supplement our own live algae production during the times of

year when our shellfish are ‘eating us out of house and home.’ This allows us to grow more and larger shellfish early in the season before moving them to the nursery.”

Reed Mariculture produces algae from continuously-harvested cultures, so the algae are always in their healthiest, rapid-growth phase. After harvesting by centrifuge, the algal cells are re-suspended in a proprietary medium of buffer salts, to stabilize cell integrity and retain full nutritional value. No pasteurization or other heat treatments are used. Shellfish Diet is a refrigerated product with a shelf life of 12-14 weeks. According to the FAO *Manual on The Production and Use of Live Food for Aquaculture*, “The density of harvested algal cultures generally ranges between 80 and 250 mg of dry weight per liter.” The biomass dry weight of Shellfish Diet 1800 is 8% (80



OVERCOMING  
THE BIVALVE  
HATCHERY ALGAE  
BOTTLENECK  
WITH ALGAE  
CONCENTRATES

g per liter), so one liter of Shellfish Diet is typically the equivalent of 320 to 1,000 liters of algal culture. In contrast to algae cultures, the consistent biomass densities of Instant Algae products make them particularly well-suited for automated dispensing to larviculture tanks via a dosing pump.

Because the algae cells in Reed Mariculture's Instant Algae products are so highly concentrated, some care is required when dispensing the products into culture tanks. This extremely high concentration can sometimes result in clumping if the product is added directly to seawater without sufficient mixing. This may happen because the cells are in such close contact that if polysaccharides on the cell surface interact with calcium ions naturally present in seawater, cells can stick together. It is therefore best to first dilute Instant Algae into around 10 volumes of fresh water (which must be free of calcium or

iron, which can also cause clumping), or even better a sodium chloride solution. Pouring the diluted product through a 20 micron screen will further ensure complete dispersion of the algal cells.

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#### About Reed MARiculture

Reed Mariculture Inc. was founded in 1995 by the Reed family to grow "tank-raised" bivalve shellfish. Over the next three years they worked on developing tank-raised shellfish technology, while also developing and refining the technology for large-scale production of marine microalgae, the essential feed for shellfish. In 1998 Reed Mariculture discontinued its shellfish operations to focus on producing microalgae for shellfish and finfish hatcheries. Today the company markets its algae-based feeds to aquaculture research institutions and commercial hatcheries in 86 countries.

#### More information

Please visit [www.reedmariculture.com](http://www.reedmariculture.com) for more information about Reed Mariculture products.

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# Young company with big plans for micro-algae



Hatcheryfeed's Roger Abbott visited a young and ambitious new Dutch company based in the Flevopolder region north-east of Amsterdam, which is aiming to conquer the algae supply market for hatcheries in southern Europe, as well as parts of Asia and the USA with its high quality micro-algae *Nannochloropsis gaditana*, within the next five years.

AlgaSpring, based in Almere, is already supplying hatcheries in Greece, Turkey, Spain and Italy and co-founder René Jongbloed said the company's products, which are sold under its NutriSpring label, are proving popular for the cultivation of rotifers, especially in Greece. The company is GMP+ and ISO22000 quality certified for food and feed safety.

René explained that AlgaSpring was launched in 2011, and started large-scale production in 2013. Housed in a huge greenhouse, it employs five people and is now producing 50 tons of micro-algae products a year mainly for aquaculture, with a small proportion going to the human food market, where it is sold as a food supplement and food ingredient.

### Unique production

AlgaSpring uses "ancient seawater" obtained from subterranean wells containing micro-nutrients and salinity for the marine micro-algae to grow on. This water is pumped up from deep wells at the company's facility, ensuring high biosecurity and lowest heavy metal contamination.

**YOUNG  
COMPANY HAS  
BIG PLANS FOR  
MICO-ALGAE**

For the production of the micro-algae, the company uses its own unique system. The foundation of this system is a hybrid technology where the process of micro-algae production is started in separate start-up photobio-reactors. The continuous outgrow takes place in the large greenhouse systems that cover an area of 12,000 m<sup>2</sup>, which is fully enclosed to prevent contamination.

After filtering and harvesting, the algae liquid is kept in 5,000-litre cooling tanks at 3° Celsius. The algae liquid is further processed to make its GMP+ certified products, which are rich in EPA-phospholipids. NannoStar, fresh frozen liquid concentrate for rotifer feed contains 35 billion cells per ml. The Liquid 40 product for green water technique contains 40 billion cells and

*Hatcheries will be able to have our products in their own cooled storage or in a storage that AlgaSpring installs at the hatchery. This system guarantees that hatcheries are never out of stock ...*

20% dry weight. Alternatively, the *Nannochloropsis gaditana* is freeze dried for non-liquid applications or storage.

AlgaSpring has chosen to produce the micro-algae in the most natural conditions with the lowest use of energy. René stated that the company does not use additional lighting, heating or cooling for the algae production. The



Co-founder of AlgaSpring, René Jongbloed, keeps a close eye on quality.

natural light conditions and the mild Dutch climate are sufficient to provide a good and economically feasible production.

Admitting they are “the new kids on the block in aquaculture,” René believes that there is a big opportunity for his business in what he sees as a growing market, because: “Hatcheries need a special rotifer feed that promises sustainable high quality, high performance and stability that is readily available.” The company’s special production process provides this as a basis.

#### **Better storage and distribution**

The company has looked thoroughly into the effectiveness and efficiency of rotifer feed and green water products

and found much room for development in both, René said.

“A rotifer feed has to come as close as possible to the natural feed on which they thrive. *Nannochloropsis gaditana* as marine micro-algae species meet this criterion to the full. The whole nutritional profile of *Nannochloropsis gaditana* closely matches the marine organisms to which they are fed. There is also room to improve efficiency.

“Liquid rotifer feeds are now transported by air halfway across the globe to be used as rotifer feed, which consists for 87% water. Both our NutriSpring products have a much lower percentage of water (higher dry weight content) and are produced in Europe.

“They have a much longer shelf life compared to other liquid products.

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COMPANY HAS  
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MICO-ALGAE**

Besides achieving a longer shelf life (up to 18 months for NannoStar) and the convenience for hatcheries, it allows us to ship our products in bulk to Southern Europe and to have a central storage facility there. From this central storage, products are distributed to hatcheries at a fraction of the current shipping cost.

“We are also thinking about storage at hatcheries in combination with a “pay-as-you-use” system. Hatcheries will be able to have our products in their own cooled storage or in a storage that AlgaSpring installs at the hatchery. This system guarantees that hatcheries are never out of stock for the rotifer feed or green water product. They can hold storage for several months, a

campaign or up to a full hatchery season.”

René pointed out that the long shelf life of the AlgaSpring products makes all this possible and the hatcheries only pay for what they use. They do not have to pay for product in their storage.

This company, which is already looking ahead to set up new production and storage facilities in Greece and other Southern European countries to ensure rapid secure supplies for customers in southern Europe, seems set on capturing a sizeable slice of the hatchery feed market for itself in the not too distant future, always focusing on its customers’ needs.

As René explained: “Listening to customers and focusing on their (future) needs is essential in any business. It is important that AlgaSpring is ‘in the field’. For our local presence we have our own technical and sales support staff in Greece and Italy. For Turkey and Spain we co-operate with other companies. Essentially we think it’s important to co-operate - co-operate with our customers, as well as other companies.”

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# Shrimp hatchery probiotics boost gut performance and profits

Beneficial bacteria are key to improve PL survivability and profit in shrimp hatcheries.

By Benedict Standen, Product Manager at BIOMIN

## Aquatic embryos: great for microbial colonization

Aquatic animal embryos are well-suited to bacterial colonization. Tank water will typically harbor rich microbial communities that can come into contact with the eggs. Thus, embryos are colonized by microorganisms rapidly; this community is termed the 'epibiota'. From a microbiology standpoint, most research has focused on pathogens in hatchery systems, as opposed to beneficial bacteria.

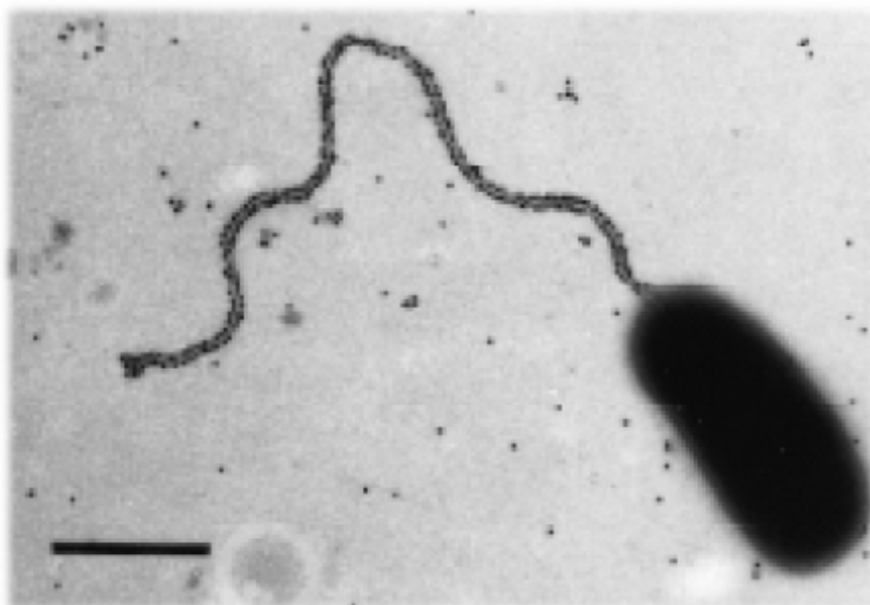


Figure 1: Electron micrograph of a *Vibrio parahaemolyticus* cell. Scale bar = 1µm

## Disinfectants lack precision

Pathogens can be bacterial, viral or fungal and have different pathologies. For example, *Flexibacter* can dissolve the chorion via enzymatic means whereas other species can cause mortalities by overgrowth (and ultimately hypoxia), e.g. *Saprolegnia*. Initially, aquatic animals are extremely vulnerable as their immune system is not fully developed or functional and their gut

is virtually sterile. Therefore, it is of paramount importance that regardless of their type, pathogenic insults are minimized. In an attempt to reduce the microbial load during early life stages, embryos are often disinfected. However, since these techniques do not discriminate between good and bad bacteria, disinfection can be counterintuitive as the beneficial bacteria are also killed. It is these beneficial bacteria

which would otherwise form the first line of defense. By removing beneficial bacteria, disinfectants increase an organism's susceptibility to disease and slows down the development and function of a proper gut structure, needed for nutrient absorption and growth. Therefore, when larvae hatch, it is important that the microbial balance is restored, and favors 'good' bacteria over 'bad'.

### Probiotics for gut performance

Probiotics, or beneficial bacteria, can reduce the abundance of pathogens directly, or, enable larvae to fight pathogens themselves by stimulating the development of the immune system. Intestinal probiotics can be given a strategic advantage if they are applied at an early stage, in effect by arriving at, and colonizing the gut first. Consequently, they are a popular choice for hatchery managers. A number of trials demonstrate how probiotics can support pathogen control, environmental stress resistance, survivability and a reduction in production costs.

*It is important that each commercial formulation is supported by sound scientific principles, is safe to use in larvae as well as effective*

### Probiotics for *Vibrio* control

Controlling *Vibrio* spp. is a major challenge for hatchery operations (Figure 1). Two recent trials demonstrate that probiotics can reduce the abundance of potentially pathogenic *Vibrio*, and consequently improve survival. In the first, Pacific white shrimp nauplii were split into two treatments: control nauplii were fed with live algae and *Artemia* until PL10, and a commercial feed until PL15. Shrimp in the probiotic

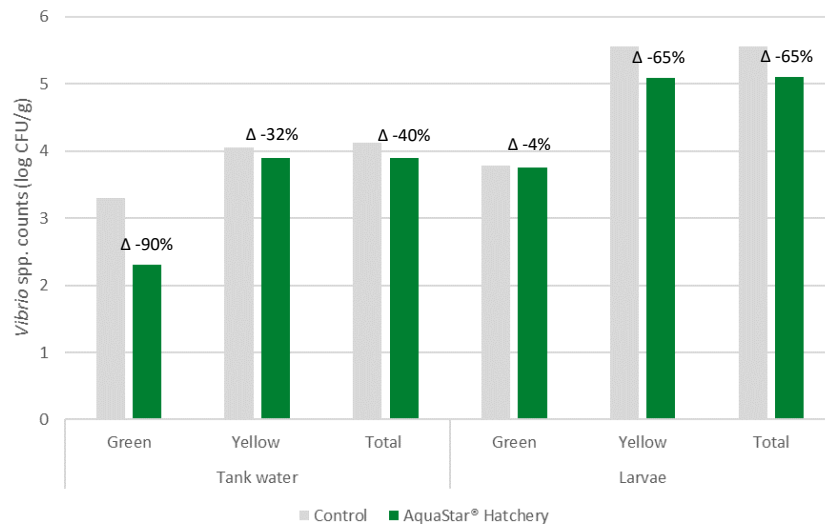


Figure 2: *Vibrio* counts in tank water and shrimp larvae from control and probiotic treatments.

treatment received a similar dietary regime but with AquaStar® Hatchery (BIOMIN GmbH) supplemented via the water (at 2.5 ppm). *Vibrio* spp. were

enumerated using widely recognized culture based techniques. Results showed that *Vibrio* counts were significantly reduced in the probiotic treated water. This could explain the survival re-

sults, where a 20% improvement was demonstrated at the nauplii phase, and a 30% improvement at PL15.

Similar results were obtained in the second trial. Although *Vibrio* spp. were detected in control and probiotic tank water, AquaStar® Hatchery appeared to reduce the total *Vibrio* abundance by 40% (Figure 2). *Vibrio* spp. were further differentiated according to their colony appearance. In the probiotic group, there were 90% fewer green colonies (*V. parahaemo-*

*lyticus*, *V. harveyi* and *V. fischeri*) and 32% fewer yellow colonies (*V. alginolyticus* and *V. anguillarum*). A similar pattern emerged when analyzing *Vibrio* loading in shrimp larvae. *Vibrio* counts were generally higher in the larvae when compared with the water, indicating an accumulation effect. However, the probiotic reduced the total *Vibrio* count by approximately 65%. This reduction was mainly down to fewer yellow colonies, whereas green colonies were only reduced marginally (-4%, Figure 2).

### Probiotics for ammonia stress resistance

Ammonia can build up quickly in hatchery systems, particularly those using live foods such as *Artemia*, as dead organisms add to the organic load. Furthermore, hatchery systems have very little water turnover, to prevent loss of the live food, allowing

toxic metabolites to accumulate. Concentrations of ammonia >0.2mg/l may interfere with the animals ability to respire, and causes high mortalities. It is likely that physiologically immature larvae, are more sensitive to low levels, when compared to their adult counterparts. A short-term ammonia challenge (20 ppm ammonium chloride added to PL tanks) was carried out to investigate whether the probiotic could alleviate this stress. After 24 and 48 hours, the survival of shrimp was significantly higher in the probiotic treatment when compared to the control group (increased by 13% and 29%, respectively; Figure 3).

#### Probiotics in low salinities

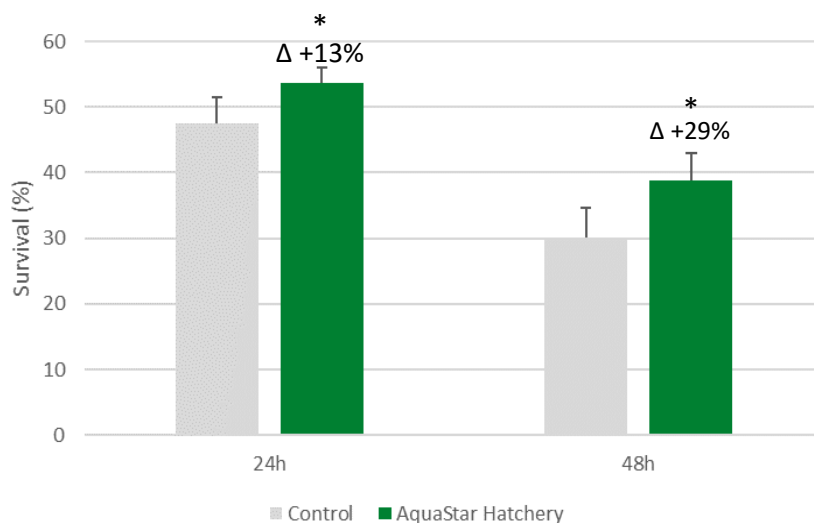


Figure 3. Effect of probiotic supplementation on the survival of shrimp PL's after 24 and 48 hours exposure to ammonium chloride (20 ppm). Asterisk indicates significant difference within the same time point ( $P < 0.05$ ).

Obtaining salt water for shrimp production can be a challenge, especially for inland hatcheries. Consequently,

many producers use water with a lower salinity, which is not ideal for most shrimp species. However, probiotics

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can help alleviate this stress. For example, during a salinity stress test, shrimp produced at 0.3 ppt showed significantly higher survival rates (>96%) when produced with AquaStar® Hatchery, compared to those produced without the probiotic.

### Probiotics make economic sense

Probiotic applications can be very successful, and consequently, there are a number of commercial probiotics available. It is important that each commercial formulation is supported by sound scientific principles, is safe to use in larvae as well as effective. A recent trial demonstrated the increased effectiveness of AquaStar® Hatchery in shrimp, when compared to another commercial probiotic product. After 12 days of culture (DOC), regardless of dosage, the survival was considerably higher in AquaStar® treatments, when compared to the other probiotic (Figure 4). Even at a lower dosage (1 ppm), AquaStar® Hatchery supplementation resulted in higher survival when compared to the second product applied at 2 ppm. Higher survival, and consequently higher productivity has clear economic benefits; these can be visualized in Figure 4. Ultimately, an effective probiotic can lower operating costs and increase survivability—improving a hatchery's profitability.

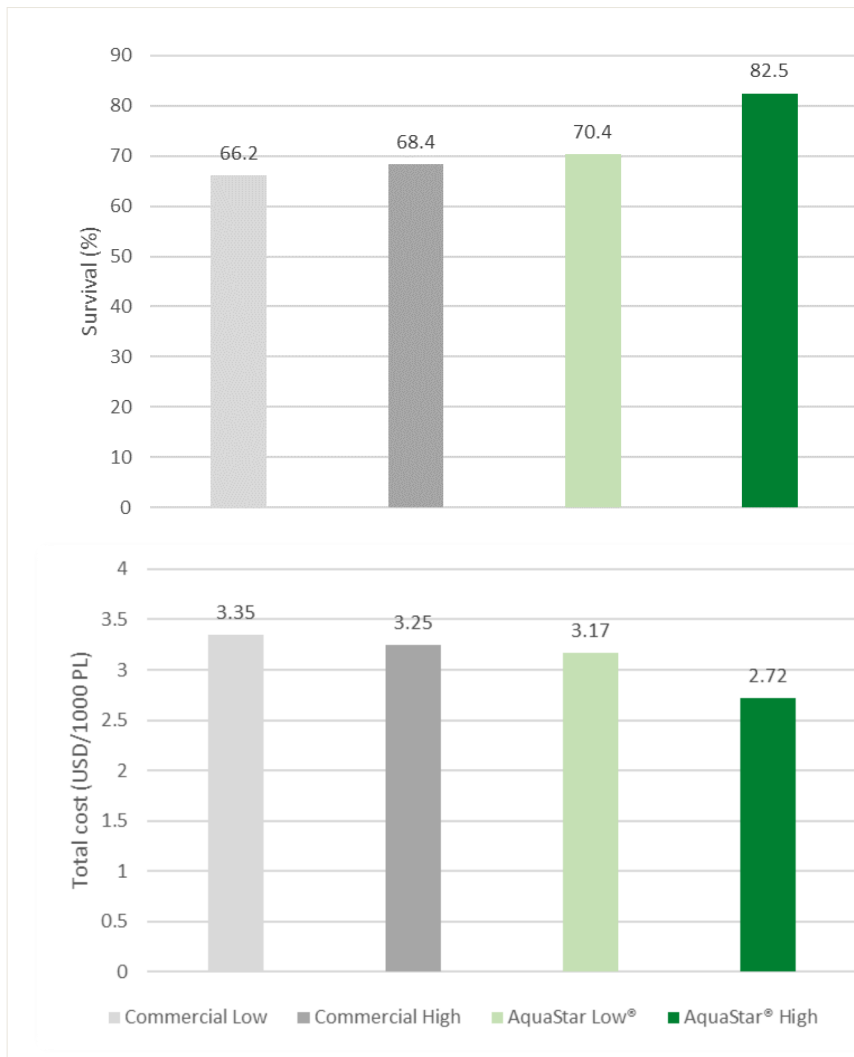


Figure 4. PL survival after 12 DOC (a) and the total cost to produce 1,000 PLs (b) when using different probiotic applications.

### Conclusions

It is clear that probiotics can support aquatic animals during the vulnerable hatchery phase by controlling pathogens, improving disease resistance and alleviating environmental stressors (e.g. ammonia, low salinity). This results in improved survival, increased production and ultimately higher profitability of hatchery operations.

#### More information

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# Prophylactic Bacilli protect shrimp against vibriosis

By Niu Yufeng, Margriet Drouillon and Peter Bossier

Aquaculture Ghent University, Laboratory of Aquaculture & Artemia Reference Center, Belgium

Faculty of Bioscience Engineering - Department of Animal Production, Ghent University, Belgium

Prophylactic Bacilli can protect aquaculture larvae against pathogenic or opportunistic vibrios by interfering with their virulence and/or by enhancing the immune response of the aquaculture larvae.

### Aquaculture and diseases

Despite the technological improvements that allowed the rapid expansion of aquaculture over the past decades, diseases are still a major constraint (FAO, 2012). Unpredictable massive mortalities still occur in early life stages of aquatic organisms in the hatchery, because of the proliferation of (opportunistic) pathogenic microorganisms in the culture systems. Estimates by the FAO of global disease losses in aquaculture are in the range of several billion US\$ per year (Subasinghe et al., 2001). In the shrimp production sector alone, the annual losses due to diseases caused by (luminescent) vibriosis are around hundreds of millions of dollars. More re-

cently, a devastating disease, Early Mortality Syndrome (EMS) or more technically known as Acute Hepatopancreatic Necrosis Disease (AHPND) caused by pathogenic *Vibrio parahaemolyticus*, has resulted in enormous economic loss to shrimp production industry. Till present, the global loss in shrimp farming due to this disease was estimated over billions US\$ (GAA, 2013). The huge economic loss due to bacterial disease has led to the use of substantial amounts of antibiotics, pesticides and chemicals, an approach which is currently being banned. As a consequence, alternative treatments to control and prevent infectious diseases, including the use of probiotics, are gaining increased attention.

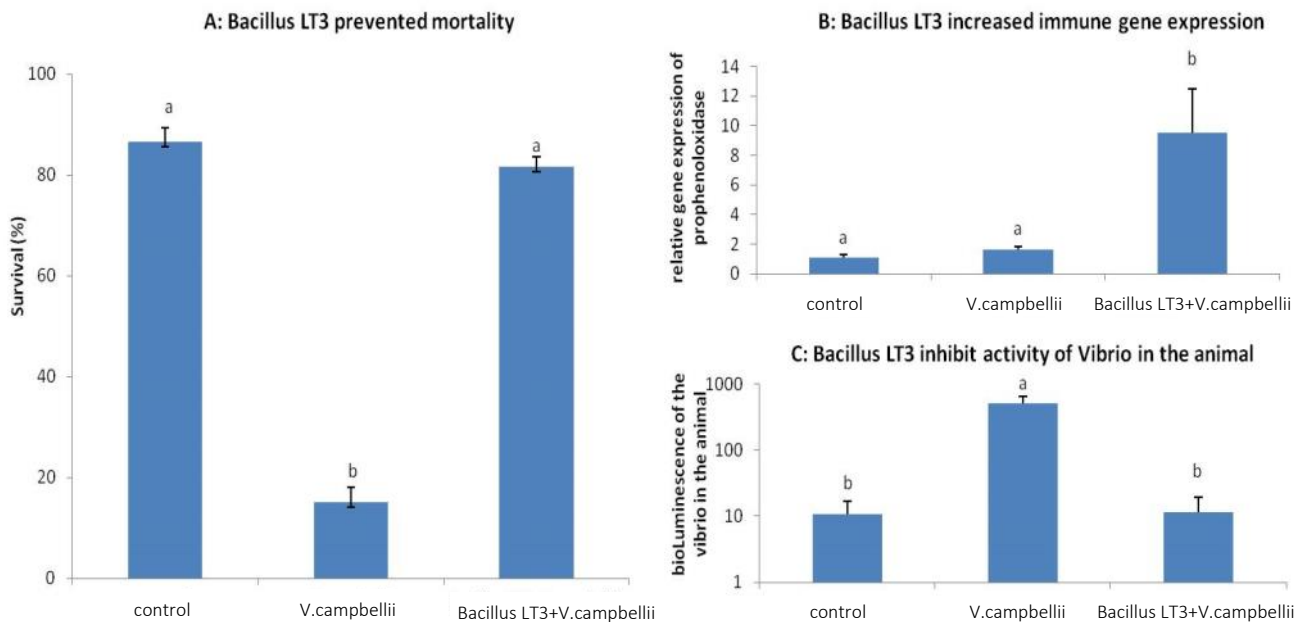


Figure 1. Validation of bacilli candidate in GART model. A: Bacillus LT3 significantly prevented the mortality of *Artemia* after the challenge of pathogenic *V. campbellii*; B: *Bacillus LT3* increased the immune gene (prophenoloxidase gene) expression of the *Artemia* after the challenge; C: Bacillus LT3 inhibit the activity of *V. campbellii* that invaded the *Artemia*. The bioluminescence of the vibrio was significantly reduced by adding the Bacilli. (Niu et al., 2014)

Probiotics are living organisms which provide health benefits beyond their mere nutritive value when administered in adequate amounts. Bacteria belonging to the genus *Bacillus* are amongst the most intensively studied group of bacteria for use as probiotics in aquaculture to control diseases. Recently, the Lab of Aquaculture & Artemia Reference Center of Ghent University has isolated a collection of prophylactic bacilli strains that can significantly prevent massive mortality of aquatic animals from pathogenic vibrio infections, particularly in shrimp.

#### Screening of the prophylactic bacilli

Our research team has been isolating specific bacilli from the intestinal tract

of healthy shrimp and fish to select those bacilli that possess prophylaxis against pathogenic vibrios. Series of in vitro and in vivo screening tests were performed to verify the effect of candidate prophylactic bacilli. Hundreds of candidate isolates were first screened using the gnotobiotic *Artemia* model (GART model Published in [Hatcheryfeed Magazine 2015](#)) to investigate their prophylactic function before being tested on actual commercial species, such as shrimp. The GART model is a high throughput screening platform that provides gnotobiotic (i.e. bacteria-controlled) experimental conditions. It rules out the interference of natural microbiota on the experiment, improves the reproducibility of the results and guarantees accurate interpretation of the

results. In the GART model, the brine shrimp (*Artemia*) larvae were first fed with the candidate bacilli and then challenged with pathogenic vibrios. The screening showed that several isolates can significantly improve the survival of *Artemia* after a challenge with different types of vibrios (Fig.1 a). It is also interesting to note that some isolates can improve the survival of *Artemia* by enhancing the immune response of the *Artemia* (Fig, 1 b) and, simultaneously, reduce the activity of the pathogen (Fig. 1c)

#### Validation of prophylactic bacilli in a shrimp experimental model

The bacilli isolates that showed the most promising prophylaxis in the GART model, were evaluated further

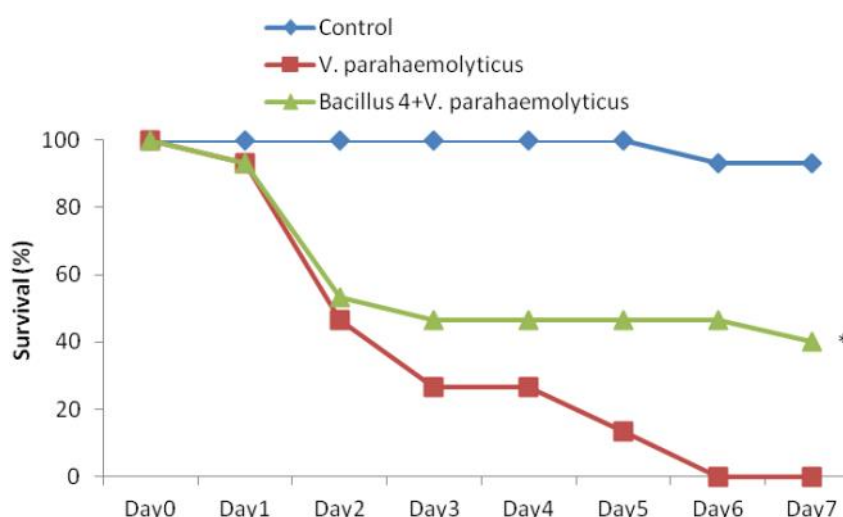


Figure 2. Validation of the bacilli in shrimp model. The strain Bacillus 4 improved the survival of the shrimp for more than 40% comparing to the control group where the bacilli was not used.

in a shrimp challenge model. During the tests, the shrimp post larvae were incubated with the bacilli for a short period prior to a challenge with pathogenic *Vibrio parahaemolyticus*. The pathogenic *Vibrio parahaemolyticus* strains were obtained from EMS-infected shrimp ponds and were proven to induce massive mortality of the shrimp post-larvae. It was exciting to see that certain isolates can remarkably prevent the mortality of the shrimp (Fig 2). Compared with the group where the shrimp did not receive prophylactic bacilli, the survival in the treated group was 40% higher. At present, the reports on effective probiotics against EMS disease are very limited, even rarer with strong scientific support. Our results suggested great potential of using these bacilli as a preventive approach against EMS in shrimp production.

#### Multifunctional prophylactic bacilli

In addition to the prophylaxis against EMS pathogens, some of these prophylactic bacilli have strong capacity of degrading a group of small signal molecules, naming N-acylhomoserine lactones (AHLs). These molecules are involved in the cell to cell communication of many opportunistic vibrios, regulating their virulence. The prophylactic bacilli that disrupt (degrade) these molecules can exert broad spectrum interference on the virulence regulation of opportunistic and pathogenic Vibrios in the culture environment, improving the survival of the cultured animals.

#### What's next?

These (multifunctional) prophylactic bacilli showed great potential to be used as direct fed microbials (DFM) for disease control purposes in aqua-

culture, particularly in shrimp production. Future work will focus on the optimization of administration strategies of these bacilli and the validation of their function on other aquaculture species. In the meantime, we warmly welcome any form of collaboration for obtaining more substantial data on the function of these bacilli in aquaculture practice.

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#### More information and References

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# Gut health in early stages of fish and shrimp: small investment, big returns

The European ban on the use of antibiotic growth promoters in livestock, and the subsequent search for alternatives, has revealed the importance of gut health and the development of a stable, favorable gut microbiota, on feed efficiency, overall performance and productivity.

Photo: Tilapia nursery in Thailand

By Peter Coutteau, PhD, Maria Mercè Isern I Subich, DVM, Alexander van Halteren, and Sam Ceulemans, Nutriad International, Dendermonde, Belgium.

In aquaculture, increasing attention has been given lately to the role of the gut microbiota in the optimal functioning of the digestive system of fish and shrimp. Fish and shrimp are highly exposed to exchanges of microflora between the environment and the digestive system. This increases the risk for the proliferation of an unfavorable gut microflora or frequent destabilization of the microflora, which can affect the optimal functioning of the digestive system. Furthermore, the digestive system of fish and shrimp is the main entry port for bacterial, viral and parasitic infections, which remain a major risk for the profitability of aquaculture production. The transfer of fish and shrimp fry from the hatchery environment to the nursery and subsequent growout system, induces drastic changes in the gut microbiota of fragile stages which are highly vulnerable to opportunistic bacteria. Promoting a stable, well-balanced gut microbiota in early life stages is a key factor for maximizing production yield and profitability in the growout operation.

Sustainable approaches to modulate the gut microflora in aquaculture include the use of a wide variety of natural compounds capable of modulating the microflora towards a favorable composition such as probiotics, prebiotics, organic acids, yeast extracts and phytobiotics. These strategies may have synergistic effects, for example phytobiotics can enhance the establishment of probiotic bacteria and therefore enhance the efficacy of probiotic inoculations.

#### Natural growth promotion in the absence of disease

A synergistic blend of phytobiotics was selected for their bacteriostatic and bactericidal properties against pathogenic and potentially pathogenic bacteria in vitro. This blend was capable of promoting growth significantly

in feeding trials with healthy specimens of different species of fish and shrimp growing under controlled lab conditions (Fig. 1). These lab results have been confirmed in a number of studies under field conditions with different species of fish and shrimp (Coutteau et al., 2010; Cuellar-Anjel et al., 2011; Valle and Coutteau, 2015; Sampaio Gonçalves et al., 2016).

#### Reduced impact from bacterial pathogens and parasites on survival and productivity

Functional feeds containing gut health promoters deliver with every meal an adequate concentration of natural antimicrobial activities into the digestive system. These feeds are a key component of any strategy to prevent diseases in aquaculture, particularly in the rearing of young stages where

opportunistic bacteria are still a major cause of mortality and vaccination is not possible yet. However, the success of this approach will depend on the efficacy of the gut health promotor. The gut modulating feed additive ideally is heat stable and can therefore be easily incorporated into industrial aquafeeds and be present in every meal from the starter feed onwards, without requiring major adaptations of the production protocols.

This is particularly important in hatchery and nursery stages where feed particles are too small to incorporate additives at the farm and the feeding frequency will affect greatly the duration of the exposure of the gut microbiota to the gut health promotor. Natural feed additives combining different action mechanisms such as direct bactericide/bacteriostatic properties as well as Quorum Sensing inhibition properties at concentrations below MIC, are most promising to control bacterial diseases caused by opportunistic bacteria such as *Vibrio spp.* The inclusion of such botanical feed additive under standard industrial conditions at the feed mill improved survival under production conditions in a semi-intensive shrimp farm in Panama and Ecuador with 18 to 24% compared to the control group during two independent production cycles (Cuellar-Anjel et al., 2011; Valle and Coutteau, 2015; Fig. 2). In these production trials, the main disease chal-

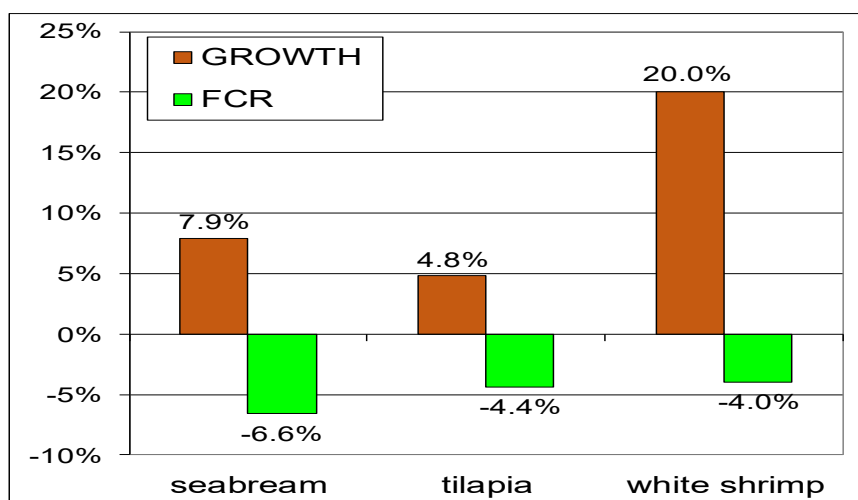


Fig. 1: Percentage improvement of growth and feed conversion ratio (FCR) due to supplementing a phytobiotic growth promotor (SANACORE® GM) to a practical feed of different aquaculture species. Data show the effect on growth (for fish : SGR, %/day; for shrimp g/week) and feed conversion ratio (FCR) relative to the performance of the non-supplemented control group in a feeding trial with healthy animals (Ceulemans et al., 2010).

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lence at the farm consisted of White Spot Disease and Vibriosis. The gut health promotor was applied from the first starter feed onwards and continued throughout the entire cycle till harvest. Other strategies include the application of gut flora modulators from PL stages up to the first 45 days of growout in the ponds in order to prevent the establishment of opportunistic pathogens during the initial phase of grow out.

#### Controlling bacterial pathogens in hatchery

Loc et al. (2015) was able to confirm the effect of a synergistic phytobiotic

product in a controlled challenge trial with *Vibrio parahaemolyticus* (Early Mortality Syndrome, Acute Hepato-

pancreatic Necrosis Disease, EMS/AHPND strain) under laboratory conditions; showing 62-107% increased

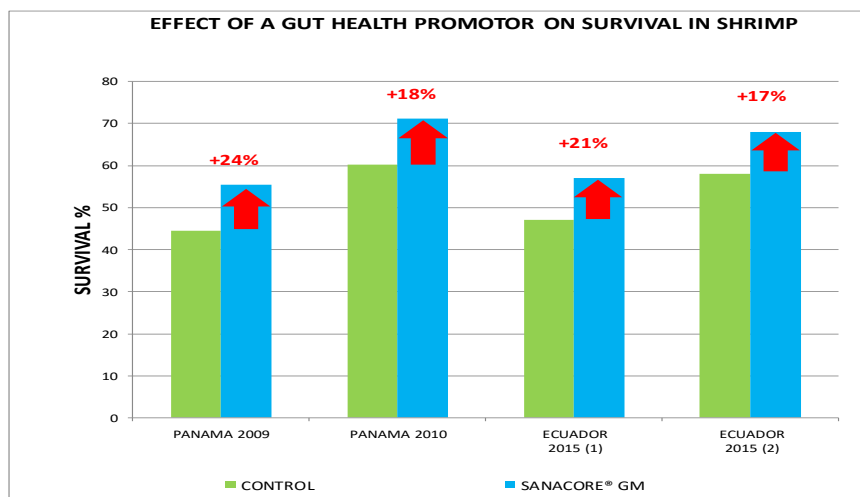


Fig. 2: Effect of functional feed additive with combined anti-bacterial/QS inhibition action on survival of *Penaeus vannamei* in various production trials in Panama and Ecuador (data from Cuellar-Anjel et al., 2011; Valle and Coutteau, 2015; Valle unpublished data).

survival in shrimp that had received the additive during 3 weeks prior to the experimental infection, compared to unsupplemented control groups. Furthermore, the addition of the phytobiotic product in the diet resulted in consistently lower *Vibrio* counts in the shrimp's digestive system compared to the control (Fig. 3), illustrating the capability of gut modulating additives to protect the shrimp's gut flora throughout a *Vibrio* challenge. Gut health modulation is a promising tool to control the count of opportunistic bacteria such as *Vibrio* spp associated with the gut of hatchery and nursery stages. The inclusion of natural products with bactericidal and quorum quenching activities is an important factor in current bio-security protocols

.....

*... the establishment of a healthy and robust gut microbiota in early stages of fish and shrimp has become a new focus area for the development of a new generation of larval and postlarval feeds*

to prevent the vertical spread of opportunistic bacterial pathogens such as *Vibrio* spp, *Photobacterium* spp, *Flavobacterium* spp, *Tenacibaculum* spp. Furthermore, a healthy gut microbiota enhances the overall health status and immune defenses, which may explain the positive effect of gut modulators on reducing the impact of cer-

tain endo as well as ectoparasites on productivity in fish.

### Conclusion

Technological developments for hatchery and nursery feeds have made tremendous progress during the past decade and increasingly meet the nutritional requirements and optimal physical characteristics for feeding fish and shrimp fry. The producers of most aquaculture species are facing increasing disease challenges which demand for enhanced biosecurity and prevention strategies from early stages onwards. As a result, the establishment of a healthy and robust gut microbiota in early stages of fish and shrimp has become a new focus area for the development of a new generation of larval and postlarval feeds. Lab and field studies demonstrated the potential benefits for aquaculture in terms of productivity and economics of specific feed additives developed to stimulate the establishment of a healthy gut microflora.

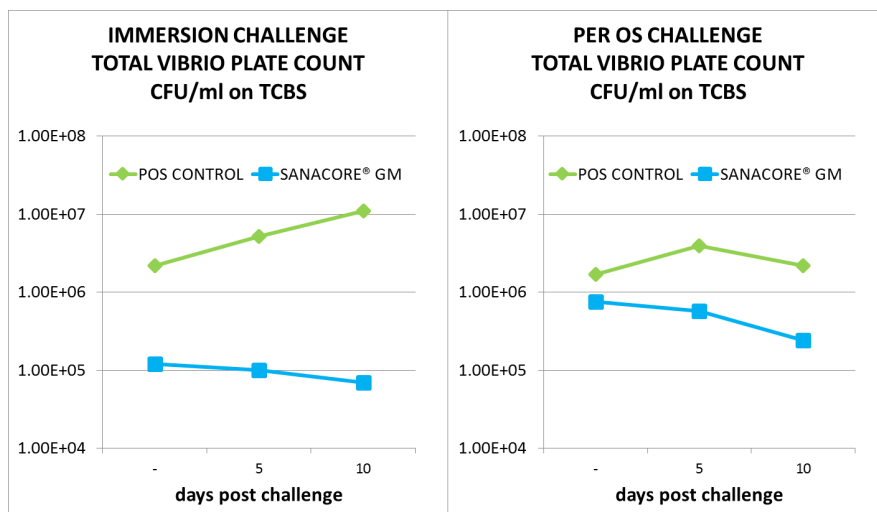


Fig. 3: Effect of a gut health modulator on total *Vibrio* count originating from the digestive tract in shrimp challenged with an EMS/AHPND causing strain of *Vibrio* parahaemolyticus following two types of experimental infection (immersion and per os). Data show total *Vibrio* count of positive control group (challenged, fed on control feed) and treatment (challenged, fed control feed supplemented with a gut health modulating additive) during 10 days of post-challenge follow-up during which the respective feeds were continued. Day 0 shows results after 21 days of pre-challenge acclimatization on the different dietary treatments, prior to challenge.

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### More information

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# Pelleted polychaetes

## A natural component in hatcheries and shrimp farms

Feeding broodstock and shrimp with wild harvested worms has its downside: for the environment, for the shrimp and ultimately also for profit margins. OddGeir Oddsen proposes a solution that modernizes the way we feed broodstock, larvae and shrimp.

### **Worms on the wild side**

Wild harvested worms have been the shrimp feed of choice in the industry for many different (and valid) reasons. First and foremost, shrimp need polychaetes as a component in their food for spawning, and suitable worms are found in the areas where the shrimp are farmed. So we have easy access to a necessary protein. What could possibly go wrong?

Let's start with how polychaetes are harvested in the wild. Currently, there are no rules or regulations that dictate where worms are to be harvested, or what constitutes a sustainable harvest. And since no-one regulates anything, areas that used to be rich in worms are now heading for depletion.

In addition, use of wild harvested worms has in several instances been

associated with EMS (Early Mortality Syndrome) in shrimp farms. Wild harvested worms are likely to harbor parasites, viruses or bacteria which are transferred to broodstock and their offspring.

As I see it, these are the main problems facing the industry in relation to wild harvested shrimp feed. And I think there's an alternative that may very well let businesses improve while at the same time modernizing their practices, accessing new potential markets and being kind to the environment.

### **Potential in polychaetes**

Since we already know that worms constitute the optimal protein if one wants to ensure good maturation rates in broodstock, there really isn't going to be a reason to try to make shrimp

feed without including worms, at least not in the foreseeable future. In addition to good maturation rates, worms contain an important fatty acid profile that broodstock pass on to their offspring, and the inclusion of polychaetes in the shrimps' diets is known to improve the health of the hepatopancreas. In short: Worms are necessary. But they don't have to be harvested from the wild.

Farmed polychaetes can be tested for parasites, bacteria and viruses in order to ensure a disease-free worm stock. The worms can be raised in facilities that do not hurt the environment around shrimp farms, a factor we all know has tarnished the industry for years. Polychaetes can be farmed in bio-secure ponds hundreds of miles away from open-water shrimp farms, thereby eliminating the potential for



cross-contamination with shrimp disease.

But switching to farmed worms doesn't solve all problems: Shipping and handling wet feed requires a complex infrastructure, and it takes considerable time and effort both to freeze and thaw the polychaetes. In addition, wet feed disintegrates as soon as it enters the water, which means that many nutritional elements will go to waste. In turn, this means that shrimp farmers need to spend more money to feed their stock than necessary.

So where does this leave us?

### **Sustainable shrimp feed**

The solution is pellets. Or rather, the solution lies inside the pellets. By using farmed polychaetes as a component in pellets made especially for shrimps, many of the obstacles shrimp broodstock farmers face on a daily basis will become problems of the past. Today, the only manufacturer of pelleted feed with polychaetes as an active ingredient is the UK-based company ProChaete.

Using pellets instead of wet feed has obvious benefits. For one thing, storing and handling pellets is much easier than storing and handling wet feed.

The same goes for feeding the shrimp, a process that can be automated when using pellets. In fact, pelleted feed is optimized for automatic feeding systems. In addition, the predicta-

*Today's harvest of wild worms is unsustainable, both on a farm-by-farm-basis and on an industrial scale.*

bility with which pellets can be delivered is far greater than with wild harvested worms.

And then there are the polychaetes. When ProChaete manufacture pellets, they source their polychaetes from bio-secure farming units in Europe. In addition to lowering the risk of shrimp disease significantly, there are other benefits of using worms that have been tested for diseases and parasites. More and more retail chains look for transparency and corporate social responsibility throughout their entire supply chain. Big retailers will not consider using suppliers who impact their environment negatively. This means that using sustainably harvested, disease-free worms in pelleted feed could help shrimp farmers move into new markets.

### **Modern maturation**

So does pelleted feed work when the object is to mature broodstock as effectively as possible? The short answer is yes. Trials indicate both good maturation rates and good fertility.

### **Food for the future**

I believe that hatcheries and shrimp farms need to adapt to an environment-friendly future if they want to survive. Today's harvest of wild worms is unsustainable, both on a farm-by-farm-basis and on an industrial scale. When we almost deplete the nearby environment of resources, the prices of those resources are going to skyrocket, as in every other industry. Thus switching to a predictable feed source has financial incentives as well. By using pelleted feed that contains polychaetes, hatcheries and shrimp farms are taking the first steps in building business models that can stand the test of time.

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### **More information**

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# A sustainable fishmeal replacement for shrimp feed

By Ylva Sundstrom, Alejandro E. Nyul, Sara I. Hash and Suresh Menon,  
Menon Renewable Products, Inc., San Diego, California, USA

Menon Renewable Products, Inc. (MRP), USA has developed a game-changing technology that converts agricultural inputs into an enhanced organic, high protein fish meal replacement – MrFeed®. MrFeed® ingredient can readily replace some or all of the fish meal in shrimp aquaculture feed while delivering improved survivability, yields and value.



Figure 1. Feed trials were conducted in cages suspended in ponds.

MRP is tackling some of the greatest challenges facing the aquaculture and fish feed industry today – providing a feed ingredient that allows aquatic species to grow fast and healthy, while simultaneously reducing the requirement for fish meal. MrFeed® is produced using MRP's proprietary fermentation process which provides the feed supplement with highly beneficial prebiotic and enzymatic properties. These com-

pounds offer significant health benefits to the shrimp by providing enhanced palatability and promoting healthy digestion and absorption of nutrients in the gut of the shrimp.

Overall feed requirements vary greatly depending on the species, the life stage of each species as well as the growth density. The MRP production process is versatile in that it has the capability to produce a variety of MrFeed® ingredient formulations,

Table 1. MrFeed® Shrimp Pro45 contains all the essential protein, vitamin and fat components that are vital for healthy development and growth of shrimp while providing a sustainable alternative to fish meal. Formulations can easily be modified to enhance any specific amino acid, vitamin or mineral requirement.

<b>MrFeed®</b>		
<b>Parameter</b>	<b>Value</b>	
Moisture	10.0%	
Protein	45.6%	
Fat	3.8%	
Fiber	3.2%	
Carbohydrates	31.3%	
Ash	6.1%	
<b>Total</b>	<b>100.0%</b>	
<b>MrFeed® (Vitamins)</b>		
Vitamins A	1200 IU/100g	
Vitamin B1	50 mg/Kg	
Vitamin B2	40 mg/Kg	
Vitamin B3	100 mg/Kg	
Vitamin B6	50.2 mg/Kg	
Vitamin B12	20 mcg/Kg	
Folic Acid	10 mg/Kg	
Vitamin E	223 IU/Kg	
Vitamin K	40.0 mg/Kg	
Vitamin D3	2000 IU/Kg	
Vitamin C	250 mg/Kg	
Pantothenic acid	100 mg/Kg	
Biotin	1 mg/Kg	
Choline	400 mg/Kg	
<b>Amino Acids</b>	<b>Fish Meal</b>	<b>MrFeed®</b>
Arginine	3.82%	3.25%
Histidine	1.58%	1.16%
Isoleucine	2.50%	2.00%
Leucine	4.44%	3.39%
Lysine	4.64%	3.09%
Methionine	1.63%	0.57%
Phenylalanine	2.43%	2.21%
Threonine	2.56%	1.76%
Tryptophan	0.60%	0.57%
Valine	3.03%	2.15%

each varying in level of protein and other essential nutrients. MrFeed® Shrimp Pro45 is MRP's first available product containing all the essential protein, vitamin and fat components that are vital for healthy development and growth of shrimp (figure 1).

*Tests conducted in cages suspended in ponds demonstrated that up to 50% replacement of fish meal and fish oil by MrFeed® resulted in higher shrimp survivability and weight gain than the control feed.*

MrFeed® Shrimp Pro45 is specially formulated for shrimp aquaculture with a blend of proprietary inoculum, nutrients and trade secrets that optimize shrimp aquaculture results, delivering improved yields and value along with reduced operating costs. MrFeed® enhances the overall performance of any standard feed product, while simultaneously reducing the need for fish meal in the total feed, thus providing a sustainable feed solution to the market. MrFeed® Shrimp Pro45 was evaluated for its impact on survivability and weight gain of shrimp at a major shrimp feed producing company. Tests were conducted in cages suspended in ponds (figure 1). This study demonstrated that up to 50% replacement of fish meal and fish oil by MrFeed® resulted in higher shrimp survivability and weight gain than the control feed (table 2).

**A SUSTAINABLE  
FISHMEAL  
REPLACEMENT  
FOR SHRIMP  
FEED**

Table 2. Feed trials demonstrate that shrimp fed diets in which up to 50% of fish meal and fish oil were replaced by MrFeed® displayed higher survivability and weight gain than shrimp fed the control feed.

Feed Trial Results		
	Control	MrFeed® Shrimp Pro45
Starting weight	3 g	3 g
Weight gain (g/42 days)	15.0 g	16.4 g
Days	42	42
FCR	1.05	0.95
Survival %	66.2	68.9

Proper nutrition provides greater tolerance to stress and infections. The effect of MrFeed® has been tested in various growth stages of shrimp, and the product has been demonstrated to provide shrimp with increased survivability and higher tolerance to disease exposure when compared to shrimp fed standard commercial feeds. A study performed at the University of Arizona revealed that shrimp fed a diet including MrFeed® were protected against the effects of exposure to EMS-causing *V. parahemolyticus*, as compared to shrimp fed the commercial diet alone. Details of the full study are described in *Aquafeed*, Vol 7, Issue 4, Dec. 2015.

In summary, MrFeed® is a sustainable, high protein aquaculture feed ingredient that provides balanced nutrition and enhanced health benefits for aquatic species. The ingredient is produced and shipped exclusively from MRP's state-of-the-art facilities in North America, which operates under approved government permits and within AAFCO guidelines for animal

feed. In addition, use of MrFeed® offers the following advantages for shrimp farmers:

- Lower operating costs and competitive price advantage over those shrimp farming operations using traditional shrimp feed formulations
- Eliminates reliance on uncertified fish for fish meal and oil inputs used in today's shrimp feed
- Allows products to qualify for sustainability certifications
- Provides a marketing advantage to retailers (Eg. Wild Oats) who want suppliers to tie into their sustainability initiatives versus competitors using traditional fish meal based feeds
- Reduces the carbon footprint of the shrimp farming industry

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**More information**

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# Pacific Mussels as a feed for *Penaeus vannamei* broodstock

By Mark Damien Ingley, GB Aqua Inc.

Mussels (*Mytilus sp.*), are cultured in Pacific coastal waters, harvested, shucked, washed and then flash frozen in their raw, natural state. They have become widely established as a valuable brood stock feed for Pacific White Shrimp.

Trials were conducted over the course of one year during 2011 at BLK hatchery (of CPP) near Lampung, Indonesia to evaluate the effectiveness of replacing both live and frozen marine Polychaete sea worms with GB Aqua Pacific Mussels.

The control had a diet consisting of (% of biomass) 14% Squid, 3% *Artemia* Biomass, 6% Live Polychaetes and 6% Frozen Polychaetes; producing an av-

erage of 167,400 nauplii per female. In trial tank #1, 6% Frozen Polychaetes were substituted with 6% Frozen Pacific Mussels and in trial tank #2, all Polychaetes were eliminated and replaced with 12% Frozen Pacific Mussels.

Trial tank #1 ended the trial producing an average of 245,200 Nauplii per female (46.48% increase) while trial tank #2 ended the trial with an average production of 229,100 Nauplii per female (36.86% increase).

Ovaries in the females took on the dark orange carotenoid pigment coloration of the Pacific Mussels as did the newly hatched nauplii (see Table 1).

Nauplii from the brood stock fed the Pacific Mussels were reported

as being more robust and active with higher energy levels than those of the control. Further observations included an improvement of survival rates of the metamorphosis from Nauplii to Zoea, Zoea to Mysis and Mysis to Post Larva, however no studies were performed to quantify these observations.



Figure 1.  
Above: Female Ovaries fed with GB Aqua Pacific Mussels.  
Below: Female Ovaries fed control diet (no GB Aqua Pacific Mussels).

PACIFIC MUSSELS  
AS A FEED FOR  
*PENAEUS*  
*VANNAMEI*  
BROODSTOCK



Figure 2.

Nauplii from brood fed with GB Aqua Pacific Mussels.

Nauplii from brood fed control diet (no GB Aqua Pacific Mussels).

Table 1 Trial results of GB Aqua Pacific Mussels

Parameter	Unit	Perlakuan 1 (19,21) Mussels 12% <i>Artemia</i> Biomass 3% Squid 14% Total 29%	Perlakuan 2 (20,21) Mussels 6% <i>Artemia</i> Biomass 3% Squid 14% Polychaeta Life 6% <b>Total 29%</b>	Control (24) Polychaeta Frozen 6% <i>Artemia</i> Biomass 3% Squid 14% Polychaeta Life 6% <b>Total 29%</b>
Number of Broods	PC	77	77	75
Doc	Day	60	60	60
Broods Mature	PC	659	757	692
Broods Sampling	PC	3,081	3,393	3,063
Spawner	PC	237	228	227
Total Eggs Produced	X 1,000	79,500	86,600	74,900
Total Nauplii Produced	X 1,000	37,700	44,500	38,000
Av of Mating Rate per Day	%	7.6	7.7	7.4
Av Fertility Rate	%	58	62.5	61.3
Av Hatching Rate (Total)	%	47.4	51.4	50.7
Av Mortality Female	%	15.6	16.5	14.7
Av Nauplii/Spawner	X 1,000	229.1	245.2	167.4
Av Nauplii/Female Ablated	X 1,000	489.6	577.9	506.7

In conclusion, while the elimination of both Live and Frozen Polychaetes from the feeding regime with the replacement of GB Aqua Pacific Mussels improved the production of Nauplii over the control, broodstock which

were fed with some Live Polychaetes produced the most Nauplii.

#### More information

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# Industry events



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## August

16-18: **AFIA Preventive Controls for Animal Food course**

Auburn, AL, USA

[Details](#)

17-18: **TARS 2016: Shrimp Aquaculture & the New Normal**

Phuket, Thailand

[Details](#)

19 - 21: **11th International Conference on Recirculating Aquaculture & 2016 Aquaculture Innovation Workshop**

Roanoke, VA, USA

[Details](#)

21-26: **23rd Annual Practical Short Course on Aquaculture Feed Extrusion, Nutrition & Feed Management**

Texas A&M University, TX, USA

[Details](#)

24-25: **ICFA 2016**

Negombo, Sri Lanka

[Details](#)

## September

6 - 8: **VIV China**

Beijing, China

[Details](#)

13-15: **AFIA Liquid Feed Symposium**,  
Memphis, TN, USA

[Details](#)

19-22: **GAA's GOAL Conference**,  
Guangzhou, China

[Details](#)

22 - 25: **Aquarama 2016**  
Guangzhou, China

[Details](#)

20 - 23: **Aquaculture Europe**  
Edinburgh, Scotland

[Details](#)

27-28: **Seagriculture 2016**  
Aveiro, Portugal

[Details](#)

## October

12-15: **Biomim World Nutrition Forum**

Vancouver, Canada

[Details](#)

19-21: **AquaSG'16 conference**  
Temasek Polytechnic, Singapore

[Details](#)

## November

15 - 18: **Eurotier**

Hanover Germany

[Details](#)

28-Dec 1: **LAQUA 2016**

Lima, Peru

[Details](#)

## December

13-15: **AlgaEurope 2016**  
Madrid, Spain.

[Details](#)

## 2017

### Mar

15-17: **VIV Asia 2017**

Bangkok, Thailand

[Details](#)

### Jun

26-30: **World Aquaculture**  
Cape Town, South Africa

[Details](#)

### Aug

8-10: **2017FishAdapt: Global Conference on Climate Change Adaptation for Fisheries and Aquaculture**

Bangkok, Thailand

[Details](#)



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