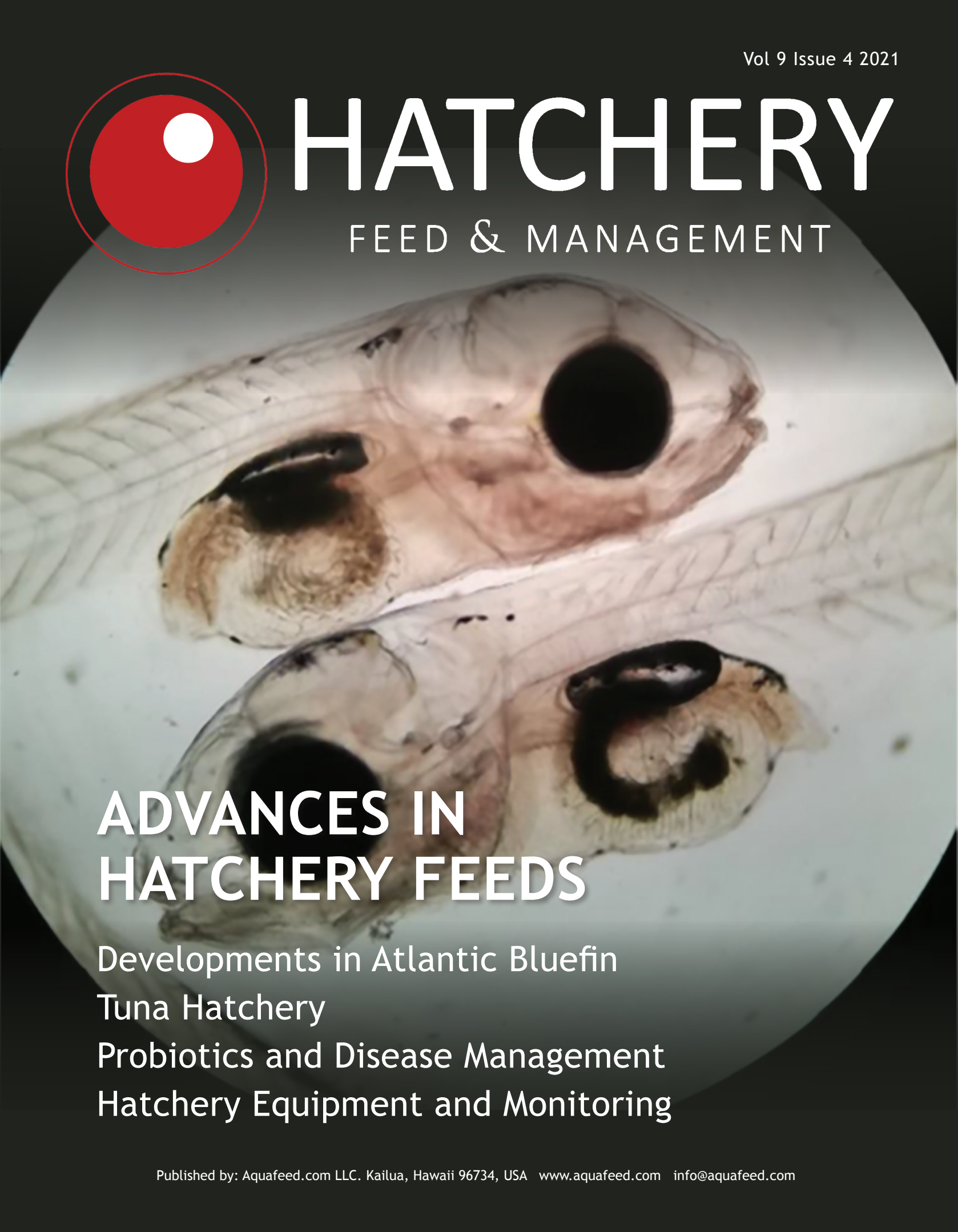
Vol 9 Issue 4 2021

HATCHERY

FEED & MANAGEMENT



ADVANCES IN HATCHERY FEEDS

Developments in Atlantic Bluefin Tuna Hatchery

Probiotics and Disease Management

Hatchery Equipment and Monitoring

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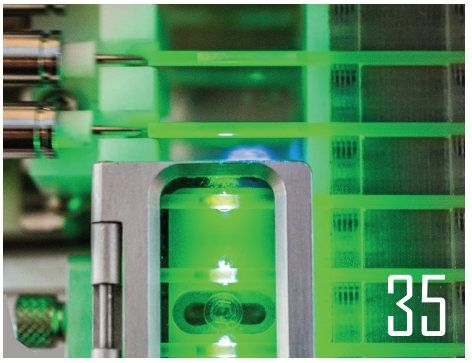


NEWS REVIEW

HATCHERY FEED & MANAGEMENT

Highlights of recent news from Hatcheryfm.com

News as it happens in the Newsroom at Hatcheryfm.com - sign up for our free weekly newsletter for updates

GenoMar to supply genetically improved tilapia in Colombia

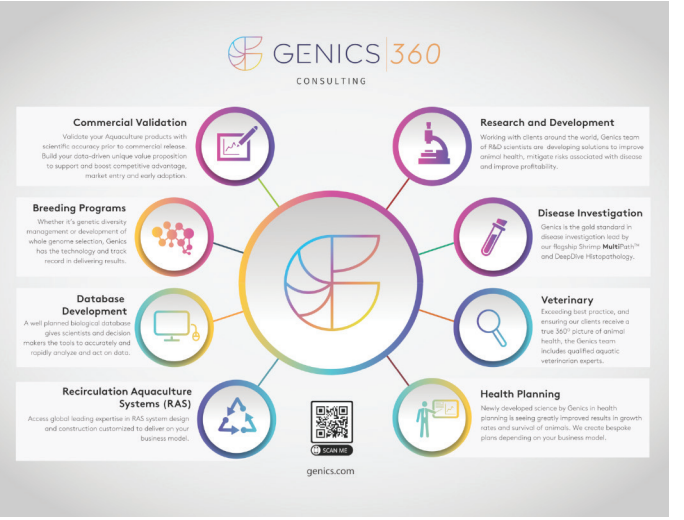
GenoMar Genetics AS signed a joint venture agreement with Agroavícola Sanmarino S.A., a Colombian poultry genetics distribution company belonging to the Italcol group. The agreement includes the establishment of a new company in the next few weeks, named GenoMar Genetics Colombia. The joint venture will distribute tilapia genetics in the form of fingerlings and juveniles from a high-quality biosecure land-based facility in Colombia's Huila province.

AquaGen opens breeding center in Scotland

AquaGen Scotland officially opened the Holywood Breeding Center, near Dumfries. The company acquired the hatchery in March 2019 and has invested more than £5 million. The acquisition of the Holywood Breeding Center enables the company to provide a reliable supply of eggs from AquaGen broodstock, which are reared in and selected for best performance in the Scottish farmed environment.

Genics expands consulting services taking a 360-degree view of the shrimp business

Genics has expanded its value proposition to include consulting. Known for its success in the very early detection of pathogens in shrimp through its flagship offering Shrimp MultiPath™, the company has opened a broad range of other services to all clients through their Genics360 Consulting model that, until now, has been only available to a select few companies upon request. Genics360 services include commercial validation, health planning, veterinary and RAS.

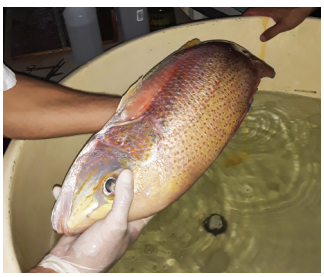


International project to develop breeding program for rohu carp in Myanmar

Xelect partnered with Fresh Studio, with funding from animal feed supplier De Heus and the Dutch government, to boost food security in Myanmar through Powering Aquaculture Progress project, a five-year initiative to modernize the local aquaculture industry.

Fresh Studio and Xelect are collaborating on the development of two flagship hatcheries, an applied R&D farm and the genetic tools required to kickstart a centralized program for rohu carp. These will act as the basis for a modern, centralized breeding program that will supply high-quality broodstock for seed production at hatcheries throughout the country.

First commercial spotted rose snapper genetic program underway



Central American aquaculture producer, Martec, and Xelect signed a partnership to create the first large-scale genetic breeding program for spotted rose snapper (*Lutjanus guttatus*). The partnership will be critical as Martec ramps up its production to 10,000 tons per year.

Finnforel to build first selective breeding center for rainbow trout in Finland

The project, which is being implemented in close co-operation with Natural Resources Institute Finland, will help secure the local supply of fish eggs and fry. Since the early 1990s, Natural Resources Institute Finland has carried out research in rainbow trout genetics, breeding and animal health. This project marks the first time that the results of this work are being applied and commercialized to this extent.

MDM, Trome partner for distribution of pumps in Europe

MDM Inc. and Trome BV signed an exclusive distribution agreement to promote MDM's Sequence®, Advance®, Genesys® and C-Shell® pumps in European aquaculture. MDM, a US-based manufacturer of non-metallic pumps and Trome, a Belgian manufacturer of drum filters, have been collaborating for 18 months to assemble pump and motor units in Europe.

BioMar to expand capacity in Ecuador

BioMar unveiled a comprehensive investment plan for its business unit in Ecuador to further support an amplified product portfolio for high-performing shrimp feed in Ecuador and follow up on strong sales figures. The investments are part of the preparation for the introduction of new advanced shrimp feed concepts, which are planned for launch in the market from 2022.

Benchmark Genetics to support Regal Springs tilapia breeding programs

Benchmark Genetics entered long-term strategic cooperation to support Regal Springs' breeding programs for premium natural grown tilapia. The agreement secures Regal Springs access to Benchmark's team of experts and advanced support systems for selective breeding programs and the company's extensive experience.

RAS feeds partnerships

Grobest Group and Universal Aquaculture (UniAqua) partnered to collaborate on the development of the world's first next-generation Functional Performance Shrimp Feed for UniAqua's proprietary Hybrid Biological Recirculating System™. In initial trials, Grobest functional performance feeds have outperformed competitive products by yielding healthier, tastier shrimp and significantly better water quality.

Cargill partnered with Salmon Evolution to supply 100% of Salmon Evolution's feed volumes for the Norwegian Indre Harøy facility which is scheduled to commence production in March 2022. Cargill has further committed to allocate significant resources and R&D capacity to develop sustainable feed solutions tailored to Salmon Evolution's operational targets.

Skretting plans to build a specialized state-of-the-art feed plant for land-based salmon in Florida to serve Atlantic Sapphire's US operations with market-leading feeds specialized for Bluehouse farming.

Paine Schwartz makes strategic investment in Hendrix Genetics

Paine Schwartz Partners, a global leader in sustainable food chain investing, acquired 50% of Hendrix Genetics. Hendrix Genetics' co-founders will maintain a 50% ownership position in the company and work in partnership with Paine Schwartz to drive the business forward.

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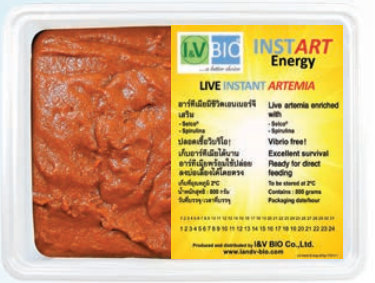

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First-ever rotifer substitution diet

Steven Debono, Tania De Wolf, Jessica Teske, INVE Aquaculture

Rotifers are the first feed for most marine fish species currently cultured around the globe. Although many hatcheries have mastered the art of rotifer culture, it remains an important but variable parameter in achieving predictable larval rearing results with regards to quality and survival. To counteract this variability, marine hatcheries need to innovate and standardize their operation to a higher level.

At INVE Aquaculture, part of Benchmark, we have the tradition to innovate and develop new technologies and products that simplify and support the industrialization of marine fish hatchery production towards the desired high-quality levels. INVE developed Natura pRo and ExL feed line, a game changer that enables hatcheries to reduce their rotifer demands by more than 50%, improving survival rates and overall fry quality. It offers hatcheries unmatched solutions as:

- Increased predictability and quality of the fry production.
- Increased flexibility of the production system.
- Reduced production costs and risks.

Natura pRo and ExL is the result of INVE Aquaculture's unique knowledge based on many years of research ranging from ingredient selection, feed production techniques to larval rearing protocol development.

The challenge of introducing dry feed from early stages

Dry feed introduction from the start of exogenous feeding in marine fish larvae has always represented a major bottleneck due to various reasons:

- Underdeveloped digestive system of marine fish larvae limits the digestibility of compound feeds with high nutrient density.
- Performance in terms of survival and fry quality.
- Keeping water quality parameters stable with low water exchange.
- Feed palatability and assimilation.
- Complexity in implementing in commercial hatcheries day-to-day operations.

INVE, however, successfully realizes the vision of introducing dry feed from the first feeding onwards using the combination of Natura pRo and ExL. This combination addresses the above challenges via a multidisciplinary approach. They ensure not only a high level of rotifer substitution but also simplicity in the protocol application enabling hatcheries to improve their biological performance and economical result.

Natura pRo and ExL consist of two formulations building on each other targeting rotifer substitution by at least 50%. Furthermore, they are supporting the nutritional and technical needs from the start of exogenous feeding until post-weaning.

The Natura pRo <100 µm & 100/250 µm

The Natura pRo formulation is designed with special attention to ingredient selection targeting optimal nutrient assimilation and palatability, and, thus, guaranteeing the attractiveness of the feed for larvae from the start of exogenous feeding. Our feed processing technique applied in this formulation results in a dry feed with a narrow particle size distribution ensuring more than 90% feeding success in 48 hours (based on customer data) from the first application. Last but not least, this technological application also ensures excellent stability of the feed particle avoiding the leaching of nutrients. Moreover, keeping water quality stable and having excellent behavior in the water mimicking rotifers ensures that fish larvae feed efficiently from mouth opening onwards. This has been validated in several field trials. The strategy during this phase is to reduce the usual daily rotifer quantity by at least 50%.

From our experience, we learned that using the powerful combination of a few high-quality rotifers with Natura pRo continued by ExL results in different realities at commercial scale but confirms improved survival and quality performance when compared to standard larval rearing protocols.

The Natura ExL 200/400 µm & 300/600 µm

Ensuring optimal results when substituting a critical feed item like rotifers without any compromise on quality and performance implies that nutrition in the following stages is of the highest standard. The ExL formulation supports high survival rates and easy weaning even at high survival rates. The ExL formulation has been meticulously formulated to boost fry robustness, ensuring that larvae are at least as robust as the ones fed with standard rotifer quantities.

Overview of trials at commercial scale

After years of product and protocol development, we achieved a consistent result, enabling hatcheries to move to the next era of modern marine fish hatchery production. The following results are an overview of various trials rearing seabream (*Sparus aurata*) at commercial scale in Europe. These trials compared a standard larval rearing protocol (LFC) and the Natura pRo and ExL feedline and protocol with more than 50% rotifer substitution.

Malformations are one of the biggest challenges experienced by hatcheries and have serious repercussions on production and revenue. Its causes are multifactorial and range from genetics, water quality husbandry techniques and, last but not least, nutrition, which is the base for any living organism to develop optimally.

Scientific evaluation has shown a positive trend in reducing the occurrence of morphological abnormalities (Fig. 1). Natura pRo and ExL are supporting producers to achieve improved results via top-notch nutrition.

Survival rates have increased constantly over the past 20 years. With Natura pRo and ExL one can not only maintain such high survival rates. Moreover, applying the protocols correctly can ensure even significantly higher rates compared to present practice, even under demanding conditions (Fig. 2).

Generally speaking, growth performance is at least similar or improved under the Natura pRo and ExL protocol (Fig. 3). Bear in mind, substantial replacement of rotifer quantities is realized, highlighting the quality of the feeds in terms of digestibility and assimilation.

Fry robustness is an important quality indicator in modern fish hatcheries operations as it indicates the fry condition and their tolerance to stress. Its widely

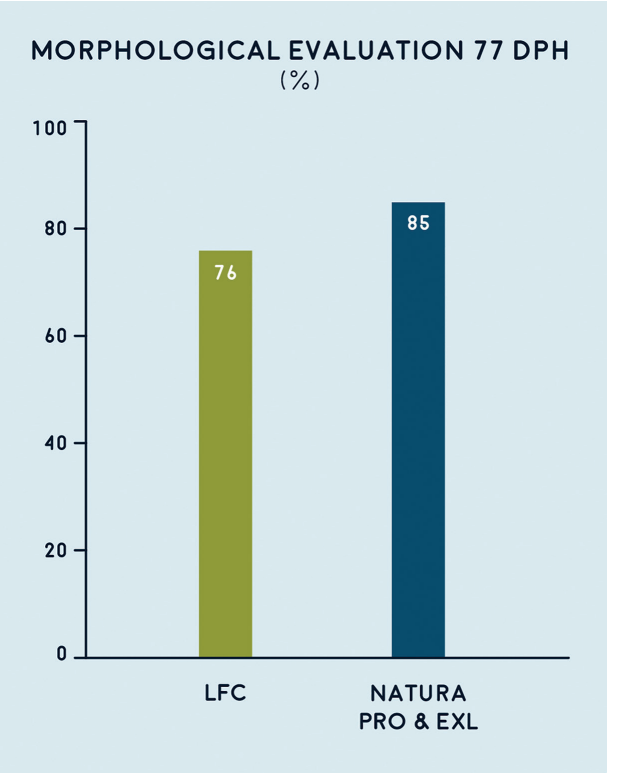

Figure 1. Reduced occurrence of morphological abnormalities in seabream with a protocol with more than 50% rotifer substitution.

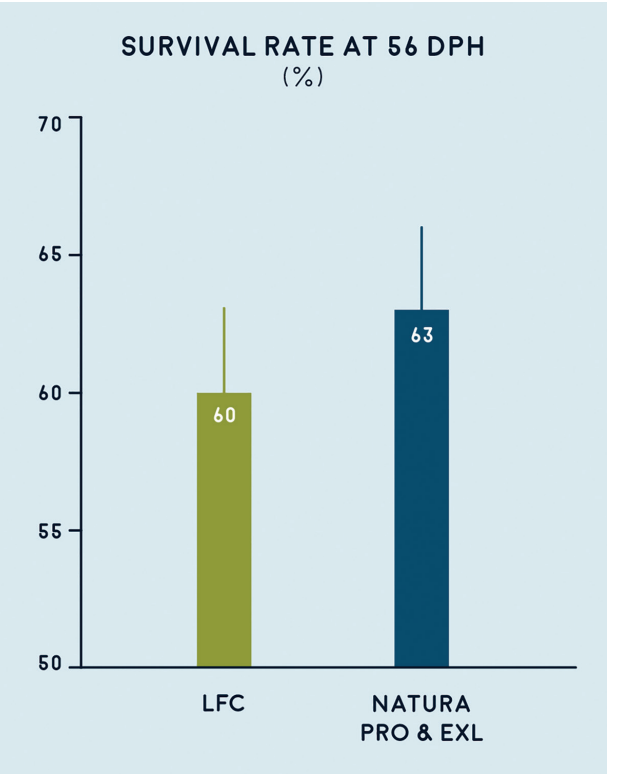

Figure 2. Higher survival rates with rotifer substitution compared to present practice in seabream (LFC).

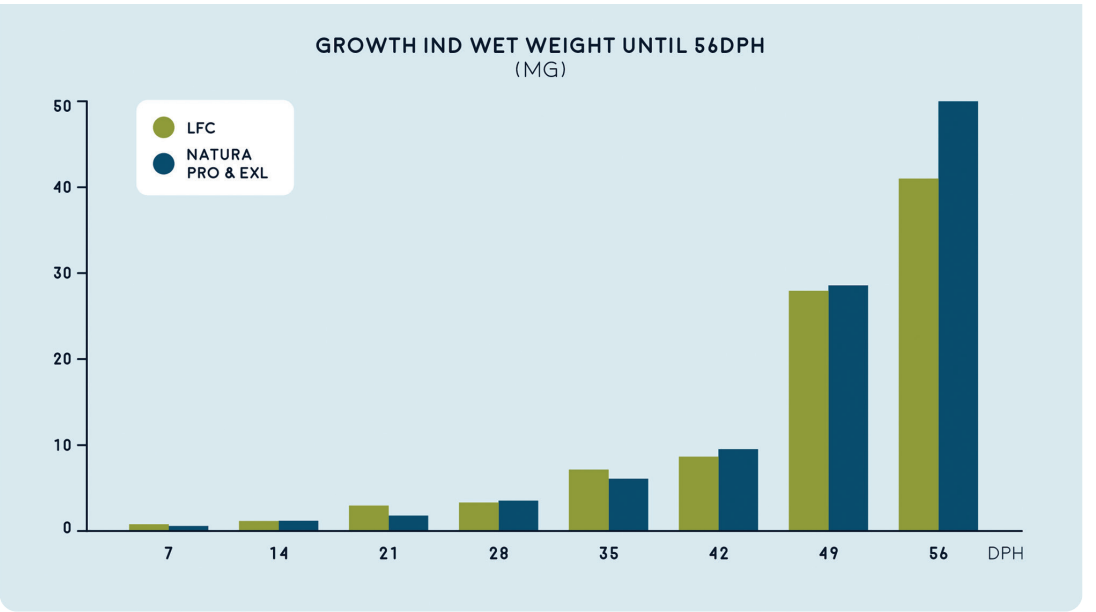

Figure 3. Growth and wet weight in seabream reared with standard (LFC) and rotifer substitution larval rearing protocol (Natura pRo & ExL).

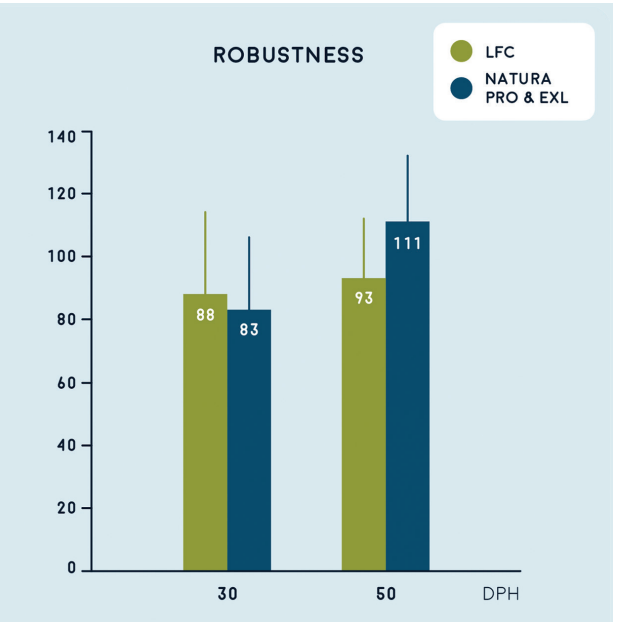

Figure 4. Similar or better fry robustness (salinity stress test) obtained in seabream reared using a standard and rotifer substitution protocol.

accepted that nutrition plays an important role in determining the stress response of the animals. In this case, Natura pRo and ExL provide similar or better fry robustness (salinity stress test) despite reducing rotifer consumption by more than 50% (Fig. 4).

All in all, rotifer consumption can be easily reduced by more than 50% (Fig. 5). This is highly remarkable given that the biological performance is excellent. These results enable hatcheries to move towards a more efficient and standardized production and industrialization.

Conclusions

Our trials revealed even higher replacement is possible. Under commercial testing conditions, INVE's IARC center in Italy developed protocols that can achieve up to 80% of rotifer substitution with no compromises on fry quality, survival, and growth performance.

Rotifer substitution in commercial hatcheries is a process. At INVE Aquaculture, we believe that the main target is to improve the fry quality and, simultaneously, simplify and standardize hatchery production. This philosophy is imprinted in our approach. We have worked with various hatcheries to challenge rotifer substitution under various commercial conditions.

The results verify advantages in terms of productivity and production efficiency when compared to standard hatchery procedures. The implementation of a rotifer substitution protocol with Natura pRo & ExL is easily integrated into standard larval rearing protocols facilitating the daily operations in the hatchery and allowing for its industrialization.

Considering the results, Natura pRo and ExL managed to reduce feed item cost per million fry

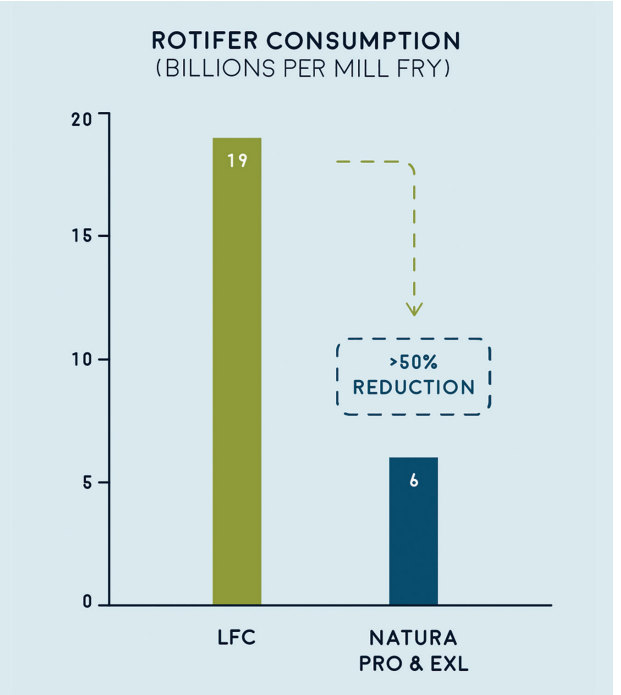

Figure 5. Protocols that can achieve up to 80% of rotifer substitution with Natura pRo & ExL.

by at least 7% based on real-world economic models. At INVE we firmly believe that it is via efficiency and innovation that the industry will achieve better profitability and predictability.

INVE Aquaculture, part of Benchmark, is widely known as a partner that provides complete solutions fine-tuned to local conditions. Successful rotifer substitution can be achieved easily, and with Naturara pRo and ExL we provide a solution for protocol customization to achieve the unthinkable in your hatchery.

More information:

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A game changer for marine nurseries

Joana Amaral, BioMar

Recirculating aquaculture systems (RAS) are gaining popularity among marine nurseries when water and space are limiting factors, and a controlled environment is necessary. High densities, fast growth and frequent handling of fry make nursery operations a challenging process, especially in RAS.

Moreover, early-stage marine fry can be extra fragile to handle and have additional challenges with life at sea. In recent years, marine hatcheries have experienced the need for increasing land-based fry production. This resulted in a change of mindset and an accelerated shift from traditional flow-through systems to more advanced technologies such as RAS, especially for the nursery and pre-ongrowing phases.

RAS farming technology demands skilled manpower and extra attention to the fish and the system. These unique conditions require an optimum, stable and controlled environment and call for a specially designed feed that helps both the fish and the farmer thrive despite challenges.

BioMar has established solid expertise within the RAS segment based on extensive research and

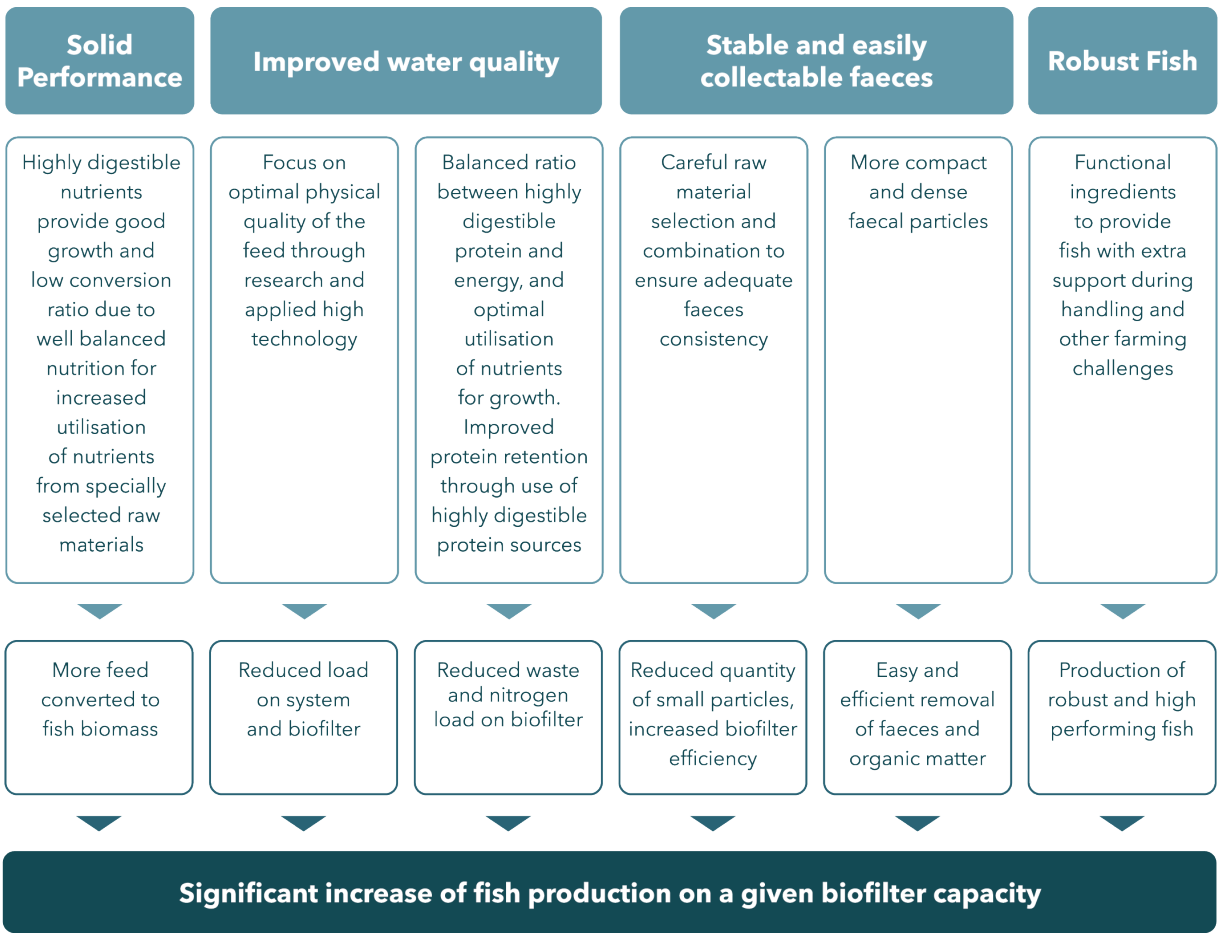

Figure 1. LARVIVA ORBIT is the solution for maximizing efficiency and performance of marine RAS nurseries by providing increased advantages and minimizing challenges in production.

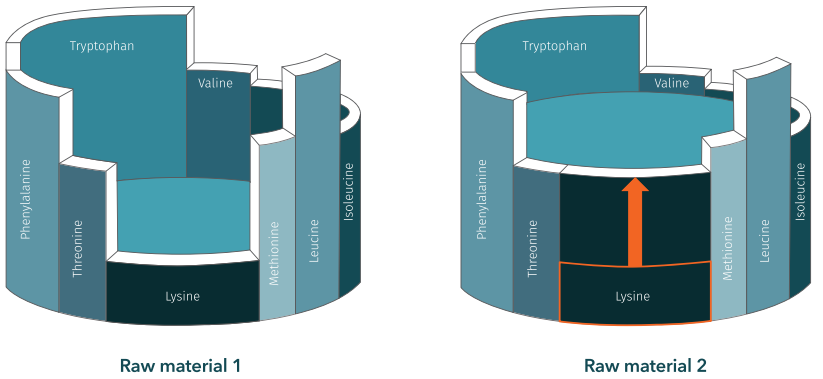

Figure 2. The "Barrel Theory" of amino acids. Careful selection of raw materials for an optimal amino acid profile ensures that the necessary "building blocks" are present in optimal ratios, balanced to requirements, maximizing performance and reducing waste.

development, and experience gained from years of fruitful collaboration with RAS farmers around the world. Now all this expertise has been transferred to marine nurseries.

Game changer for marine nurseries

LARVIVA ORBIT is the innovative feed concept specially designed for marine nurseries operating on RAS. LARVIVA ORBIT is the solution for maximizing efficiency, performance and profitability of marine nurseries by providing increased advantages and minimizing challenges in production (Fig. 1).

Principles of LARVIVA ORBIT

Digestibility matters

In recirculating aquaculture systems, the digestibility of proteins and lipids play an important role in ensuring maximum performance of fish and the system. When energy comes from the breakdown of protein, the result is unutilized nitrogen, which is released as ammonia through the gills and urine. LARVIVA ORBIT enables the proteins to be better used for growth, instead of being catabolized in excess. The optimal protein to energy ratio (DP:DE) assures a balance of energy and protein for growth without excess protein waste and thus reduced nitrogen load on the system.

Raw material selection

Careful selection of raw materials is crucial as they have a direct impact on physical pellet quality, dust levels and water stability (Fig. 2). All these criteria are fundamental to control the optimal condition of fish and their impact on the water treatment systems.

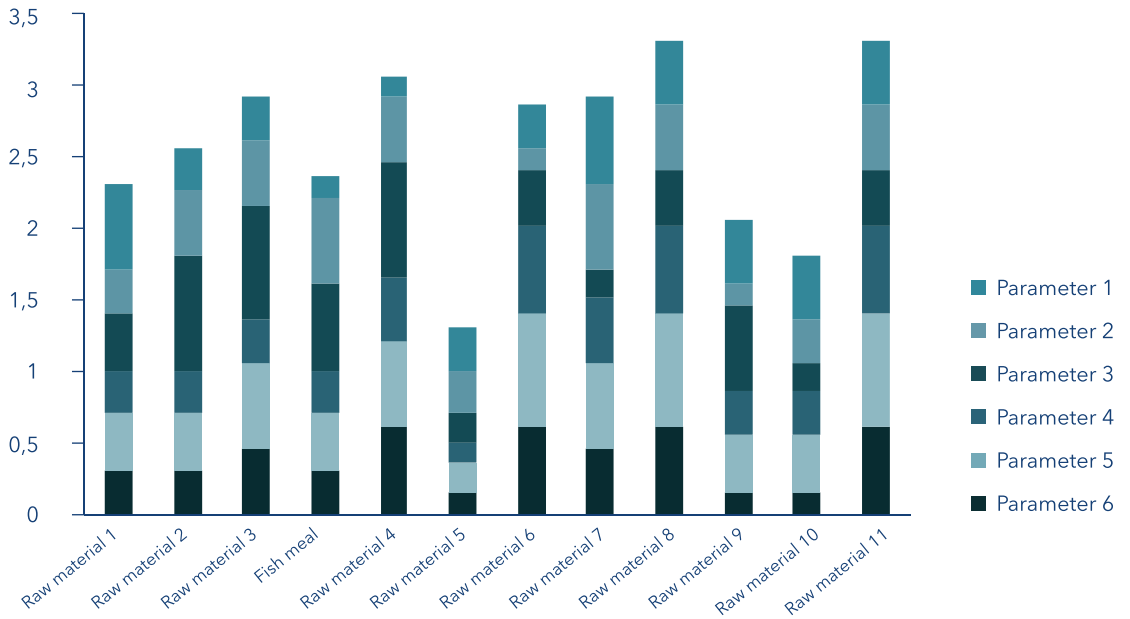

Figure 3. Raw materials are graded: the higher the value, the more suitable it is for use in ORBIT feeds. Optimized diets result in the lowest possible impact on the production unit.

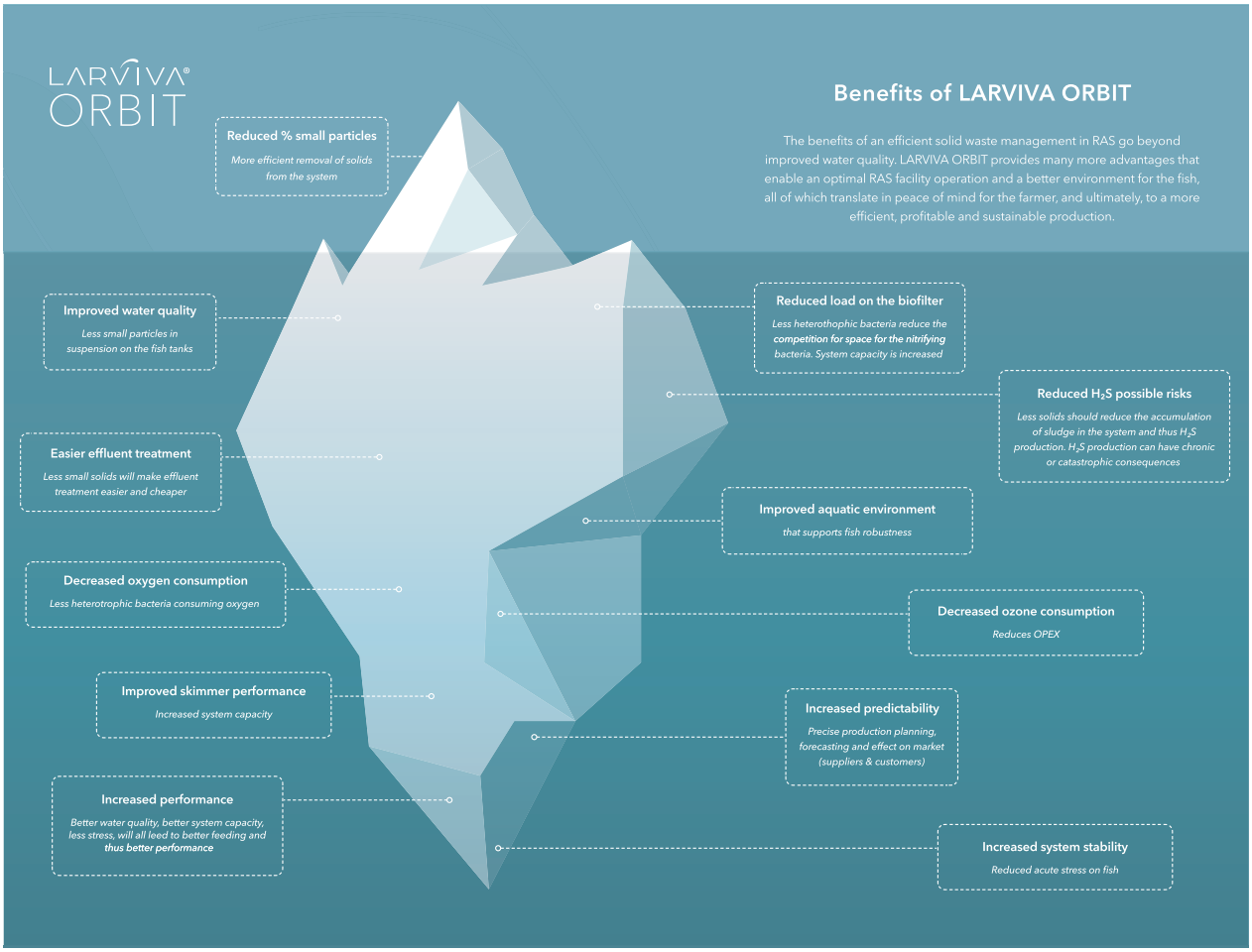

Figure 4. Benefits of LARVIVA ORBIT.

BioMar's state-of-the-art raw material evaluation methodology allows selection, grading and scoring any raw material used for RAS feeds in terms of fecal stability. All ORBIT feed formulas are optimized using this methodology, in order to select the optimal raw material composition to ensure maximum fecal stability, providing the lowest possible impact on the RAS unit, without affecting the performance (Fig. 3).

Feces matters

In recirculating aquaculture systems, it is vital to ensure that solid waste can be collected in an easy and efficient way to be eliminated from the system. Non-used feed and feces are the main contributors to the solids on the system.

While uneaten feed can be minimized by a correct feeding strategy and distribution, the impact of the feces is directly linked to their stability in water and thus particle size generation. The finer the particles, the more difficult it is to remove them efficiently.

The reduction of fine particles is a key parameter for the successful management of any RAS. The impact of fine particle reduction goes deeper than the immediate consequences that are visible to the eye, and much further than the direct effectiveness of solid removal filters, screens, or other devices.

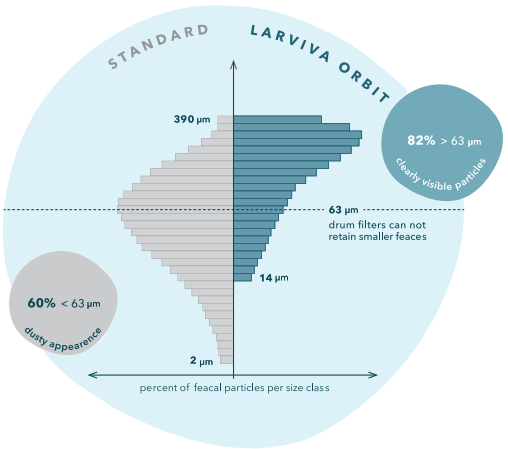

Figure 5. Trials show a reduction of small particles.

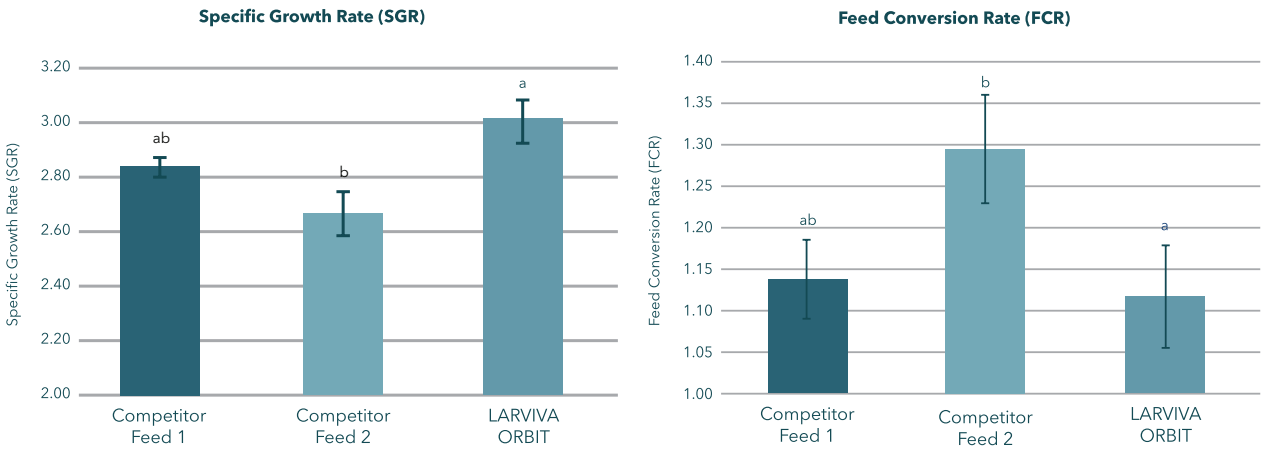

Figure 6. Trials performed in Mediterranean fry species in semi-commercial conditions.

A well-documented solution

Trials performed with Mediterranean fry species in semi-commercial conditions have shown that LARVIVA ORBIT matches or exceeds productive performance of other commercially available diets (Fig. 6).

It is a game changer feed concept that provides an efficient organic matter management. Large scale trials in commercial conditions have shown a consistent reduction of the percentage of smaller particles (Fig. 5).

A fusion of two well-recognized feed concepts

LARVIVA - Best start in life

LARVIVA is a complete range of premium feeds developed for the very first life stages. LARVIVA products are developed with specially selected, high-quality and sustainable raw materials, unique functional ingredients and nutraceuticals, produced with state-of-the art production technology. They cover all nutritional needs of the larvae, fry and broodstock, providing strong and sound growth as well as excellent physical quality.

ORBIT - Balance and harmony

ORBIT is the world's first specially designed feed concept for advanced aquaculture technologies, e.g. recirculating aquaculture systems (RAS).
It aims to maximize the performance by providing balance and harmony for fish and the biofilter bacteria. It is recognized for being ideal for intensive farming systems where optimal solid waste and improved water quality are required.

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New nature-inspired diet to help hatcheries wean faster and cleaner

Eamonn O'Brien, Skretting

With many marine hatcheries actively seeking ways to improve the sustainability of their operations, Skretting's new hatchery diet, GEMMA Neo, offers first-to-market ability to reduce the sector's dependence on traditional feed ingredients, while also providing unprecedented production system performance and weaning flexibility.

Applying the company's latest aquafeed technology know-how and incorporating new sustainable raw materials rich in the essential long-chain omega-3 fatty acids EPA and DHA, GEMMA Neo builds on the success of its predecessor, the industry-leading GEMMA Wean. The result is a unique diet that offers independence from some fishery-based raw materials alongside the ideal nutrient composition and physical characteristics for marine fish larvae.

Not only does GEMMA Neo give Skretting much more flexibility in terms of formulation, but because the omega-3s fatty acids are contained within the cells of the raw materials and marine zooplankton, it does not leach into the production system as quickly or to the same degree as conventional fish oil-based larval diets.

This cellular encapsulation also ensures feed particles remain intact for longer, which in turn facilitates maintenance and lessens the loads placed on systems. Hatchery tanks remain very clean, and because the diet degrades very slowly, there are almost no negative effects on water quality.

Eamonn O'Brien, product manager of LifeStart at Skretting, explains, "GEMMA Neo is inspired by what happens in nature. In the wild, marine larvae will feed on a mix of different food sources with

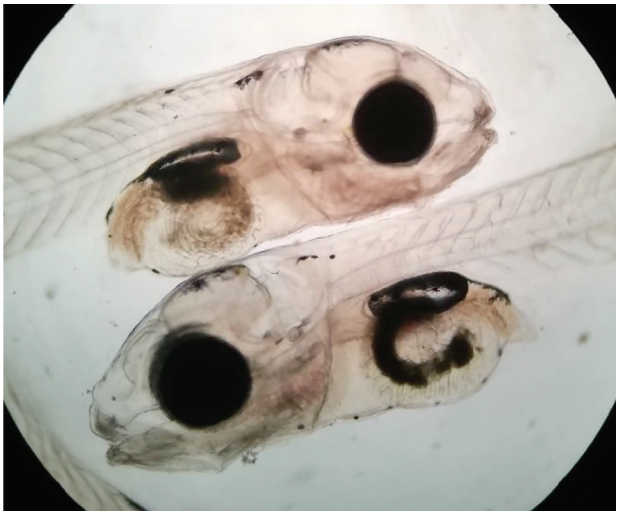


Figure 1. 22-days old sea bream larvae with rotifers (up) and Gemma-Neo (down) clearly visible in the gut.

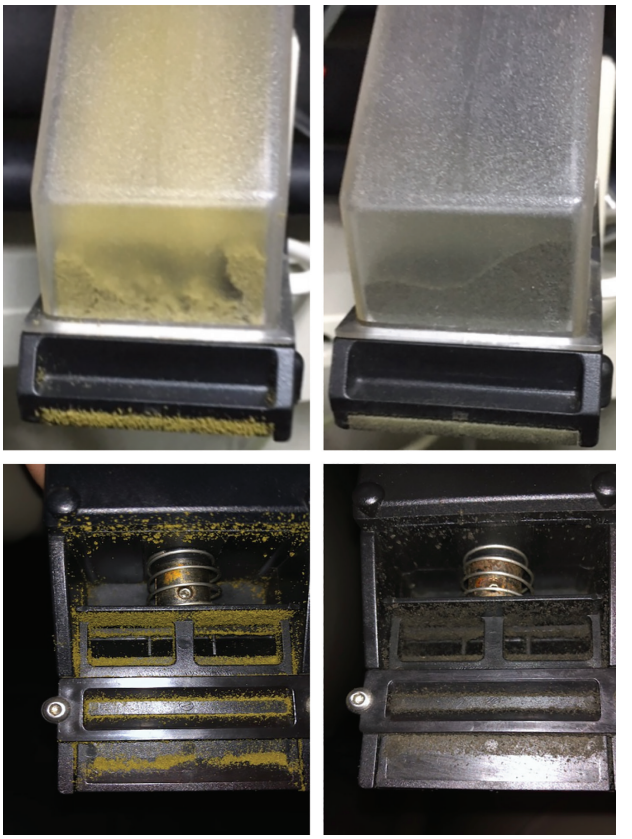


Figure 2. Better free flowing characteristics with Gemma Neo (right) prevent obstruction in the hopper and deposits around the solenoid and plates of the feeder system.

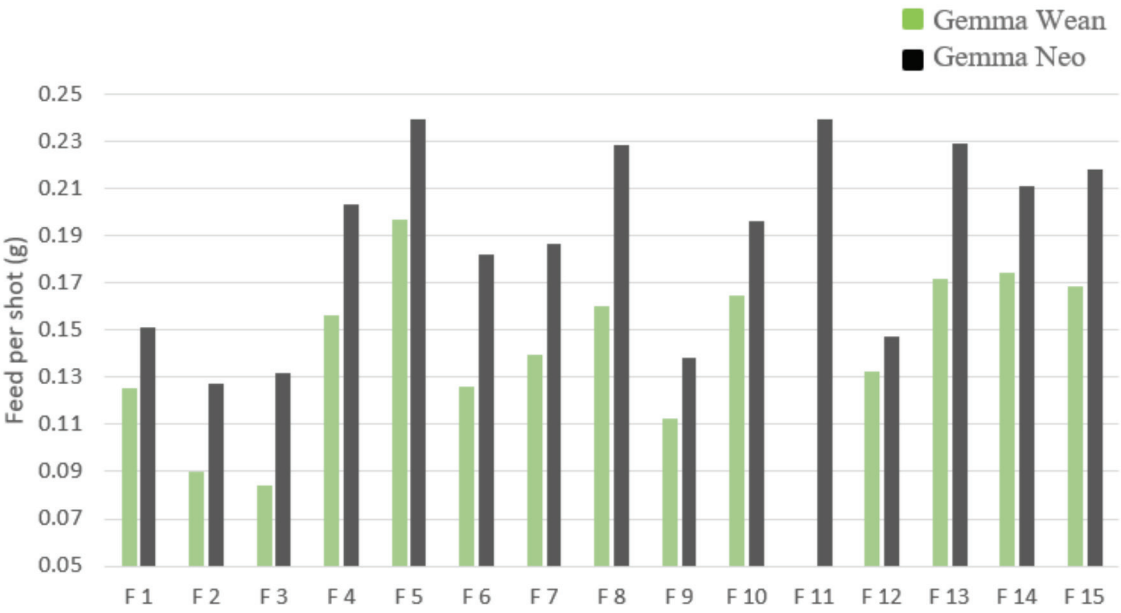


Figure 3. Feed amounts delivered during 15 consecutive shots with Gemma Wean 0.1 and Gemma Neo 0.1 using an AMD feeder.

different protein profiles. With the new solution, we have replicated those instinctive feeding behaviors through diversification of beneficial raw materials, delivering an incredibly stable diet that the larvae find very appealing."

In control of the weaning process

In making use of new raw material ingredients that the larvae naturally want to consume, GEMMA Neo presents hatcheries with the opportunity to introduce the diet at their own discretion, potentially accelerating the weaning or shortening co-feeding processes and taking the difficulties out of sourcing and delivering live foods.

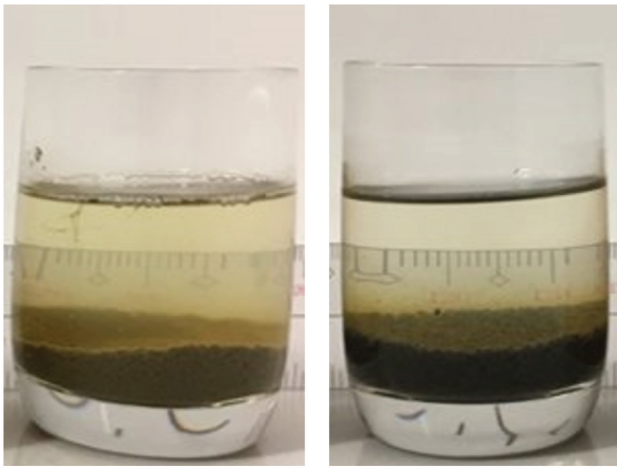
To assist the weaning process, the color of the GEMMA Neo is very dark. This allows hatchery staff to visually ascertain whether the fish are eating Artemia and/or eating the diet, which helps to determine whether it is time to start to wean or increase the feed amount or frequency (Fig. 1). The speed of such transitions depends on the species being produced.

GEMMA Neo's formulation is also ideally suited to the recirculation systems that are increasingly being introduced within hatcheries. It ensures that the water in these systems remains stable and geared towards improved performance. This has been achieved by making the diet much more stable and free-flowing

Figure 4. Gemma Neo spreading after hitting the water surface of a bass larval rearing tank.

than other hatchery diets with no tendency for clumping even in small particle sizes in automatic feeding devices. The free-flowing properties reduced rat-hole and bridge forming in the hopper and increased the feed delivery in AMD feeders (Fig. 2, 3).

When delivered, GEMMA Neo spreads very quickly on the water's surface and floats longer before gradually starting to descend, making the diet much more available (Fig. 4).



Gemma Wean Gemma Neo

Figure 5. Improvement of water stability of Gemma Neo compared to Gemma wean results in less foam formation, more transparent water and reduced-fat leakage.

"Commercial hatcheries have seen a great deal of change in the last ten years due to the unprecedented demand for larger volumes of better-quality young fish. With GEMMA Neo – a much more appealing and palatable diet for these larvae – we are enabling these production systems to meet their customers' expectations, and to do that much more sustainably through a much broader spectrum of beneficial ingredients," O'Brien said.

Early evaluations from the market have also been very positive, with hatcheries endorsing GEMMA Neo's ability to uphold the nutritional quality of its predecessor while being very clean and easy to use (Fig. 5.)

"Our customers, who greatly appreciate GEMMA Wean, have shown a great deal of interest in the new diet. Many have noted that GEMMA Neo not only provides excellent nutrition but also has specific physical characteristics that ensure better water quality. They have also confirmed that it offers excellent compatibility with RAS systems and automatic feeders," said Arturo Scopece, technical advisor at Skretting Italy.

Decades of ingredient innovation

Skretting's ambition to provide aquaculture with the opportunity to be independent of wild fishery-based ingredients dates back more than two decades. Through pioneering research, it has developed the expertise to replace traditional marine-based feed components with alternative ingredients while ensuring that aquatic species retain their flavor and nutritional properties.

Because fishmeal and fish oil are natural, well-balanced sources of high-quality protein for aquaculture feed formulation and carry large quantities of energy per unit weight, Skretting will continue to source these two raw materials from sustainably certified fisheries. Both will be used in the formulation of its feeds at varying levels depending on specific nutrient requirements, customer needs as well as fluctuating prices and availability. However, through its ongoing R&D and utilizing new ingredients, Skretting is committed to being increasingly flexible with regards to its raw material inclusions across the fish and shrimp farming sectors.

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Next level of tuna aquaculture

Jan Giebichenstein, Paul-Daniel Sindilariu, Next Tuna

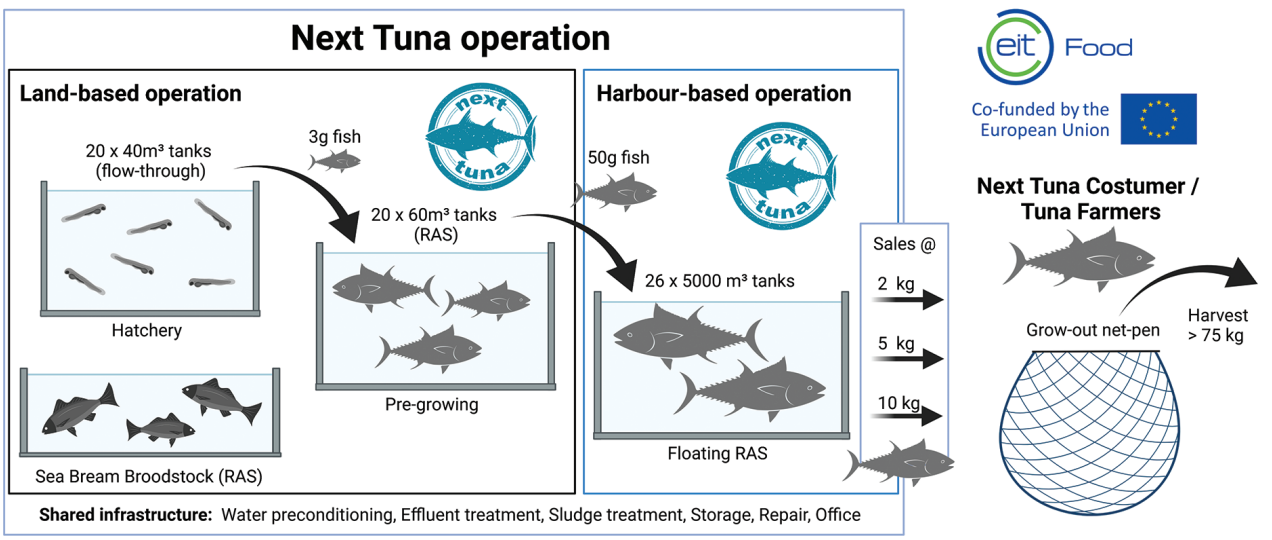

Figure 1. Schematic set-up of Next Tuna production facility. Created with BioRender.com.

Next Tuna is dedicated to sustainably producing and commercializing Atlantic Bluefin Tuna (ABT) in the Mediterranean Sea. We aim to be the first company to start the ABT reproductive cycle in an aquafarm at an industrial scale. Using new and innovative methods, we can meet the demand for high-quality ABT and mitigate the pressure from commercial fishing on this valuable species. To tackle this ambitious goal, Next Tuna combines state-of-the-art development in recirculating aquaculture systems (RAS), the latest improvements in feed technology, and novel knowledge on ABT biology.

Financed by EIT Food and private investors, the first goals of Next Tuna are to develop a commercial production concept for ABT and develop a shovel-ready project at the Mediterranean Coast to license and build our facility starting 2023. For us, tuna is teamwork. Our partners include the Spanish Institute of Oceanography, IEO (Spain), Seafarming Systems (Norway), Wageningen University and Research (Netherlands), CFEED AS (Norway) and CM Aqua (Denmark). In this article, we focus on our innovative production concept, specifically our hatchery design.

Next Tuna: The production concept

Next Tuna is setting out to produce a total of 1,200 tons of Atlantic bluefin tuna juveniles per year in a size range of 2-10 kg to stock existing ABT grow-out farms all around the Mediterranean Sea. The ambitious goal is based on a three, up to four times per year reproductive cycle including broodstock derived from a species-specific breeding program in recirculating aquaculture systems.

The hatchery, including first feeding and weaning up to a fish size of 3 g, as well as the pre-growing up to a size of 50 g, will be located in a land-based facility, while the on-growing to sales size of 2-10 kg will be performed in floating RAS in an allocated harbor area (Fig. 1).

Next Tuna hatchery and live feed production

The Next Tuna hatchery consists of a hatchery as such, where the ABT eggs are incubated, and fish start with

Figure 2. Example of an overhead crane planned to be used in the Next Tuna hatchery for fish movement.

external feeding. In the first five years of the project, the hatchery is dependent on ABT eggs from offshore net pens. It is planned to have different ABT egg collection locations all over the Mediterranean (Spain, Malta, Croatia, Greece/Turkey) to prolong the supply of new ABT eggs. The goal here is to stock the first feeding tanks twice per season. The first feeding is generally planned with two different tank sizes (10 m^3 and 40 m^3 tanks) in a flow-through system.

Eggs from different locations bring different challenges, including a small percentage of other species. We expect to better meet this challenge in smaller tanks. However, bigger tanks reduce the risk of floating and sinking deaths or wall collisions. In the different systems, ideal handling should be worked out including potential grading and improved transport. One of the biggest challenges in ABT rearing is fish handling and to tackle this, two independent systems will be implemented:

- The top part of the tanks will be connected with a flexible channel system to guide the fish into the next tank avoiding the need of touching the larvae.
- The whole tanks can be moved together with the supporting plate below the tanks using a 40-ton overhead crane (Fig. 2). The bottom of the tank is equipped with an over-dimensioned bottom outlet to slowly release the fish into a new tank. This avoids physical handling of fish and adds a lot of flexibility in regard to fish density control. Another benefit of multiple movable units is a modular approach that will make it easy to refurbish and improve the systems for the specific needs of this species.

We expect to improve survival and animal welfare, as well as increase the flexibility within the system. Next Tuna believes that feeding the highest quality food from the beginning is a key factor for succeeding in the commercialization of Atlantic bluefin tuna. A strategic partnership with CFEED AS, the world's largest copepod supplier, will help us to feed the ABT larvae with their natural prey. This will increase survival and growth, reduce deformities, and enable a more uniform growth pattern among the larvae to ultimately reduce potential cannibalism.

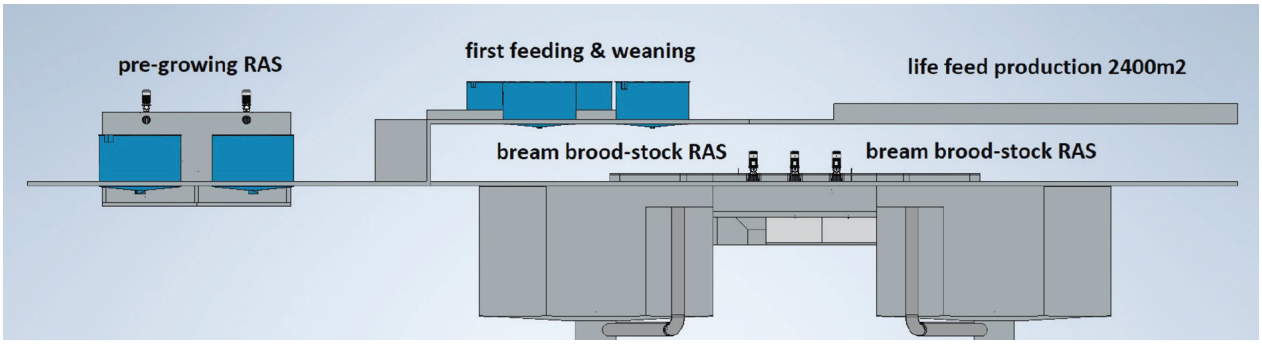

Figure 3. Next Tuna hatchery system side view with top level live feed production, middle level first feeding and weaning, lower level left-hand side pre-growing RAS and right-hand side independent seabream broodstock systems in the basement.

A total area of 2400 m^2 is planned for live feed production including live algae, copepods and Artemia. In addition, the whole ground floor of the hatchery facility is dedicated to seabream yolk-sac larvae production as a necessary food source for the larvae before starting to feed on micro particular diets. This area will be completely independent without direct contact with the tuna hatchery to avoid potential contamination and increase biosecurity (Fig. 3).

ABT larvae will remain in the same tank for first live feeding as well as for the dry feed weaning (Fig. 1). These tanks are operated in flow-through until fish reach 3 g.

Depending on size and age (around 30 days post hatching (dph)), fish will be moved to the pre-growing RAS tanks, and, if possible, pre-graded. We expect production of at least 5,000 fish per 40 m^3 weaning tank. The whole tank will then be moved by crane and emptied into the pre-growing 60 m^3 RAS tanks. Here, fish will be reared up to a size of 50 g. The 60 m^3 tanks will as well be built modular and with the option to be transported by crane to the floating 5,000 m^3 RAS tanks in the harbor.

Conclusion

The hatchery is the central element of the planned aquaculture production of Next Tuna. For the starting period, we will operate the hatchery with eggs collected from the wild until our own broodstock derived from our breeding program is ready for spawning. This initial production phase, with only a limited degree of hatchery utilization, is used to fine-tune the hatchery production concept and protocol. The fine-tuning is required to achieve the full production capacity later with our own Next Tuna reproduction scheme.

The Next Tuna team believes that ABT reproduction requires cooperation. We are encouraging feedback and suggestions from the audience and looking forward to making sustainable Atlantic bluefin tuna aquaculture a reality.

Acknowledgements

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Marine probiotics to improve shrimp larvae microbiota constitution, health and growth

Carine Le Ker, Emilie Giudicelli, Fanny Giudicelli, Marine Akwa

Importance of native microbiota constitution for growth and health purposes

The microbiota is known to interact with its host and contribute to a number of key host processes including nutrition, development, immunity and behavior. It is therefore involved in the regulation of shrimp's health and disease. Its composition changes with different factors: stage of development, feed quality, environmental conditions and presence of pathogens.

In the hatchery, drastic sanitary conditions are applied to control pathogens occurrence and protect fragile larvae for which defense metabolisms are not all in place yet. Everything is disinfected: water, live feeds, eggs, nauplii, so there is no native microbiota constitution. Consequently, a lower survival of shrimp's larvae is observed in production compared to wild animals with global native microbiota. The low beneficial microbes' exchanges throughout the feed or water are an open door for any opportunist pathogens that will quickly proliferate in those non-occupied spaces.

A solution to the imbalance of shrimp's microbiota in production is the introduction of beneficial bacteria to restore bacterial diversity.

Marine bacteria as a well-adapted solution for aquaculture production

Not all bacteria can be successfully introduced in shrimp microbiota or its water environment. Shrimp production is commonly performed in salted water, so probiotics (i.e. live microorganisms) used in shrimp production must be active at a salinity range of 2-35‰. Most of the probiotics on the market are of terrestrial origin and, therefore, not fully adapted to the aquatic living environment or the host itself. Stressed terrestrial bacteria in salted conditions slow down or even stop their metabolism and reproduction, and consequently stop playing their probiotic function. An illustration is the use of terrestrial Pediococcus (isolated from natural-pasture Gramineae) in salmon feed supplementation. The terrestrial probiotic did not persist in salmon microbiota following the transfer from freshwater to seawater. However, benefits have been observed over time (Jaramillo-Torres *et al.*, 2019).

Probiotics encapsulated in algae, a dedicated way of administration

An underestimated parameter in aquaculture development is the administration of probiotics in the targeted animal tract. At the hatchery and nursery level, probiotics are usually added to the water. For the water biocontrol, it's the appropriate way of administration but for the shrimp tract's colonization, adding probiotics in water is a non-targeted use. As probiotics are quickly dispersed in water, very high dosages are required to expect an adequate quantity goes to the larvae's tract.

To offer an administration independent to the feed and water, Marine Akwa developed a new technology: the encapsulation of probiotics in algae. Algae are highly palatable and digestible by shrimp. Thanks to this encapsulation, we guarantee the consumption of probiotics and the targeted release of adapted marine probiotics in the tract. Our selected marine probiotics are able to grow and compete in shrimp's mucus and are released at the right place to act as probiotics (i.e. production of enzymes, organic acids, surfactants, antibacterials, quorum quenching, growth factors, immunostimulants, etc).

In addition, algae residues strengthen the probiotic actions thanks to their prebiotics (stimulating probiotics and beneficial commensal bacteria), immunostimulants and phytobiotics (improving shrimp metabolism). Trial research in hatchery and nursery stages showed synergic beneficial effects of both marine bacteria and algae content.

Efficiency of marine probiotics solution in nursery stages

A trial under controlled conditions was conducted in China on shrimp from PL5 to PL30 with marine probiotics encapsulated in algae, added independently to the feed (15 minutes before). After the 20-day trial, the use of marine probiotics significantly increased final body weight by 15%, weight gain rate by 15% and specific growth rate by 4% compared to the control (Table 1). Marine probiotics also increased the survival rate by 6% (not significant).

When stress was applied by decreasing the salinity down to 1‰ (n= 100 PLs), survival of PL was 15% higher when marine probiotics solution was used (Fig. 1). When a challenge was done with *Vibrio parahaemolyticus* (carrying the phage responsible for the Early Mortality Syndrome), mortalities observed in control were more than twice higher than those observed in the treated group. Thus half-life was increased by 28% with the use of marine probiotics encapsulated in algae. In addition, a significantly lower amount of *Vibrio* populations was counted in hepatopancreas of supplemented challenged shrimp (Fig. 2).

Looking at PLs metabolisms and associated gene expression, significant (up to 10 times more) higher expression of digestive enzyme genes (peptidase, amylase, lipase, and protease) was observed in shrimp treated with marine probiotics. Significant (up to 5 times more) higher expression of immune-related genes (antimicrobial peptides (crustin) and prophenoloxydase (proPO) in hepatopancreas, antioxidant reaction (dual oxidase), clotable protein (mucin like peritrophin) and antimicrobioal peptides (penaeidin-3α) in the intestine) was observed when feed supplemented with AkwaBiotic was used (Fig. 3).

	Control	AkwaBiotic	
Final body weight (g)	0,177 + 0.0071[a]	**0,204 + 0.017[b]**	+15%
Weight gain rate (%)	3249 + 132[a]	**3751 + 314[b]**	+15%
Specific growth rate (%/d)	17,55 + 0,19[a]	**18,24 + 0,39[b]**	+4%
Survival rate (%)	82,69 + 2,78[a]	**87,81 + 2,31[a]**	+6%

Table 1. Growth parameters with and without use of AkwaBiotic at PLs nursery. Groups not sharing a letter are significantly different (t test, α = 0.05).

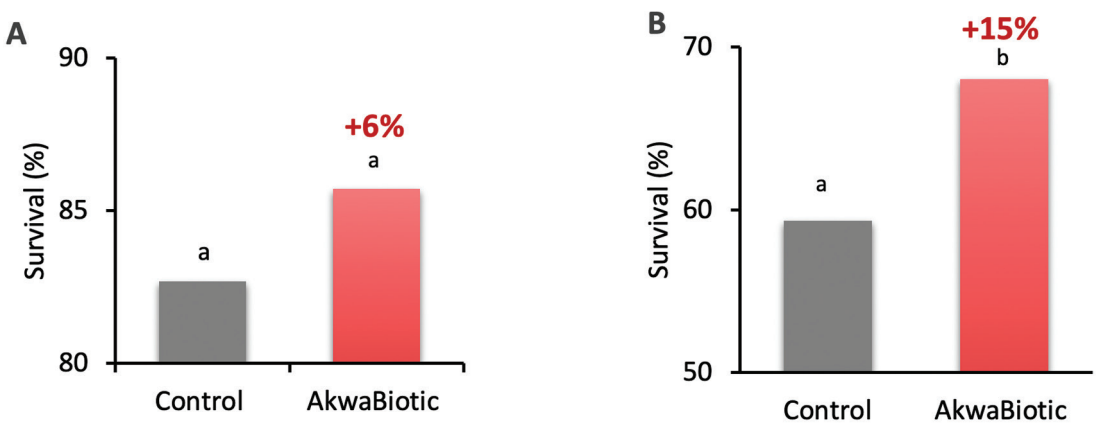


Figure 1. Survival without (A) and with (B) the application of salinity stress on PLs (n=100 in stress conditions) treated or not with AkwaBiotic. Groups that do not share a letter are significantly different (t test, α = 0.05).

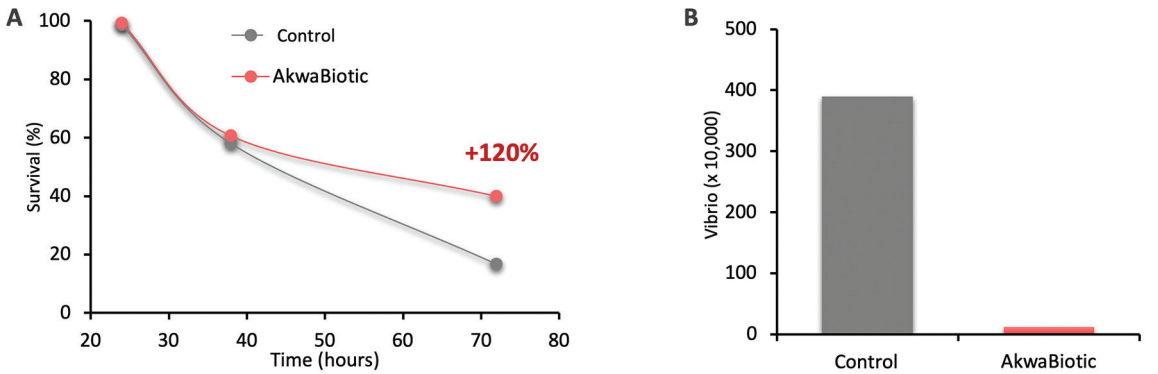


Figure 2. (A) Survival with and without a challenge with *Vibrio parahaemolyticus* on PL shrimp (n=100) treated or not with AkwaBiotic. (B) *Vibrio* colonies in hepatopancreas from challenged PLs shrimp (B).

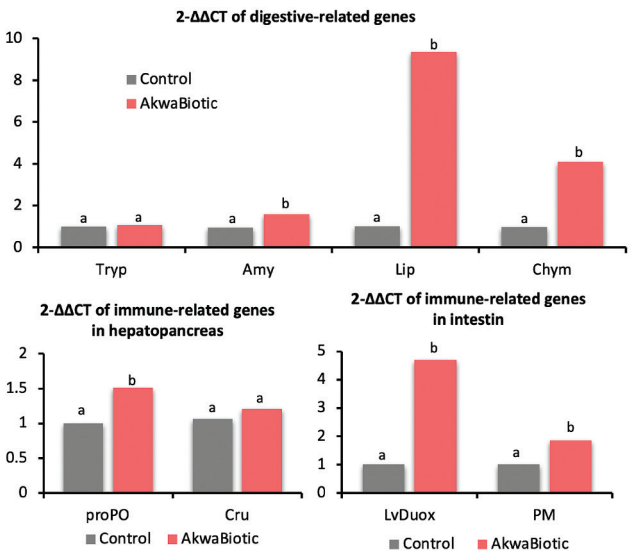


Figure 3. Gene expression related to digestion. Trypsine, amylase, lipase, chymotrypsine in hepatopancreas and immunity; crustin and prophenoloxydase in hepatopancreas; dual oxidase; mucin like peritrophin and penaeidin-3α in intestin in shrimp treat or not with AkwaBiotic. RT-qPCR analyses on n=50/triplicate. Groups not sharing aletter are significantly different (t test, α = 0.05).

Benefits of marine probiotics solution in hatcheries

Trials in five different hatchery productions in Ecuador were performed to assess the benefit of marine probiotics solution. The marine probiotics solution increased survival by 21% in hatchery n°1, 59% in hatchery n°2, 53% in hatchery n°3, 12% in hatchery n°4 and 18% in hatchery n°5.

In hatchery n°1, the increase of survival was linked to a decrease of counted *Vibrio alginolyticus*. This species was previously associated with mortalities on this farm (Fig. 4). In hatchery n°2, the situation was so critical in the control tank that the farmer decided to add the probiotics there too. It has been saved and the use of the marine probiotics solution avoided economical losses in this hatchery (Fig. 5).

In the hatchery n°3, the use of organic acids was substituted by the use of marine probiotics encapsulated in algae, and better results on both survival and growth were observed (Fig. 6).

In addition, no endoparasites were observed in treated larvae from hatchery n°2, in contrast with control (without probiotics) where treatment with organic acid was required to reduce endoparasites proliferation (Fig. 5). In the same way, in hatchery n°3, less hepatopancreas tubules deformations were observed in larvae when feed was supplemented with the marine solution (Fig. 6).

Marine probiotics encapsulated in algae also improved larvae growth and homogeneity. PL/g was respectively lowered by 22%, 33% and 35% in hatcheries n°1, n°4 and n°5 and weight gain increased by 35% in hatchery n°2 and size by 22% in hatchery n°3 with the use of marine probiotics (Fig. 4 - 8). Looking at homogeneity in larvae production, the use of marine solution decreased disparity size and reduced the larvae size range by a factor of 2 in hatcheries n°2, n°4 and n°5 (Fig. 5, 7, 8).

In addition, the use of marine probiotics solution showed better quantity and quality of lipids stock, higher fattening, increased muscle mass and branching gill, and also increased pigmentation of PL chromatophores (Fig. 7, 8).

Lastly, the farmer from hatchery n°4 noticed that shrimp larvae supplemented with AkwaBiotic were more active, presented higher mobility than normally observed, swim against the current, jump out of water. All those phenotypes are good health indicators for hatcheries.

A

	PL/g	Survival (%)
AkwaBiotic 1	**250**	**81.1**
AkwaBiotic 2	**220**	**79.1**
Control 1	285	73.3
Control 2	240	75.0

B

	PL/g	Survival (%)
AkwaBiotic 1	**1,028**	**80.6**
AkwaBiotic 2	**1,240**	**86.3**
Control 1	1,170	71.3
Control 2	1,100	71.3

C

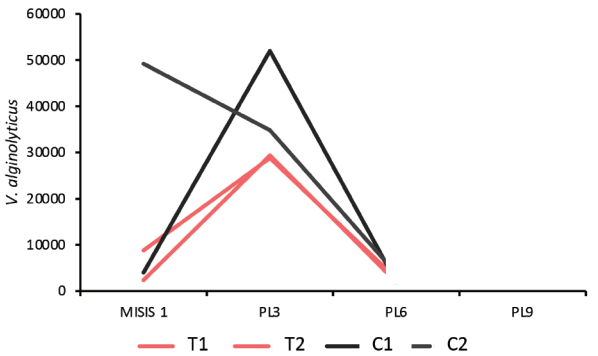
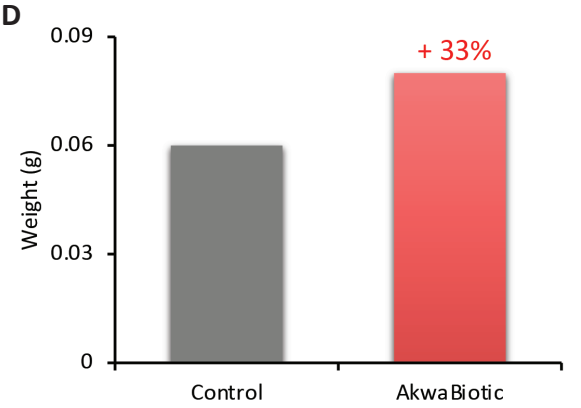


Figure 4. Benefits from AkwaBiotic observed in hatchery n°1. (A) Benefits at harvest from AkwaBiotic, in addition to additives already used in hatchery protocol. (B) Benefits at PL3 before transfer, in substitution of tract probiotics additives used in hatchery protocol. (C) Tracked *Vibrio alginolyticus* previously associated with mortality.

D



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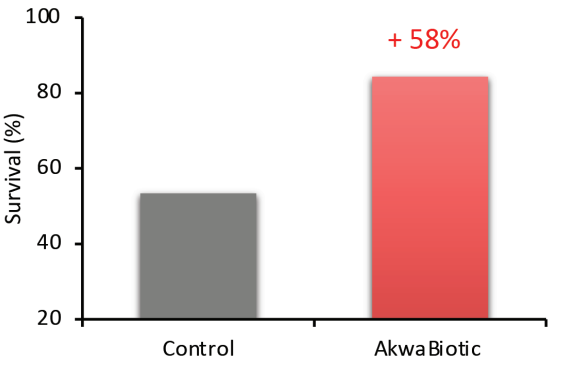


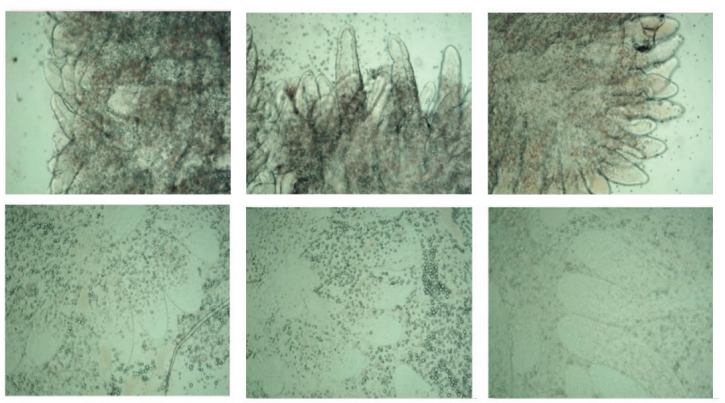
Control	AkwaBiotic	HATCHERY
5%	0%	Empty digestive tract
8 sizes	4 sizes	Larvae disparity
90 %	0 %	Growth delay

Critical !

AkwaBiotic

AkwaBiotic	GROWOUT
2 %	Empty digestive tract
4 sizes	Larvae disparity
2 %	Growth delay

F



Control
Moderate amount of G2 lipids
Tubules strangulations and deformations at apical zones
Endoparasites and gametocytes in intestines

AkwaBiotic
Moderate amount of G2 lipids
No tubules deformation
No endoparasites

Figure 5. Benefits from AkwaBiotic observed in hatchery n°2. (D, E, F).

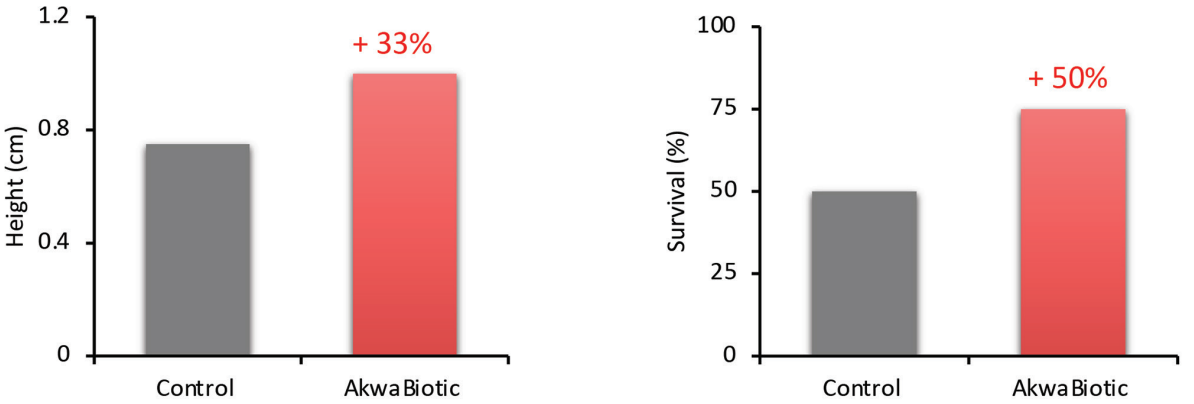
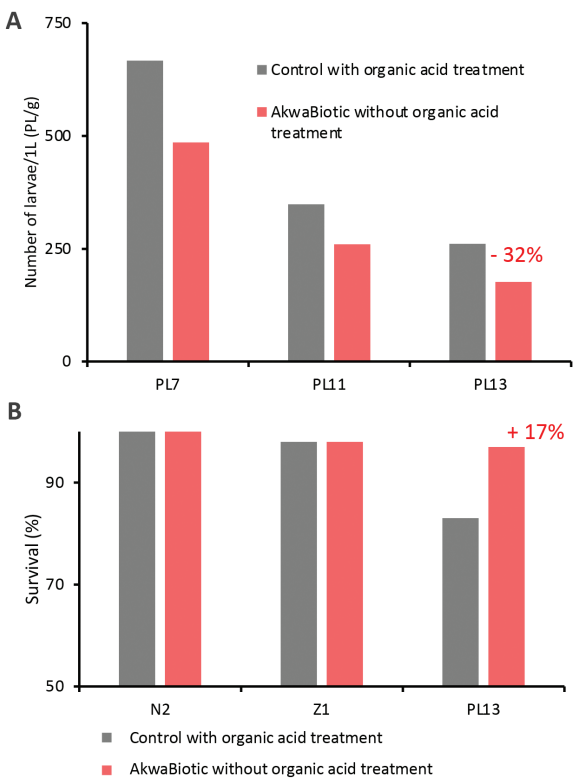


Figure 6. Benefits from AkwaBiotic observed in hatchery n°3.

	Control	AkwaBiotic
Size	small	**medium**
Size range (mm)	6-13	**8-11**
Number of sizes	4	**2**



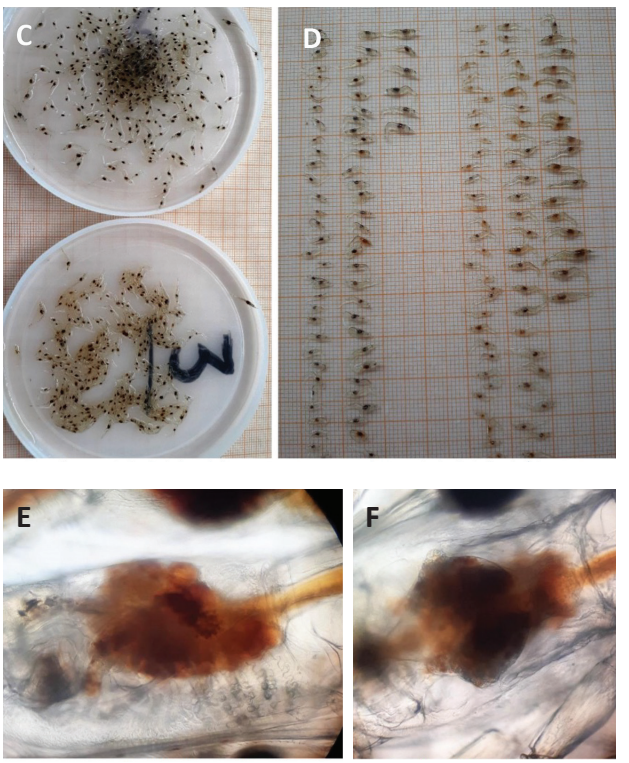
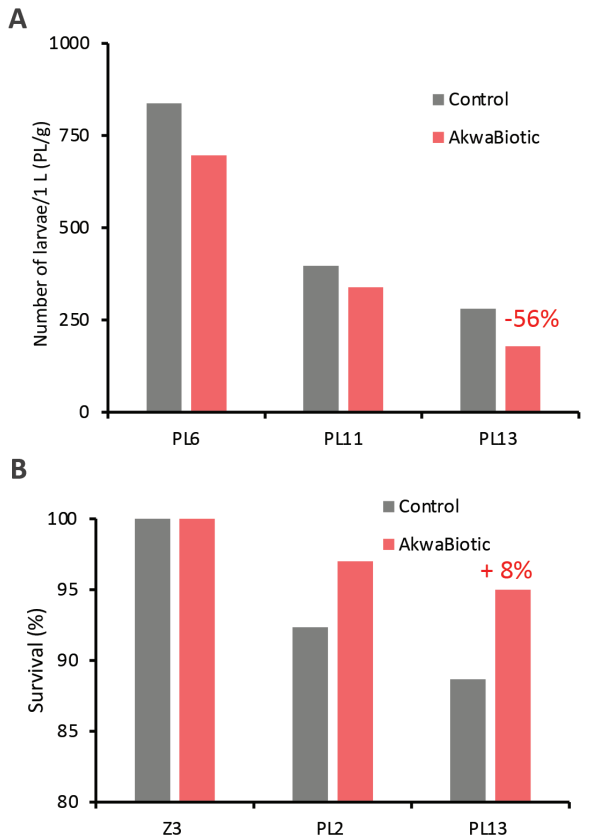


Figure 7. Benefits from AkwaBiotic observed in hatchery n°4. (C) AkwaBiotic (up) and Control (down) larvae. (D) AkwaBiotic (left) and control (right) larvae. (E) Gill lamellae development. (F) Lipid content in hepatopancreas.

	AkwaBiotic	Control
Size	**medium**	small
Size range (mm)	**8-12**	5-12
Number of sizes	**2**	4



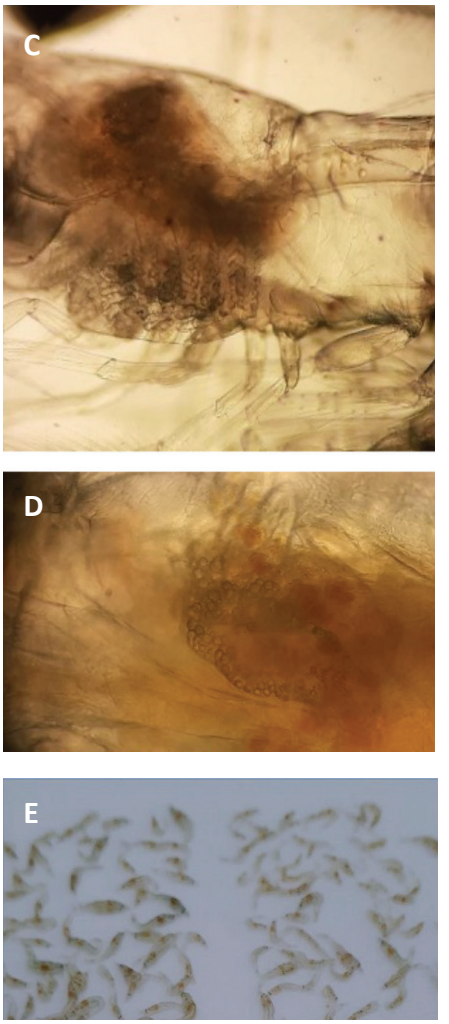


Figure 8. Benefits from AkwaBiotic observed in hatchery n°5. (C) Development of gill lamellae. (D) Lipid content in hepatopancreas.

Conclusions

Under controlled conditions, AkwaBiotic solution promotes growth and resistance to biotic and abiotic stress of PL shrimp. Marine probiotics encapsulated in algae enhance digestive and immune metabolism expression, partially explaining the higher feed efficiency and resistance of larvae when AkwaBiotic is used. At the field in hatcheries, marine probiotics encapsulated in algae have demonstrated their ability to:

- Improve survival and growth.
- Enhance homogeneity in size and weight.
- Decrease hepatopancreas tubules deformations and endoparasites occurrence.
- Enhance lipids accumulation and fattening, pigmentation, muscle mass and gills branching.
- Decrease *Vibrio* concentration or/and their virulence.

All those benefits were observed thanks to the synergic effects between marine probiotics and algae contained in AkwaBiotic.

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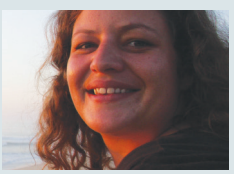

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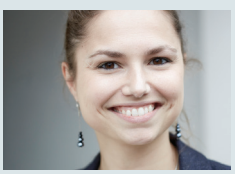

A practical guide to pathogen screening and detection

Robin Liew, Lab-Ind Resource Sdn Bhd/Geneseq Sdn Bhd

The aquaculture industry suffers disease losses to a tune of US$6.0 billion per annum (World Bank, 2014), making disease detection, prevention and management a critical component of the success of the industry.

Biosecurity practices aim to minimize the potential introduction of infectious diseases and spread to animals in a facility (hatchery and farm). A measured implementation of biosecurity practices reduces the likelihood of pathogens and disease animals from leaving a facility, which results in wider spread to untouched sites and even other susceptible species. Unregulated movement of live animals like brooders, post-larvae and fingerlings is the main cause for disease spread.

This article highlights the common principles of pathogen screening applicable across the aquaculture sector, using shrimp pathogen screening and detection as an example.

Disease triangle concept

To understand biosecurity is to appreciate the disease triangle (Stevens, 1960) concept, a model explaining how disease happens through the causal interaction between host species, pathogen and environment as seen in the diagram below (Fig. 1). Biosecurity practices seek to manipulate and control these relationships.

Pathogen screening and transmission mode

The ultimate objective for pathogen screening is prevention. It aims to identify and keep out asymptomatic animal carriers and vectors from entering and leaving by "ring-fencing" the production system. To achieve this, we must first understand pathogen transmission modes to design an effective screening strategy. Three transmission modes are worth noting: vertical, horizontal, and vector transmission (Fig. 2). Vertical transmission happens when the pathogen is transferred from brooder to progeny during the

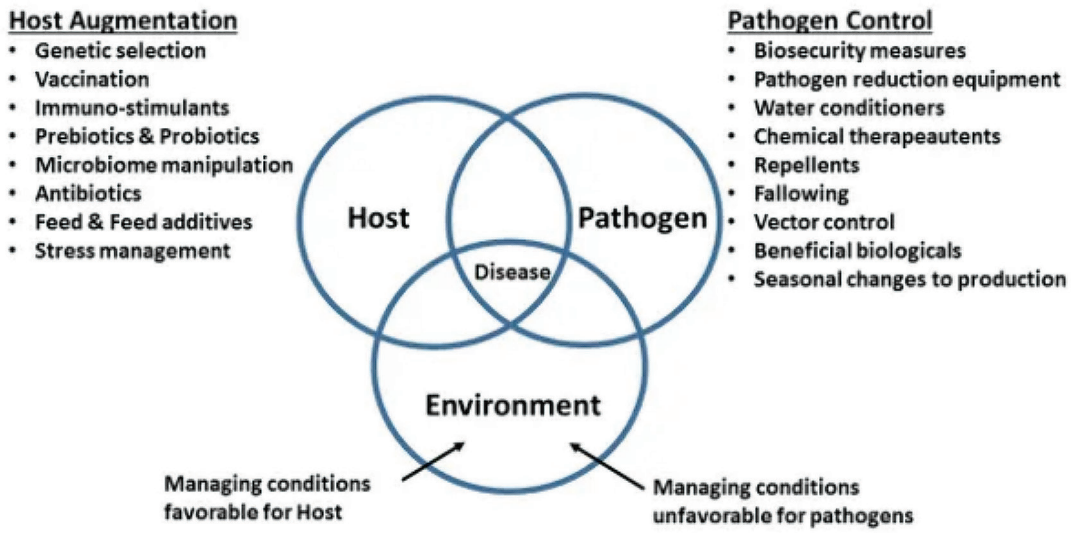

Figure 1. Manipulating disease triangle. Source: Lucy Towers, Eliminating the need for antibiotics on fish farm, 14-08-2015. Thefishsite.com.

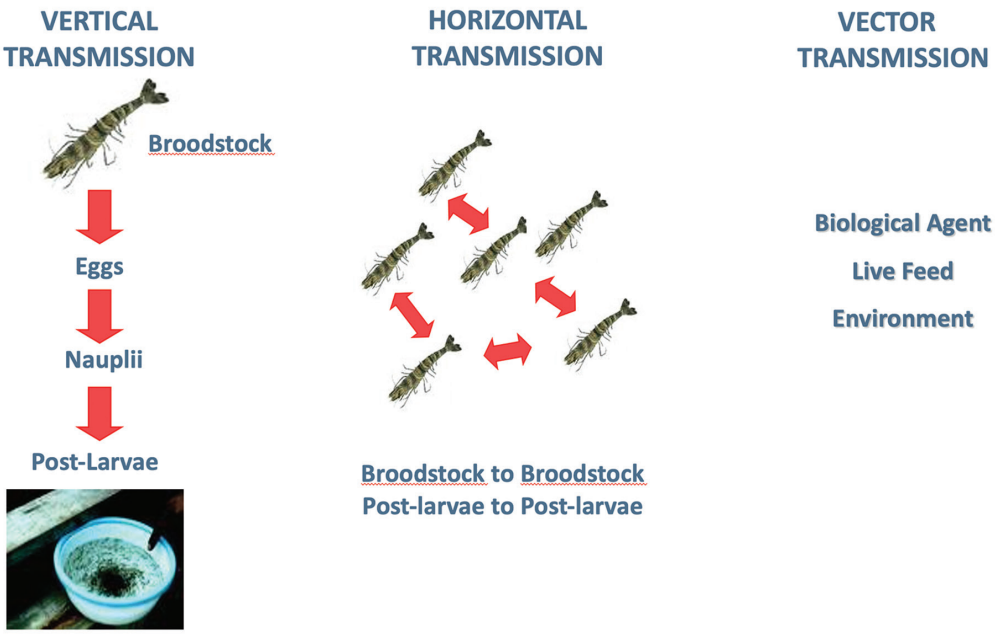

Figure 2. Transmission modes: vertical, horizontal and vector.

egg-laying process. Horizontal transmission occurs between brooders (in holding/maturation tanks) or between progenies (in larval tanks) through contaminated fecal matter or cannibalism. A third route, vector transmission, can happen through live feeds, polychaetes, Artemia or in water body harboring *Vibrio* spp. carrying PirA/PirB toxin plasmid (causing AHPND), parasite Enterocytozoon hepatopenaei, or human factors.

What is best for pathogen screening: molecular or immunological methods?

In general, Nucleic Acid Amplification Techniques (NAATs) are best for pathogen screening. The reason for this is that these methods can identify the presence of the pathogen's unique nucleic acid signature at very low loads, even at less than 10 copies/reaction, while carriers are in the asymptomatic phase. NAATs include conventional and real-time Polymerase Chain Reaction (PCR), and the newer isothermal method, Loop Mediated Isothermal Amplification (LAMP).

For the protein or immunological method, pathogen antigens (typically from surface or envelop proteins) are captured by antibodies. The result is interpreted from the visible lines on the test strip. The advantage is its low cost, rapid turn-around time, and point-of-care (on-site) application. The drawback is that the technique suffers from the lack of sensitivity and non-specificity, leading to greater cases of false negatives or false positives (typically, a result of environmental interferences). However, the immunological method is a powerful tool for diagnostics when animals exhibit appearance or gross clinical symptoms.

The wrong choice of methodology, at the wrong time, can be costly to the hatchery and farm.

Sampling techniques

A frequent and common question is How should sampling for testing be conducted? For broodstock in a hatchery, we can pool specimens from five brooders, from the same holding tank, as 1-test-composite-sample. Pooling more than five individuals may cause a "dilution effect" where the already low pathogen load is further diluted below the method detection limit resulting in a false-negative result. If the test is positive, every individual is tested by destroying the carrier.
After an interval, the same individuals are pooled, and the composite sample is retested.

For larval tanks with thousands of animals, we can deploy a random or bias sampling technique. The techniques are defined as follows:

- *Random sampling* is based on a statistically significant sampling method developed by Ossiander &

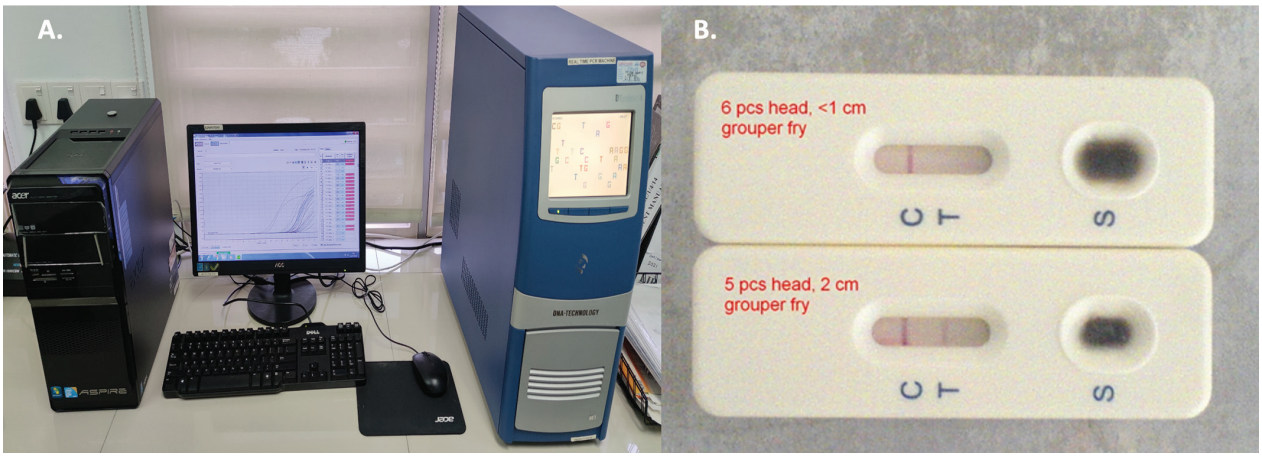

Figure 3. (A) Nucleic Acid Amplification Technique (NAAT) real-time PCR is the preferred pathogen screening tool. (B) Immunological Lateral Flow Device is useful for on-site diagnostic.

Wedemeyer, 1973. To detect a positive sample in a 2% infected population (1,000,000 x 2% = 20,000 infected individuals), we test 150 random individuals. The same positive result is consistent in 19 out of 20 subsample repeats, to give 95% confidence limit. For animals at the larval stage, we pool 150 individuals into three composite samples of 50 each. For the population to be disease free, all three pooled samples must be negative (Table 1).

- *Bias sampling* is the intentional collection of weak or dead animals. Random samples are taken from different tank locations and pooled into a small container. The water in the container is stirred vigorously clockwise. Strong and healthy post-larvae will swim energetically counter current. The weak or dead animals will settle to the center-bottom of the tank. These are collected and pooled together to be tested.

The larval/post-larval population is subjected to multiple tests at different developmental stages (for example, PL 3, PL 11, etc.) Repeated testing increases the probability of identifying any carriers or pathogens before it moves out of the facility. For the grow-out system, bias sampling is a more practical approach.

What pathogens should be screened?

The World Organisation for Animal Health's (OIE) Aquatic Manual has a pathogen list with new emerging

Table 1. Random sampling guide with population size, disease prevalence and sampling size to detect one positive with 95% detection confidence limit.

	Prevalence (%)						
Population size	**0.5**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**10.0**
50	46	46	46	37	37	29	20
100	93	93	76	61	50	43	23
250	192	156	110	75	62	49	25
500	314	223	127	88	67	54	26
1,000	448	256	136	92	69	55	27
2,500	512	279	142	95	71	56	27
5,000	562	288	145	96	71	57	27
10,000	579	292	146	96	72	29	27
100,000	594	296	147	97	72	57	27
1,000,000	596	297	147	97	72	57	27
>1,000,000	600	300	**150**	100	75	60	30

pathogens to be included. Which approach, full battery or "a la carte", would be the best testing strategy? Ideally, all pathogens must be screened but economics make this impractical.

For hatcheries working with SPF (Specific Pathogen Free) or HH (High Health) animals, "a la carte" testing is practical. The test choices depend on potential species susceptibility and pathogen prevalence in the region. For both *P. monodon* and *P. vannamei* broodstock and larvae, they should be checked for WSSV, AHPND, and EHP. In addition, IMNV is tested for *P. vannamei* but not *P. monodon*.

Hatcheries depending on captured wild stocks must implement full pathogen screening as the natural populations are known to be contaminated becoming carriers. A recent report demonstrated wild *P. monodon* infected with IMNV and DIV1 is yet grossly normal (Srilasa *et al*., 2021).

Conclusion

Pathogen screening and disease detection are central to aquaculture biosecurity. It is a preventive multi-layer "ring-fencing" strategy for early detection and disease management. By understanding the science and applying common sense, we hope the industry practitioners can effectively integrate disease screening measures into their operations.

Our laboratory offers extensive molecular aquatic pathogen screening services to SPF producers, hatcheries, farms and processors across ASEAN, South Asia, and Africa. Our bespoke solutions include test kits, laboratory set up, training (end-to-end solution), method development and validation.

References available on request.

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How microbiome analytics de-risk the aquaculture industry

Rachelle Jensen, Luminis Water Technologies

As the aquaculture industry races to meet growing demand, water quality issues and disease prevention have moved into center focus. The Singapore-based tech firm, Luminis Water Technologies, seeks to take the aquaculture industry to a new level of efficiency by introducing a range of next-generation microbiome analytical solutions and water sampling kits that target a wide range of pathogens and offer unprecedented visibility into water health. The use of their proprietary algorithms can help fisheries diagnose mystery ailments that previously have decimated stock and driven operating costs up.

As the world population grows, the pressure on aquaculture and the ocean's ability to meet protein needs will only increase. Luminis offers a range of solutions to help aquaculture operations optimize their systems and better manage disease outbreaks.

Currently, the gold standard in pathogen screening is traditional bacterial culturing on selective media, qPCR, and antibody tests which quantitatively determine the presence of a target gene or protein. Positive ID of a specific pathogen is quick and quantifiable. However, all of these approaches have their limitations. Any attempt to troubleshoot or identify an unknown pathogen is not possible. Beyond these limitations, buying a qPCR machine introduces a significant investment with prices starting at $5,000 per machine, plus the added investment in training up technicians.

Screening offered by qPCR and plating is an effective technique used to determine the presence of a target pathogen. However, in the case of trying to understand co-infections by multiple pathogens, reaching a conclusive answer as to the cause of the disease cannot be determined.

After the disease has taken hold, treatment can include antibiotics, but the pressure is on to deliver sustainably farmed fish and shrimp since antimicrobial resistance (AMR) was named one of the most important human and animal health-threatening issues worldwide (Ernesto *et al.*, 2020).

Figure 1. Fish lesions sampled.

Early disease warning

The onset of disease is complex and not always caused by just one pathogen. There are underlying conditions that create an opportunity for pathogens, usually present in low latent numbers, to suddenly boom in population. By analyzing the microbiome behavior before a disease outbreak, microbiome analytics can spot the beginning of an imbalance, usually flagged by a specific marker species, which opens the door for

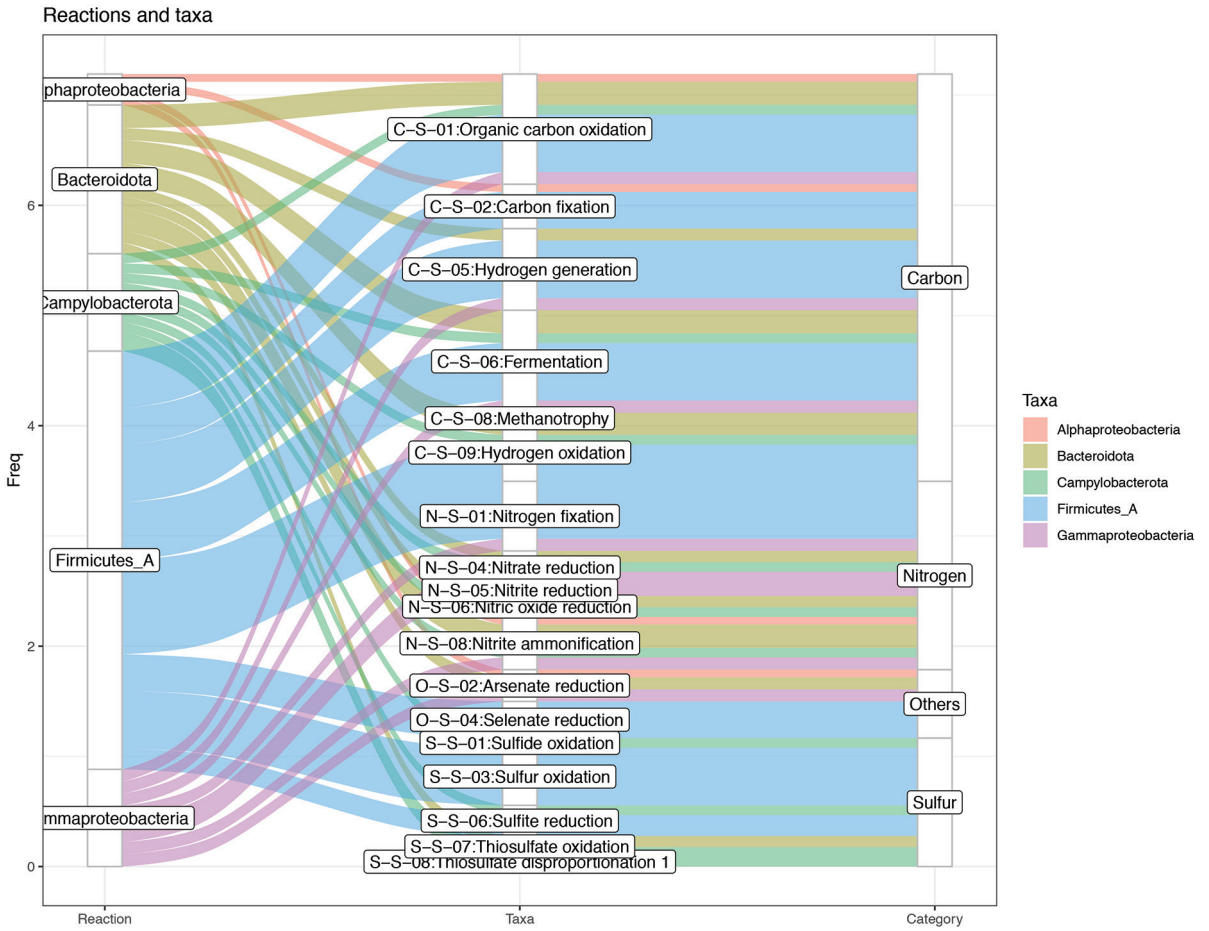

Figure 2. Technical analysis of the microbiome.

pathogen proliferation. Once the vacuum is created, pathogens can rush to fill it.

When disease takes hold, the visible signs of well-known diseases like *Vibrio harveyii* or White Spot are easy to see with the naked eye. In shrimp, the hallmark white spots appear. In the case of *Vibrio*, skin ulcers are immediately apparent. By the time visible signs of disease are noticeable, the time to save stock is extremely short.

Moreover, the actual infection may present as one type of pathogen but may, in fact, be caused by a cluster of microbes, including non-pathogenic ones that "help" the pathogen to take hold and thrive.

For example, we evaluated a diseased grouper from a fish operation that suffered from lesions attributed to a *Vibrio* outbreak (Fig. 1). All the visual pathology was in concurrence with the symptoms of vibriosis. However, the stock proved hard to treat, and the lesions advanced. Upon swabbing the lesions and using next-gen sequence analysis on the lesions, we discovered that the fish suffered from co-infection of *Vibrio* with *Tenacibaculum* and *Clostridium* (Fig. 2). Therefore, had this fish been diagnosed only with qPCR or plating, crucial underlying information would have been lost.

The benefits of microbiome analytics

Next-gen analysis offers more than just disease determination and unknown pathogen ID.

By harnessing the power of microbiome analytics, fish and shrimp farmers can capitalize on a crucial window of opportunity that lies between imbalances and a full-blown disease event. Other benefits include: screening quarantined stock for parasites and pests such as leeches, track feed and probiotics efficiencies, identifying causative agents that lead to reduced water quality, and verifying if a treatment actually killed all the pathogens or just reduced the load.

But perhaps the most important benefit is the ability

to make informed targeted decisions that can lead to decreased operating expenses and increased yield.

Sustainability 2.0: Snapshots of health

Not only can analytics provide a crucial window of opportunity when it comes to saving stock from disease outbreaks, but Luminis also offers unprecedented "snapshots of health" of any sample.

Their range of screening tests undergoes full genetic sequencing and analytics in-house at the Luminis sequencing center. The kits are portable and easy to use. There are three flagship products: fishGENius, shrimpGENius and aquaGENius. Each offers a full panel of all species present, relative abundance, and flagged species that are either outside of normal parameters or potentially problematic. The results can be accessed by the customer through a data portal that also contains comparisons with historical data from the same customer.

Our kits can screen for a wide range of pathogens, including *Vibrio, Aeromonas, Edwardsiella, Pseudomonas, Flavobacter, Mycobacter, Streptococcus, Renibacter*, including common anaerobes, *Clostridium* and *Enterobacter*, and *Francisella*, along with every single species of microbe in that given sample.

Understanding "who" is in any sample and "what" they are doing leads to better, more precise and targeted decision making, which ultimately leads to the smarter and more sustainable use of water resources. Luminis Water Technologies is dedicated to providing data-driven and actionable solutions that will help tackle some of the most pressing problems in water management and the aquaculture industry today.

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State of the industry in salmonid sperm cryopreservation

Soledad Francke, IMV Technologies

During the last decade, genetic programs in aquaculture farming have experienced exponential development. Sperm cryopreservation represents a critical backup to accompany those advances, with the imperious need to move toward real automation of the process.

The use of frozen semen allows to have genetic material for desired crosses, reduce seasonal effects, optimize the use of genetically valuable males and thus, substantially accelerate the genetic improvement of the farm. This process is used routinely in the main salmonid genetic companies and increasingly in other species, such as cleaner fish or lumpfish and marine species. In some cases, the level of frozen semen usage reaches more than 75% of the production of milt within some companies. However, there are still some important steps to take before cryopreservation becomes routine in production.

Cryopreservation challenges

Achieving an acceptable fertility level with frozen semen has room for improvement, especially considering cryopreservation results can have variability within sub-species and even within males.

Some publications suggest variations in cryopreservation results between different males in Atlantic salmon, based on seminal fluid composition. For other aquaculture species like sea urchins, researchers work with freezing curves and different cryoprotectant combinations for each type of sea urchin.

The difference has been studied and works in land-based animals such as horses. Specialized stud farms with high-value stallions may use different freezing curves for each stallion. Achieving excellence requires research and testing for fine-tuning and optimization of freezing curves and techniques.

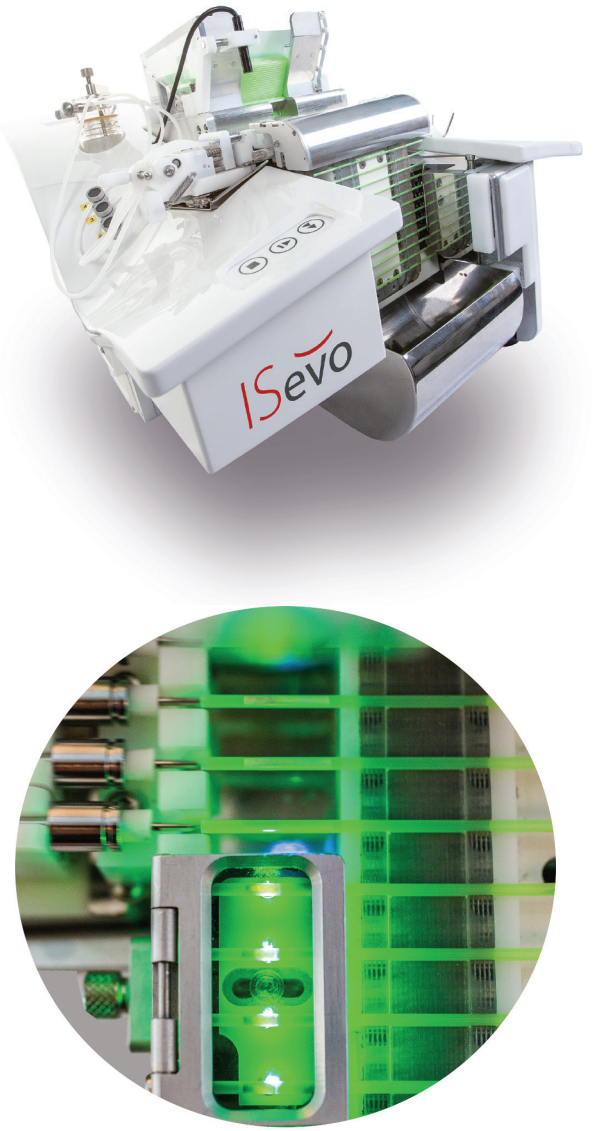

Figure 1. ISEVO Alpha. Straw filler with fluorescence detection.

It is also suggested that tolerance to freezing may be a hereditary condition within some lines. Therefore, in preparation for mass cryopreservation, it will be necessary to include the selection of those

Figure 2. Digitcool Alpha, most advanced technology for automated freezing.

individuals with a greater ability to be cryopreserved and fine-tunning work to find the right process for certain strains.

Another challenge has to do with the need to freeze the increasing volumes of semen. Most production sites do not have the room to freeze all their requirements manually. For this final challenge, there are solutions.

Cryopreservation technology

IMV Technologies, a leader in cryopreservation and artificial insemination biotechnologies, has developed equipment that allows processing, packing and freezing a large throughput of semen in one day. Standard equipment can produce enough straws to fertilize more than 5 million eggs. High technology not only optimizes performance but also aims to improve biosecurity and the quality of the process.

In addition to this, IMV Technologies offers semen straws that are not toxic to the sperm, are resistant to cryopreservation and can be securely sealed to prevent cross-contamination of samples or the loss of genetic material. Options to the filling process include fluorescent detection of misfiled straws and technology that reduces the waste of valuable material.

Freezing is a critical part of the process. Highly valuable genetic material should not be frozen over nitrogen vapor. The procedure is highly variable and virtually impossible to control or document. Controlled rated freezers, including fully programmable systems from IMV Technologies, inject precise volumes of nitrogen into an insulated chamber to meet a theoretical, user-defined temperature curve. This ensures the homogeneity, safety and monitoring of each freezing process, as well as operator safety.

Finally, there is no clear regulation for the commercial exchange of semen worldwide. While the exchange of genetic material is controlled by the OIE, a milt-specific process must be enacted, considering cryopreserved straws can be stored for a very long period. In contrast, eggs are incubated and immediately utilized in production.

For example, there are European regulations for the commercial exchange of bovine semen in straws. These may include formats for the identification of each straw that ensure traceability of the semen sample. This ensures traceability and supports the sanitary safety (and biosecurity) of commercialized milt samples.

Conclusions

While there is still some distance to go before cryopreservation of milt becomes commonplace as it is for land-based animal farming operations, there is a significant amount of knowledge and state-of-the-art technology available for those companies that want to apply them into successful genetics and breeding programs.

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Nanobubble technology offers extensive benefits for aquaculture

Kelly Coleman, on behalf of Moleaer

Aquaculture professionals are well aware that water quality is of paramount importance in productivity, product quality and long-term sustainability for both hatchery and grow-out stages of production. Limited resources and effects on water quality from climate change present daunting challenges for the growth of global aquaculture.

Innovative nanobubble technology shows immense promise in addressing those challenges. Founded in 2016, Moleaer's nanobubble technology increases dissolved oxygen (DO) levels and water quality by reducing ammonia levels and suppressing waterborne pathogens, with proven successful applications in wastewater treatment and hydroponic agriculture. Moleaer has raised $20 million in capital to date, with recent fundraising of $9 million led by S2G's Ocean and Seafood fund. The groundbreaking company is expanding its focus to the aquaculture industry, with demonstrable benefits for increased productivity and sustainability.

"The aquaculture market is driven towards intensification, as evidenced by the growth of RAS, and more than ever requires better water treatments and more efficient gas utilization," said Warren Russell, chief commercialization officer at Moleaer. "We believe that Moleaer's nanobubble technology offers a unique value proposition by easily integrating with existing systems to significantly reduce operational costs and promote sustainability for the industry."

How it works

Nanobubbles, 2,500 times smaller than a grain of salt, are generated by injecting compressed oxygen into rapidly flowing water, which further spreads and dissolves bubbles. Whereas traditional aeration methods like diffusers only dissolve between 1-3% oxygen per vertical foot of water, Moleaer's nanobubble generators have an oxygen transfer efficiency of up to 85%, allowing for reduced oxygen use and energy savings.

Figure 1. High flow Nexus 1000 nanobubble generator delivering 15 kilos of oxygen per hour in a Canadian RAS hatchery.

The tiny size of nanobubbles allows them to remain suspended in water for long periods of time until they dissolve, with properties closer to colloidal particles (small particles suspended in fluids) than larger bubbles that rise to the surface and pop. Nanobubbles act as a battery that delivers both oxygen and treatment continuously to the entire body of water. This makes supersaturated oxygen levels simple and economical, providing oxidative benefits and producing scouring effects to clean surfaces without the use of chemicals.

Moleaer's Nexus™ nanobubble generators are modular, pumpless systems designed to work in-line

with existing liquid flows. They offer unmatched flexibility and can be installed horizontally, vertically and run parallel in sequence and can be applied to high-flow gas injection, low-head recirculation, side stream injection and modular systems.

Increased productivity

Since introducing Nanobubble technology to the aquaculture industry in 2020, Moleaer has partnered with universities for research and commercial operators for demonstration case studies, with promising results for more efficient use of resources and substantially increased productivity.

An academic study conducted in Indonesia compared shrimp reared at raceway ponds using nanobubble and diffuser aerators found that the higher oxygen levels from nanobubbles improved the growth environment by specifically reducing total bacteria, virus, and diseases, increased feed conversion efficiency and survival rate, and resulted in doubling the total harvest (Tables 1, 2, 3).

A major producer in Canada also experienced increased productivity in the hatchery when their low head oxygenator (LHO) system was retrofitted by adding Moleaer's high flow nanobubble generators. While the LHO is an efficient, low-cost oxygenator, in this case, they were not able to meet the oxygen demand of the biomass in their system, resulting in low DO levels and suboptimal feed conversion ratios. Within thirty days, the hatchery observed increased DO levels by 15% while reducing their overall oxygen use by 17%, achieving a 22% increase in biomass growth during the test period.

Table 1. Mean weight and length (±standard deviation) of *P. vannamei* reared at raceway ponds using nanobubble and diffuser aerator. *t*-test for equality of means

	Mean	SD	t
Weight (g)			
Nanobubble	15.10	1.79	2.96*
Diffuser aerator	12.70	1.83	
Length (cm)			
Nanobubble	13.10	1.10	2.72*
Diffuser aerator	11.55	1.42	

n = 10. *Significant at the 0.05 level.

Table 2. Survival rate and feed conversion rate in *P. vannamei* pond using nanobubble and diffuser aerator.

Parameter	Nanobubble	Diffuser aerator
Survival rate (%)	95	78
Feed conversion rate	1.1	1.5

Table 3. Total harvest and productivity in *P. vannamei* pond using nanobubble and diffuser aerator.

Parameter	Nanobubble	Diffuser aerator
Total harvest (kg)	436	222
Productivity (kg/m^3)	8.7	4.4

Improved product quality

Because nanobubbles act like solid particles, they have a scouring property that helps remove biofilm, pathogens and algae from surfaces. The nanobubbles also have natural coagulation properties, further reducing contaminants and improving water quality and cultured animal health, especially in RAS environments. Research at a commercial shrimp farm in Singapore found that nanobubbles reduce *Vibrio* and other pathogens, resulting in fewer deaths and better yields of healthier shrimp.

Figure 2. Two Nexus 500 nanobubble generators installed horizontally at the Canadian RAS hatchery to provide inline nanobubble gas injection.

A key issue in RAS aquaculture is a "muddy" off-flavor in the finished product caused by the accumulation of geosim and MIB compounds in recirculated water. University research has validated that nanobubbles reduce these off-flavors through oxidation and volatilization, resulting in a superior tasting product with less purging time and reduced critical weight loss.

In ocean aquaculture, nanobubble technology can support therapeutic treatment for sea lice, allowing for extended treatment time and calmer, unstressed fish, which ultimately results in a higher quality finished product. At a salmon farm in Chile, Moleaer's nanobubbles significantly improved sealice treatment by safely increasing treatment time by 90 minutes, producing the best result ever of a single treatment at that operation.

Improved sustainability

Moleaer's nanobubble technology can be an effective, eco-friendly tool in the efficient management of water resources for RAS aquaculture in terms of cost savings realized by increased efficiency, and improved water quality, less disease and healthier fish. All these factors lead to improved long-term sustainability for hatchery and grow-out RAS operations.

The technology can also improve sustainability for ocean aquaculture by remediating degraded near-shore sites, repairing damaged seabeds and cleaning up sludge from under net pens. Freshwater farm sites in lakes and ponds, which can experience periods of heavily eutrophicated waters from accumulated waste, can also greatly reduce their environmental impact.

Increased profitability

Russell admits that Moleaer faces challenges in aquaculture industry acceptance of the nanobubble technology, as it is a new, unknown concept and is more expensive than conventional aeration systems. But beyond simply increasing oxygen levels, the unique properties of this technology ultimately present an opportunity for increased profitability. With up to 60% reduced oxygen use and over 40 to 50% reduced electricity consumption, combined savings in energy costs and oxygen use reduction alone allow for a typical Return on Investment of 12 to 18 months. That does not account for increased biomass and higher yields, which can be significant.

Moleaer's goal in aquaculture is to help operators use oxygen more efficiently, lower operational costs while simultaneously enhancing productivity.

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New real-time monitoring technology assists hatcheries in successful productions

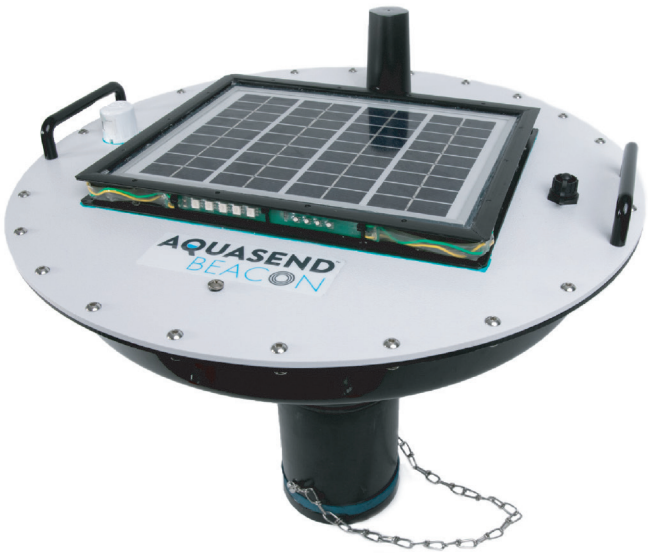
Ted Lucena, Aquasend

The measurement of dissolved oxygen, temperature and other production attributes are an essential component of successful hatchery practices. Numerous studies and industry experts credit the monitoring of these attributes to growth, survival, feed efficiency, water chemistry requirements and the overall effect on hatchery production. What can be a manual process often limited by employee availability, accuracy and the ability to efficiently move from tank to tank or pond to pond is now being monitored remotely and in real-time.

The Aquasend Beacon™ collects and radio transmits water oxygen and temperature measurements for real-time water-quality management. The Beacon is solar powered, fouling resistant and designed to withstand the harshest of weather and farming environments. The innovative technology includes an optical oxygen sensor developed by Aquasend's parent organization, Precision Measurement Engineering, Inc. (PME).

The optical oxygen sensor contains an optode that measures lifetime-based luminescence quenching of a thin membrane. The sensing foil contains a coating that has a variable fluorescence that depends upon oxygen concentration in the surrounding water. The sensor can compute oxygen concentrations in mg/L.

The Beacon's optical oxygen sensor is factory calibrated to maintain accuracy for more than 12 months. Its LED lights correspond to prescribed water-quality levels and provide a visual notification of changing conditions. The sensors are integrated with a cloud-based data platform that allows farmers to receive preset alerts and alarms in real-time from any global location via GPS positioning, by phone, tablet or laptop.



Three benefits of hatcheries implementing consistent monitoring methods

Development and testing of the Beacon has been an ongoing process dedicated to deployment and data collection in varying hatchery and farming environments. Throughout the testing, data collection and distribution process, three distinct benefits have been identified by farmers and industry experts.

Benefit one

Collecting data to be used for consistent growth and using it daily, weekly and monthly to access and determine farm best practices. It is important to monitor oxygen and temperature continually and log data over time to determine optimum stocking rates, fish health and other feeding rates. The Beacon can protect against catastrophic low oxygen levels and chronic sub-optimum conditions that impact fish health and growth.

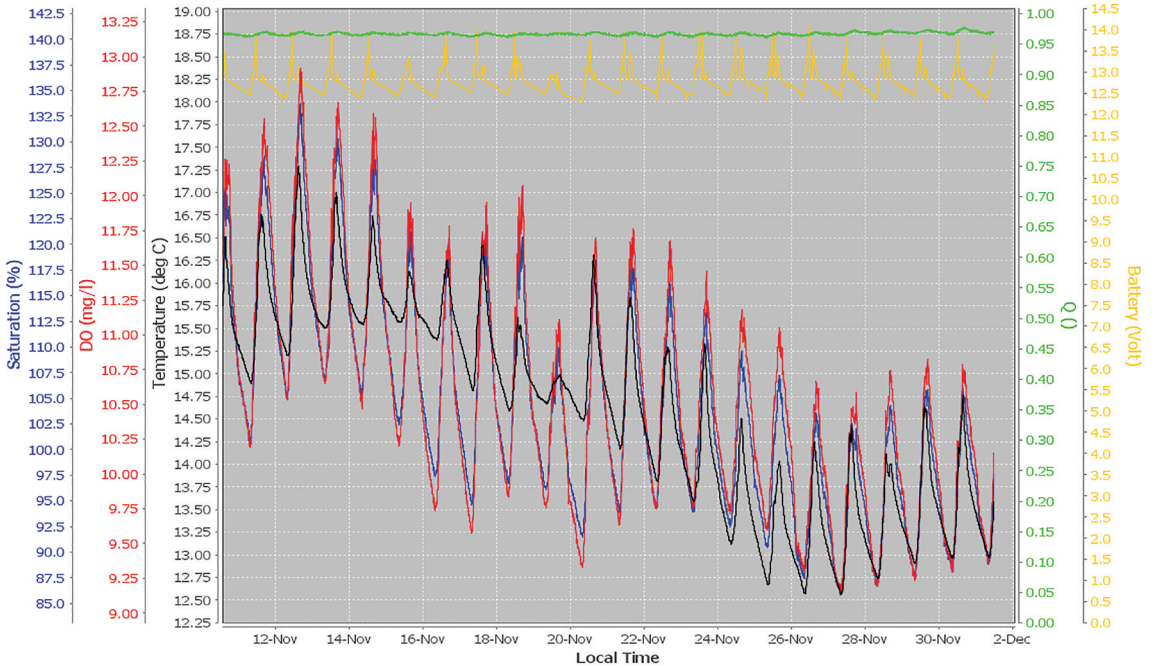


Figure 1. Overall trend data obtained while monitoring water temperatures and verifying the aeration system.

Benefit two

Understand oxygen levels in ponds and tanks and the impacts of these levels on production numbers and successes. Typically, when oxygen depletes, farmers do not feed. Not feeding equals less growth and lower weights at harvest. By knowing the precise oxygen conditions, feeding rates can remain optimal and can ensure faster growth. By ensuring optimum conditions, survival rates or livability will increase. It is noted by farmers that when lost growth is averted, Beacon costs can be recouped in one season and potentially from one tank or pond. ROI is fast and can be calculated.

Benefit three

Insurance for farmers and peace-of-mind to not lose crops. Crop insurance is expensive or simply not available for an aquaculture project. The Beacon alerts staff of lethal low oxygen conditions and prevents fish deaths.

By installing a reliable state-of-the-art monitoring system, test farms have indicated the following calculable results: labor is reduced, feed cost savings are realized, and fish growth and survival rates increase.

On-going research and development

A literature review of hatchery-specific research confirmed that the percentage of dissolved oxygen available is monitored from fertilization until the start of feeding and beyond. These studies, of varying species, identify the impact of dissolved oxygen levels in various research settings, from cold water recirculating aquaculture systems to low oxygen levels and the correlation to hypoxic stress and hatchery environments where dissolved oxygen levels were controlled to determine the effect of dissolved oxygen concentration on the development and survival of eggs and fry.

Using this research and countless interviews with farmers, industry professionals and through the deployment and data collection phase of the Beacon, farmers expressed the value of the data but indicated the solar-powered design of the Beacon to be prohibitive in covered hatchery environments. Using this information, the Aquasend team has developed a Beacon 2.0 design that is hard-wired and includes real-time data collection, alerts and access.

Currently deployed at a hatchery in California, USA, the Beacon 2.0 is performing to the standards of the Beacon. Beacon 2.0 can be used in both tanks and ponds where power is available. Product design parameters are currently being tested for mobility and readings can be collected from 8 feet from the water's edge or side of the tank.

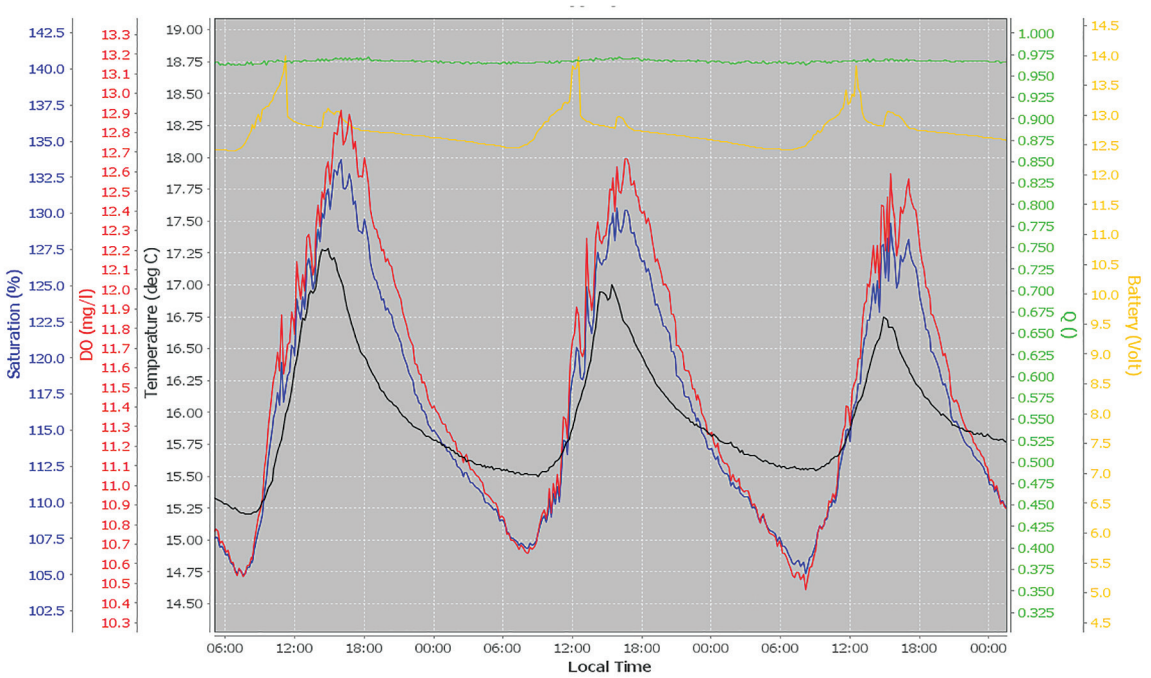


Figure 2. Hourly trend data obtained while monitoring water temperatures and verifying the aeration system.

Data collection and interpretation made easy

Collected data can be accessed in varying intervals. Data in Figures 1 and 2 were collected from a test location with the long-term goal of monitoring water temperatures and verifying the aeration system was working properly. The farmer monitored each variable for eight to ten weeks prior to the introduction of hatchlings.

Figure 1 describes the long-term approach to data collection. This 20-day interval reveals a downward trend in water temperatures and dissolved oxygen that informed the farmer conditions would soon be adequate for the introduction of hatchlings.

Figure 2 describes an hourly, detailed view of each variables' daily cycle within the hatchery. This data was used by the farmer to determine the most efficient and effective feeding and aeration schedules for the hatchery. The real-time data also allows the farmer to gain immediate feedback on any changes that are made to operations.

Data can not only be viewed in real-time via the Aquasend dashboard but data can also be exported and sent to customers for detailed analysis and longitudinal insights.

Conclusion

From instantaneous data to the ability to correlate collected data and make cost saving and revenue increasing decisions for your overall production process, the Aquasend Beacon™ is revolutionizing a hands-off approach to monitoring. This state-of-the-art technology provides much needed peace of mind, while simultaneously accessing multiple areas of the growing cycle where beneficial interventions and adjustments can be made. Consistent, real-time monitoring of dissolved oxygen and temperature, two major attributes, whose fluctuation can be costly, is a proven necessity.

References available on request.

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RAS systems for tilapia hatcheries

Bas Weinans, Aquaculture ID

Figure 1. Labor-intensive collection of tilapia eggs and fry.

Tilapia is one of the most farmed fish species in the world and for good reason. It is easy to farm, grows quickly (excellent strains available), is a herbivore (low-cost fish feeds possible), and, of course, a tasty fish. Despite the advantages, there are also disadvantages such as the relatively low selling price (small margins for the farmer) and the fact that it is a mouthbrooder, meaning a high number of broodstock is needed to achieve a decent production, in contrast to, for example, carp or catfish that give tens of thousands of eggs per broodstock.

RAS is not always the best choice

Since tilapia is a tropical fish, it is mostly farmed around the equator. Tilapia culture can, of course, also be done in other more temperate regions of the world, but a heated recirculation system is required. Since the margins are small, this is often not profitable, with the exception of a few niche markets, for example, in densely populated areas where customers are willing to pay a good price for fresh fish, whether or not combined with aquaponics.

Tilapia is, therefore, mainly farmed in areas where the tilapia would also do well in nature. If sufficient water of good quality and suitable land is available, pond cultivation and cage cultivation may be a better choice than cultivation in a recirculation system.

Experience of Aquaculture ID

Aquaculture ID is a company with a lot of experience in RAS technology. It has over 30 years of experience in the construction of RAS systems for commercial

Figure 2. Easy harvesting and good track of production statistics.

farming of different fish species and has worked for customers in Africa, Asia and Latin America since 1997.

We often see our customers running grow-out farms based on ponds and/or cages with a simple hatchery set-up supplying the fingerlings. These hatcheries mostly consist of ponds for the broodstock, either with or without an incubation section and a section for fry/fingerlings.

The incubation area often consists of jars with tanks through which freshwater flows continuously (raceway). Water from a stream or lake is often used, introducing organic particles and/or bacteria and parasites in the eggs and fry tanks. In addition, the water temperature highly depends on the season, which has a negative effect on the hatching rate.

After the eggs have hatched and the yolk sac has been absorbed, fry are transferred to the next phase. Aquaculture ID has seen many different hatchery systems over the years, such as flow-through systems, ponds, hapas within ponds and biofloc systems. Despite the farmers doing their best and achieving reasonable results, they often have unexpected losses and/or reduced growth in the grow-out phase.

Figure 3. Easy handling of tilapia fry using small RAS systems.

RAS technology as a sensible choice during the hatchery phase

The first weeks are the most delicate part of a tilapia's life. There is almost no immune system developed and fry are therefore very vulnerable to all kinds of diseases during that time. This is why Aquaculture ID strongly recommends RAS technology for hatcheries.

RAS systems provide optimal water quality to broodstock, eggs and growing fry and fingerlings. Due to the use of concrete or plastic tanks with limited size compared to ponds and raceways, observation and feeding of fish are optimized. In addition, these systems allow for optimal handling of fish. Grading is performed easily, resulting in batches that are very uniform in size. The overall survival rate, health and condition of fingerlings produced in a RAS hatchery is superb.
Of course, using RAS technology alone is not enough for a successful hatchery. A RAS hatchery can only be a success with the availability of:

- Good genetics, using broodstock from a well-performing genetic strain improves the growth and health performance of your fish.
- High-quality fish feed. Broodstock and young fish in the hatchery should be fed with high-quality fish feed to give them a head start in life. Quality fish feed is not only important for fish but also for the operation of the RAS systems as low-quality fish feed spoils the filter of the RAS system, reducing water quality.

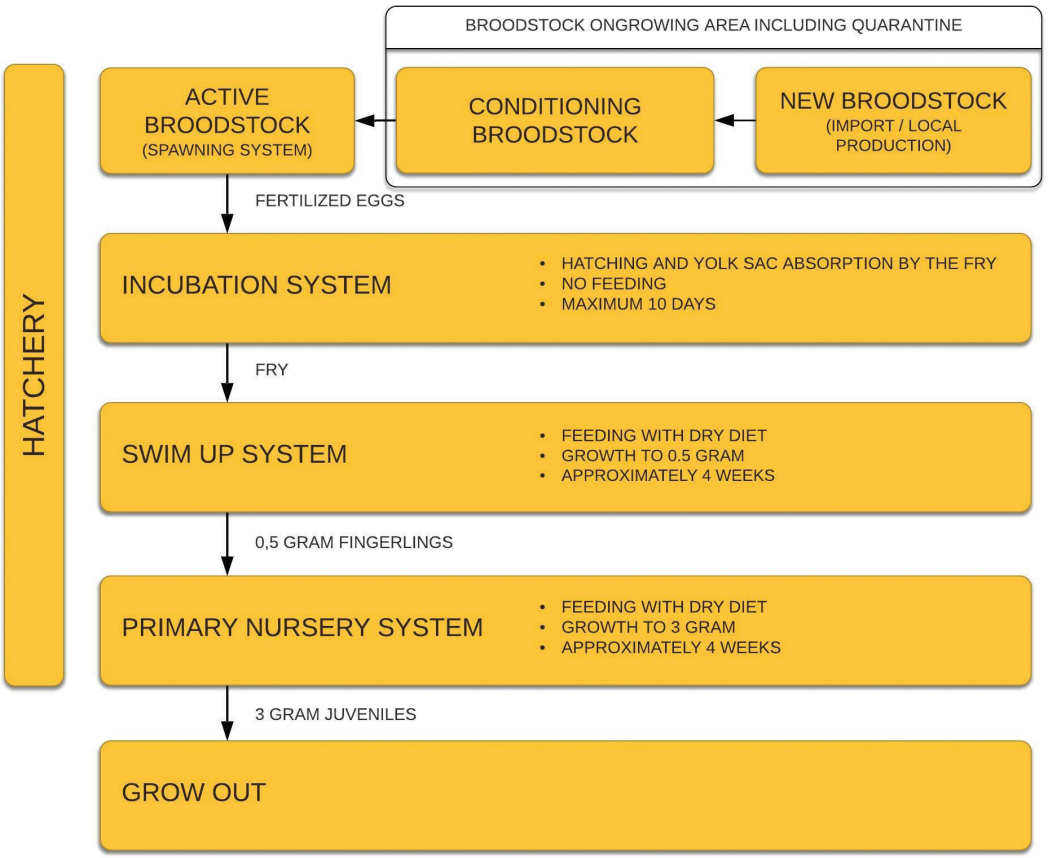

Figure 4. Tilapia production schedule.

- Proper management and staff. A successful hatchery cannot work properly without good management and well-trained staff.
- Constant energy and water source. RAS systems require 24/7 electricity and a good source of high-quality water.

A good set-up RAS hatchery requires higher investment and has higher running costs compared to other techniques used, but the benefits of producing high-quality fingerlings pay off. We do believe a RAS hatchery is only feasible from a production capacity of 500,000 fingerlings per year.

The tilapia production schedule

Aquaculture ID has divided the hatchery into the following four phases (Fig. 4):

- Active broodstock in spawning system
- Incubation phase
- Swim-up phase
- Primary nursery phase

Phase 1: Active broodstock

The active broodstock phase can be performed in three ways:

- Hapas in ponds.
- Hapas in concrete tanks using flow-through or raceway technique.
- Hapas in concrete tank using RAS technique.

Broodstock is stocked in these hapas with 3 females and 1 male per m^2. Every ten days, all female broodstock are checked for eggs and eggs are flushed out if present. Broodstock in hapas allows for easy harvesting of eggs, compared to broodstock swimming freely in a pond or tank. Any prematurely released eggs or fry can also be easily removed from the hapas during each harvest moment, maintaining a clean and optimal spawning environment.

The most ideal setup is using concrete tanks since egg harvesting is much easier compared to ponds. Having a RAS broodstock system strongly reduces water use compared to flow-through and pond

operation. Next to this, the water quality in the system is controlled.

Phase 2: Incubation

Aquaculture ID advises using a RAS incubation system. The system has multiple jars that are connected to a filtration system with a high-capacity UV-C device. UV-C strongly reduces the occurrence of bacteria, fungi and parasites in the system water, providing a perfect environment for the delicate eggs and young fry. After every batch, the incubation system is disinfected with chemical agents like hydrogen peroxide.

Phase 3: Swim-up phase

As soon as fry absorb their yolk sacs and are free swimming, they are transferred to the swim-up phase. These systems are complete RAS systems, but simpler in setting up. Next to a mechanical and biological filter, there is also a high-capacity UV-C device that is incorporated. Tanks are only 500 liters, which makes observation, feeding and handling much easier than in, for example, a hapa in ponds. The stocking density is much higher, compared to more extensive techniques. Ideally, fry are grown to a weight of approximately 0.5 grams.

Phase 4: Primary nursery

Often fry from the swim-up systems are transferred to hapas in the grow-out ponds or cages, being released from those hapas as soon as they reach 3 to 5 grams per piece. It may however be interesting to grow fry in a RAS primary nursery system to a similar weight or even larger. With the benefits of a RAS system, fingerlings grow fast to the desired weight and can be released to the grow-out facilities at a larger size. Being stronger, these fingerlings have a better chance of surviving the harsher conditions in grow-out compared to the ones released at 0.5 grams.

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Recirculating aquaculture system and freeze-dried microalgae for bivalve hatcheries

Vincent Vermeylen, Nancy Nevejan, Ghent University**, Luc Roef, Maarten Muys,** Proviron

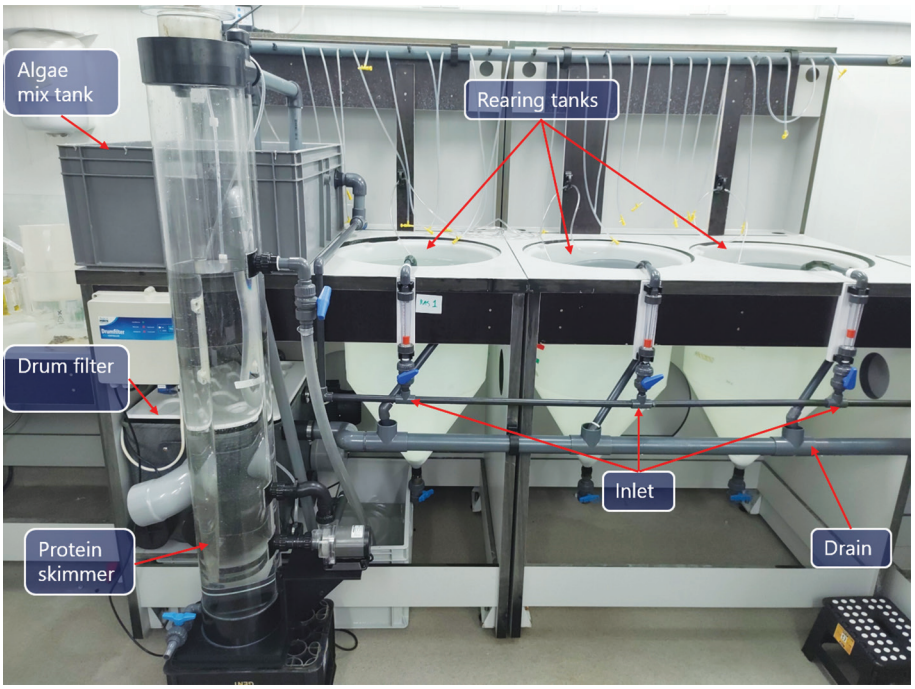

Figure 1. Overview of a RAS used for the rearing trials.

Bivalve aquaculture is a major source of seafood. Oyster farms rely on the availability of seed for their activities, supplied by wild catch or hatcheries. However, both sources are facing a wide range of issues, leading to variable quality and quantity of starting material. An alternative method for growing oyster larvae and seed in a recirculating aquaculture system or RAS may promote a steadier supply. Compared to traditional flow-through systems (FTS), RAS offers many advantages, including better control of water quality parameters, a higher biosecurity level and a more stable microbial community in the rearing tanks. These features reduce exposure to stressful conditions and allow the growing of healthier oysters. This is the reason why many finfish and shrimp producers already implement RAS technology, especially for the sensitive larval phases. RAS is also a very sustainable approach for growing aquatic food, as it uses only a fraction of the water needed in FTS.

Unfortunately, very little is known about the behavior and performance of bivalves in a closed recirculating system. Another challenge for bivalve hatcheries is inhouse microalgae production, which is costly in terms of facilities and labor and is not always reliable. Replacement of live algae with "off the shelf" freeze-dried algae could be a solution. Within the BlueMarine^3.Com project, we aim to study the feasibility of running a multi-species hatchery that relies entirely on recirculation and reduces live algae requirements, while integrating the production of crustaceans, seaweed and bivalves.

RAS system in function of bivalves

Cupped oyster (*Crassostrea gigas*) spat at size T2 was obtained from Aquacultuur Oostende (Ostend, Belgium), while live and freeze-dried microalgae were supplied by Proviron, a company producing microalgae in their proprietary closed vertical flat panel photobioreactor (Hemiksem, Belgium). As this was the

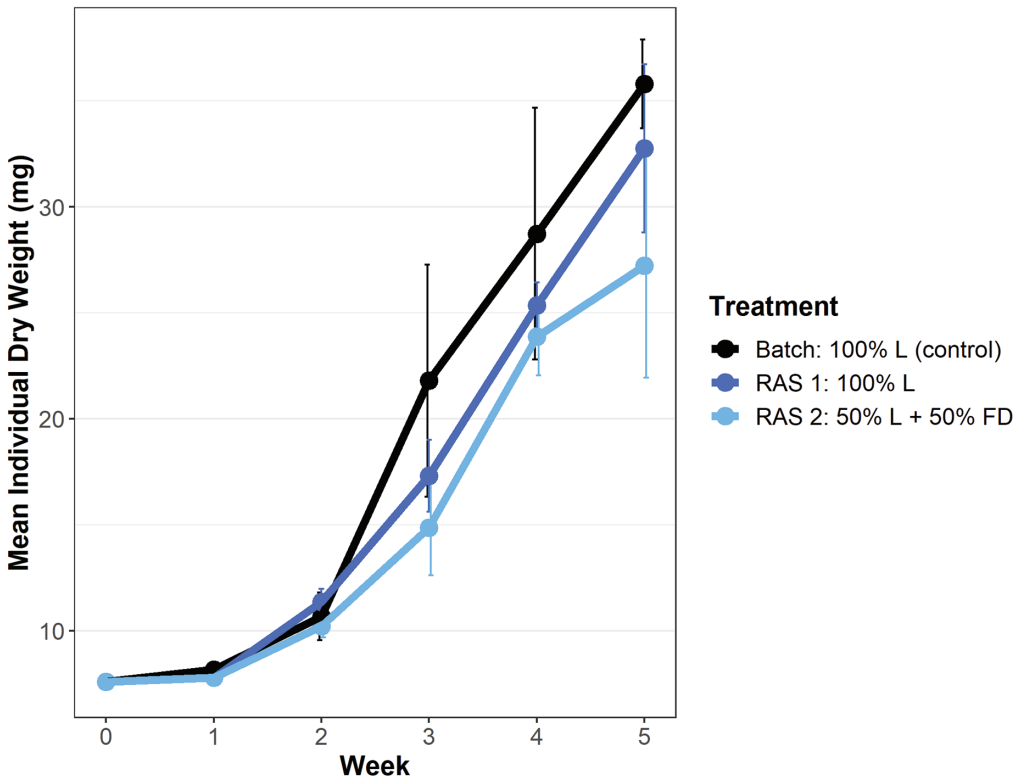

Figure 2. Mean individual dry weight (in mg) of cupped oyster spat fed diets with live (L) and/or freeze-dried (FD) algae. Final dry weights (week 5) did not differ significantly between the treatments (ANOVA, all p>0.05). Error bars represent standard deviations.

first large-scale trial on bivalves in RAS, working with spat was preferred over larvae, due to their higher robustness, ease of handling and monitoring.

The 5-week lasting rearing trial was performed at the Laboratory of Aquaculture & Artemia Reference Center (Ghent University), in two independent recirculating systems. Each RAS consisted of three cylindroconical 100-liter rearing tanks, a drum filter, protein skimmer and header tank, totaling 500 liters per system (Fig. 1), with water upflow in the rearing tanks and a daily water renewal rate of 5%. The control setup consisted of three separate 100-liter batch tanks, with a 100% water renewal rate every second day. Oyster spat was kept in sieves hanging in each tank. All oysters were fed a mixture of *Isochrysis galbana*, *Chaetoceros muelleri* and *Tetraselmis chuii*.

Since in-house microalgae production often represents a relatively large cost, we investigated at the same time the feasibility to replace 50% (on the basis of algae dry weight) of the live algae (L) in RAS with their freeze-dried (FD) counterparts (IsoPrime, ChaetoPrime and TetraPrime, respectively). The oysters in the control treatment (batch) and the other RAS received 100% L algae.

Fecal production was observed in all treatments, indicating effective ingestion of freeze-dried microalgae by the spat. The final oyster dry weight did not differ significantly between the treatments (Fig. 2).

Figure 3. Cupped oyster spat used in the trials at the Laboratory of Aquaculture & Artemia Reference Center (Ghent University).

These results demonstrate, for the first time, that it is feasible to grow cupped oyster spat in a closed recirculating system, and that a 50% replacement of live algae with their freeze-dried counterparts is possible for oyster spat, with no significant difference in final dry weight compared to a 100% live algae diet in RAS. In each RAS, we also observed a slow but steady drop in calcium and carbonate levels, both crucial building blocks in shell growth.

Upcoming trials will further study the potential of replacing live with freeze-dried microalgae, automated feeding protocols and the feasibility of rearing oyster larvae in a RAS, while diet composition and algae production and process techniques are continuously optimized.

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MAY

3 - 5:	Aquaculture UK	www.aquacultureuk.
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31 - June 2:	VICTAM International – VIV Europe, The Netherlands	www.victamintermational.com

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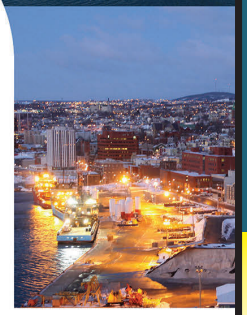

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