Vol 8 Issue 3 2020

HATCHERY

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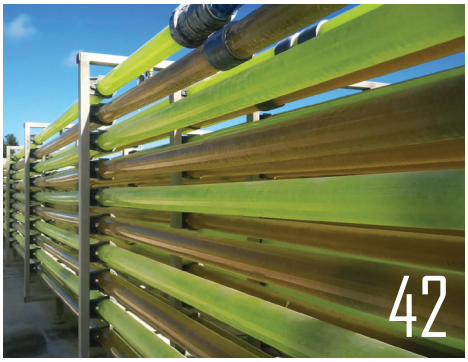
Contents



HATCHERY FEED & MANAGEMENT VOL 8 ISSUE 3 2020

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NEWS REVIEW

HATCHERY FEED & MANAGEMENT

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Hendrix Genetics starts shrimp production at its breeding facility in Ecuador

Hendrix Genetics began the first cycle of shrimp production at the Macrobio hatchery, a state-of-the-art shrimp breeding operation in Ecuador. The hatchery is making waves in the Ecuadorian shrimp industry with a more sustainable approach to shrimp breeding. Hendrix Genetics partnered with Nutreco and Ecuacultivos to invest in upgrading the Macrobio hatchery in Ecuador in 2019. Located in the western region of Ecuador, one of the largest shrimp production regions in the world, it is a closed-cycle breeding operation that will produce clean, Specific Pathogen Resistant (SPR) shrimp. The first generation of shrimp will be ready by the end of 2020.

Genomar builds a new tilapia breeding center in Brazil

Genomar Genetics Group, a tilapia genetic product distributor and a subsidiary of EW Group, is building a tilapia breeding and genetics center in the state of Tocantins, Brazil. The hatchery will be first-of-its-kind for tilapia species in the Latin American region and will have sufficient capacity to manage GenoMar's breeding programs as well as produce parent stock for its distribution operations. The new facility is expected to be operational by the 2020 first quarter.

Til-Aqua International sells its genetics to interested parties

The COVID-19 pandemic has brought logistic issues to many aquaculture companies due to limited available flights and closed borders. Specifically, the import restrictions that some countries have taken regarding the tilapia lake virus had already severely limited Til-Aqua International current operations.

As a result, the company decided to drastically change course so as not to waste 25 years of genetic effort and sell its genetics to interested parties. "We believe that transferring our YY technology to countries for local YY production is the best option. These parties would own YY-males and YY-females and would receive the information on how to maintain these lines," said Eric Bink.

BioMar scales up RAS and fry feed production in Denmark

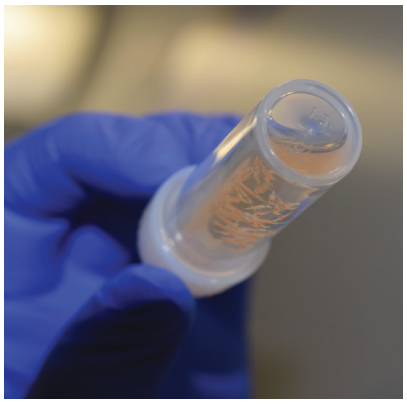
BioMar's new production line in Brande, Denmark, is now fully operational. The line is dedicated to RAS and fry aquaculture feed production and is the result of a DKK 100 million investment that has enabled BioMar to increase the annual capacity of the Danish facility by 25%.

The company said that aquaculture feeds from the new line have been tested through the last couple of months and has shown impressive results. The improved process control technology on the new line will further strengthen BioMar's ability to focus on more physical quality parameters such as sinking speed and water stability.

Genics' improved pathogen detection platform adds Decapod iridescent virus

Australia-based Genics aims at creating the world's most advanced pathogen detection company delivering global food security while executing its vision to help farmers reduce risk, boost productivity and sustainability to meet increases in global food demand.

The company launched Shrimp MultiPath Xtra, the new state-of-the-art early warning system for farmers that enjoys all the data collection innovation found in the original Shrimp MultiPath with the much-anticipated addition of Decapod iridescent virus 1 (DIV-1) which emerged in East Asia around 2014 and is now causing high mortalities in shrimp production and economic losses in many regions of the world.



Xelect's new genetic due diligence service to support aquaculture investor decisions

For decades, aquaculture mergers and acquisitions have focused on the financial and infrastructure aspects of a transaction. Until now there's been no clear way to evaluate what lies at the heart of a company's value: its broodstock. Xelect introduced a new kind of due diligence for aquaculture, genetic due diligence.

The team's new BioAudit combines specialist genetics skills with deep industry know-how to provide independent and confidential genetic audits, alongside biological due diligence. This unique service will give investors and buyers a wealth of new insights to help them make more informed decisions.

New oxygen gas generator reduces dependency on bulk gas deliveries

Peak Gas Generation, parent brand of Peak Scientific and a global leader in on-site gas generation for manufacturing and processing industries, launched its first oxygen gas generator, the i-Flow O2, to add to its already successful i-Flow range of nitrogen gas generators.

Engineered based on proven Pressure Swing Adsorption (PSA) technology, i-Flow O2 utilizes a synthetic zeolite molecular sieve, producing high-quality industrial-grade oxygen gas, with up to 95% purity and a maximum flow rate of 576 L/min. As with all i-Flow products, i-Flow O2 offers a modular and expandable gas generator system, designed to deliver an on-demand supply of oxygen gas with a future-proof capacity to increase oxygen supply should a facility require this moving forward as operations grow.

Redd Zone introduces a portable egg eyeing station

Redd Zone developed a Portable Egg Eyeing Station that provides an oxygen-rich environment for the operator to eye up salmon or trout eggs of any species using up-welling, recycled, temperature modulated water. Using primarily off-the-shelf components keeps the cost down while maintaining a very stable and easily operated unit. Whether deployed to accelerate or decelerate development, the unit can be used for thermal marking of otoliths or even for triploid treatment.

Signify's LED lighting helps optimize salmon growth from hatchery to on-growing stage

Signify launched the new Philips Sea cage 340W fish light which helps optimize growth results for land- and marine-based cultivation of salmon and also shows proven results for other fast-growing fish species like seabass and seabream. In tanks, the light distribution addresses the need for lighting in every part of the tank ensuring light can reach where it needs to reach, without the fish or tiny particles obscuring the light.

New feeding solutions for fish hatcheries

Vard Aqua developed a new range of feeding solutions, the Exact Series, for accurate and predictable feeding. These new solutions aim to make sure fish get the right quantity of feed at the right time, and make work at the facility a whole lot simpler. The Exact series consists of two feeding solutions, the Exact Feeding Robot and a new version of the independent Exact Mini Feeder.

Development of hatchery technology for European eel aquaculture: first results on larval feeding and growth in culture

Jonna Tomkiewicz and **Sebastian N. Politis,** DTU
and **Sune Riis Sørensen,** Billund Aquaculture

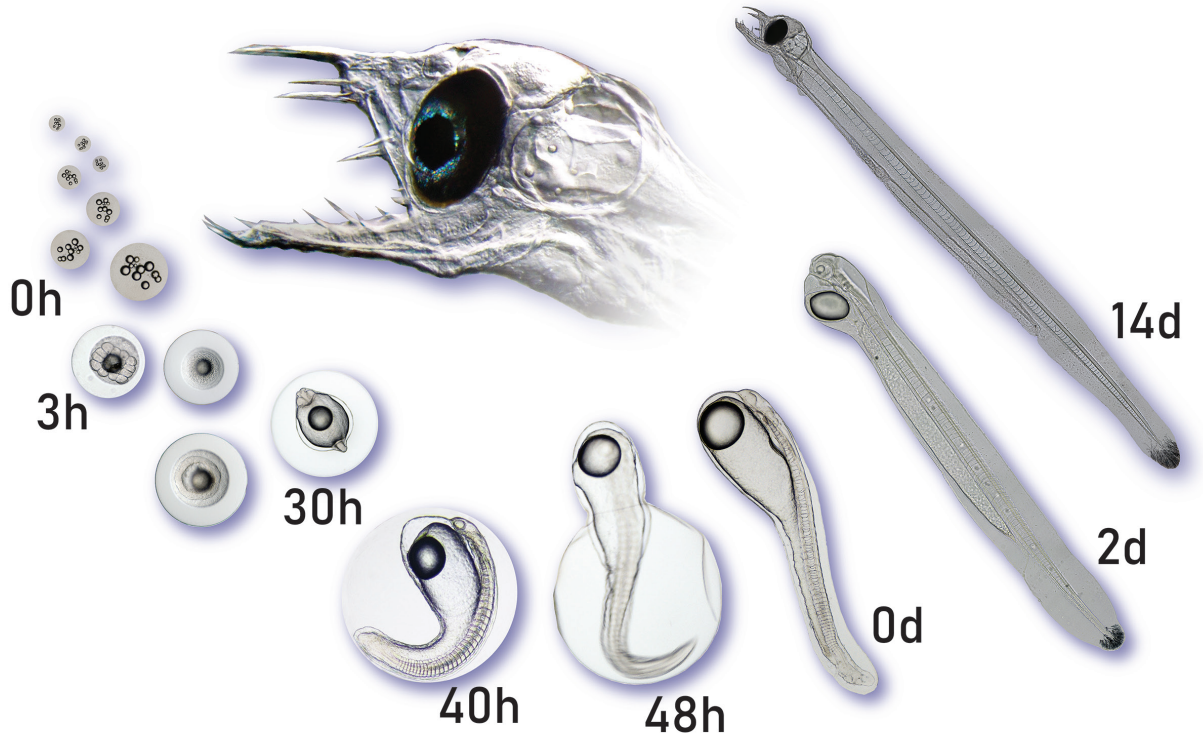


Figure 1. European eel development from egg via embryonic stages to hatch in hours and yolk sac larvae in days after hatch at 20°C – ultimately reaching the feeding larval stage.

Future growth and sustainability of eel aquaculture extensively depends on closing the life cycle in captivity. A general decline of the natural population of European eel (*Anguilla anguilla*) has brought together industry and scientists across the continent in an effort to develop breeding and hatchery technology, enabling captive production of glass eels, the juvenile stage used as fry in eel aquaculture. The vision is an eel industry relying on closed life cycle propagation, ensuring high-quality, year-round hatchery production of glass eels to sustain commercial farming and facilitate breeding programs.

Life cycle challenges for controlled propagation

One of the main challenges to the establishment of eel hatchery technology is the complex catadromous life cycle of Anguillid eels. Here, knowledge about their natural reproduction and spawning habitat is

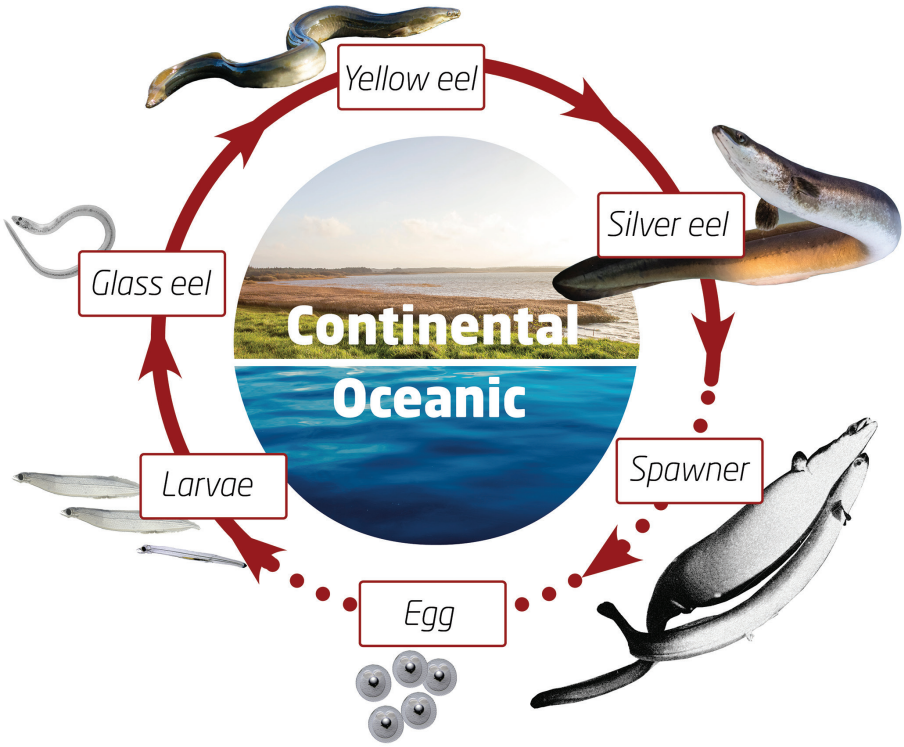


Figure 2. Life cycle of European eel including life-history stages related to the oceanic and continental phases. The solid line of the inner circle represents the known part in nature, while the dotted line shows the still unknown part.

negligible, while insights into the ecophysiology of their egg and early larval stages solely rely on experimental findings (Fig. 2). The main obstacles are that eels do not reproduce naturally in captivity due to intricate hormonal control mechanisms related to their spawning migration and their distinctive, oceanic leptocephalus larvae that are unprecedented in larval culture.

As natural conditions cannot be consulted, the development of reliable culture technologies for different life history stages relies on targeted experimental research and analytical techniques, generating the knowledge-base needed. This involves broodstock dietary requirements and assisted reproduction protocols for high-quality gamete production, standardized fertilization methods as well as technology and techniques for egg incubation and larval culture, including the development of suited rearing systems and feeds.

First step attaining viable offspring production

Hatchery technology development for the European eel took off in the early 2000s, inspired by results obtained by researchers in Japan, who for the first time successfully produced glass eels of the Japanese eel (*A. japonica*) in captivity. Offspring production methods evolved from physiological studies, which applied hormonal administration to overcome the intricate hormonal control that prevents gamete development and natural spawning in continental habitats. Adapting the assisted reproduction protocols developed for the Japanese eel to the European eel soon led to the production of viable eggs and the first larvae.

As assisted reproduction protocols often result in variable gamete quality, low fertilization capacity and/or embryonic developmental failure, much research in eel reproduction has targeted endocrine functions and hormonal therapies in order to enhance gamete quality. Improving those protocols and enhancing factors such as fish nutritional status and rearing conditions have been important broodstock management measures, enabling the production of large numbers of viable embryos.

From pioneering larval production to a prototype hatchery

Recognizing the opportunities, Danish scientists and the aquaculture industry took up the challenge and established a pilot facility in 2005. The research concept, developed and used in consecutive research projects, applies a systematic incremental approach linking basic science to application and technology development for implementation in full-scale production. Here, a knowledge-base covering the reproductive phase and early life history stages is gradually substantiated through controlled experimental tests and dedicated analysis of samples and results. The insights gained are used to develop products, enhance protocols for application development, establish standardized full-scale production and engineer culture systems.

Figure 3. European eel larvae. Upper panel: 16-day-old eel larvae in feeding culture. Middle panel: 32-day-old leptocephalus larvae with its characteristic laterally flattened body and head morphology with prominent eyes and protruding teeth. Lower panel: illustration of 40-day-old larvae showing the characteristic undulatory swimming pattern typical for leptocephalus larvae.

During the period 2005-2008, the team succeeded in producing viable embryos and larvae thereby uncovering the development until the feeding stage. The embryos hatched at ~48h, while the yolk sac larval phase lasted around 12 days at 20ºC (Fig. 1). Still, larval production was limited and in subsequent projects, improvement of techniques was targeted in collaboration with international partners in the EU FP7 project PRO-EEL. For the broodstock, the focus was on enhancing assisted reproduction methods and tailoring broodstock diets for farm-raised eels, benchmarked by wild-caught silver eel counterparts. For the offspring, the development of standardized fertilization procedures, incubation and larval culture techniques, as well as monitoring methods, was given priority. Gradually, improvements led to increased egg production and frequent mass hatching of larvae, creating a demand for more space to accommodate the development of larval culture technology.

In 2016, when three partners DTU, Billund Aquaculture and STMI Aqua Systems established a prototype hatchery, research and technology development entered a new phase. The 650m^2 hatchery got its name after the innovation project EEL-HATCH during which, it was designed and completed. The new facilities allowed an upscaling of technology for increased offspring production and larval culture. Since then, designing and testing larval rearing systems and dedicated experimental work on identifying abiotic and biotic thresholds and optima have increased survival to levels, where hundreds of thousands of larvae enter feeding trials.

First results obtaining feeding larval culture

The present focus is on the feeding larval stage, the leptocephalus larvae, with their distinctive morphology unique to the Elopomorphs (Fig. 3). Still, the natural food of eel larvae, despite the genetic analyses of gut content, is subject to debate, while slurry diets based on shark egg yolk so far yield the best results for a sustained culture of Japanese eel larvae. In order to establish on-growing of European eel larvae in the ITS-EEL project, a team involving researchers from SPAROS, DTU, Billund Aquaculture and STMI Aqua Systems strives to develop suited larval feeds and feeding techniques. The results are promising.

This year, feeding and growth of European eel larvae in culture was obtained for the first time in history. Prior to feeding, the larvae maximally reach a length of 7-8 mm and a body area of 3-4 mm^2. In the feeding experiments, larvae with full guts grew to a length of 12 mm and the body area increased to 8 mm^2 (Fig. 3). These first results on growth and increased survival extended the longevity of European eel larvae to a record of 55 days. In the coming time, the focus will be on developing suitable feeds by enhancing the nutritional composition and digestibility of the feeds.

Within the scope of this research, the provision of feeds and culture technology, including rearing systems and microbial management, also played an important role.

Conclusion and perspectives

In little more than a decade, European eel hatchery technology has developed from reproductive trials characterized by low gamete quality and limited embryonic viability into an up-scaled production with an established culture technology that sustains larval culture and first feeding experiments. The development of suited larval feeds, rearing conditions and systems that can support growth and survival until metamorphosis into glass eels appear to be within reach. Still, a wide range of obstacles remain, which need to be overcome to develop closed-cycle propagation and commercial production of glass eels. Nevertheless, the results represent a promising step towards closing the life cycle in captivity and support future prospects for sustainable eel aquaculture and management of this species. Ultimately, captive breeding and hatchery production of glass eels would remove constraints on eel aquaculture production, linked to the stock decline and trade restrictions and in turn regenerate commercial activity that can revive the highly profitable market for the European eel.

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Watch a video of European eel larvae produced at the EEL-HATCH facility - first results on feeding, growth and transformation into the leptocephalus stage in culture.

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Using DNA tools to produce resistant shrimps

Nicolas Robinson, Nofima and **Morten Rye,** Benchmark Genetics

Healthy whiteleg shrimps. Photo: Marcela Salazar, Benchmark Colombia.

Breeding for disease resistance in shrimps usually implies infecting animals with the pathogen, and selecting either the survivors (mass selection) or, in order to keep the SPF status, select the non-infected siblings (family selection). As we can imagine, selecting the survivors gives us more certainty in the selection than using the siblings, but what if we could select the non-infected animals with the best genetics for survival? In recent years, the use of genomic tools has helped us increase the accuracy of the selection of non-infected animals by identifying the carriers of the markers associated with resistance. We have used this technology to boost the resistance of *Penaeus vannamei* to White Spot Syndrome Virus (WSSV). Using genomic selection, we have demonstrated that we can rapidly increase the level of disease resistance in our lines, without compromising the biosecurity of our breeding nucleus.

Global viral pandemic in shrimp

White spot syndrome virus disease still causes annual multibillion dollar losses for shrimp farmers all around the world. The disease spreads quickly and preventative measures for contagion have proven ineffective, as it has not been possible to use a vaccination strategy for boosting the immune response against the virus (Feng

et al., 2018). Stimulation of the innate immune system shows some promise but has so far remained unproven in the field. We know that some animals are inherently better able to resist or tolerate the virus than others, but we still do not understand the specific mechanisms underlying these differences. It is possible to breed animals with higher resistance to WSSV through conventional family selection, but progress has been slow (Gitterle *et al*., 2005; Trang *et al*., 2019). The industry desperately needs better solutions to prevent mass mortality caused by this aggressive pathogen and to boost profits.

Genomic selection

Genomic selection is a methodology originally developed for livestock improvement employing the latest in DNA sequencing technologies, and in the last few years, it has been applied to aquaculture species (Vallejo *et al*., 2017). Breeding and genetics scientists in Nofima have worked with Benchmark Genetics on these technologies in whiteleg shrimp (*L. vannamei*). Instead of relying on pedigree relationships for estimating the breeding value of individuals, we used DNA sequence data to estimate the genomic relationships between individuals at tens-of-thousands of positions throughout the genome of the organism. This technology gives us a more precise means of predicting the breeding value of potential broodstock in the population (known as a genomic breeding value in this instance).

A major advantage of genomic selection over traditional selective breeding for a trait like viral disease resistance is that it allows us to more accurately predict which breeders have the best overall resistance genotype without exposing the candidate breeders themselves to the disease.

First application to genetic improvement of crustaceans

As part of the *GenomResist* research project funded by the Research Council of Norway, Nofima and Benchmark Genetics tested how effective genomic selection would be for improving WSSV resistance in whiteleg shrimp by designing an experiment using two source populations developed by Benchmark Genetics Colombia, one already mass-selectively bred for several generations for resistance to white spot syndrome virus, and the other bred for fast growth rate and pond

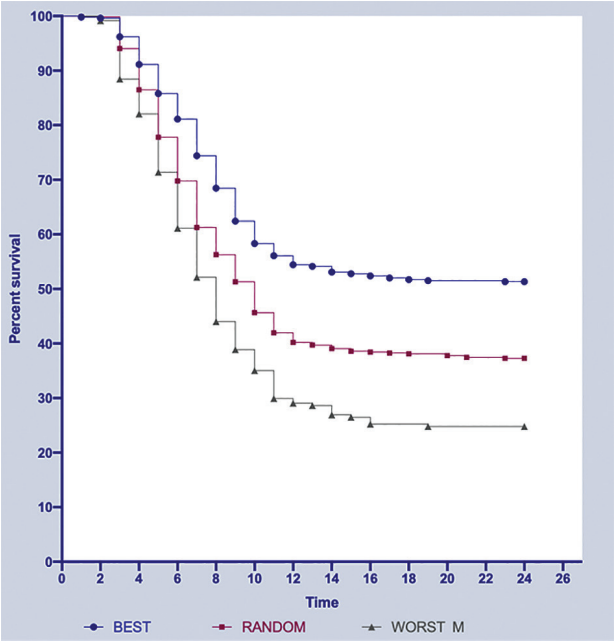

Figure 1. Percent survival plotted over days in challenge test for animals in the high blue- (closed circles), random- (closed red squares) and low- (closed grey triangles) genomic breeding value populations.

survival. Animals from both groups and their crosses were randomly separated into two groups, a test population that was challenged with the virus, and a candidate broodstock population that was kept under high biosecurity conditions. The test population was *per os* infected with WSSV, and dead and moribund animals were tissued sampled each hour for the duration of the trial and, the time of death was recorded for all. All samples were analyzed for DNA markers with a SNP chip that has strong coverage of every position across the shrimp genome, to give us accuracy for the genomic selection. Information about the survival and time of death of shrimp following the challenge test was combined with the genomic relationship data from the DNA testing and used to predict genomic breeding values for survival for individual broodstock (animals not exposed to the virus).

In the second phase of the experiment, broodstock from the non-infected group was mated to produce two different populations of shrimp, one from parents with high genomic estimated breeding values and the other with parents from low genomic estimated breeding values for WSSV. The survival of these two populations, and offspring from "randomly" mated parent stock, was compared in a challenge test. The results of

that challenge test showed that more than 80% of individuals in the best genomically selected families survived when challenged with the disease, in contrast, animals from some of the worst families survived less than 5%. Like vaccinating a population against disease, having animals with this level of resistance in the shrimp population would likely be sufficient to provide a herd effect, greatly reducing the impact of the disease. Genomic selection holds great promise for improving resistance to other diseases as well, as currently being implemented by Benchmark Genetics.

Survival booster

The results demonstrated that the average survival of shrimp families increased from 38% in the random population to 51% in the high breeding population after only one generation of genomic selection for high WSSV resistance.

Implications for shrimp production

Our collaboration has demonstrated that relatively high levels of genetic improvement can be achieved for survival to WSSV in whiteleg shrimp after just one generation of genomic selection and have shown that genomic selection can be used to improve survival to levels that have commercial relevance for the industry. Compared with conventional methods of selective breeding for disease resistance, genomic selection is significantly more sensitive at predicting, and better able to utilize, information about the underlying genetics affecting resistance. Like the effect of vaccinating members of a population, we expect that high levels of immunity in the best populations will have a "herd effect" because highly resistant animals will no longer infect other animals. The inoculum pressure is likely to be lower in commercial ponds than it was with our experimental challenge tests in tanks, and the best families in our high genomic breeding value population showed survival of over 80%. Levels of 70% survival have been shown by other researchers to be sufficient for keeping another viral disease (caused by taura syndrome virus) under control in shrimp.

By using genomic selection, we have demonstrated that we can rapidly increase the level of disease resistance in whiteleg shrimp. Benchmark Genetics now uses this tool to offer growers shrimp populations that can survive and produce in the presence of WSSV. Genomic selection also holds great promise for the improvement of other economically important traits in shrimp and other aquaculture species. The research shows that genomic selection could go some way towards solving this multibillion dollar problem for the shrimp industry in the future.

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Genomic selection for resistance to bacterial cold water disease

Keith Drynan, Troutlodge

Bacterial Cold Water Disease (BCWD), also known as rainbow trout fry syndrome or flavobacteriosis, is a serious contributor to losses in the commercial rearing of rainbow trout (*Oncorhynchus mykiss*) in almost every region in which it is farmed. The condition is caused by the bacteria *Flavobacterium psychrophilum*, a gram-negative rod-shaped bacteria, with symptoms including lethargy, inappetence and most commonly tissue erosion around the caudal fin. Behavioral changes such as spiral swimming may also accompany infection and depending on the life cycle stage of the infected group, mortality rates may vary. In addition to the acute responses, BCWD can result in chronic infections with long term impacts on the population and can flare up again after stressful events such as vaccination, grading and smoltification, as well as persistence in reducing quality of fish produced due to induced skeletal deformities.

BCWD issues

BCWD can be difficult to detect and differentially diagnose, as the bacteria can be difficult and slow to incubate on the necessary specialist agar required to generate easily identifiable yellow colonies. Unfortunately, field diagnosis is often required to prompt treatment of the population with antibiotics such as terramycin and florfenicol, although the bacteria have been noted to develop resistance over

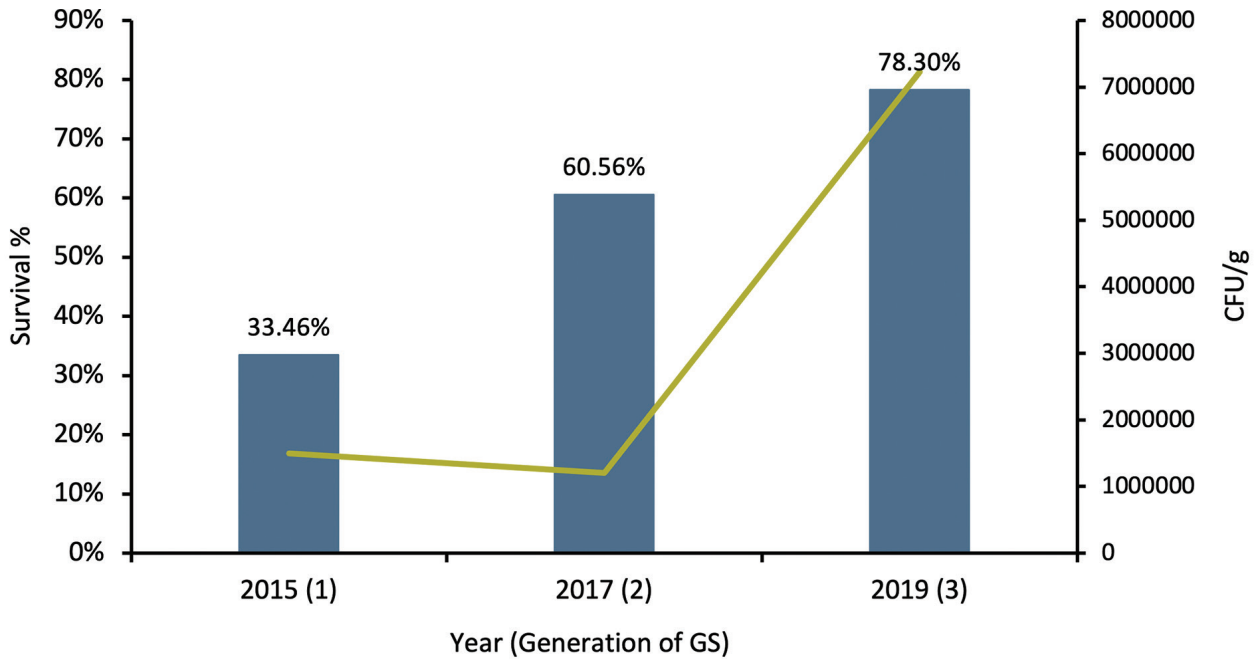

Figure 1. Results of BCWD laboratory challenges conducted by the USDA on Troutlodge's May strain. The graphic shows generational improvements in survival from 2015 to 2019 as a result of genomic selection. Generational improvements are consistent, despite increased severity of bacterial load (CFU/g) from 2015 to 2019.

multiple treatments. Delays in obtaining medicated feed can lead to an escalation of the outbreak with more serious short and long-term effects on the farm.
A significant amount of research has been done in attempts to develop protection from *F. psychrophilum*, however, no effective vaccine is commercially available. Antibiotic approaches for treatment and prevention of outbreaks have been widely used, however, medication adds to production costs in both materials and labor, and the efficacy is variable as antibiotic-resistant bacteria are continually evolving (Barnes, 2011, Bruun *et al*., 2000, Kum *et al*., 2008). For these reasons, *Flavobacterium psychrophilum* continues to be a significant burden to trout aquaculture worldwide.

A new resistant strain

Industry feedback on the difficulties caused by BCWD led Troutlodge to attempt the development of a strain with increased resistance to the condition as a partial solution for farmers. The combination of good husbandry, future vaccines and selection for resistance all have a role to play in controlling on-farm infections, with reduced antibiotic treatment decreasing the risk of developing further antibiotic-resistant strains.
Classic family-based breeding, where the average performance values of all members of each family combine to form the breeding value for the family, are good drivers for long-term improvement in a breeding population but lack the ability to single out and compare exceptional (high and low) performers within families for a particular trait.

For this reason, the R&D team at Troutlodge elected to utilize novel technology with collaboration from industry partners and the USDA. Genomic selection, as opposed to the family-based selection, allows identification of exceptional individuals within a family and is especially effective in selecting disease resistance and other traits that cannot be measured directly on broodstock animals. Troutlodge and the USDA worked together to increase selection accuracy for BCWD through the use of genomic selection and apply the technology to commercially available broodstock.
The process started in 2013 with the first of many laboratory disease challenges at the USDA's Center for Cool and Cold Water Aquaculture followed by genotyping with a 57k single nucleotide polymorphism (SNP) chip.

Following the first disease challenge, the analysis indicated that the use of genomic technology offered significant advantages over traditional breeding

approaches by drastically improving accuracy in selecting animals with resistance to BCWD (Vallejo *et al*., 2017). This approach has been taken by Troutlodge to allow the production and marketing of commercially available eggs with enhanced resistance to BCWD since 2017.

Results

Application of the technology to Troutlodge broodstocks has resulted in consistent generational improvement in the resistance to BCWD. Over three generations of selection between 2015 and 2019, survival rates in controlled laboratory challenges increased from 33.5% to 78.3%, an improvement of 134% (Fig. 1).

Laboratory challenge survival data in figure 1 represents selection results from Troutlodge's May spawning population and confirms that genomic selection can be a highly effective tool to select improvements in disease resistance. Expanded use of the technology is underway at Troutlodge, with plans for BCWD improvements in additional broodstock groups. A cooperative project involving both government and academic institutions is also underway, targeting similar improvements in IHNV resistance using similar techniques.

Troutlodge is part of the Netherlands based multi-national, multi-species breeding company Hendrix Genetics. Hendrix Genetics is also active in the production of Atlantic salmon eggs and smolts in the UK and Chile, production of whiteleg shrimp (*Litopenaeus vannamei*) post-larvae in Ecuador (Macrobio) and selective breeding of SPF whiteleg and black tiger shrimp on Ka'uai, Hawaii (Kona Bay).

References available on request

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Shrimp breeding: The importance and complexity of a successful breeding program

Robins McIntosh, Charoen Pokphand Foods Public Company

Figure 1. Culture of 60-gram *P. vannamei* in 150 days of grow-out in Malaysia.

The Americas and Asia underwent a similar decline in shrimp production efficiency due to the use of wild broodstock in hatcheries. In both cases, shrimp lost growth potential and had an increasing variance in growth rates within a pond, resulting in major size differences at harvest. Belize Aquaculture was the first industrial application of *Penaeus vannamei* Specific Pathogen Free (SPF) stocks recently developed which removed the issues brought in with wild broodstock. Survival, growth and uniformity were re-established. These same SPF shrimp were failing in larger Latin American grow-out ponds, but Belize Aquaculture was designed as intensive smaller ponds with farm and pond pathogen exclusion biosecurity measures.

CPF shrimp breeding program

In 2001, a similar situation existed in Asia, an industry that was failing with the use of wild *Penaeus monodon*. However, on this occasion, culture was based on smaller intensive ponds applying biosecurity principles. As a

Table 1. Changes in shrimp culture performance from the period when wild broodstock were "clean" to the time these wild brooders became infected and the positive change from the adoption of both SPF *P. vannamei* and SPF *P. monodon*.

	Penaeus vannamei			*Penaeus monodon*		
Year	**1985 (clean)**	**1994 (infected)**	**2019 (SPF)**	**1990 (clean)**	**2001 (infected)**	**2017 (SPF)**
DOC (days)	140	130	110	130	140	130
ABW (g)	30	12	40	33	18	40
ADG (g/day)	0.21	0.09	0.38	0.25	0.13	0.30
Survival rate (%)	65	55	80	70	50	85
Weight CV (%)	18	35	15	18	34	16

result of the positive experience in Belize, Charoen Pokphand Foods (CPF) introduced SPF *P. vannamei* shrimp from Hawaii to Thailand and tested them under the local conditions. The same recovery in pond performance was experienced which led to the decision to develop a SPF breeding program for Thailand for both, *P. vannamei* and *P. monodon*. But what would be the model of this program as no large comprehensive programs existed at the time? *P. vannamei* were imported from already domesticated stocks from various sources in the Americas, screened in a primary quarantine and evaluated for over one year before populating the nucleus breeding centers. *P. monodon* were collected from the wild from many locations of the Indo Pacific and went through a similar primary quarantine of individual shrimp.

The criteria established for the breeding program were: 1) It would need to retain genetic heterozygosity reducing the loss of genetic variance while at the same time allowing methodical improvement of important commercial traits such as survival and growth, 2) the program would always be operated under SPF nucleus breeding guidelines, and 3) to prevent disruptions from unanticipated disasters second and even third nucleus centers run independently would be created – along with back up centers for each to keep a depository of families.

To maintain the genetic variance and reduced inbreeding, the programs were established so that a large number of families would be created and evaluated each year and from those, a subset selected for the next generation. Selection would be based

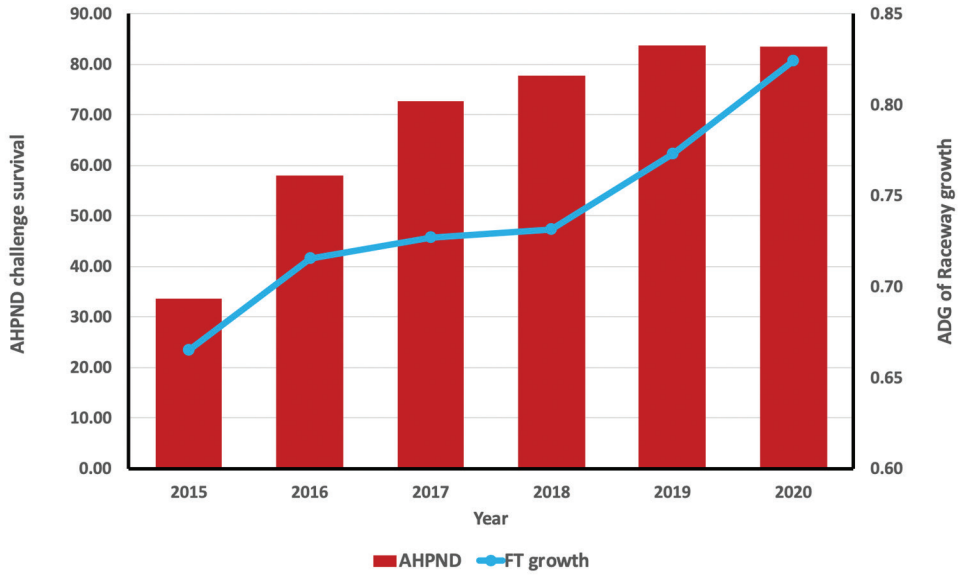

Figure 2. Development of APHND-tolerant shrimp while increasing growth rates in shrimp.

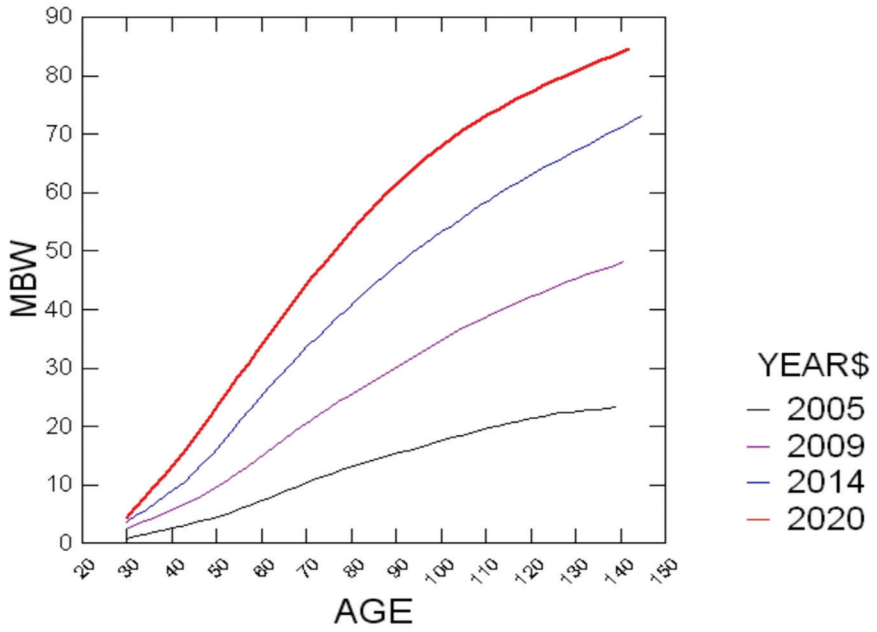

Figure 3. Historical improvement of growth of CPF *P. vannamei* shrimp reared in closed raceway conditions.

on indexing the importance of characteristics being selected, such as growth, robustness, disease tolerance to select pathogens and fecundity. Tracking of all families is done with pedigree software which allows cataloging inbreeding values and characteristic values of each family. The three nucleus breeding centers for *P. vannamei* operated by CPF creates and evaluates over 3000 families a year. For *P. monodon*, there is a single nucleus that creates and evaluates 300 families per year.

SPF shrimp facilities

To ensure that families remain SPF, strict biosecurity guidelines were created for the selection, design, and operation of the nucleus breed and broodstock multiplication centers. Facilities were located inland away from the marine environment and away from other shrimp farms. This required the development of a complete recirculating hatchery and broodstock grow-out operation. Original salts were obtained from evaporated salt farms and diluted with freshwater from the farm area. Procedures limit people's access to defined zones. Vehicles and guests are limited to an outside perimeter. Staff are allowed in after shower and change of clothes and no crustacean or marine products are allowed for human consumption. No fresh feeds of any kind are used in shrimp culture or hatchery operations; all feeds are pasteurized pellets with low moisture content.

A nucleus-breeding center has an established boundary and all areas within this boundary are declared pathogen-free by frequent surveillance of all known shrimp pathogens, both OIE and non-OIE pathogens, by realtime PCR in a lab located within the nucleus. All stages of shrimp are tested: nauplii, postlarvae and broodstock, as well as insects and aerosols. Similar criteria and designs are used for all broodstock multiplication centers that keep the same level of biosecurity based on pathogen exclusion. As important as testing is, so is constant shrimp health surveillance. Any noticeable changes, such as physical appearance, growth loss, an increase of the coefficient of variation or lower survival, will result in an investigation with a complete pathological examination to determine the cause of the change. Animals may be discarded regardless of their health status.

A good SPF shrimp program besides the nucleus and multiplication centers will require a facility to determine disease and stress tolerance by challenging families with pathogens and stressors. This facility needs to be placed outside the nucleus breeding and multiplication centers. Today, the CPF program challenges families for Acute Hepatopancreatic Necrosis Disease (AHPND, previously known as Early Mortality Syndrome (EMS)), White Spot Syndrome Virus (WSSV) and robustness in two different centers with a total capacity of 4,000 tanks. A challenge is being developed for the microsporidian parasite *Enterocytozooan* (EHP) and will become part of the program as soon as it is determined that there is sufficient heritability to make such component worthwhile.

The program is evaluated every six months for performance gains, performance tradeoffs, inbreeding and diversity indexes. Adjustments in the index or

protocols will be made if it is determined that the current protocols are not providing results, or the industry requires a rebalance of the index. This happens when a new disease becomes dominant in the industry, such as the AHPND in 2012 when the indexes were weighted heavily towards AHPND tolerance and robustness instead of growth characteristics.

Health and genetics

The introduction of SPF *P. vannamei* and the creation of advanced breeding centers pushed hatcheries to produce SPF post-larvae through the training of farmers in basic biosecurity which led to a revolutionary change in the world shrimp production. In 2001, Asian shrimp production was estimated at 500,000 tons (mostly *P. monodon*), but after the introduction SPF *P. vannamei*, Asian shrimp production increased to 2,800 thousand tons in 2019, a spectacular annualized growth rate of 128,000 tons. Before the introduction of SPF shrimp, the growth rate in Asia between 1986 and 2001 had been an annualized 31,000 tons.

This increase in production is causally related to the introduction of SPF technologies accompanied by suitable biosecurity infrastructure and practices (broodstock, hatchery, feed and farm management). Whereas often people refer to the genetics of the SPF shrimp being responsible for the farm gains, the most important characteristic of SPF stocks is not genetic but the health of the shrimp. SPF only refers to the health status of the animal. Without health, no genetic gains can be selected or expressed properly. Maintaining the SPF status of the shrimp stock is one of the most important tasks of the broodstock program.

But there is no doubt that the selection of improved performance characteristics such as growth and survival has led to a continuous improvement of farm efficiency. Farm survival before AHPND were averaging over 85% which represented an increase of 20% from the start of CPF *P. vannamei* program in 2002. Growth rates under optimal culture conditions (at the nucleous breeding center) have been improved from 0.2 g/day to over 1.0 g/day and the expression of that growth on farms from 0.18 g/day to over 0.8 g/day. Harvest sizes have continuously increased from 2001 until today with many farmers in Thailand now routinely harvesting 40-50-gram shrimp and as an anecdote, one farm in Malaysia having achieved a harvest of 150-gram *P. vannamei*

shrimp. Two disease episodes have been successfully managed by the development of SPF shrimp with higher tolerances and soon WSSV and EHP will also be controlled with newer strains of tolerant SPF shrimp.

Conclusions

Operation of a correct and proper shrimp broodstock program is complex and requires technology, heavy investment and discipline over many years to achieve and maintain both improvements and the SPF health status of the shrimp. Such programs cannot be scaled down to a farm level as they require the sale of many hundreds of thousands of broodstock to pay for such efforts. The value in the broodstock business is not as much in the broodstock themselves, but in the values created at the hatchery, farm and marketing levels. Finally, it should be noted that any company that runs a shrimp broodstock business, particularly when exporting shrimp, takes on a huge responsibility in ensuring that customer farms and countries do not receive pathogens, both known and unknown, in any shrimp broodstock that are sold.

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Genetics: The five insider secrets your competitors don't want you to know

Marie Smedley and **Mark Looseley,** Xelect

"Genetics in aquaculture is complicated, expensive, and it's only for the elite few." A few years ago, that statement may have been true. However, aquaculture has transformed beyond recognition in recent years. If you still think genetics isn't for you, then perhaps you're speaking to the wrong people?

The reality is that breakthroughs in technology, computing power and knowledge mean that a practical, cost-effective genetics program is now available for producers of all sizes and species. At Xelect, we're already working with almost all the main species, on every continent except Antarctica.

Some producers don't tend to publicize the fact they're running a genetics program for the simple reason that it's giving them a major competitive advantage. Take Atlantic salmon, for example. The most well-managed family genetics programs can give growth gains of around 15% per generation - a rate unimaginable in terrestrial livestock breeding. Genetics is rapidly moving from the preserve of the few to the norm for any serious producer. Here's why.

Genetics programs give you a huge return on investment

For any producer with their own broodstock, a genetics-based breeding program is the secret weapon to success. You will be able to breed fish proven to thrive in your production environment and you will be able to do this sustainably year after year. If, for instance, high mortalities were costing you $1 per kilo of fish harvested, and you select for 10% improvement, this would equate to $0.1 saving for every kilo. There aren't many investments you can make that deliver that kind of return!

Gains are cumulative, increasing every generation

A good genetics partner will work with you to identify the traits that matter most (such as growth, or disease resistance) and build a bespoke breeding program. The returns you'll get depend on how many traits you're trying to increase, and – of course – on environmental conditions. However, a well-run program in a stable environment will mean that your improvements build cumulatively from one generation to the next. For example, the 10% increase in disease resistance above could be more than 20% in the second generation, and you would now be saving more than $0.2 per kilo in mortalities.

It's costly to not use genetics

Every year that you're not using genetics you're falling behind those who do. A competitor that's previously implemented trait selection can quickly begin to over-take the market. Every year without genetics management is a future loss in genetic gain. Even more of a concern to fish producers are the adverse effects of inbreeding. High levels of inbreeding can translate to sudden performance loss. Worst of all, once you've lost your genetic diversity you cannot get it back without purchasing new broodstock sources.

It's more affordable than you think

Historically, genetics has only been available to the largest of producers due to its associated cost. However, recent reductions in the cost of laboratory services, combined with advances in computing power and analytical techniques mean that genetic services are now accessible to almost any producer. At the very least, a simple evaluation of inbreeding risks will stop you sleepwalking into a genetics dead-end through inbreeding.

You can, of course, choose to build your own in-house genetics team – however, that would require a major internal investment. At Xelect, we like to think that our customers effectively have fractional ownership of a dedicated genetics team, and they can easily scale up and scale down to meet their needs.

This is just the start

With aquaculture moving as fast as it does, the industry has more applied knowledge and greater depth of insight than ever before about different species, technologies, and the commercial application of genetics.

Let's look at one example of what we think will be the next big breakthrough to revolutionize aquaculture.

Next gen genetics: Why cutting-edge statistics is a game-changer

All genetics selection programs rely on the fact that fish tend to be more similar in performance to their close family members than they are to unrelated animals. In a traditional family-based selection program, we would use pedigree records to identify how closely fish are related (as full siblings share 50% of their genes, half-siblings share 25% and so on) as an indicator of how well they'll perform.

In reality, however, there's still significant variation even within these groups. More advanced genomic selection programs bridge that gap by using large numbers of DNA 'markers' to compare the similarity of two individuals directly. This allows us to assess an individual's genetic value much more accurately.

In addition, the most important commercial traits (for example harvest weight, fillet quality, mortality and disease resistance) are carefully measured on live fish under production-like conditions on an "evaluation" broodstock of fish (called "sentinels"). Whereas traditional family-based selection programs give a genetic "value" to candidate fish based on the performance of their relatives, genomic selection allows us to consider the genetic makeup of each individual, resulting in bigger gains for the producer.

Unfortunately, the many thousands of DNA marker tests on thousands of broodstock candidates and sentinels means that – for most producers – genomic prediction programs are simply not feasible because of their cost.

For aquaculture to meet the increasing demands for sustainable, cost-effective products we need to continue to innovate. At Xelect we've been collaborating with world-leading academic partners at the Roslin Institute to implement new, cutting-edge, statistical approaches that reduce the need for such a high number of genetic markers while retaining the improved trait prediction accuracy you'd expect from the genomic selection. The new highly-efficient techniques use known pedigree relationships along with much smaller sets of DNA markers to deliver 80-90% of the genetic gain of expensive traditional GS techniques but at a fraction of the cost.

These approaches have the potential to revolutionize genomics-based selection, bringing the benefits within reach of most small and medium-sized producers. But this is not just a pipe dream. We are already rolling this out with a number of our long-standing customers, validating the technology and fine-tuning our approach.

Early results are extremely encouraging and our genomic prediction programs will, we believe, soon become the new benchmark for genomics programs.

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Cryopreservation: a valuable tool for hatcheries

Steffen Wolla, Cryogenetics

Cryopreservation is a technique that preserves biological material in liquid nitrogen (-196°C). This extremely low temperature makes sure that all biological and biochemical processes are paused, allowing the storage of biological material, such as the male gametes, indefinitely (Özkavukcu & Erdemli, 2002). The use of cryopreservation of genetic material has provided several opportunities in modern breeding programs, which has led to a rapid breeding progress for both livestock and fish. Breeding companies get access to a more predictable and efficient method for transferring important genes between populations, which in turn allows an increased and more controlled exchange of genetics. Therefore, more rapid breeding progress can be achieved, as well as more efficient distribution of important genetic traits. Additionally, the use of cryopreservation provides more assurances and serves as a backup in case a disastrous event occurs, such as natural catastrophes, viral infections or technical errors resulting in the death of fish.

Milt patented packaging

Cryogenetics is a biotechnology company specializing in reproduction and cryopreservation services within aquaculture with production laboratories situated in all major salmonid producing countries, such as Norway, the UK, Canada, the USA, and Chile. What distinguishes Cryogenetics' services from traditional cryopreservation is their patented packaging, the SquarePack® (Stengel, 2010). This allows a higher volume of the male gametes to be frozen in each package, facilitating the reproduction process at the hatcheries. This is because one SquarePack® has the capacity to fertilize a much higher volume of eggs than straws, making the work process less difficult. Additionally, Cryogenetics' protocol for using cryogenically frozen milt has shown very high fertilization rates in salmonid species (Grevle *et al*., 2015; Kommisrud *et al*., 2020).

Advantages of cryopreservation

Cryopreservation might be something most would associate with nature management of threatened species, but it is becoming an increasingly popular tool in many hatcheries' breeding strategies. Generally speaking, the use of cryopreservation as a tool at the hatcheries may be divided into two main strategies:

(i) gene banking of genetically important males in the breeding nucleus (ii) and cryopreservation of production milt for better utilization and efficiency. In these cases, a high diversity of genetically important males from different lines are selected and a small portion of the gametes are cryopreserved, or a fewer number of males are selected to have their entire gametes homogenized and cryopreserved, respectively.

Implementing this technology at the hatcheries also eliminates the need for reproductive synchrony, in that male gametes will be readily available at all times when female fish undergo spawning. This means that high-quality milt can be used before and after the normal spawning season. For salmonid species, it is important to note that males within the breeding nucleus subjected to cryopreservation are generally stripped for milt, whereas it is recommended that males selected for further use in production have their gonadal tissues extracted and homogenized to maximize the utilization of their genetic material.

Optimizing milt harvest

There are several ways to harvest the male gametes in fish, in which the most prevalent method is stripping the fish during spawning. Though this might be sufficient for the amount required for establishing a gene bank of the breeding nucleus, a much higher amount of milt would be needed to achieve a more sustainable utilization of the males in production.

Cryogenetics has therefore established protocols for clean extraction and handling of the gonads in salmonid species, to achieve a much higher harvest volume of milt per male fish. Following the culling of the fish, both gonads can be excised from the abdominal cavity. The quality and maturation stage of the gonads can then be evaluated visually before the tissue undergoes homogenization. The homogenate, or the milt, is then diluted with AquaBoost® SpermCoat. This chemical remedy assures that the sperm cells remain viable until they undergo cryopreservation.

AquaBoost® is a line of chemical remedies developed by Cryogenetics that can be used during the different stages of the reproduction process at the hatcheries, such as (i) activating the sperm cells upon fertilization, (ii) short-term storage of fresh milt, (iii) or dilution for better utilization of fresh milt.

Cryogenetics

Cryogenetics is the leading provider of cryopreservation services of the male germplasm within pisciculture. Through their high focus in research and development, the company has been able to expand their product and service portfolio over the last decade to (i) streamline production at the hatcheries, (ii) create gene banks of the breeding nucleus, (iii) provide tools and storage solutions in nature management regarding conservation issues, (iv) develop protocols for the proper handling of milt and eggs during the reproduction process.
The company has so far mainly focused on pisciculture, including salmonids, lumpsucker, Atlantic halibut, Atlantic cod, sablefish, blue catfish, white sturgeon, and others. Additionally, the company is eager to explore possibilities with other important aquaculture species to find new ways to implement innovative and sustainable solutions during the reproduction process. Each breeding company, or hatchery, is different, which is why Cryogenetics also offers audits and consultations regarding the reproduction process.

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Trends in breeding and genetics

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What's in a number?

When operating a hatchery, in most instances the number of mature fish (or shrimp, or abalone, or whatever you are working with) you have on hand is very different from (and always significantly greater than) your "effective" number of breeding individuals. The concept of the effective population number, or Ne, reflects the relative contributions of maternal and paternal alleles to the subsequent generation. Three key factors are typically involved in estimating Ne: the actual number of broodstock on hand, the variability in reproductive contribution among individuals, and the degree to which the sex ratio deviates from 1:1.

A lower effective population number almost always equates to reduced genetic variation in the offspring you are producing. If all the offspring you produce are going out the door, a lack of genetic variation becomes someone else's problem. But if YOUR next generation of broodstock will be selected from that pool of candidates, the effective population number becomes a consideration.

Table 1. Predicted reduction in heterozygotes over time.

	Percent Heterozygous Individuals		
Generation	**N_e of 100**	**N_e of 50**	**N_e of 20**
H0	0.2000	0.2000	0.2000
H1	0.1990	0.1980	0.1950
H2	0.1980	0.1960	0.1901
H3	0.1970	0.1941	0.1854
H4	0.1960	0.1921	0.1807
H5	0.1950	0.1902	0.1762

Most hatcheries are practicing some type of selection, even if it's only domestication selection. An inescapable cost inherent in any selection program is the loss of genetic variation within a population over time. In every generation, the genetic variation found in those animals that don't "make the cut" is lost. Forever. As a result, the number of mature animals on hand begins to become important in terms of selection intensity and progress. But numbers of broodstock on hand from generation to generation can also have some less apparent impacts on fitness and performance.

Two important phenomena related to this are inbreeding and genetic drift. Drift involves the eventual loss of genetic variation due to the random sampling of gametes over time, becoming more of a concern in very small populations. In breeding populations with Ne values of 50 or less generation after generation, drift can be assumed to be an ongoing process. And, while drift is a serious issue under certain circumstances, loss of genetic variation due to uncontrolled inbreeding is a much more constant threat.

Inbreeding is often portrayed as some sort of foul genetic magic (especially by consultants), but it's not always a bad thing. In fact, most genetic improvement in domesticated animals and plants over the past 10,000 years has been the result of controlled inbreeding. And therein lies the rub … that word "controlled." When inbreeding is left to run its course without direction, inbreeding depression often results. The expected increase in the inbreeding coefficient (F) within a population in one generation of random mating is calculated as $\Delta F = 1/(2N_e)$. So what? Well … the smaller the effective population number, the greater the incremental increase in inbreeding per generation, and once inbreeding accumulates, it cannot be reduced simply by increasing population size.

Let's look at how some of these abstract concepts can impact a hatchery population. You have a known number of males and a known number of females. When you want to assess the potential accumulation of inbreeding, or even drift, can you count on these numbers to provide the information you need? Probably not, unless you're stripping every fish and keeping track of the number of offspring each one produces.

Consider species like red drum, meagre and many other marine fishes that are coaxed into spawning in groups under controlled conditions. Some males may produce more milt, or more motile sperm, and thus contribute disproportionately to the larvae that result. Similarly, some females may produce more eggs, or eggs of higher quality with greater fertilization or hatching rates. The extent to which each individual contributes to the total spawn cannot be accurately ascertained by simple observation. Now, all this can be made a bit easier if you have access to molecular technology that allows you to determine the sire and dam for any given individual … but at what cost?

Even under the simplest of circumstances, a simple formula must be employed to get a better handle on the relative, or "effective" number of breeding individuals that will be represented in the offspring you produce in your hatchery. The formula, in this case, is $N_e = 4N_mN_f/(N_m+N_f)$, where Nm and Nf are the numbers of breeding males and females, respectively. Simple – you can calculate it on your phone … or a spreadsheet … or a napkin.

Let's consider the implications of Ne, beginning with the simplest of examples. Let's say we have 50 males and 50 females randomly mating, and every animal contributes equally in terms of gametes and offspring. So, $N_e = 4N_mN_f/(N_m+N_f) = 10{,}000/100 = 100$, equivalent to the actual count. Life is easy when all the mathematical assumptions are met, and under this scenario, inbreeding accumulation can be assumed to be $\Delta F = 1/(2N_e) = 0.0050$ per generation. No worries – nothing a little selection can't overcome.

However, the assumption of random mating can rarely be satisfied, and that of equal contributions … well, never, really. If only 20 of those 50 males and 35 of those 50 females actually spawn (as is typical in group

spawning of tilapia, penaeids and many other aquatic species), we have $N_e = 4N_mN_f/(N_m+N_f) = 2800/55 = 50.9$, or roughly half of our actual broodstock count. Inbreeding accumulation per generation using these numbers would be $\Delta F = 1/(2N_e) = 0.0098$, or roughly 1 percent. Not necessarily cause for concern, but definitely something to keep an eye on.

So, let's go back to that original number of 100 broodstock, with the assumption of random mating and every animal contributing equally, but this time with 20 males and 80 females (as might be the case in many hatcheries working with various aquatic species at a 1:4 sex ratio). Our calculation yields the following result: $N_e = 4N_mN_f/(N_m+N_f) = 6400/100 = 64$. So, even though the output of offspring will increase significantly (in this example by roughly 60%), the genetic variation will still be reduced. Our expectation of inbreeding increase per generation is $\Delta F = 1/(2N_e) = 0.0078$. And remember, in most situations not all of these animals (even the outnumbered males) will participate in spawning activity, so in reality, the effective population number will be lower.

I know what you're thinking (well … some of you). What if we try to remedy this exclusion of individual breeders by taking our 20 male: 80 female breeding population and divide it in such a way that each male has his own exclusive "harem" (in this case 20 tanks or hapas, each with one male and four females)? The Ne equation must be modified slightly to accommodate this type of mating design (doubling the Nm component in the denominator), but the results might surprise you. The following estimate is generated for this scenario: $N_e=4N_mN_f/(2N_m+N_f) = 6400/120 = 53.3$. So even if the assumption of all individuals contributing gametes equally can be met, this type of breeding management reduces Ne even further than what would be expected if all the broodstock were maintained together, not to mention the requirement for additional infrastructure and labor. And our expected increase in inbreeding coefficient is $\Delta F = 1/(2N_e) = 0.0094$.

Many other factors can influence the Ne estimates we attempt to calculate, but the math and details are beyond the scope of this article. Some may result in significant divergence from the actual numbers on hand, but for most applications, the simple equation incorporating sex ratios, $4N_mN_f/(N_m+N_f)$, is a great place to start when developing your own estimate. Just be sure to work your way down from there.

A magnet to Artemia quality

Geert Rombaut, INVE Aquaculture

***Artemia* is the most widely and successfully employed live feed in larviculture of marine fish and shrimp. Yet, current harvesting procedures for *Artemia* nauplii are often inefficient and unsustainable. With the SEP-*Art* technology, INVE Aquaculture (part of Benchmark) helps hatcheries become more efficient in producing high-quality *Artemia* nauplii in a more sustainable and eco-friendly way without any toxic chemical waste.**

Artemia – The power bar of the aquaculture sector

For children, brine shrimp or, as they are commonly known, sea monkeys, are one of nature's many curiosities – creating life from seemingly sterile eggs through the mere addition of salty water. For the aquaculture sector, however, the genus *Artemia* means serious business: the ability to produce dormant eggs, i.e. cysts, that hatch into live nauplii on demand makes *Artemia* a convenient live feed for cultured fish and crustacean larvae. Moreover, the hatched nauplii of *Artemia* are a rich source of protein, lipids and carbohydrates, have a suitable size range and stimulate the feeding response of marine species (Leger *et al*., 1986). In addition, capitalizing on the non-selective filter-feeding behavior of *Artemia*, it is possible to conveniently manipulate the biochemical composition of live nauplii. The filter-feeding crustacean can rapidly bioaccumulate nutrients deficient in its own nutritional profile, which are critical for the development of marine fishes and crustaceans (Sorgeloos *et al*., 2001). While *Artemia* has been known to man for centuries, its increased usage as live food for larval organisms since the 1980s enabled aquaculture production to grow beyond that of wild-caught fisheries in the last decade (SOPHIA, FAO, 2016).

Hatching *Artemia* is no child's play after all

The process of separating *Artemia* nauplii from cysts remains a significant bottleneck in the production of high-quality live feed. Double-sieving, for instance, is still commonplace, but the method is labor-intensive, inefficient and often results in *Artemia* of poor quality (Fig. 1). The method rests on the idea that nauplii will pass through the mesh of a submerged net while the cysts are caught in the net. Problematically, however, cysts smaller than the mesh size pass through and remain in the filtered mix floating among the live nauplii. During the process, the nauplii - forced to pass through the mesh of the sieve - often suffer physical abrasions. Moreover, nets clog easily, risking increased damage to the *Artemia* nauplii. Dead or damaged nauplii are less attractive prey items and together with the sinking cysts increase bacterial activities at the bottom of the tank. On top of that, leftover cysts can block the still-developing digestive system of fish and crustacean larvae.

Chemical decapsulation was introduced to remedy the shortcomings of double-sieving (Fig. 1). An alkaline hypochlorite solution is used to dissolve and oxidize the hard, brown outermost layer of cysts (i.e. chorion) to free viable embryos ready to hatch. The challenges arising from decapsulation, however, are manifold: the chemical process is complex and requires a highly trained and skilled workforce, which, to avoid any losses, executes the process in the correct way. During the exothermic process, heat is produced that may damage or reduce the viability of the embryos. Consequently, it is necessary to monitor and control the oxidation process closely as to interrupt the process at the right time. The need for chemicals and skilled labor makes decapsulation a more costly solution. Lastly, the decapsulation produces toxic byproducts not easily disposable due to the detrimental effect on the environment. Waste disposal, therefore, limits a large-

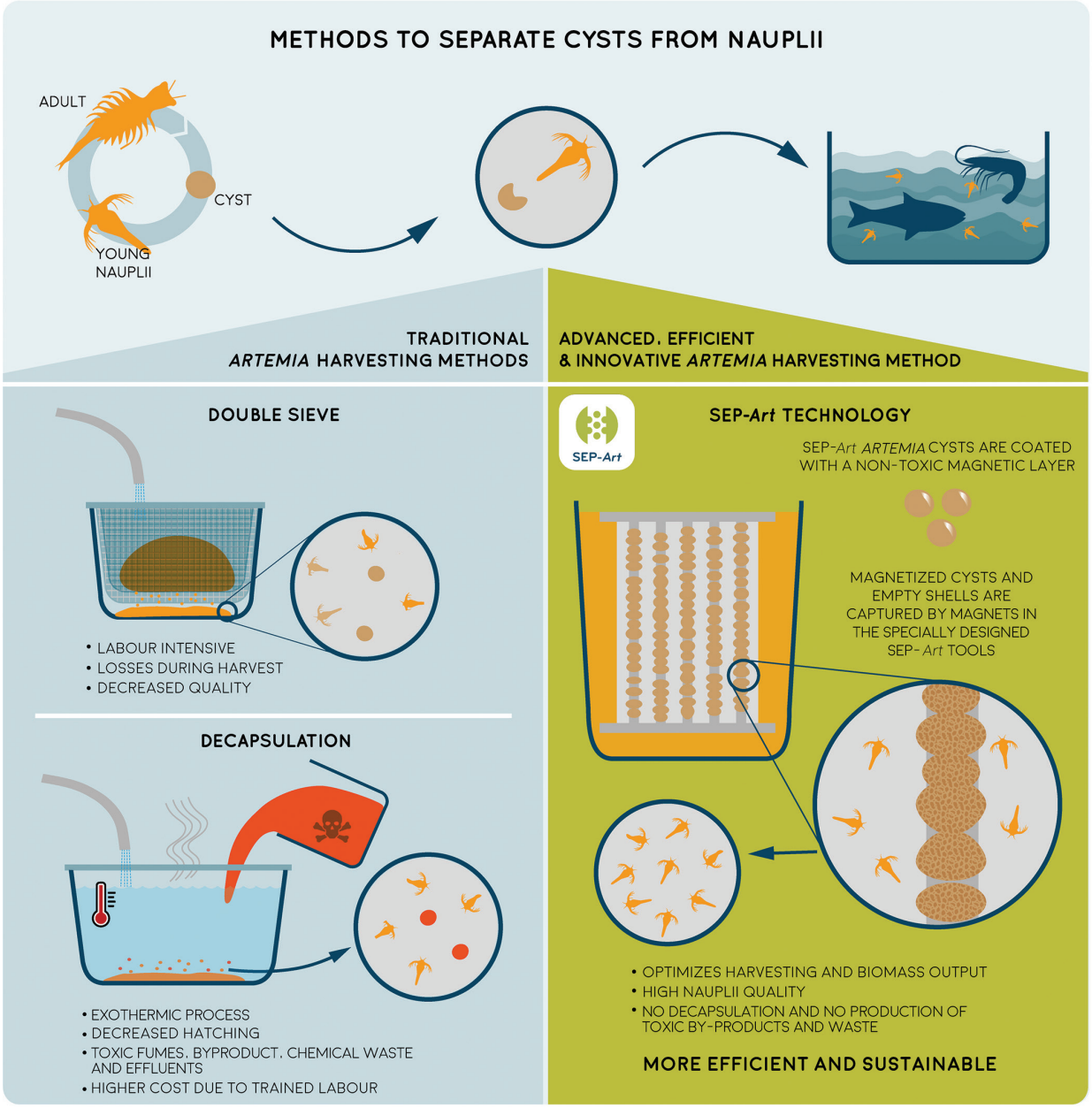

Figure 1. Methods to separate *Artemia* cysts from nauplii.

scale application of the decapsulation process. Consequently, double-sieving and/or decapsulation, no longer represent efficient and sustainable methods to produce live *Artemia* nauplii. A better alternative is critical to allow the aquaculture sector to grow further.

Traditions aside, the future is now

Production by the aquaculture industry has surpassed that of wild-caught seafood and beef and is gaining importance as the main source of animal protein in the future (SOPHIA, FAO, 2016). And as production is expected to increase even further, more efficient and sustainable *Artemia* nauplii harvesting techniques than the traditional methods are crucial. INVE has worked tirelessly for more than 35 years to develop ingenious technologies to revolutionize the aquafeed industry. This includes the patented SEP-*Art* technology, which is both a product and a method to separate *Artemia* nauplii from their cysts: magnet-sensitive SEP-*Art* cysts and a SEP-*Art* separation tool that utilizes magnets to separate the nauplii from the cysts and empty shells (Fig. 1).

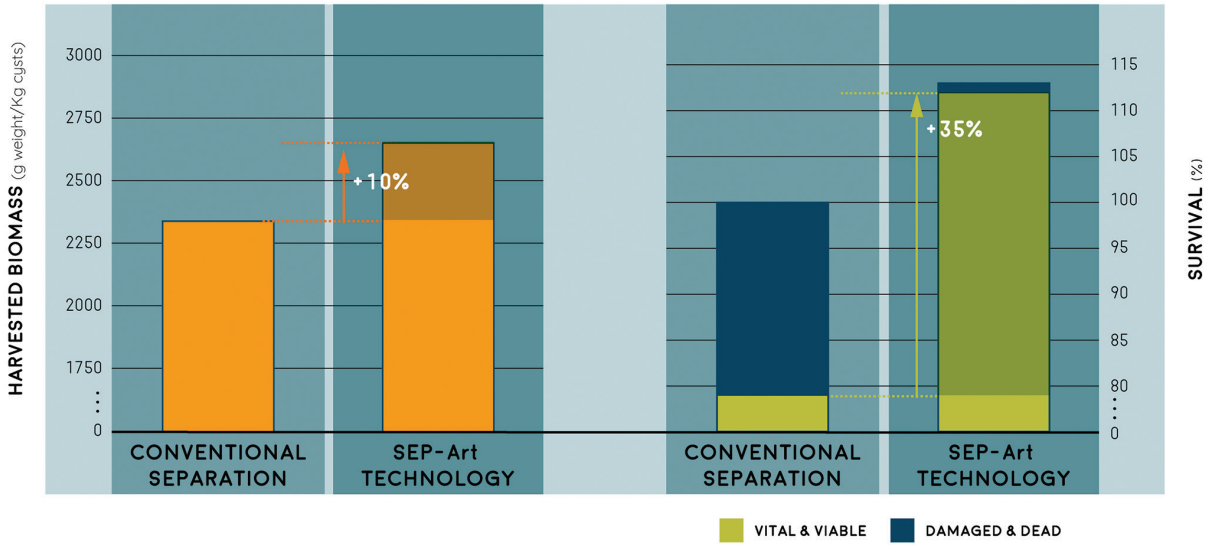

Figure 2. A comparison of the quantity (i.e. biomass) and quality (i.e. survival) of *Artemia* harvested from traditional versus SEP-*Art* methodology.

It is important to note that SEP-*Art Artemia* cysts are not a specific strain of *Artemia*, but a patented technology especially developed to coat cysts with a non-toxic layer of magnetic material with no effect on the overall hatching characteristics of the cysts. This means that the optimal hatching conditions have not been changed and the same protocols can be followed to hatch the product. Yet, harvest and collection of the nauplii have been simplified to become more efficient and straightforward. During the last ten years, the SEP-*Art* tools have been continuously improved from initial designs to three next-generation tools: SEP-*Art* HandyMag, SEP-*Art* CysTM 2.0 and SEP-*Art* AutoMag. Although the three SEP-*Art* tools differ in their degree of automation, integrated cleaning system and ease of usage, each employ the same concept of using magnetism to free *Artemia* from their cysts. The process collects the cysts on the submerged magnets for easy removal of cysts from the *Artemia* suspension. Doing this, the suspension will turn from a murky brown into a bright orange colored suspension, only containing *Artemia* nauplii at the end of the process.

In contrast with double-sieving and decapsulation, SEP-*Art* technology maximizes the recovery of the hatching output. This can lead to a 12% increase in biomass generated from the SEP-*Art* technology (Fig. 2). Furthermore, the technology does not decrease the vitality of the *Artemia* nauplii since no physical force or chemical reaction was employed during the process (Fig. 2). Simultaneously, the easy-to-use SEP-*Art* tools speed up the process of handling and harvesting, allowing even large volumes of nauplii to be handled efficiently. The tools are intuitive to use, making the technology accessible even to untrained workers. This means, using SEP-*Art* technology farmers will be able to harvest more and better quality *Artemia* nauplii in a shorter amount of time. More importantly, no chemical waste is produced making SEP-*Art* technology safer for both the user and the environment.

Live feed of the future

All in all, by employing simple physical principles INVE is innovating the aquaculture live feed sector, providing an eco-friendly alternative to traditional, ineffective, complex and even harmful *Artemia* harvesting techniques. The SEP-*Art* technology is the quintessence of a forward-thinking aquaculture sector ready to feed the world on sustainably reared seafood.

References available on request

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Algae as functional ingredients in Senegalese sole microdiets

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Figure 1. a) *Nannochloropsis* sp. cells. Photo: Allmicroalgae. b) Dried *Gracilaria gracilis*. Photo: AlgaPlus.

Algae as functional ingredients for fish larvae

Micro- and macro-algae are rich sources of bioactive compounds that can influence fish growth, nutrient utilization, resistance to diseases and, as a natural source of antioxidants, have beneficial effects in overall health (Sáez *et al*., 2020; Sharma *et al*., 2019). In addition to these factors, their nutritional composition and sustainable nature have made them great candidates for alternative feed additives in fish diets.

Fish larvae and other early stages of development are more susceptible to oxidative stress due to their high metabolic rate associated with very fast growth and other factors (Sharma *et al*., 2019). This leads to pressure on fish fitness and may cause high mortalities due to larvae´s limited capacity to respond to daily challenges derived from a complex environment and dietary changes. Here, the application of algae as a feed ingredient may have a positive influence on larvae's early resistance, immune capacity and physiologic output.

Within the goal of a constant search for new alternative and sustainable ingredients for optimal fish health and performance the VALORMAR project has developed and tested new microdiets that may innovate the hatchery feeds market, bringing to the table diets that not only increase sustainable standards in a cost efficient manner but also have the potential to benefit fish development in the long term, resulting in more robust fish.

Micro- and macro- algae blends increase sole post-larvae performance

A trial was performed to assess if the inclusion of algae blends (Fig. 1) had a positive effect on fish growth, immune and oxidative response on weaned post-larvae of Senegalese sole (*Solea senegalensis*). Larvae were reared in a recirculating system from 15 to 63 days after hatching (DAH), using an initial density of 3,000 larvae/m^2. After weaning (27 and 28 DAH), co-feeding with Artemia and a commercial diet, 34 DAH post-

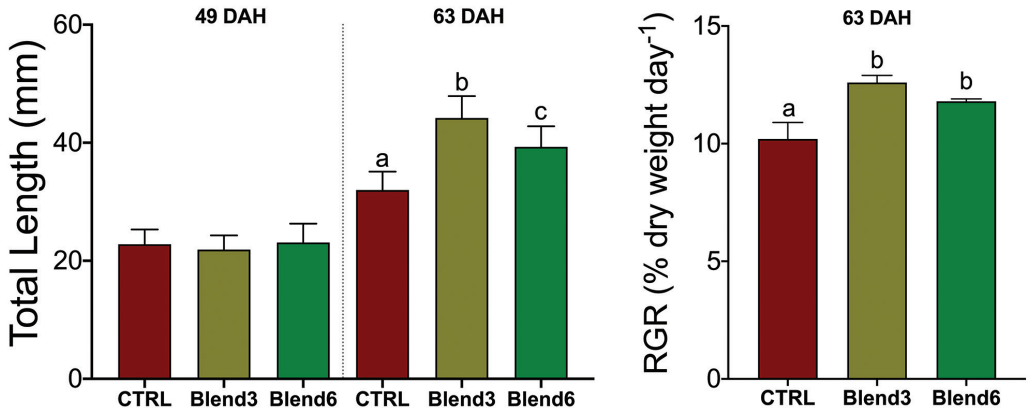

Figure 2. *S. senegalensis* post-larvae total length (TL) at 49 and 63 DAH, and relative growth rate (RGR) throughout a 29-day feeding trial. Different letters indicate significant differences.

larvae were offered experimental diets supplied throughout the day through automatic feeders. Diets were a) control commercial diet (CTRL), b) commercial-like diet with 1.5% *Nannochloropsis* sp. and 1.5% of *Gracilaria gracilis* biomass (Blend 3) and c) commercial-like diet with 3% *Nannochloropsis* sp. and 3% *Gracilaria gracilis* biomass (Blend 6). Responses were evaluated at 49 and 63 DAH in a 29-day feeding trial. Differences between groups were assessed by ANOVA after assurance of parametric conditions, considering significant when $p<0.05$.

After 29 days, fish fed with both algae blend supplemented diets had better growth performance (Fig. 2) than fish fed the control diet, with higher emphasis on the fish growth response when fed Blend 3 diet. Interestingly, the immune and antioxidant analysis indicated higher responsiveness also in post-larvae fed with diets supplemented with algae blends. At 49 DAH, lysozyme activity was higher when fish were fed with Blend 6 when compared to both Blend 3 and control diets. However, at 63 DAH lysozyme activity in fish fed with both algae blends presented a higher lysozyme activity, with higher prominence on fish fed with Blend 6 (Fig. 3). Both at 49 and 63 DAH, peroxidase activity was higher in fish fed with Blend 6 whereas the control diet and Blend 3 presented no differences between each other (Fig. 3). Accordingly, there was an overall positive response of these two key components of the fish innate immune response in post-larvae fed with the inclusion of algae.

Despite no statistical differences observed regarding tGSH and SOD enzymatic activity (Fig. 4) at 49 DAH or 63DAH, a tendency of higher tGSH and SOD enzymatic activity when fish were fed diets with algae blends was observed, supporting a positive effect of the functional ingredients. Both micro- and macro-algae are a source

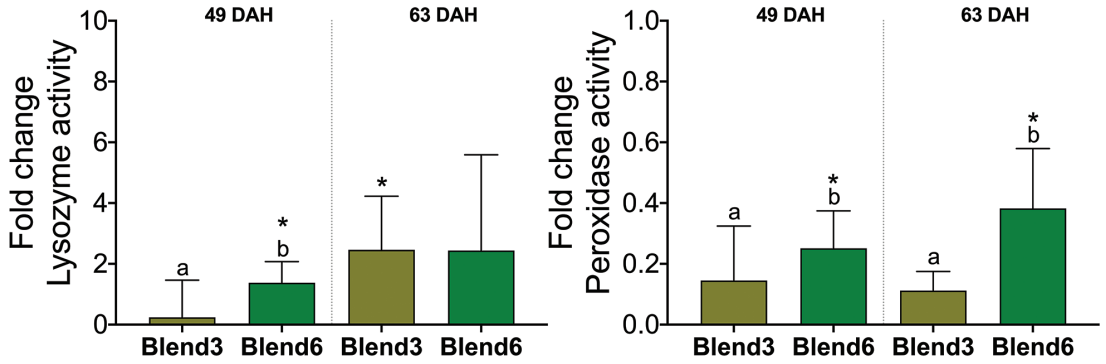

Figure 3. Fold change lysozyme and peroxidase activity of *Solea senegalensis* post-larvae at 49 DAH and 63 DAH during a 29-day feeding. Different letters indicate significant differences between diets and * indicate differences from the control group at the same DAH.

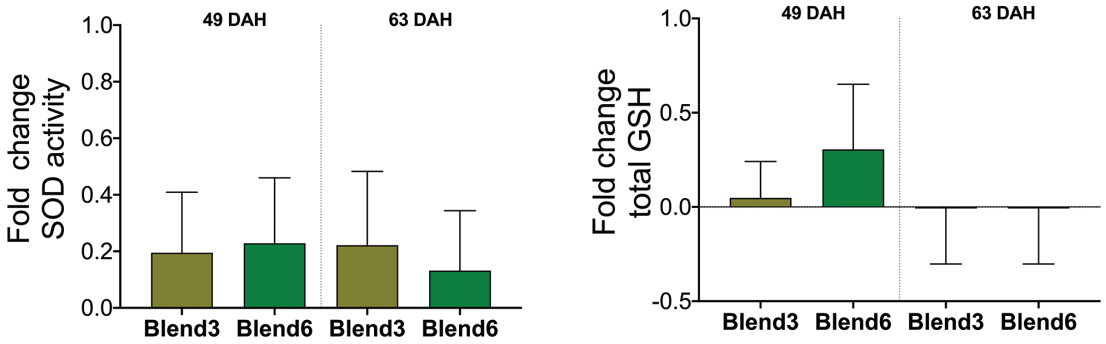

Figure 4. Fold change superoxide dismutase (SOD) activity and total glutathione (tGSH) of *Solea senegalensis* post-larvae at 49 DAH and 63 DAH during a 29-day feeding trial.

of marine-derived polysaccharides which can improve different aspects of the innate immune system, such as lysozyme enzyme which is an important index of innate immune that occurs in mucus, lymphoid tissue, and serum and is involved in a wide range of protective mechanisms, including bacteriolysis, complement-mediated opsonization, phagocyte and antibacterial activities (Safavi *et al.*, 2019).

Another important enzyme of the immune system is peroxidase that acts as an essential microbicidal agent and maintains the redox balance of the immune cells (Guardiola *et al.*, 2018). Moreover, studies have shown that the natural antioxidant enzymes present in both micro- and macro-algae play an important role in the antioxidant defense system by removing free radicals and decreasing the toxicity of drugs and chemicals in biological systems (Safavi *et al.*, 2019). Therefore, these natural emergent ingredients may be key in improving not only fish immunity as well as their oxidative response throughout the delicate post-weaning stage.

Conclusions

Both diets, with the inclusion of macro- and micro-algae, have displayed great potential in terms of fish growth as well as immune and oxidative responses due to their combined bioactive properties. Therefore, the application of Blend 3 presents higher potential since it triggers relevant functional responses in fish at a lower cost. This study supports the usage of algae blends as a new alternative and sustainable feed ingredients in the early life stages of fish.

Acknowledgments

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References available on request

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Regional developments

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New finfish species developments in Southeast Asia

Figure 1. Larval rearing tanks in a typical small-scale marine finfish hatchery in Bali, Indonesia. These hatcheries produce a range of species, depending on demand.

While China remains the dominant force in aquaculture production in Asia, aquaculture, and particularly mariculture, is expanding in many Southeast Asian countries. Several features of hatcheries in Southeast Asia stand out in comparison with the hatchery sector in the Northern Hemisphere: the large number of small hatcheries producing a range of fish species, and low levels of vertical integration. Hatcheries in Southeast Asia typically have simple production systems – concrete larval rearing tanks, with larger concrete tanks for microalgae and rotifer production. Influent water filtration systems are usually basic, comprising a gravity sand filter and rarely includes effective water treatment systems. For microalgal and rotifer culture, culture water is usually sterilized with sodium hypochlorite.

Even though many hatcheries are small, the cumulative impact of their production can be substantial. Indonesia is the major producer of milkfish (*Chanos chanos*) fingerlings. The center for hatchery production of milkfish is the Buleleng Regency in northern Bali. A 2017 survey of hatcheries in Buleleng revealed that there are 347 hatcheries, of which 17 are "complete" (i.e. they hold broodstock) and the remainder (~95%) are categorized as "small-scale" or "backyard". All 347 hatcheries have around 5,000 larval rearing tanks, and the 17 "complete" hatcheries have 73 broodstock tanks, mostly holding milkfish broodstock. Annual exports of milkfish fingerlings from these hatcheries between 2014 and 2016 ranged from 1.5 to 2.5 billion fingerlings per annum. Most (70–80%) milkfish fingerlings are exported to the Philippines,

Figure 2. Small-scale hatcheries are an important source of employment in rural northern Bali. Here local women are employed to count and grade fingerlings, in this case grouper.

which is a major producer and consumer of milkfish, and smaller numbers are exported to Japan, Malaysia, Singapore, Thailand and Vietnam.

Fingerling production in many parts of Asia is highly compartmentalized. In Indonesia, grouper fingerlings produced in hatcheries in Bali or East Java are often shipped to northern Sumatra to undergo a nursery phase in brackishwater ponds. This phase lasts about six weeks, after which the fish are shipped to grow-out cages. In Taiwan, fingerling production may be separated into several different phases, such as initial larval rearing, late larval / early juvenile rearing, nursing, etc. These different phases are often undertaken by separate hatcheries or farms.

Another feature of hatchery production in Southeast Asia is the diverse number of species produced, including snappers, groupers, Asian seabass and pompano. Many of the larger hatcheries in Bali, as well as hatcheries in other parts of Indonesia, notably the Situbondo area of East Java, produce groupers (Family Serranidae, Subfamily Epinephelinae). In the past, the grouper species in highest demand has been the tiger grouper (*Epinephelus fuscoguttatus*) because of its robustness in culture, and good market price in the live fish trade. More recently, hybrid groupers have begun to dominate aquaculture, and consequently hatchery production. The advantages of hybrid groupers are reported to be improved resistance to disease in sea cage culture (mostly caused by protozoan parasites) and faster growth than the parent species. In the case of giant grouper (*Epinephelus lanceolatus*) hybrids, hybridization seems to provide an opportunity to utilize the faster growth of the giant grouper without the difficulties of breeding this species in captivity. A recent study in Indonesia has shown that a proportion of hybrid groupers are triploid, which may also explain the higher growth rates exhibited by hybrids.

To hybridize groupers, sperm is stripped from male giant grouper and chilled and/or cryoprotected to extend its storage life. Eggs are stripped from female tiger grouper (or other species depending on local preference) and artificially fertilized with the giant grouper sperm. Cryopreserved sperm may be shipped to other areas, e.g. from East Java to Bali, to allow production of hybrids even when giant grouper broodstock are not immediately available.

A problematic aspect of hatchery production in most of Southeast Asia is the cost of production. Because most farming operations are not vertically integrated, farmers are free to shop around various fingerling suppliers in order to obtain the cheapest fish. This in turn forces competing hatcheries to reduce costs and cut corners, often at the expense of fingerling quality.

Disease remains a significant problem in hatcheries in Southeast Asia. Viral nervous necrosis is a common cause of mass mortality, as are occasional outbreaks of protozoan parasites. Because of the focus on reducing production costs to provide the cheapest fingerlings, most hatcheries have poor biosecurity infrastructures and operations. The close physical proximity of large numbers of hatcheries also promotes cross-contamination because effluent and intake pipes from different hatcheries are often close together.

Although most hatchery production in Southeast Asia is from operations that could be categorized as small or medium scale (SME), there is increasing development of large, industrial-scale integrated farms, including hatcheries. These operations are usually focused on mass production of one or two species, such as Asian seabass/barramundi (*Lates calcarifer*) and pompano (*Trachinotus* spp.). Prior to the coronavirus pandemic, there were several ventures planning large-scale integrated farms and hatcheries in Southeast Asia. It seems likely that, in a post-COVID world, Southeast Asia will see increasing development of large-scale integrated finfish mariculture operations in many countries, while retaining the smaller and more diverse SME hatcheries, nurseries and grow-out farms.

Algae cultivation via a novel photobioreactor and harvest apparatus for sustainable aquaculture

John S.F. Barrett, Pure Biomass

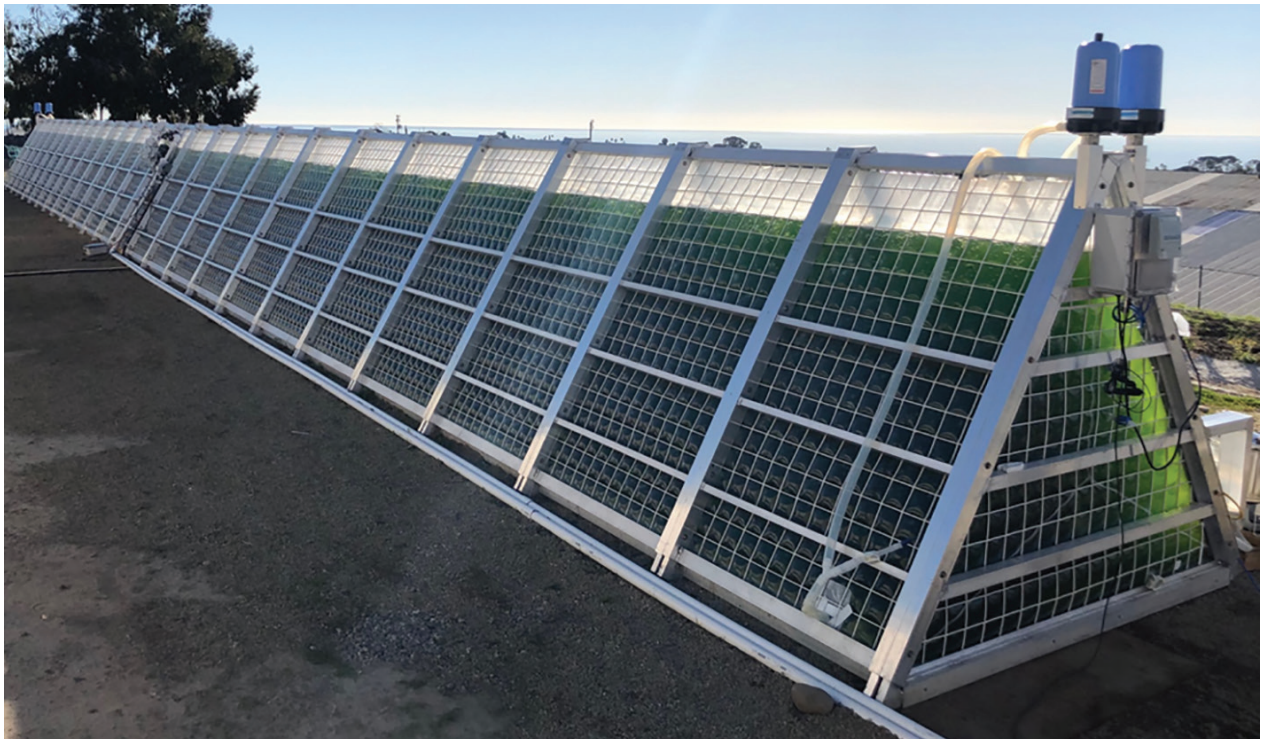

Figure 1. A full-scale (55,000 L) TriPAR™ system, growing *Haematococcus pluvialis* at the Pure Biomass research site in San Diego, CA.

Aquafarmers and feed manufactures have considered cultivation of microalgae as an alternative source of aquaculture nutrition. Algae feedstocks can be incorporated in both grow-out and hatchery operations and for the cultivation of either finfish or shellfish. In shellfish hatcheries, the use of algae supplementation is already well-established, and live algae feeds are an essential factor for increasing survival rates during the oyster's metamorphosis from suspended larvae to adherent spat.

At Pure Biomass, we are excited about the potential of live algae and algae-based feeds to improve aquaculture yields while also restoring sustainability to the world's marine fisheries. To meet this opportunity, Pure Biomass is focused on delivering state-of-the-art, yet affordable, algae cultivation systems to help aquafarmers improve the quality, reliability and productivity of their algae production process. Because algae cultivation can make up 30-40% of a hatchery's operating expenses,

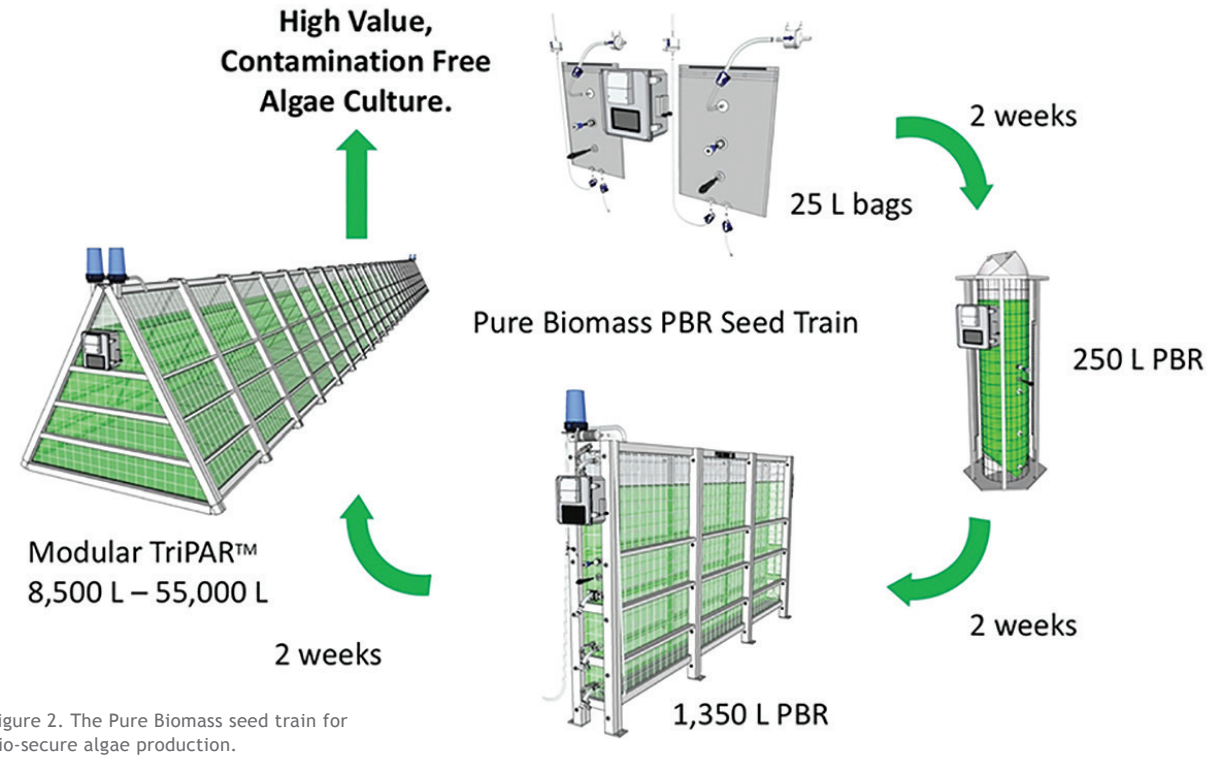

Figure 2. The Pure Biomass seed train for bio-secure algae production.

improvements in algae production can lead to significant savings and a better bottom line for the business.

Triangular prism algae reactor

The centrepiece of the Pure Biomass algae production platform is our newly developed TriPAR™ (Triangular Prism Algae Reactor) intended for large-scale, sustainable algae production. The TriPAR™ is a closed photobioreactor containing 8,500 L within a space of around 10 m^2 (6.7' x 16') by 6' high. The inner reactor body is formed from a large plastic bag, which is surrounded by an aluminium support cage in the shape of a long, triangular prism. Inside the reactor, a robotic harvest apparatus allows for the daily removal of concentrated biomass while maintaining culture sterility. Operation of the system is completely automated, including media charging, pH control, nutrient feeding and biomass harvest. The unit can be operated as either a semi-continuous or fully-continuous system. If more production capacity is required, the TriPAR™ can be linearly expanded in 4 ft. sections up to a volume of 55,000-L (104' long) (Fig. 1).

Benefits of the TriPAR™ include: minimal (or no) requirement for concentrated CO_2 supplementation, no requirement for a surrounding greenhouse, reduced energy loads for heating and cooling, and significantly improved water conservation compared to traditional reactors, all while maintaining culture purity and high productivity on a modest areal footprint. To achieve these benefits, the TriPAR™ leverages several technological features. 1) The large volume of the TriPAR™ provides a large thermal mass that reduces the need for cooling. 2) The counterintuitive, low-density (g/L) method of cultivation allows for a high efficiency of CO_2 capture, requiring a minimum amount of energy to drive mass transfer, while increasing productivity ($g/m^2/d$). 3) The reactor geometry is designed so that the air bubbles flow continuously along the inner walls, constantly mixing the culture and scouring the surface free of biofilm. This allows for unfettered light penetration into the reactor, and largely removes the reservoir for contamination that a biofilm provides. 4) The style of mixing generated by the rising bubbles closely mimics optimal algae growth conditions found in nature, reproducing the cyclical pattern of light exposure that maximizes photosynthetic efficiency. 5) By taking advantage of gravity, upon turning off the air, the harvest apparatus, which is completely novel to photo-bioreactors, allows for the removal of settled biomass without compromising sterility.

The potential result is a 20x reduction in water consumption associated with harvest and refill compared to existing technology. 6) The plastic bag used for process containment lowers the frequency of contamination events leading to better crop purity and more process uptime. In the event that a contamination does occur, the downtime required for process cleanout is greatly reduced by the ability to simply install a new culture bag, which also reduces the likelihood of a repeat contamination due to inadequate cleaning. These five features, as well as improvements in nutrient delivery and automation, comprise a comprehensive algae production system that can greatly improve algae production for hatchery operations.

In addition to our full-scale TriPAR™ reactors (8,500-55,000 L), Pure Biomass also provides seed train equipment (25-1350 L) needed for inoculum expansion and/or smaller production operations (Fig. 2).

Algae quantity and quality

In the aquaculture industry, both algae quality and quantity are key factors. The quantity of algae produced, which depends on both the volume of the cultivation equipment and the productivity of the process (cell dry weight/L/day), is important, because algae cultivation can be a large part of operating expenses (especially for hatcheries). Algae quality is also an important attribute due to the sensitivity of animal development to the balance of nutrients supplied by its diet. Ensuring proper animal nutrition is often so important as to warrant the cultivation of 3-5 different algal species in a single hatchery. This is done in order to provide the right blend of nutrients during each stage of an animal's developmental cycle. Because the TriPAR™ is a closed system, algae producers are able to achieve high culture purity by excluding unwanted algal strains found in the local environment. In addition, the use of process automation and instrumentation, for pH control, nutrient delivery, and batch cycle management, ensure reliable, high productivity cultivations and that process conditions are maintained at the correct values to maximize the nutritional value of each algae strain.

Algae production costs

Currently, the costs of algae-derived protein and oil are higher than their fish-derived equivalents.

In early 2020, Pure Biomass installed an array of 250-L and 1,350-L systems at Star Algae in Vietnam as the principal seed platform for their 1-hectare algae greenhouse. Star Algae will use the facility to produce live algae feeds for supplying SE Asia's burgeoning seafood demand. To construct the process, Pure Biomass provided the seed-train equipment, including 25-L bags, 250-L bioreactors and 1350-L bioreactors, that Star Algae will use to grow the seed needed to inoculate their production scale ponds (>50,000 L each). When operating at full capacity, the facility, which utilizes both natural sunlight and artificial LED illumination, will be able to produce high-quality algal feed to the Asian aquaculture industry.

However, the limited capacity of ocean fisheries and rising demand for aquaculture products, in conjunction with continued improvements in algae cultivation technologies will, in time, eliminate the price difference between these two feed options. As this trend continues, those aquaculture operations that have already invested in algae cultivation will be in a more favorable position to capture the expanding aquaculture market.

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Microalgae as a nutritional and bioactive source for aquafeed

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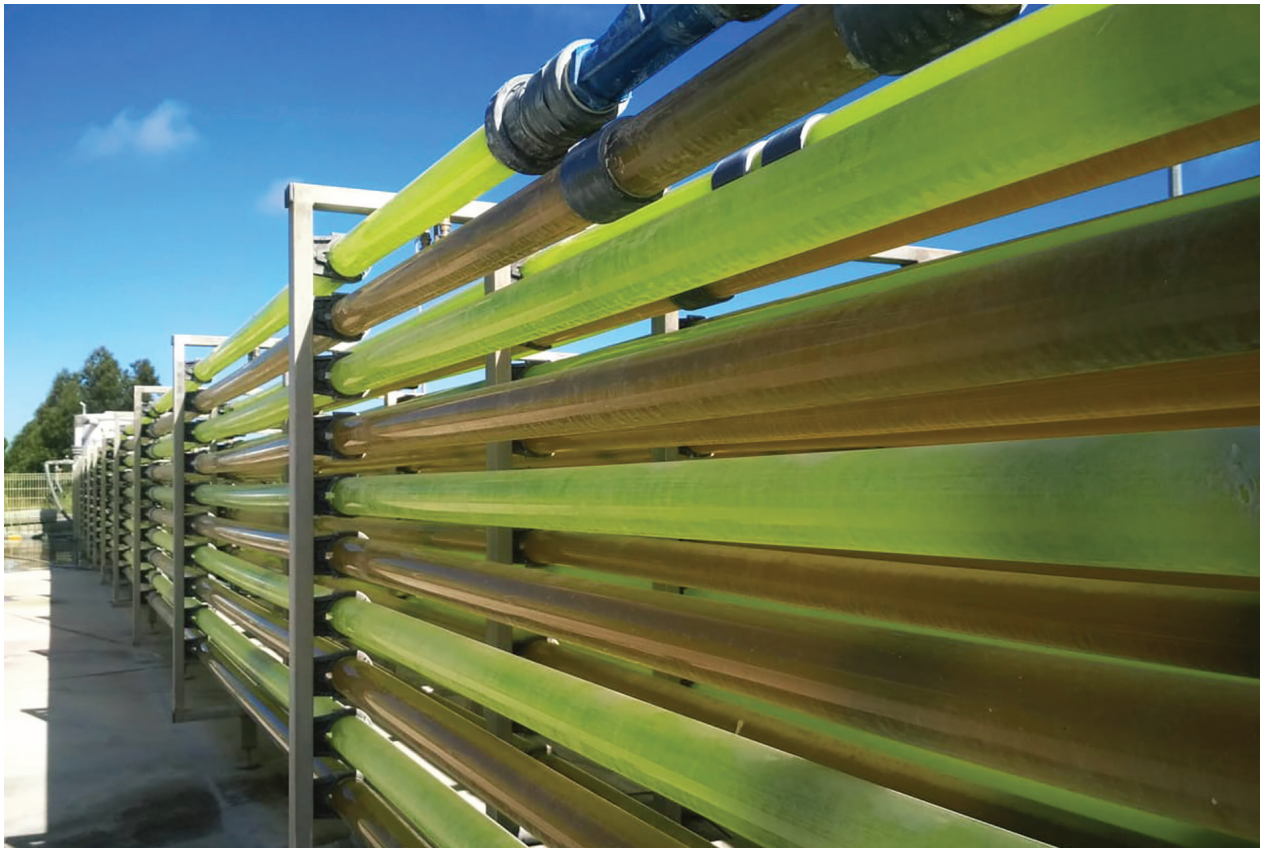

Figure 1. Tubular photoautotrophic bioreactors cultivating *Nannochloropsis oceanica* and *Phaeodactylum tricornutum* at Allmicroalgae´s facilities.

For effective aquaculture, a good quality fish seed is essential, however, seed production is typically intensive and health management of both, brood and seed fish, can be critical (Little *et al*., 2002). If a hatchery seed is not of good quality, it may compromise cultivation, slow growth rate and induce poor survivability or disease resistance (Shah *et al*., 2018). One of the main challenges to achieving this high-quality fish seed is the quality of feeding, the adequate supply of nutrients, both in terms of quantity and quality (Carter, 2015).

The traditional fishmeal is industrially produced from fish, such as sprat and sand eel, which are non-appreciated for human consumption. Due to the increasing production of fish feed for the growing aquaculture industry, the use of fishmeal in aquafeed is becoming both environmentally and economically unsustainable. Commercially produced microalgae could become a sustainable alternative fish feed ingredient, once they are sustainably cultivated and contain the necessary nutrients in the required quantities.

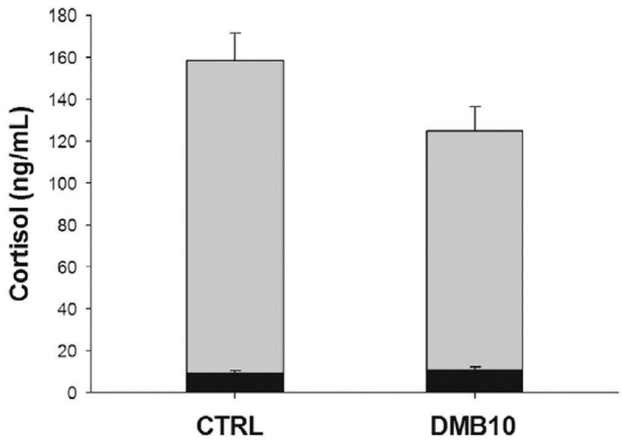

Figure 2. Changes in plasma cortisol of *Sparus aurata* exposed to acute stress, fed with CTRL (control) and DMB10 (10% inclusion of deffated *Tetraselmis chui* biomass). Black bars represent the basal values of cortisol in the plasma, while grey bars represent the cortisol response of stressed fishes. Asterisk denotes significant differences ($p < 0.05$). Bars are means ± standard deviation ($n = 15$) (Pereira *et al.*, 2020).

The potential of microalgae as fish feed

Microalgae have long been identified as a promising alternative to replace fishmeal and fish oil, ensuring sustainability in the aquaculture sector. However, the success of this adjustment has been mainly dependent on the microalgae cultivation sector. Microalgae are a biologically diverse group of microorganisms that can biosynthesize a plethora of bioactive compounds. Their nutritional content and capacity of growing in bioreactors make them key players in the industries of food and animal feed, cosmetics, pharmaceutical, chemical, bioenergy, biofertilizers and CO_2 sequestration. They represent one of the most promising organisms in the world, and its industrial cultivation may occur in open or controlled closed cultivation systems (Cavalho *et al.*, 2006), depending on the species/strain to be produced, compounds of interest and quality of the final product.

The most challenging and crucial issue for large-scale microalgae cultivation is maintaining, at the same time, the quality of the final product and industry's economic sustainability. Heterotrophic growth of microalgae allows reaching higher cell concentration in a shorter time, enabling greater output masses and, therefore, more cost-effective pricing and greater batch-to-batch nutrient consistency (Barros *et al.*, 2019). Allmicroalgae, a Portuguese industrial microalgal producer, has expanded its production capacity by adding to the already existing photobioreactors (Fig. 1), the fermentation process. This technology enabled the development of a sustainable industry for microalgae production.

The solar exposition in Portugal enhances the phototrophic cultivation, allowing the algae to develop attractive key nutrients. The controlled-environment in closed photobioreactors minimizes the bioaccumulation of heavy metals or any other contaminants, potentially harmful to aquaculture (Barros *et al.*, 2017). Together, freshwater and marine microalgae offer new opportunities in the aquafeed industry as a valuable fishmeal supplement.

Microalgae feed applications and trials

Microalgae-based aquafeed benefits from its high level of purity, small particle size (2-10 µm), and the nutritional composition of the microalgae in minerals, vitamins, amino acids, carotenoids and essential fatty acids. Depending on algal strain/species and growth conditions, the composition of the biomass may vary. Different microalgae, with specific attributes suitable for the aquafeed industry, are produced at the Allmicroalgae production unit.

Chlorella vulgaris

Chlorella vulgaris is one of the most common cultivated algae in the world, used extensively in a diverse array of applications. Autotrophic *Chlorella vulgaris* produced at Allmicroalgae facilities is highly rich in protein (57.18%) and chlorophyll (23.98 mg/g DW) (Barros *et al.*, 2019). Starch is its most abundant polysaccharide and the polysaccharide extract has shown an immunostimulatory activity once it can dose-dependently activate C19-lymphocyte cells (Ferreira *et al.*, 2020). This alga has a high digestibility rate when ingested by juvenile *Dicentrarchus labrax* (Batista *et al.*, 2020).

Tetraselmis sp.

Tetraselmis sp. is a protein (40.5 to 42.7% DW) and carbohydrate-rich (41.23 to 46.52% DW) microalgae grown by Allmicroalgae (Trovão *et al.*, 2019). Its incorporation into juvenile gilthead seabream (*Sparus aurata*) feed induces a three-fold increase in initial body weight in 61 days. When compared to conventional feed, the animals keep their final body weight, daily growth index, feed conversion ratio, protein efficiency

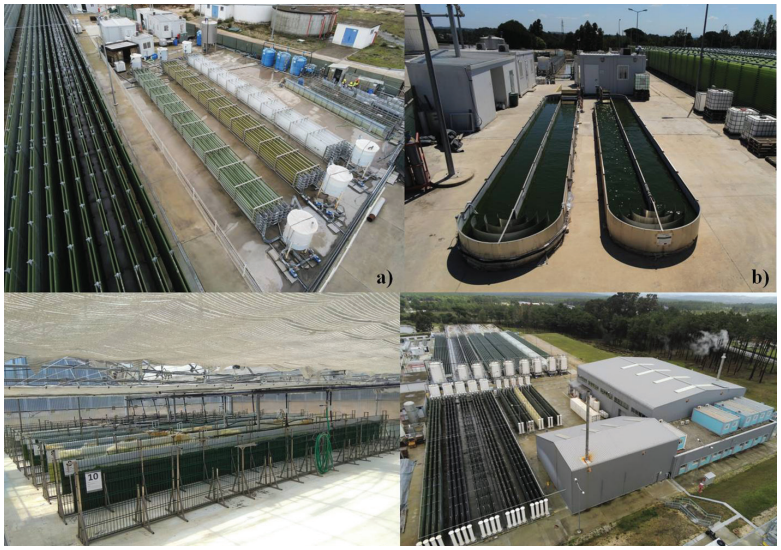

Figure 3. Allmicroalgae facilities. a) Photobioreactors, b) Open raceways, c) Flat panels, d) Panoramic view of the company.

ratio, whole-body composition and nutrient retention, and is a good alternative to the soybean meal (Pereira *et al*., 2020). In fish fed with a diet supplemented with *Tetraselmis* sp., an exposition to acute confinement stress induces a significantly lower plasma cortisol response (120 ± 23 ng/mL) than those fed with the control diet (160 ± 33 ng/mL) ($p < 0.05$) (Fig. 2).

Nannochloropsis oceanica

Nannochloropsis oceanica is the microalgae most used as feed or feed supplement in aquaculture due to its fatty acid profile. Allmicroalgae´s *Nannochloropsis oceanica* has 12.7 to 19.0 % of lipids in total biomass, with around 30% of omega-3 (Cunha *et al*., 2020). Its thick and bilayer cell wall confers this alga a very robust structure, which is a natural protective form of EPA source for animals (Alves *et al*., 2018). But besides the cell wall, *N. oceanica* has nutrients that are bioavailable for fish (Batista *et al*., 2017). European seabass juveniles (*Dicentrarchus labrax*) when fed with a *N. oceanica*-based diet presented an increased muscle cohesiveness and moisture content (Batista *et al*., 2020), which are two major properties for commercial value and consumer acceptance.

Other microalgae

Besides all the referred ones, Allmicroalgae produces an array of different microalgae which have shown to have a nutritional profile suitable for aquaculture feed applications, such as *Phaedactylum tricornutum*, with around 30% DW of lipids (Quelhas *et al*., 2019) or *Chlorococcum amblystomatis*, with 55.7% DW of protein and 40.01 mg/g DW of chlorophylls a and b (Correia *et al*., 2020).

Together, all these different microalgae options can be applied in hatcheries feeding practices as a sustainable optimized ingredient and might be an interesting supplement to boost the immune system of fish, reducing, for instance, the use of antibiotics (Cerezuela *et al*., 2012).

Allmicroalgae expands access to algae-based ingredients

Allmicroalgae produces 100% natural ingredients and exclusive-microalgae blends for the food segment and feeds such as aquaculture, farm animals, birds or pet food. Optimizing production and maximizing the biochemical and functional characteristics of its products are at the heart of the company's R&D activities and are always in line with the needs of its business partners and industry developments. With a commitment to transparent operations and the highest safety standards, Allmicroalgae is certified for European Organic Production as well as Halal, ISO 22000, ISO 9001 and ISO 14001. It produces microalgae via auto- and heterotrophic pathways where fermenters, flat panels, photobioreactors and open raceways enable a production on a large scale (Fig. 3).

The controlled close-environment production at a large scale, the variety of microalgae species to offer, and the European Organic Certification, strengthened by continuous academic involvement, reinforce the value of Allmicroalgae as an expert in fish nutrition.

References available on request

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Submersible robotic solutions for aquaculture

Andrew Lawrence, Deep Trekker

With total annual aquaculture production rapidly approaching 50% of the world's seafood production, the importance of aquaculture on the global food market cannot be overstated. As with any food resource, sustainability and animal welfare ought to be top priorities. Keeping infrastructure and equipment in proper operating condition and ensuring compliance with health and welfare standards are of paramount importance to Deep Trekker.

Deep Trekker is proud to offer a variety of submersible robotic solutions and aquaculture-specific tools. Our products provide operators with a safe and reliable way to perform a wide variety of husbandry tasks around the farm. With a Deep Trekker ROV, operators can get eyes underwater quickly and easily to inspect tank wall integrity, build-up around drains, or perform pipe inspections. Offering a safe, convenient and economical alternative to physically entering tanks, our robots are changing how technicians maintain their land-based facilities.

Deep Trekker offers two different submersible ROVs: the DTG3 and the REVOLUTION, both of which are perfect for convenient and efficient underwater inspections and fish monitoring. The DTG3 is a mini observation-class vehicle built to provide operators with the ability to deploy in 30 seconds or less. The REVOLUTION is a completely reimagined vehicle with greater payload capabilities, deeper depths and advanced stabilization. The DTPod submersible camera is ideal for permanent installation and around-the-clock monitoring. The DT640 Utility Crawler is the ultimate payload-carrying submersible crawling robot and is the ideal tool for tank cleaning.

Observe fish behavior

By using an ROV or submersible camera to observe key behavioral indicators, technicians can make educated judgments about the current state of health and welfare in their pens. Common diseases among fish are the source of billions of dollars in loss annually. As prevention is the best treatment, maintaining exceptional fish husbandry and care practices will go a long way in preventing costly diseases.

Side swimming, for example, is an anomaly occasionally exhibited by various fish species under intensive culture conditions, side swimmers swim in a coordinated manner but are oriented abnormally in the water column so that their dorsal and ventral aspects are perpendicular to the direction of gravity (Branson and Turnbull, 2008). Side-swimming has been observed in a variety of aquaculture settings, including flow-

through raceways and circular tanks in recirculating aquaculture systems. Symptoms of side-swimming and other ailments can be caught early by monitoring fish behavior with Deep Trekker's ROVs or DTPods. With early detection, health challenges can be identified before they impact your bottom line.

Reduce feed costs

The feed you choose has a direct impact on the health and welfare of your fish. Particular feed regimes are strategically adopted based on water temperature, prevalence of particular diseases, type of biofilter used, legislation concerning phosphorus and water quality standards. Specialty feed types, especially starter feeds and those used in RAS, tend to be extremely expensive due to the many supplemental vitamins, minerals, prebiotics and amino acids therein. For these reasons, it's imperative that farmers maximize the utility of these feeds. A Deep Trekker ROV or DTPod is a vital tool for any effective feed management strategy.

Feeding to satiation is a very common practice, but it really only allows you to see when fish at the surface have fed adequately. Weaker or smaller fish below the surface may not be able to compete for feed at the surface, and by the time feed has reached them it may be minimal, or it could have floated into the drain. A DTPod can be suspended or permanently mounted in a particular location and then rotated 360° on two axes to ensure you are getting a full picture of how satiated all of your fish are. Recently we have seen a considerable amount of interest from land-based facilities that want to place DTPods permanently at drains to check feed waste and monitor fish health. The video can be remotely monitored and controlled by connecting the DTPod to your local area network so you can view it anywhere.

Reduce stress

Being able to closely monitor feeding may cut down on the amount of sampling and grading necessary, saving vital time that can be spent on more productive tasks. Sampling and grading also raise cortisol levels in fish which has an effect on growth. Many conventional flow-through sites may benefit from video monitoring due to the added complication of dealing with unfavorable water temperatures at certain times of the year, which further compounds the problem of stress.

Environmental sensing

Both Deep Trekker ROVs and the DTPod have built-in temperature, depth and dissolved oxygen sensors available, allowing users to get an accurate environmental profile of each pen in minutes. Our DO sensor integrates directly into the ROV and controller screen to allow measurements to be taken at any point in your tank and then your profiles can be graphed using the spreadsheet our controller produces.
We partnered with Aquatroll to integrate their probes directly onto our ROVs and DTPod so you can test up to four different parameters (phosphorus, ammonia, etc.) simultaneously. This can be useful for informing your current feeding practices or locating areas in the tank with undesirable environmental conditions.

Maintain clean tanks

The DT640 Utility Crawler can be equipped with a variety of vacuum heads that are perfect for cleaning waste from tank bottoms, and when equipped with our magnetic wheels, it's capable of cleaning the vertical side walls of ferrous aquaculture tanks. This minimizes the amount of sediment stirred up which helps maintain water quality. The DT640 can also be equipped with a powerful pressure washer for empty tanks and hard to clean areas of your facility. Using the DT640 for these jobs is a far safer and more hygienic alternative to conventional methods of cleaning.

Conclusion

The entire Deep Trekker team is committed to ensuring that all of our aquaculture customers have the best submersible equipment on site at all times. We strongly believe that aquaculture has the potential to change the world in profoundly positive ways and we are determined to continue to contribute in any way we can.

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Industry Events

Send your meeting details to:
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OCTOBER

6 - 8:	GOAL Virtual Conference	www.aquaculturealliance.org

NOVEMBER

10 - 11:	US Microalgae Industry Summit, Orlando, USA	www.wplgroup.com

DECEMBER

1 - 3:	AlgaEurope 2020, Rome, Italy	algaeurope.org
3 - 5:	Aqua Fisheries Cambodia, Phnom Penh, Cambodia	aquafisheries-expo.com
9 - 11:	ILDEX Vietnam 2020, Ho Chi Minh City, Vietnam	www.ildex-vietnam.com

2021

FEBRUARY

9 - 12:	EuroTier, Hanover, Germany	www.eurotier.com
21 - 24:	Aquaculture America, San Antonio, USA	www.was.org

MARCH

10 - 12:	VIV Asia 2021, Bangkok, Thailand	vivasia.nl
22 - 25:	LACQUA, Guayaquil, Ecuador	was.org

APRIL

12 - 15:	Aquaculture Europe 2020, Cork, Ireland	www.aquaeas.org

MAY

5 - 7:	International Genomics in Aquaculture Symposium, Granada, Spain	www.gia2020.es
19 - 20:	World Aquaculture and Fisheries Conference, Tokyo, Japan	worldaquacultureconference.com
18 - 21:	Aquaculture UK, Scotland, UK	aquacultureuk.com

JUNE

14 - 18:	World Aquaculture 2020, Singapore	was.org

SEPTEMBER

7 - 10:	Asian Pacific Aquaculture, Surabaya, Indonesia	www.was.org
26 - 29:	WAS North America & Aquaculture Canada, St. John's, Newfoundland, Canada	www.was.org

OCTOBER

4 - 7:	Aquaculture Europe 2021, Madeira, Portugal	www.aquaeas.org

DECEMBER

11 - 14:	Aquaculture Africa, Alexandria, Egypt	www.was.org

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