Vol 10 Issue 1 2022

HATCHERY
FEED & MANAGEMENT

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Status of Artemia cyst use

Equipment

Published by Aquafeed Media S.L.U. www.hatcheryFM.com info@hatcheryFM.com

HATCHERY FEED & MANAGEMENT

VOL 10 ISSUE 1 2022

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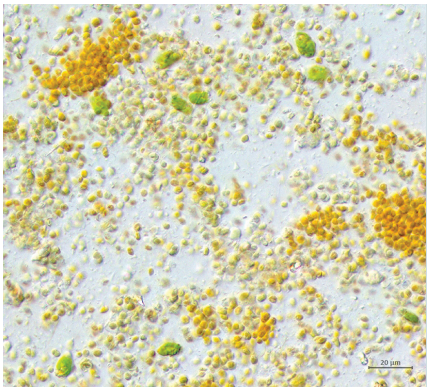

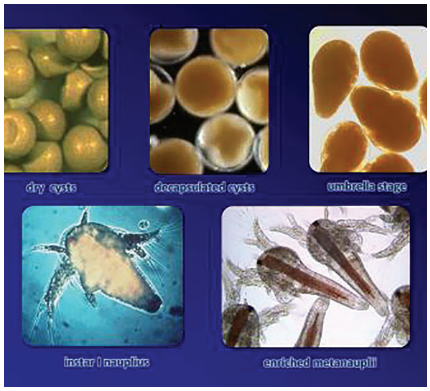

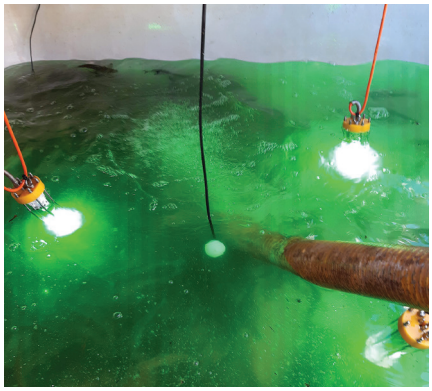

Maryke Musson is General Manager of Kingfish Zeeland.

INTERVIEW *with* ***Maryke Musson***

HFM: Please tell us about yourself. What has been your journey in aquaculture? How did you get to where you are today?

MM: I studied marine science and my ocean career started as part of a small team building and stocking the Two Oceans Aquarium in Cape Town about 27 years ago. This is where I gained incredible experience in fish husbandry, animal welfare, aquatic life support systems and creating inspiring stories to share science with the general public and where I developed "fish fingers".

With a deep interest in sustainability and conservation and great support from thought leaders and funders believing in sustainable food production, we forged ahead and pioneered the hatchery production and commercial production of mulloway/dusky kob (*Argyrosomus japonicus*) in South Africa. This was a fantastic journey, getting involved in and ultimately leading all aspects of a vertically integrated RAS facility, from design and construction, to R&D, broodstock management and hatchery production, to developing best-case production plans and stock management and ultimately, processing and marketing of a sustainable certified product fresh to the market.

After almost 17 years in aquaculture development, I was lured back to aquarium science as general curator of the Two Oceans Aquarium, and eventually CEO of the Two Oceans Aquarium Education Foundation. I was then offered the opportunity to jump back into the fast-paced world of next-generation fish production at The Kingfish Company here in Zeeland, the Netherlands where we are on a mission to produce the perfect fish using innovative and disruptive technologies

while working towards being carbon neutral. It has been quite a fishy journey indeed.

HFM: The Kingfish Company runs a land-based yellowtail kingfish farm in the Netherlands. What have been the main challenges in setting a RAS for this species?

MM: Yellowtail kingfish is a species that is so well suited to land-based RAS. They naturally shoal in very high densities, in fact, in the wild, they actually swim in a formation that is very similar to what we see in our large rearing tanks. They prefer higher densities, which is generally required in making RAS profitable. This species is very energetic, grows incredibly fast and requires the best possible water quality, so ensuring life support systems that can maintain the perfect rearing conditions for yellowtail kingfish is vitally important. We give our fish a much higher tank turnover rate for instance compared to what you would see in RAS for any other species. But it is absolutely worth it as it truly is a fish like no other, very versatile with exceptional taste.

Access to broodstock and fingerlings remains a limiting factor in general yellowtail kingfish farming, and we have been fortunate to increase our hatchery output consistently over the past four years. We are pioneering the production of this species in RAS, so there is little information out there compared to species that have been farmed for many years already.

HFM: Kingfish Zeeland is one of the few commercial yellowtail kingfish farms globally with year-round juvenile production. What are the main challenges faced in terms of broodstock management and reliable spawns?

MM: The main challenge would be access to broodstock to start with and then going through a selection process to ensure the best possible genetic diversity and traits in current and future generations. We have closed the cycle already and have our new generation cohorts in production. We also only use environmental cues to achieve all-year spawning activity from our various broodstock groups, so we do not make use of hormone inductions. Our broodstock management has matured, along with our broodstock, and we thus have access to fertilized eggs year-round produced by our selected fish which is a great advantage of course.

We have worked closely with Wageningen University to develop an optimal breeding program for our needs here in the Netherlands as well as in Maine, and we have seen very positive early-stage results in our new generation cohorts.

HFM: What have been the key points for a successful larval rearing process?

MM: The larval rearing of yellowtail kingfish has been more challenging than most other species I have worked with, and I believe that the success achieved in our hatcheries here at Kingfish Zeeland is directly linked to the incredible dedication by our hatchery biologists and attention to detail. These fish require around-the-clock care and you have to combine proven rearing protocols with a bit of flexibility and "gut feel" to ensure success. There are still a lot of factors we continuously look at to achieve consistent quality, and we continue to make great progress. Offering the best rearing environment, nutritional plans and weaning schedules are key, but as we all know, broodstock quality contributes greatly too, so we make sure our broodstock gets five-star care, 24/7.

HFM: What have been the main nutrition advances you have achieved for broodstock and larval stages?

MM: We have developed our own broodstock formulation with the inclusions of high-end maturation pellets which has resulted in high fecundity and optimal broodstock health. Nutrition is an ongoing program and we continue to look at where and how we can improve. With larval rearing, we have looked at the digestive system ontogeny and enzymology to optimize our weaning program while offering the best nutritional profile at exactly the right time to ensure the best larval rearing success. I believe feed optimization will always be a focus area as we pursue the best performance while reducing the reliance on marine resources.

HFM: The farm uses 100% green energy. What are the main goals achieved in terms of hatchery sustainability?

MM: We are very committed to our sustainability goals and working towards carbon-neutral production while also monitoring our social impacts and governance performance. All our external supply

electricity is indeed certified green with guarantees of origin and we also produce solar energy on-site, while having to still rely on some additional heating capacity during peak winter months to maintain an optimal rearing temperature.

We implemented our Plan Blue, monitoring our ESG impacts and achievements, and from an environmental impact perspective, created our "25 x 25" campaign, where we committed to reducing our carbon emissions per kg of product produced by 25% by 2025, as well as reducing our residual waste, increasing our recycling efforts and reducing our Fish In Fish Out ratio all by 25% by 2025. We have already made great progress on these commitments. We also have ASC and BAP certifications in place, with the most recent completion of the BAP Hatchery certification. In the hatchery specifically, we look at the best possible heat exchange and retention. We incorporate sustainability practice in all our design and operational plans, so it is top of mind in everything we do.

We also use the UN's sustainable development goals to guide our activities and our 2021 focus areas were innovation, responsible production and consumption, climate action, clean energy and life below water.

Finally, where would you like to see the aquaculture industry and hatchery technology in the next ten years?

MM: I am really excited about the smart farming opportunities that precision technology and AI offer. If we continue to innovate technologies to help us manage risks, predict challenges and act as early warning signs, our biologists and researchers could see improvement results much quicker. Hatcheries all look for consistency, and when you work in an environment with so many variables and increased production pressure it becomes challenging to allocate time resources to practical research and innovation. Combining technology skills and real-time monitoring and AI with biological care and welfare practices, we will see ongoing improvements with regards to quality, sustainability and consistency.

I am also seeing continuous production growth in developing countries where food security is the driver. Being able to offer healthy, low-carbon protein while growing equal employment opportunities and reducing poverty and hunger in these countries truly inspire me.

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NEWS REVIEW

HATCHERY FEED & MANAGEMENT

Highlights of recent news from Hatcheryfm.com

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Benchmark Genetics Chile receives organic ova certification

The company completed the organic egg certification process in accordance with the production method standard of the European Union. The company plans to deliver the first batch to Cooke in April, has scheduled a second batch of certified organic eggs for September and will continue with a stable quarterly production regime in the future.

Earth Ocean Farms, CAT partnership to improve genetics of red snapper

The Center for Aquaculture Technologies (CAT) partnered with Earth Ocean Farms (EOF) in Baja Mexico to unpack the genetic mysteries of Pacific red snapper (*Lutjanus peru*) to boost productivity and promote faster growth. The development of this customized breeding program is the first of its kind for Pacific red snapper. It will assist EOF in selecting specific traits in the snapper stock that show higher production yields through improved growth performance and phenotypic traits.

Spring Genetics, Genetika partnership to supply tilapia broodstock in Peru

Spring Genetics signed a five-year contract for the supply of broodstock to Acuicola de la Selva S.A.C. (Genetika) in Peru, a market leader in the production of tilapia fingerlings and native Amazonian fish in the Latin American country.

Spring Genetics currently supplies tilapia broodstock and fry in Canada, the U.S., Haiti, Dominican Republic, Guatemala, El Salvador, Colombia, Perú and Brazil, together with Benchmark Genetics that delivers tilapia in Chile, Costa Rica and Honduras.

Skretting introduces starter feeds for rainbow trout

The company unveiled new starter and grower feeds for rainbow trout to help farms meet their full potential. Built around Skretting's expertise, the latest offering provides farmers with solutions that can be better utilized than conventional feeds. Nutra Sprint is a new high-performance starter feed that supports first-feeding trout fry, while Celero accelerates growth in the grow-out phase and enables farmers to produce premium quality fish with a high harvest yield.

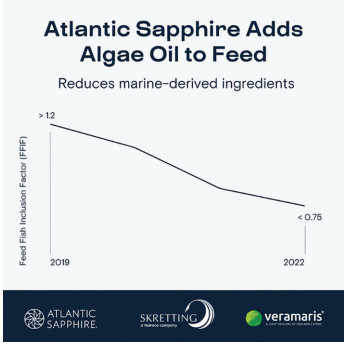
Molofeed invests in new R&D hatchery facility

The company will invest in a new R&D facility in Natal, Brazil. Molofeed aims to develop advanced hatchery nutrition solutions and new hatchery-related technologies for shrimp and finfish in the new RAS facility. The new state-of-the-art research facility was designed to give complete control of environmental conditions, allowing multi-factorial and multi-species research, focusing on tropical species' early stages.

Electrocoagulation technology to reduce nitrites in RAS

NaturalShrimp's patented electrocoagulation technology has proven its ability to remove ammonia within RAS before it converts to nitrites. The company's technical team has now discovered that the EC system could also reduce nitrites without the need for a biofilter in RAS that might experience a buildup of nitrites.

Atlantic Sapphire to include algal omega-3 ingredients in RAS feeds



The largest global land-based aquaculture company, Atlantic Sapphire ASA, partnered with Skretting to include Veramaris ASC-MSC certified sustainable algal oil in its feed from Q4 2021, reducing the fish oil content in its salmon feed by approximately 25% and accelerating the process of eliminating the use of marine-derived feed ingredients by 2025.

I&V BIO opens Artemia center in Bangladesh

The center, located in Cox's Bazar, is ready to deliver fresh and live Artemia, on a daily basis, in the country. I&V Bio supplies hatcheries, nursery and grow-out farms daily with Instar 1 Artemia Nauplii. The company started the concept in 2012 in a hatchery in Thailand. Since then, I&V-BIO expanded with operational centers in Thailand, India, Indonesia, Vietnam, Bangladesh in Asia and Ecuador.

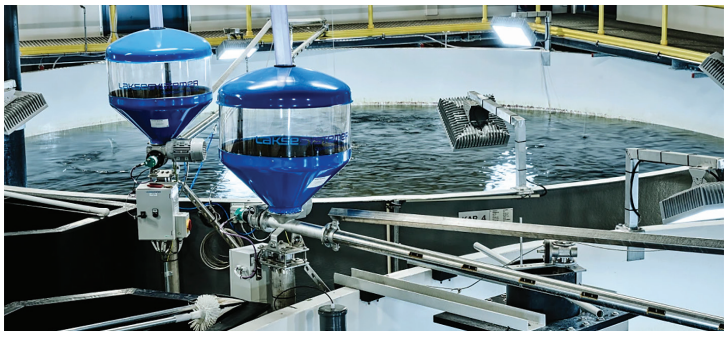
Trial showcases improvements in barramundi feed efficiency with genetics

Vietnam-based barramundi pioneers, Australis Aquaculture, have been running an advanced genetics-backed breeding program with Xelect since 2018. Its latest trial suggests that significant gains are possible, with high weight maintenance groups showing a 12% improvement in FCR over low weight maintenance individuals. Because this trait is also "heritable", a significant part is down to genetics. Therefore, fasting evaluations can be performed routinely to select the most feed-efficient broodstock in Australis' breeding program.

PHARMAQ Analytiq opens lab in Scotland to enhance aquaculture diagnostic capabilities

PHARMAQ Analytiq Scotland opened a molecular biology laboratory at Solasta House on Inverness Campus, following the acquisition of Fish Vet Group in 2021. The new laboratory complements the existing digital pathology, microbiology and environmental laboratories already in Inverness and will deliver faster results to Scottish farms using PCR to safeguard the health of fish populations.

Joint venture to deliver autonomous feeding systems for RAS



Norwegian technology provider, Laksesystemer, and Canadian AI solution provider, ReelData, signed a joint venture agreement for the delivery of fully autonomous feeding systems for land-based aquaculture. The companies will provide feed systems for land-based aquaculture with ReelData's ReelAppetite, autonomous appetite adjustment system.

Advanced maturation feeds for biosecure production of robust nauplii

Mark Rowel Napulan, Ramir Lee, Peter Van Wyk, Chris Stock, Craig L. Browdy, Zeigler Bros.

Attractability, palatability and consumption are key attributes for replacement live and fresh feeds.

The global export of specific pathogen-free (SPF) *Penaeus vannamei* broodstock was estimated to be 1.4 million animals in 2019. This equates to USD 77 million in trade at US D55 per animal. The majority of *P. vannamei* broodstock is exported from the Americas to Asia where 80% of world shrimp production is carried out. The use of SPF shrimp broodstock in Asia is driven by the regional use of small, biosecure pond systems.

Shrimp maturation protocols in Asia are dominated by the use of live and fresh feeds due to their perceived benefits in improving the fecundity of broodstock. These natural feeds, however, pose significant biosecurity risks. Polychaetes, an important dietary component in shrimp maturation, are confirmed carriers of *Enterocytozoon hepatopenaei* (EHP) and pathogenic *Vibrio parahaemolyticus*, the early mortality syndrome/acute hepatopancreatic necrosis disease (EMS/ AHPND) causing bacteria. In fact, EMS/AHPND was first reported to have been transferred from China to Thailand through the live polychaete trade, subsequently spreading throughout Asia.

Spawners fed Redi-Mate (left) show significantly improved ovary coloration indicating enhanced pigment uptake into ripe oocytes.

Imported live SPF polychaetes from Europe have gained popularity and are often used as supplements for cost efficiency. Recently, many large companies have invested in the production of live SPF polychaetes. These types of production systems have high fixed and variable costs.

Polychaete farms must be frequently monitored to maintain SPF status and so far, no standard third-party certification bodies exist to verify the status of these facilities. Moreover, the nutritional quality of SPF polychaetes varies depending on feeds used and culture techniques.

Understanding the nutritional requirements of broodstock during maturation and spawning is complex and remains obscure. Feed companies are racing to develop alternative biosecure artificial diets to supplement live SPF polychaetes and to replace a portion of non-biosecure fresh feeds. In the end, a new nutrient-dense artificial diet that has greater attractability delivers better performance and improved biosecurity can be a great benefit, helping the industry to move forward.

New specialized maturation diet

Leading global broodstock providers invest significant resources to ensure their animals are free from pathogens which means they rely exclusively on manufactured diets. It is only upon arrival at the customer's facilities that higher-risk diets enter the feeding protocol.

Disease occurrence due to breaches in biosecurity becomes a cyclical pattern and a continuing challenge to the shrimp industry. Major diseases have been shown to enter shrimp maturation systems via fresh and live feeds. Practically, there is a need to pursue a balance between using biosecure feeds combined with lower risk fresh diets that satisfy the shrimp nutritional requirements while achieving maturation expectations. Strategies for risk management include using the following:

- Low risk verified SPF fresh feeds cleaned, treated and frozen (e.g. squid).
- Frozen polychaetes for a short period of time have been shown to kill some pathogens (although extended freezing can cause leaching of critical enzymes and nutrients).
- Live SPF polychaetes.
- Biosecure manufactured diet that is proven to supplement or replace fresh/live feeds.

Zeigler Bros., Inc (USA) has invested years of research to replace live and fresh feeds and improve biosecurity in shrimp maturation systems by developing a specialized semi-moist fresh feed replacement diet that reduces costs, boosts performance and increases hatchery profitability. Zeigler's Redi-Mate, a semi-moist artificial diet, has been specifically designed for maturation and spawning penaeid shrimps and has delivered excellent results as shown in the field and commercial studies in both Asia and Latin America.

The Latin American approach: Replacement

It must be noted that most shrimp maturation facilities in Latin America do not use live polychaetes, but rely instead on frozen polychaetes, krill, bivalves, squids etc. Redi-Mate has been tested in commercial trials in large hatcheries in LATAM and has successfully replaced fresh feeds with the highest risk of introducing pathogens.

To demonstrate the efficacy of this semi-moist artificial diet, a commercial trial in Central America was established. Three maturation tanks were fed a control diet consisting of squid, mussels, Artemia biomass, frozen polychaetes and a dry maturation diet, while the other three tanks were fed a diet in which 59% of fresh feed was replaced with Redi-Mate.

Table 1. Feeding rates of constituent feed items for the control and Redi-Mate tanks expressed as a percentage of biomass fed per day [(dry weight fed per day/biomass of maturation tank) x 100%].

Feed item	Control		Redi-Mate	
	Dry weight %	% of diet (dry weightbasis)	Dry weight	% of diet (dry weightbasis)
Squid	2.20	31.43	1.80	25.71
Mussels	1.20	17.14	0.70	10.00
Artemia biomass	0.70	10.00	0.40	5.71
Dry diet	1.84	26.29	0.00	0.00
Polychaetes	1.06	15.14	0.00	0.00
Redi-Mate	0.00	0.00	4.10	58.57
Total per day	7.00	100	7.00	100

Table 2. Summary of maturation production results from a trial in a commercial Latin American hatchery.

Parameters	Control	Redi-Mate	% Difference
Spawning/night (%)	12.30	11.50	-6.30
Egg/spawn	240,136	240,434	0.12
Nauplii/spawn	114,271	116,882	2.28
**Nauplii/female per night	13,871	13,352	-3.74
Broodstock survival (%)	78.6	97.5	24.03
Total nauplii x 10^6	147.60	158.20	7.14

*% Difference = ((ValueRedi-Mate-ValueControl)/ValueControl)x100%
** This value is the Nauplii Production Index. The unit is nauplii per female per night. This is the total number of nauplii produced over a period of time divided by the number female production nights. It is sometimes expressed as nauplii per 100 females per night, in which case the numbers in the table would be multiplied by 100.

In the experimental tanks, fresh and live feed inclusion rates were significantly reduced by the incorporation of Redi-Mate in the protocol. Complete (100%) replacement of frozen polychaetes was achieved along with reducing the amount of mussels, Artemia biomass and squid at levels of -43%, -43% and -20%, respectively. The semi-moist artificial diet Redi-Mate was accepted well by shrimp which readily consumed more than 5% of their biomass per day on a wet weight basis. Table 1 summarizes the daily feeding rates based on dry weight for each component of the maturation diets of the control and experimental tanks.

The results in Table 2 show that Redi-Mate, when fed at close to 60% of the entire diet while replacing 100% of the polychaetes and nearly 50% of the mussels and Artemia biomass in the diet, supported excellent levels of nauplii production. Moreover, 24% higher survival of the broodstock was observed as pathogen introduction with frozen polychaetes was eliminated by the replacement strategy.

The Asian approach: Supplementation

In Asia, spawn size and fecundity of breeders have been emphasized over biosecurity. Zeigler tested replacing up to 50% of live polychaetes in commercial maturation systems demonstrating Redi-Mate efficacy in reducing costs of live SPF polychaetes without compromising on production.

Table 4 shows that in this trial, the semi-moist artificial diet Redi-Mate demonstrated no significant difference in performance against a control that used 100% live polychaetes. It was displayed in the trial that this semi-moist artificial diet can potentially replace up to 50% of live polychaetes without compromising reproductive performance or fecundity. Across Asia, hatcheries are now increasing the use of supplementation strategies after a series of successful trials.

Dry weight versus wet weight

The real value of a maturation feed must be measured by the nutrients it provides. Moisture has no nutrient value, so the real cost and benefit of maturation

Table 3. Feeding rate of live polychaetes versus Redi-Mate trial in Asia.

Commercial Trial (Asia)	Control	Redi-Mate
% Feeding rate (wet weight)	18%	9%
% Diet (wet weight)	100%	50%
No. pairs	150	150%

Table 4. Performance of Redi-Mate demonstrating 50% live polychaetes replacement.

Parameters	Control	Redi-Mate	% Difference*
Initial weight	61 g	61 g	0%
Final weight	67 g	71 g	5.60%
Average nauplii/spawn	144,000	138,000	-4.30%
Mating rate	13%	15%	13.30%

Table 5. Dry weight of commonly used maturation feed.

Maturation feed	Amount of food	Percent dry weight	Actual dry weight fed
Polychaetes	1 kg	17%	170 g
Squid	1 kg	20%	200 g
Redi-Mate	1 kg	73%	730 g

feed must be measured in terms of dry weight. To understand and compare different feeding regimes used in various hatcheries around the world in terms of the actual nutrients fed, feed amounts and feed costs, each must be measured in terms of dry weight.

As shown in Table 5, 1 kg of polychaetes would provide only 170 g of nutrient dry weight. Similarly, 1 kg of squid delivers about 200 g, while 1 kg of Redi-Mate makes up 730 g of dry weight. Technicians may feel that consumption of the compound feed is lower than that of a fresh feed because of the amount that they are feeding. The fact is that shrimp must consume 3.5 to 4 times as much polychaetes or squid to get the same nutrient content as that of Redi-Mate. Similarly, costs should be measured in terms of dollars per kilogram of dry weight.

The real cost of ingredients must be considered relative to nutrient delivery. The key is the consumption of essential nutrients and how these nutrients are being balanced in the formulation. When it comes to manufactured feed, if the shrimp do not eat, it will not provide any benefits. In developing Redi-Mate, considerable attention was paid to attractability. Moreover, it includes antioxidants and Vpak to boost immunity, making it an optimal pathway to deliver supplements and gut health additives.

Maturation feed calculator,a technical support tool

To simplify the development of feeding protocols when using prepared feeds, the Zeigler Aquaculture Research Center (Z-ARC) team has developed a maturation feed calculator. It is based on wet weight to dry weight ratios of different kinds of feeds. Users input the number of female and male broodstock and their approximate average body weight, to calculate the biomass. Then, the manager enters the feeding regime in terms of percent body weight per day of different maturation feed types used.

In Figure 1, this hatchery is using 10% polychaetes, 15% squid and 5% mussels. The program calculates the percent body weight per day of dry weight fed; in this case, 5.7% per day. The calculator recommends the right amount of Redi-Mate to replace a certain fresh feed, based on the dry weight. To achieve the best results, the semi-moist artificial diet must be fed at rates of 2.5% to 3% per day based on total biomass. In this example, we recommend reducing the polychaetes from 10% to 7% wet weight and squid from 15% to 12% wet weight per day and replace with Redi-Mate. The overall dry feed rate remains at 5.7%. Zeigler's technical team is ready to discuss current practices and make recommendations on how to incorporate Redi-Mate into the hatchery's maturation system.

Variability in the industry: East vs West

The Zeigler team has been using the maturation calculator to support shrimp hatcheries globally. Interestingly, much has been learned on the variability in practices from one hatchery to another. For example, in LATAM, live polychaetes are not fed and in Ecuador, most hatcheries do not use polychaetes at all. Many use frozen Artemia biomass or even beef liver. On the other hand, in Asia, nearly all hatcheries use live polychaetes

Population	
Numbers of female breeders per tank	105
Average weight- females (g)	45
Numbers of male breeders per tank	110
Average weight- males (g)	38
Broodstock biomass (g)	**8,905**

Control tank			
Feed type	**%BW (wet weight)**	**g/day (wet weight)**	**%BW/day (dry weight)**
Polychaetes	10.00%	891	1.70%
Squid	15.00%	1,336	3.00%
Mussels	5.00%	445	1.00%
Daily Total	30.00%	2,672	**5.70%**

Redi-Mate tank			
Feed type	**%BW (wet weight)**	**g/day (wet weight)**	**%BW/day (dry weight)**
Zeigler Redi-Mate	3.01%	268	2.11%
Polychaetes	7.00%	623	1.19%
Squid	12.00%	1069	2.40%
Daily Total	22.00%	1,960	**5.70%**

Figure 1. An example of the maturation feed calculator interface.

to increase spawn size. A key index for understanding feeding rates is the total dry weight of maturation feeds offered for given biomass of broodstock per day. This number informs us if the maturation system is being overfed or underfed. Feed intake can be related to the health and productivity of the broodstock hence, depending on the type of feed consumed, it will manifest later in overall performance.

Chris Stock (right) demonstrates the use of the maturation feed calculator to a customer in Indonesia.

In Table 6, Ecuador has a variable range from 6.64% (dry weight) down to 4.14%, while in Indonesia, the amount of feed used ranges from 6.35% to 5.1%. On average, most systems are between 5.5 and 6% dry weight per day based on total biomass.

Wean and train

To successfully achieve adequate inclusion rates for prepared diets, the following steps are recommended when acclimating broodstock:

- Feed 100% prepared diet for the first 3-5 days. Whenever possible, try to provide the same feed last provided by the broodstock supplier.
- On days 4-6, begin incorporating fresh feed from 1 feeding per day to 3-4 feedings to achieve a ratio of four meals fresh feed to two meals of prepared diet.
- Alternate fresh feeds and artificial diets between feedings.

For older broodstock, it is necessary to train them back to accepting pellets. During the initial introduction, it is advised to skip one meal before introducing Redi-Mate to enhance appetite. Ensure to feed the semi-moist artificial diet right after water exchange, leaving no signs of fresh feed such that only Redi-Mate's attractants dominate.

Table 6. Variability in % feed rate (dry weight) in maturation systems.

	1	2	3	4	5
Equador	6.64%	5.98%	5.59%	5.11%	4.14%
Most hatcheries do not feed polychaetes, most feed frozen Artemia biomass, some mussels, liver					
Indonesia	6.35%	6.30%	5.84%	5.40%	5.10%
Most hatcheries use live polychaetes, frozen squid, mussels, no Artemia biomass					

An industry perspective

Hatcheries in Asia pay premium prices for quality SPF broodstock. For the industry to be sustainable, it is imperative that growers balance biosecurity demands with the desire to maximize spawn size and nauplii production numbers. The long list of diseases that have decimated shrimp aquaculture for decades, along with new emerging threats Taura syndrome virus (TSV), white spot syndrome virus (WSSV), EMS/AHPND, EHP, shrimp haemocyte iridescent virus (SHIV), viral covert mortality disease (VCMD) etc. must motivate our industry to do better.

Experience provides ample evidence that maturation is the first entry point of disease. With new and improved prepared maturation diets now readily available, managers have the option to achieve biosecurity without sacrificing productivity. Development of well-considered, cost-effective feeding regimes incorporating attractively prepared feeds like Redi-Mate that delivers nutritional and health advantages have been shown to provide higher broodstock survivability, better longevity and higher quality nauplii.

We all must challenge industry norms and profitability based on short-term objectives. By developing and applying new maturation protocols and technologies that enhance biosecurity, we can ensure consistency, responsibility, and sustainability through all phases of shrimp production.

This article was published in Aqua Culture Asia Pacific magazine issue November/December 2021.

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Plant extracts support oxidative balance and promote growth in fish larvae and postlarvae

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Nutrition has tremendous impacts on the growth, survival and health status of farmed fish, with both short- and long-term effects. Larvae rearing still holds a constrain in the development of marine fish production, as survival rates are often low or highly variable and growth potential is, in most cases, not fully utilized. Moreover, larval stages are sensitive periods, and correct ontogenesis will influence the quality of the juveniles.

Therefore, optimizing microdiets formulation is a possible strategy to promote farm's profitability. Microdiet optimization may encompass both fulfillment of the nutritional requirements of the fish species and also the inclusion of bioactive compounds. The use of plant extracts in fish diets has been gaining attention, due to the fact that they are economically attractive, environmentally friendly and present a wide spectrum of bioactive molecules. Polyphenols comprise the most abundant group of bioactive compounds and are present in a variety of plants, including seeds, spices and microalgae. The molecular structure of these phenolic compounds confers the antioxidant capacity. In addition, they can regulate the activity of the endogenous antioxidant system or synergize with it, promoting fish allostasis.

This article summarizes a PhD study (Xavier M.J., 2021) aiming to provide a nutritional approach, with the use of plant extracts, to respond to the current challenges of marine hatcheries. Therefore, the potential of these natural additives as promoters of digestive capacity, oxidative status, and muscle growth in fish larvae and post-larvae was evaluated. The goal was to promote growth performance and health status at the early stages of development of two fish species, Senegalese sole and gilthead seabream, important for South-European Aquaculture.

Screening of plant extracts as potential additives for microdiets

A dietary screening of different plant extracts with high antioxidant capacity, such as curcumin, green tea and grape seeds, was performed in Senegalese sole. This initial trial was carried out at CCMAR facilities (Faro, Portugal) with 45 days after hatching (DAH) Senegalese sole postlarvae reared in a recirculating aquaculture system (RAS) and fed with experimental inert diets for 25 days. Postlarvae were fed with one of four diets: a commercial diet (CTRL treatment) and the other three

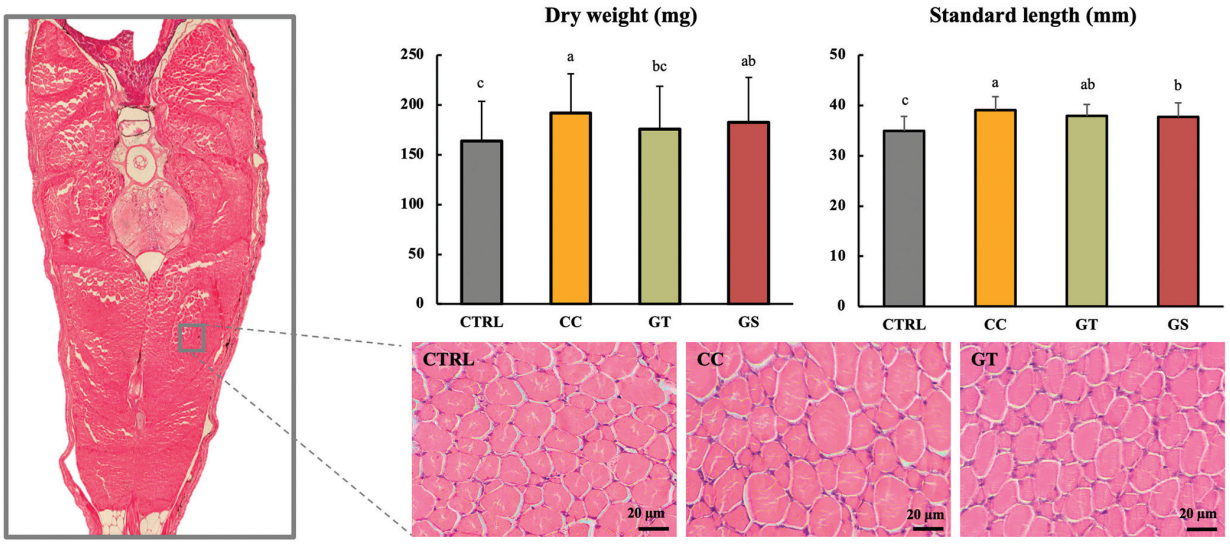

Figure 1. Dry weight and standard length of Senegalese sole at the end of the growth trial (70 DAH) fed the distinct dietary treatments (CTRL, CC, GT and GS). Different subscript letters indicate significant differences.

supplemented with curcumin (CC), green tea (GT) and grape seed (GS) extracts. The experiment was done in triplicates and abiotic conditions were maintained at optimum values for maximum sole growth. At the end of the growth trial, postlarvae were then submitted to a thermal shock for one week. The system water temperature was raised from 21 to 25°C.

Overall, the results showed that at the end of the growth trial (70 DAH), the inclusion of dietary curcumin and grape seed extracts were able to significantly promote higher sole growth, 17.3% and 11.7%, respectively, than CTRL diet (Fig. 1). The analyses of postlarvae muscle morphometry showed that dietary curcumin increases the growth of sole by improving the total number of muscle fibers, as well as the proportion of larger fiber, compared to the CTRL fish (Fig. 1).

Moreover, these postlarvae also presented a decrease in stress-related biomarkers, such as glutathione-S-transferase (GST) and heat shock protein 70 (HSP70), compared to the CTRL fish. These biomarkers are a link to the detoxification and prevention of accumulation of toxic metabolites, such as lipid and protein oxidation products, respectively. Therefore, our results suggest that dietary curcumin, due to its high antioxidant capacity, is able to spare endogenous antioxidant defenses, hence preventing sole to divert energy resources away from growth. In contrast, sole-fed green tea and grape seed diets present the highest content of both lipid and protein oxidation (lipid peroxidation (LPO) and protein carbonylation (PC)), which might have come from a potential pro-oxidant effect of these extract supplementation. However, after thermal stress, fish fed these diets seemed to revert to the previously observed pro-oxidant effect, by preventing the decrease of endogenous antioxidant defenses glutathione (GSH) and by reducing PC content. Therefore, it seems that these two plant extracts can improve fish stress resistance by improving the redox status of the fish.

Closer look at curcumin dietary supplementation

Due to the positive outcomes with curcumin supplementation in Senegalese sole postlarvae, an experiment was conducted focusing on the effects of distinct dietary curcumin levels in gilthead seabream at mouth-opening (4 DAH). The trial was carried out in RAS at CCMAR facilities (Faro, Portugal), under optimal environmental and zootechnical conditions. Gilthead seabream were fed for 27 days one of three inert experimental diets: commercial diet use as control (CTRL) or one of two supplemented with curcumin (LOW and HIGH), under an early co-feeding regime until 24 DAH. After this period larvae were fed exclusively microdiet.

The results showed that curcumin supplementations were not able to improve the growth of gilthead seabream larvae (31 DAH). However, its dietary inclusion was able to significantly modulate the fish condition factor. Larvae fed at mouth-opening with high curcumin

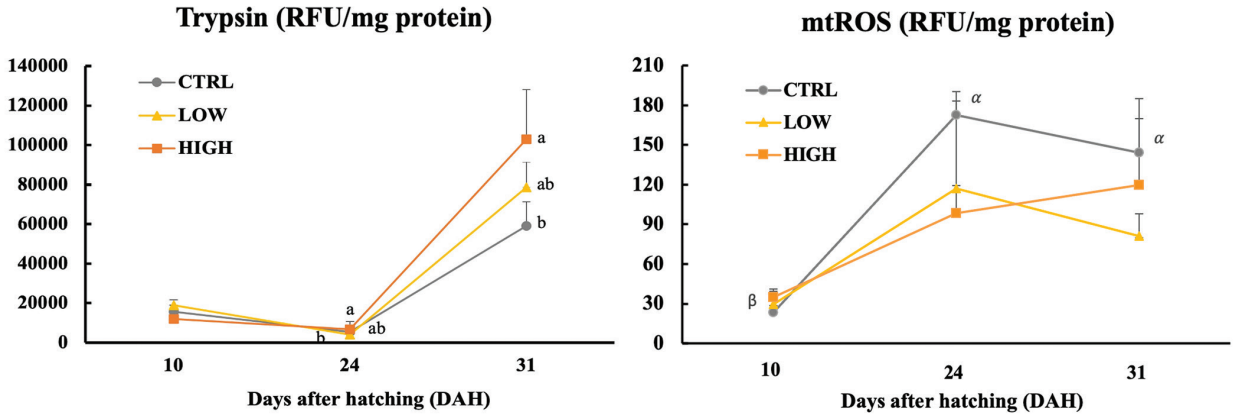

Figure 2. Activity of trypsin and mitochondrial ROS production in gilthead seabream larvae throughout development feeding the distinct dietary treatments (CTRL, LOW and HIGH). Different subscript letters indicate significant differences.

supplementation show an improvement of digestive capacity compared to CTRL treatment, by increasing the activity of proteases trypsin and chymotrypsin (Fig. 2). Moreover, gilthead larvae fed curcumin supplementation diets along development were able to maintain constant the production of mitochondrial reactive oxidative substances (ROS) production in contrast to the crescent production observed in larvae from CTRL treatment (Fig. 2). In addition, the LOW larvae showed similar feed ingestion throughout ontogeny, when compared with CTRL fed fish.

Conclusions

This work provided new evidence that dietary supplementation of natural compounds could be a nutritional strategy to enhance marine fish larvae robustness at early life stages of development. Therefore, this study contributes to improving larvae

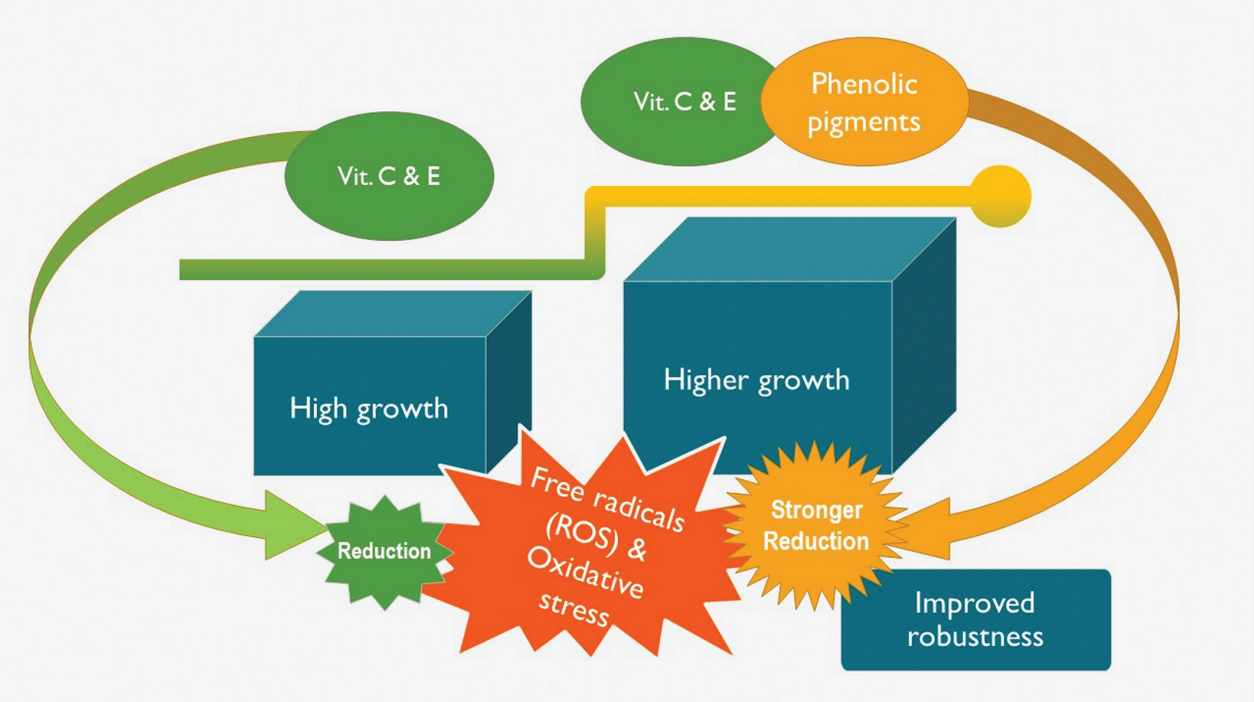

Figure 3. Phenolic pigments in plant extracts may support oxidative balance in early life stages of fish, and promote better growth and welfare.

performance and quality in marine hatcheries and promotes a more sustainable industry. Curcumin and grape seed extracts seem particularly interesting in this endeavor, and a patent on their use as growth promoters is pending. Nevertheless, it is still required to adjust the supplementation doses of the different extracts in order to optimize growth results, as each extract presents different physiological modes of action. Moreover, the supplementation doses need to be adjusted regarding the fish species, developmental stage, and also rearing conditions.

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Acknowledgments

The present study was supported by projects PATHAA, PROLAR (ref. ALG-01-0145-FEDER-029151) and UIDB/04326/2020 financed by the FCT (Portugal) and by the project VALORMAR (ref. 024517) through Compete 2020, Lisboa 2020, CRESC Algarve 2020, Portugal 2020, and the European Union's ERDF. Maria J. Xavier was supported by Grant PDE/0023/2013 (SANFEED Doctoral program, with support by FCT and SPAROS Lda., Portugal).

Figure 4. Low-shear extrusion of microdiets at SPAROS feed mill

Development of new bivalve diets with industrially produced microalgae

Patricia Diogo, Necton

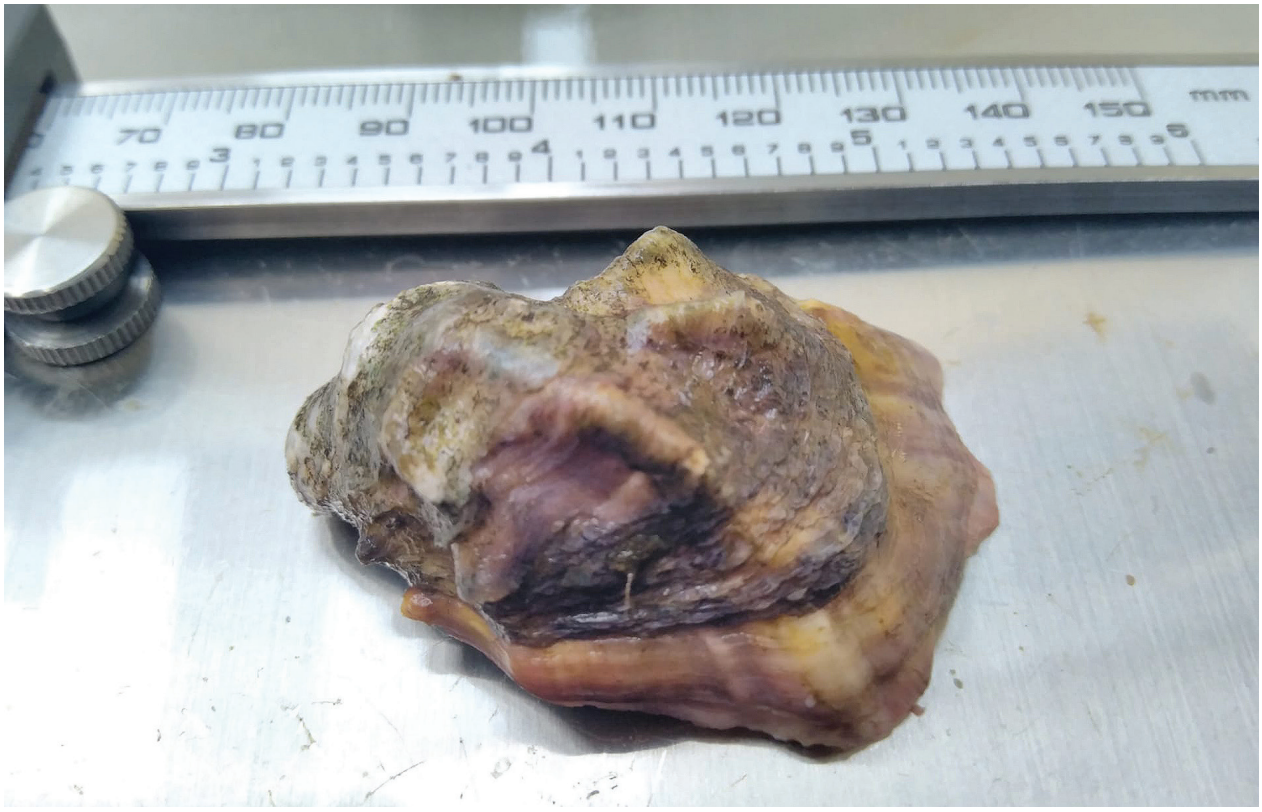

Figure 1. Juvenile *C. angulata* oyster.

Bivalves are low trophic species with high production sustainability, and commercial and nutritional value. Due to these factors, shellfish aquaculture is expanding. Mollusk production in Europe reached 655.30 thousand tons in 2018 and is forecast to reach 869.12 thousand tons in 2026 (Global Algae Paste in Aquaculture Market, 2019). Bivalves are filter feeders that consume microalgae throughout their life cycle.

However, microalgae production performed in hatcheries is a main constraint due to its high labor costs (30-50%; Willer & Aldridge, 2017), limited daily production and productivity fluctuation due to technical constraints (e.g. contaminants and culture crash). Consequently, hatcheries show an increasing usage of industrially produced microalgae.

The European microalgae paste market for mollusk hatcheries has an expected Compound Annual Growth Rate (CAGR) of 3.7% (2019-2026; Global Algae Paste in Aquaculture Market, 2019). In 2018, shellfish production (17.3 million tons) represented 56.2% of

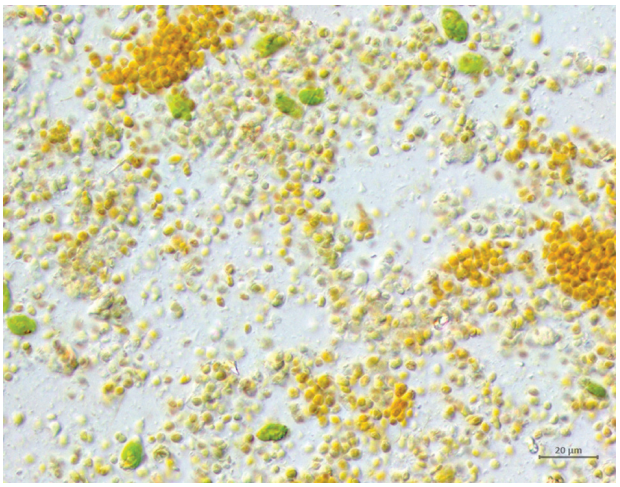

Figure 2. Microscopic observation of Diet 2 multialgal formulation.

the marine aquaculture production, with *Crassostrea* genus representing 33.2% of global mollusk production (FAO, 2020).

Development of bivalve commercial diets

The development of new commercial diets specifically designed for bivalve nutrition, respecting their nutritional requirements in terms of microalgae species and proportions, is a priority in Necton SA to fulfill aquaculture sector necessities. Necton has produced microalgae since 1997 and is particularly proficient in the scale-up and cultivation of innovative microalgae species relevant for bivalve nutrition such as *Skeletonema* sp., *Tisochrysis lutea*, *Phaeodactylum tricornutum* and *Tetraselmis chui*. The combination of these biotechnological advances, combined with the available knowledge in bivalve nutritional requirements, allowed the development of innovative formulations. Tests continue to be performed both in juvenile nutrition and broodstock maturation.

The development of PhytoBloom® SHELLbreed was performed using Portuguese oyster (*Crassostrea angulata*) as a case study. Currently, its validation in *Crassostrea gigas* broodstock maturation is ongoing. One of the main constraints in bivalve feed development is the fact that their optimal nutrition depends on a balanced combination of diatoms and flagellate microalgae species challenging to be produced, particularly on an industrial scale, such as *Skeletonema* sp. and *Tisochrysis lutea*. Consequently, there is a lack of diets formulated specifically for bivalves respecting their nutritional requirements.

Climate change

Nowadays, bivalve culture is facing challenging constraints related to global climate changes. In the past, only broodstock maturation, larval rearing and seed production were performed in hatcheries. Juveniles were commonly grown in the natural environment feeding on natural phytoplankton. However, climate changes, particularly in recent years, are affecting natural environmental conditions.

These changes affect the available microalgae species and their abundance in the natural environment; thus, affecting the animal's nutrition and growth. Moreover, these changes are reducing the natural recruitment and the aquacultures that relied on the collection of spat from the natural environment are now heavily dependent on the seed produced in hatcheries.

Altogether, these factors lead to higher necessities in bivalve hatcheries, juvenile growth with controlled nutrition (FAO, 2020) and the development of balanced commercial microalgae feed, towards the improvement of productivity and management of the sector.

Trials in oyster juveniles

Our study focused on *C. angulata* juvenile growth, a control group was grown in Mira River (Odemira, Portugal) feeding on the naturally occurring microalgae. The objective of this work was to develop commercial diets for bivalves, formulated with a blend of microalgae species commonly used in aquaculture for oyster nutrition and applied to Portuguese oyster juveniles.

Oysters (Fig. 1) were placed in nets on the natural environment as control, while oysters from the two dietary treatments were maintained in the nursery in recirculation tanks in duplicate (n=250/replica). Pilot commercial diets were formulated in a liquid concentrate containing 8% of industrially produced microalgae biomass in dry weight (DW), and juveniles were fed daily with an amount equivalent to 8% of the oyster dry meat (g) in DW of microalgae (g).

Both diets contained a blend of microalgae species commonly used in oyster nutrition (Anjos *et al.*, 2017). Diet 1 was composed by *Tetraselmis chui*, *Skeletonema costatum*., *Tisochrysis lutea* and *Pavlova* sp., whereas diet 2 (Fig. 2) was composed by *T. chui*, *S. costatum* and *T. lutea*. Biochemical analysis of proximal composition of the pilot diets was evaluated.

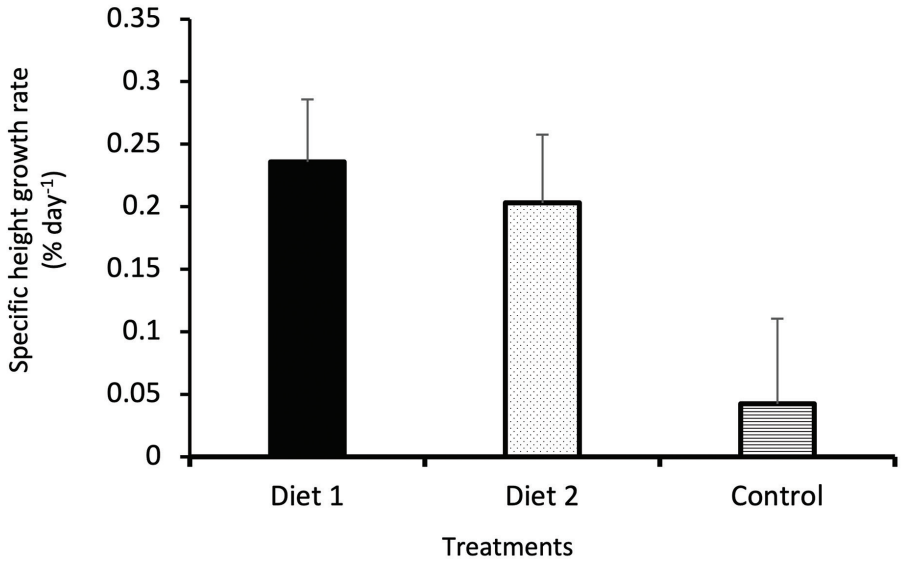

Figure 3. Specific growth rate (% day-1), in height, of C. angulata juvenile oysters.

Oysters were sampled (n=50/replica) monthly for three months for weight, growth through biometry evaluation, survival and specific growth rate (K) (Sami *et al.*, 2020). Samples were collected for further dry weight analysis and condition factor evaluation. The environmental conditions of the tanks and Rio Mira were daily monitored. Proximal composition showed the proteins, lipids and ashes content of Diet 1 (23%, 10.9%, 63%) and Diet 2 (9.9%, 2.1%, 82.2%) respectively.

Results

There were no significant differences in the survival rate in all treatments. Juveniles fed with Diet 2 showed higher and less variable wet weight and length gain than Diet 1 and control, along with a higher specific growth rate in terms of height (Fig. 3). A decision tree approach was applied through a CHAID method to the clusters obtained, with wet weight as the dependent variable (n=266). In the small sized oyster cluster group, Diet 1 and control promoted the highest wet weight below 40.6 mm, while above this length, Diet 2 improved oyster's weight. In the medium sized oyster cluster group below 40.6 mm, Diet 1 showed the highest wet weight. In the large size oyster cluster group below 40 mm, Diet 1 and control had the highest weight, while in oysters above 40mm, Diet 2 showed the highest value.

In conclusion, oyster growth with diets was more constant throughout time than in the natural environment. This fact may be related to the environmental fluctuations which can promote variability in microalgae species and abundance, impacting oyster growth.

Prototype Diet 2 promoted high and steady growth in Portuguese oyster juveniles. This product is a balanced diet based on microalgae species and proportions used in oyster hatcheries (Anjos *et al.*, 2017; Rato *et al.*, 2018). Prototype Diet 1 promoted an equivalent wet weight to oysters grown in the natural environment below 40.6 mm. Higher protein and lipids content in Diet 1 can support the high requirements for the growth of smaller oysters. To enhance wet weight gain in Portuguese oysters, a dietary protocol based on Diet 1 until 40.6 mm and Diet 2 after this length, is recommended.

This study allowed the development of innovative microalgae diets specifically formulated for bivalve growth to support the optimization of bivalve nurseries management.

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Status of Artemia cysts use and its future sustainable production

With the expansion of hatchery production, the demand for Artemia cysts has continued to increase. Annual consumption is now estimated at 3,500-4,000 tonnes, underpinning the production of over 900 billion crustacean postlarvae and fish fry by a hatchery industry valued at more than USD 2 billion, and the final production of over 10 million tonnes of high-value aquaculture species.

In the 1960s, two companies in the U.S. started marketing Artemia cysts collected from the salt ponds in San Francisco Bay, California, and the Great Salt Lake, Utah. As of the 1960s, different research institutes developed the first hatchery protocols with Artemia nauplii as a crucial live food source, initially in Japan with Japanese seabream and kuruma shrimp and soon thereafter in other parts of the world with other fish, shrimp and prawn species.

Despite initial concerns on Artemia production costs and availability, confidence increased with improved Artemia production techniques. With the creation of the Artemia Reference Center in 1978 at Ghent University and with an international interdisciplinary approach, experts in Europe and the Americas addressed several issues, such as processing cyst techniques, hatching process, storage, etc.

As of the 1980s, and especially in the 1990s, the commercial hatchery industry experienced a boom, particularly with marine shrimp aquaculture growth in Latin America and Asia, and with marine fish in Asia and Europe. Annual Artemia cyst consumption increased from less than 100 tonnes in the 1980s to over 2,000 tonnes by the turn of the century.

Currently, commercially available cysts are harvested from the Great Salt Lake in North America, from several large salt lakes and coastal salt works in Asia, and from controlled production (but still in moderate quantities) in seasonal salt works in Southeast Asia.

As has happened with several lakes in the past decade, inland salt lakes are under constant threat of drying up, and with climate change, this situation could only worsen in the future. With approximately 90% of the current Artemia production harvested from inland salt lakes, the future of the hatchery industry could be at risk and requires urgent attention. To address these issues, two workshops took place in September 2021, organized by Ghent University's Artemia Reference Center, the Network of Aquaculture Centers in Asia-Pacific, the Artemia Association of China and the Asian Regional Artemia Reference Center, to explore needs and opportunities for a new international initiative to guarantee a more sustainable provision of Artemia.

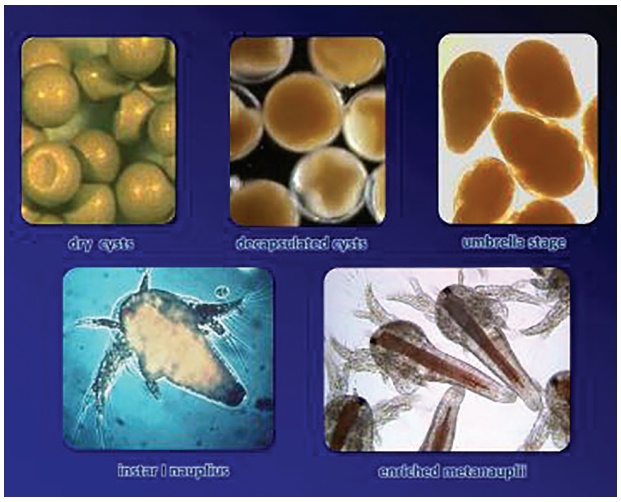


Photo credit: Artemia Reference Center, Ghent University, Belgium.

Artemia use around the world

Experts presented differences in practices in the use of Artemia cysts by fish and crustacean hatcheries in different countries.

China

China has the world's largest market demand for Artemia. The country annually consumes 1,700 MT of dry Artemia products (50% of global production)

Dry Artemia hatching and harvesting process in Thailand, presented by Montakan Tamtin, director of the Samut Sakhon Coastal Aquaculture Research and Development Center, from the Department of Fisheries in Thailand.

of which 76% are for shrimp hatcheries, 15% for *Macrobrachium* and 9% for marine fish.

In China, there are specific demands for Artemia cysts from different origins. Marine fish larvae need Artemia with better separation of the nauplii from the shell, such as Tibet and Ebiet cysts, and *Macrobrachium* larvae prefer faster swimming Artemia nauplii, such as BHB cysts. Artemia cysts with higher hatching temperature tolerant cysts are preferred in the summer in Hainan Province and lower hatching tolerant salinity cysts are preferred in regions with low-salinity seawater (1%) in certain seasons.

Hatching facilities and techniques have been significantly improved in recent years. Some hatcheries have their own Artemia facilities following standard procedures. To facilitate shell separation, at the end of the hatching period, light and aeration are turned off and a separation powder is added (H_2O_2 or sodium percarbonate, 5 ppm). After ten minutes, shells and nauplii are separated.

Artemia nauplii hatcheries are a new business model that produces Artemia by a specialized company near the hatcheries. It started in 2005 in the south and currently operates all over China. These companies annually produce 500-1,000 MT nauplii biomass, accounting for 50-80% of the total nauplii demand in China. This Artemia is sold fresh or frozen.

China is also a producer of Artemia cysts. The country annually harvests 800-1,200 MT of dry cysts, but still has to import from other countries, such as Russia, Kazakhstan and Uzbekistan, to fit the country's demand. The country also exports 400-600 MT of dry Artemia.

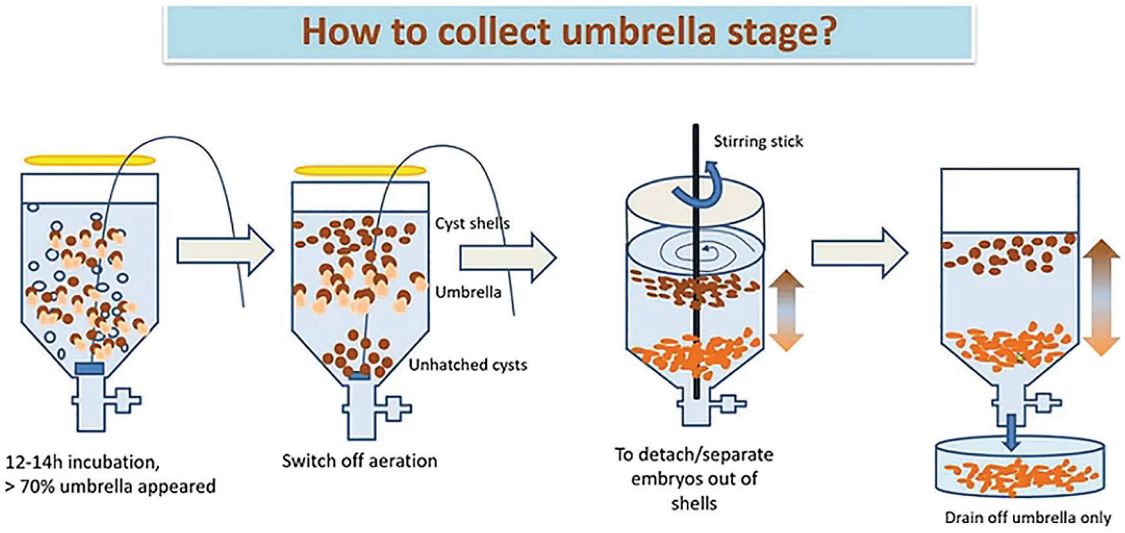
Other types of Artemia used in the hatcheries are decapsulated Artemia cysts and Artemia biomass (100,000 MT harvested annually).

Thailand

With 468 shrimp hatcheries and nurseries in 2019, the country has an estimated annual shrimp production of 66.58 billion Nauplii and 33 billion PLs. Thailand imported 634 tonnes of Artemia cysts in 2019 and 482 tonnes in 2020, 90% from China, Russia, Uzbekistan and Kazakhstan and 10% from the U.S., according to the Fish Import and Export Control Group.

Dried Artemia cysts are used following the traditional protocol harvesting Instar I or enriched Instar II. Chilled Instar I and II are also available in the market providing more quality and quantity consistency. Raw Artemia cysts are also used, as well as shell-free Artemia cysts. Shell-free Artemia cysts are 30-40% cheaper than standard Artemia cysts and are used to feed PL5 onward.

In the past for the production of *Penaeus monodon* selling at PL 15-16, enrichment techniques were



Incubation process of Artemia umbrella stage presented by Nguyen Van Hoa from CanTho University.

used, but currently, enrichment is considered not necessary for *Litopenaeus vannamei* selling at PL10. Some farms do a short enrichment adding vitamins and lipid emulsions.

Large-scale hatcheries use 90-100% of chilled Artemia Instar I&II and 10% of traditional dry Artemia cysts and medium-scale hatcheries use 40% of chilled and 60% of traditional cysts, or 100% traditional dry Artemia cysts in most eastern hatcheries. Small-scale hatcheries use 100% traditional dry Artemia cysts.

Asian seabass hatcheries rarely use Artemia since they use zooplankton from earthen ponds to reduce production costs.

India

In India, there are 401 shrimp hatcheries with a total PL production in 2020 of 70 billion. The country estimated Artemia consumption in 2020 was 325-345 MT with the U.S. as the main sourcing country. The average Artemia consumption per million PL produced is 3.8 kg. The country uses GSL Artemia following the standard procedures. Enrichment is sometimes used with micro-nutrients for frozen Artemia.

Probiotics (5-20 ppm), chlorine dioxide (2-5 ppm), or ozone (300-350 ORP) are applied during hatching to reduce the bacteria load, also improving the hatching rate by 2-5%. For nauplii, probiotics (10-20 ppm) and/or bacteriophages (5-10 ppm) or povidone-iodine (20-30 ppm) are used to reduce and control the bacteria loads.

For zoea and mysis stages, inactivated Instar I is preferred, using frozen nauplii (frozen for 6-8 hours before feeding the larvae) or heat-inactivated nauplii (boiled).

Temperature is the most challenging parameter due to the high fluctuations between day and night, especially in winter, and as its control is too expensive for Indian hatcheries. The lack of light and pH control also affects the Artemia hatching output, as well as Vibrio contaminations and the lack of skilled labor.

Vietnam

Artemia cysts in the Mekong Delta in Vietnam are mainly imported from the U.S., Russia and China. The country has a small production of Vihnchau Artemia that does not meet the country's consumption. Hatcheries use dry cysts following the standard protocol. For large-scale operations, chilled Instar I&II are also available.

A recent innovation developed in the country was the replacement of rotifer with Artemia umbrella stage in zoea stages of mud crab larvae. The replacement was made with the small Vinhchau Artemia strain. To get the umbrella stage, the same incubation process as the standard protocol is followed for 12-14 hours. At this

time, <70% of the umbrella stage appear. Aeration is stopped and a stirring stick is used to separate embryos from shells and harvest the umbrella Artemia.

Viet-Uc, the world's largest shrimp hatchery, built its first shrimp hatchery in 2001 and now operates nine shrimp hatchery units that supply 15-20 billion postlarvae per year with an Artemia consumption of 60-80 tons per year, hatching 30-100 kg of Artemia per day. The company uses Artemia dry cysts and has upgraded its Artemia production facilities to reach this production implementing room temperature and light control, larger hatching tanks and pH control.

Bangladesh

The number of monodon shrimp and prawn (*Macrobrachium*) hatcheries in operation in Bangladesh in 2021 were 56 and 6, respectively. The number of PL produced per year was 10-14 billion shrimp and 30 million prawn and the amount of Artemia cysts required per year was 50 MT.

The country uses Artemia dry cysts following different procedures close to the standard procedures, but that require improvements for better hatchery management. Nauplii disinfection treatments used before feeding larvae are chloramine-T, formalin, antibiotics, probiotics and C vitamin.

In 2020, an EU-funded Artemia project (Artemia4Bangladesh) successfully locally produced Artemia cysts and biomass with the aim to reduce import dependency and open the scope for increased income of salt farmers.

Europe

Mediterranean marine fish hatcheries hatch and harvest Artemia in their own facilities with full control of the rearing parameters. They were the first to adopt cold storage of Artemia nauplii and now use milk storage tanks. The technique of cold storage needs to receive much more attention as it allows to keep the Artemia under the most nutritious condition for a prolonged time, while making the production of Artemia less labor intensive as the hatching of the cysts can be concentrated in a more limited period using the resources in a more efficient way. Furthermore, live Artemia storage also allows more frequent feeding of the fish/shrimp larvae, eventually with automatic pumping devices.

Artemia consumption for seabass and seabream in a Mediterranean commercial hatchery (Avramar) is around 50 kg of Artemia cysts per million fish produced and 100 kg for meagre, since it is faster-growing species.

Ecuador

In Ecuador, 386 shrimp hatcheries produce 159,479 million Nauplii and 79,739 million PLs. The country imported 194,331 kg of Artemia cysts in 2020, 60% from Russia and 40% from the U.S.

The hatching procedures do not monitor the main hatching and rearing parameters. Most of the Artemia cysts (98%) are decapsulated and 2% of hatcheries use direct incubation.

Peroxide or brine salt solutions are added to the hatching tank for cyst shell separation. Nauplii can be disinfected with formalin (10 ppm) or hydrogen peroxide (50 ppm). Freshly harvested and disinfected Artemia nauplii are seldom offered to shrimp larvae but they can also be frozen in plastic bags, or heated (dipping in hot water) and then frozen for later use. Heating Artemia might kill Vibrios but also damage the nauplii.

Recommendations

Over time, the practices used by hatcheries in Asia, Europe and Latin America have diverged from the good aquaculture practices for Artemia production recommended by FAO's 1996 live food manual. There is room for improvement in optimizing the use of Artemia cysts in aquaculture hatcheries, and this should result not only in economizing its use but especially offering a highly nutritional and Vibrio-free food to the baby fish/crustaceans.

To guarantee a more sustainable provision of Artemia, both from natural sources and from controlled extractive Artemia farming integrated with salt production and other fish/crustacean aquaculture, the workshop made the following specific recommendations:

- Develop improved guidelines for biosecure production and use of Artemia in hatcheries, including an update of the FAO Artemia manual and convene regional Artemia training courses for local hatcheries, to disseminate good practices and facilitate the adoption of standardized protocols.

- In view of the large variety of species and strains of Artemia that are now available in the market, their specific characteristics should be studied to identify their most suitable application for specific species of fish and crustaceans. This could relate to their nutritional composition, synchrony in hatching or enrichment characteristics.
- Initiate strain selection and selective breeding to develop improved Artemia strains for aquaculture applications, noting the availability of the Artemia genome.
- Investigate the use of umbrella Artemia as successfully applied in the Vietnamese crab hatcheries for wider application in aquaculture, as a new source of live food in earlier larval stages, be it for shrimp or in fish.
- Reconsider a wider use of Artemia enrichment techniques in hatcheries, as it is now restricted to applications in marine fish and crab production. This method not only allows enhancement of the nutritional value of the nauplii but can also be used as a vector to deliver, for example, pre- or probiotics to the larvae.
- Investigate the impact of climate change on Artemia production in inland lakes and coastal saltworks.
- Develop science-based protocols to assure sustainable harvesting of wild Artemia sources, especially in central Asia.
- Conserve Artemia biodiversity through means such as cyst banks, species identification, “wild” vs “farmed” species, genotyping and strain characterization.
- Investigate integration of extractive Artemia farming with intensive fish/crustacean aquaculture.
- Investigate the use of Artemia biomass as high-value protein ingredient in human diets.
- Consider integration of Artemia production in artisanal salt farming in Asia and Africa, desert/arid and salt-affected areas.

An International Artemia Aquaculture Consortium (IAAC) is planned to be launched soon for further improvements.

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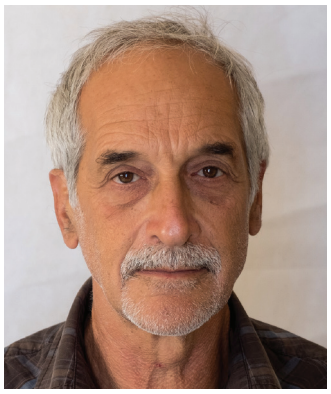
Developments in marine fish hatcheries



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Dry food or live food for larval first feeding

The manager of the fish hatchery where I was first employed had previously worked for a company that was developing a dry feed for substituting live feed. That was (can I say this) in 1987. That diet never materialized. Since then a considerable effort has been invested into developing hatchery diets. This has resulted in a range of feeds for co-feeding larvae alongside live feed, as well as reducing the live feed stage through early weaning. However, eliminating live feed through commercial substitutes has not yet happened.

Over the years, and to support the development of the hatchery industry, a very significant body of literature on feeding and nutrition in fish larvae has been generated. The development of visual, locomotory and digestion systems, as well as specific nutritional requirements for fish produced in our hatcheries on a massive scale, have been well described and there are excellent reviews that are easily accessible (Zambonino Infante *et al*., 2008; Rønnestad *et al*., 2013). This short column will examine some of the major issues that have impeded the development of inert first-feeding diets.

The world of fish is extremely diverse, and in the diverse water habitats fish have developed a range of specific adaptations. The subject of this column is those fish that are raised in our hatcheries, larvae that hatch from small floating eggs, hatch with a yolk sac and start feeding on small zooplankton. The academics call them "altricial larvae". For such a larva to start feeding, an artificial diet would need to have the specific attributes that will elicit a feeding response, be small enough for the larvae to swallow, digestible, and contain all the nutrients that the larva needs for its development.

Larvae of sablefish (*Anoplopoma fimbria*) first feeding on rotifers.

The technical process for producing a small particulate diet in the size range 60-150 µm is well established. However, providing a complete nutrient pack at this size range, from which the nutrients will not leach until it is eaten by the larvae, which could take a couple of hours, has yet to be resolved.

Larvae feeding is elicited through visual cues which is why eye pigmentation develops prior to first feeding. Light is essential for developing feeding behavior. On top of that, we also know that the larvae are attracted by the movement of their food in the water. Zooplankton actively moves and maintains itself in the water column. Inert diets on the other hand tend to sink, albeit slowly. Although it is possible to keep diets in suspension through aeration and water currents, these same currents also move the larvae and impede them from focusing, homing in and capturing their prey in one single lunge.

Things get even more complicated once the feed passes through the esophagus and into the digestive

tract. The digestive tract of the larvae at first feeding is very delicate, a tube that is constructed from a single layer of cells (epithelium) that make up the inner coating of the tube which are held together by a diffuse connective tissue. This tube is then surrounded by a layer of muscle cells that work to move the food backward through the digestive tract.

Fish do not have salivary glands that will soften and initiate the digestion of food. Instead, they have cells that release a mucus substance that protects the epithelial cells from abrasion by the food. The number of these cells and the secretion of mucus-like substances at first feeding is very low.

All this histological evidence indicates that a dry diet at this stage for replacing live food would need to be very soft and lack any sharp edges. Otherwise, its physical effect on the digestive tract would be like that of sandpaper, irritating and destroying those delicate epithelial cells.

Once the food particle is caught and ingested it needs to be digested. Marine fish larvae at start feeding do not have a functional stomach. Stomach development only takes place towards the end of the larval phase. Once the stomach develops and acid secretion takes place then, within the acidic medium that is created in the stomach, pepsin is released and the larvae acquire the capacity to break down complex proteins into short peptides and amino acids.

But at start feeding, in the absence of a stomach, the proteins are broken into long peptide chains by the enzyme trypsin. While short peptides and amino acids can be directly absorbed from the intestine into the body, the long peptides need to be actively transported into the body by the epithelial cells through a process of pinocytosis. This process is both energetically costly as well as requiring particular cellular structures (membranes) for carrying out the process.

Proteins are the building blocks that the larvae need for both development and fuel. Depending on the species, their weight increase rate is equivalent to 10% and up to 50% body weight per day as the larvae require a lot of protein. Unfortunately, having only trypsin at their disposal, their digestion of proteins is limited. One of the major advantages of zooplankton over inert diets is that they contribute both digested nutrients as well as enzymes that augment the digestion capacity of the larvae.

The search and research for first feeding diets will continue. There is a market for such a product and we prefer “off the shelf” rather than on-site production. Nevertheless, I sometimes wonder if the effort is moving in the right direction. Do we really need to replace rotifers? Rotifers have paved the way to a very successful marine fish farming sector. They are easy to culture in mass, have the ideal size range, are easily digested and can be boosted with the nutrients that are required by the larvae. We could continue using rotifers and possibly, by applying genetic tools, develop rotifer clones that synthesize molecules such as DHA, taurine and others which they are not capable of doing. Venturing into GM rotifers poses an ethical dilemma. However, as rotifers have been a core driver of the marine fish hatchery sector, should we not be improving rather than replacing them?

Selecting shrimp broodstock for small-scale indoor shrimp farming

Eric De Muylder, CreveTec

During the last couple of years, there has been an increased interest in small-scale indoor shrimp farming using RAS of biofloc systems. This interest was mainly driven by the market for fresh shrimp, with superior taste and texture compared to cheaper imported, frozen shrimp.

However, the European Union has forbidden any import of postlarvae from any other country than the U.S. Since Hawaii was not really an option, the available hatcheries that could supply the EU were limited, with interruptions in supply due to cold weather, and even destruction by a hurricane. Furthermore, it was observed that undersized postlarvae, measuring only 6-7 mm were supplied as PL11, which had difficulties coping with the long transit time, which would usually be more than 36 hours from hatchery till the farm. Poor survival rates were observed with these imported postlarvae.

Therefore, some attempts were made to produce postlarvae in the EU and three hatcheries are now in production. The postlarvae produced by these hatcheries made a big impact on survival rates, giving an economical future to growing shrimp in Europe. However, most hatcheries still rely on imported broodstock, which are believed to be genetically superior. The selection criteria for these broodstock were mainly to be SPF and faster growth.

CreveTec's farm in Belgium

CreveTec operates a biofloc farm in Belgium, which is quite unique since it has been able to use the same water for six consecutive years since its startup. The farm was initially filled with rainwater with artificial salts. The yearly analysis shows that the minerals are still balanced.

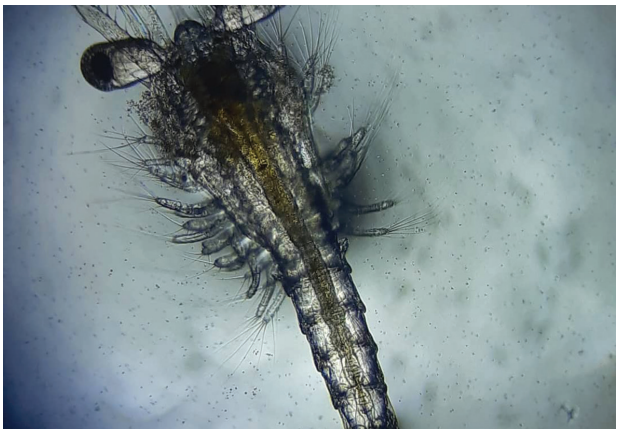
Since the production of shrimp based on imports from the U.S. was not economically feasible, attempts

were made to grow larger shrimp from the best production batches as a source of broodstock for further reproduction. After more than two years of trial and error, nauplii were produced and slowly a system was developed to produce postlarvae in a closed recirculation system. In 2021, a new maturation facility and larval rearing unit were built inside the same greenhouse where the grow-out tanks of the farm are located.

This maturation facility presently hosts broodstock from the 3th generation, producing 4th generation postlarvae.

Table 1. Average growth and survival observed during various feed trials (1-8 gram).

Period	PL Source	Average weekly growth (g/week)	Average survival rate (6 weeks)
2015-2017	Imported from the US	1.17	76%
2019-2021	Produced on the farm	1.18	85%



Operation

The water quality of the maturation tank is maintained with bioflocs, a drum filter and a biofilter. About 20% of water is added every week from the grow-out tanks to compensate for the drum filer discharge water, which goes back to the bioreactor of the grow-out farm.

The maturation of females is about 10% per day, from which 8% is fertilized properly. Eyestalk ablation is not used and broodstock is used for 6-8 months on average.

Gravid females are transferred to a larval rearing tank and spawn between 200 and 300,000 eggs, since most females are 60-75 grams. After spawning, the female is moved back to the maturation tank. Nauplii hatch in the same tank and unfertilized eggs and waste are siphoned out. No disinfection is used during the process. The tank is gradually filled with algae water. After metamorphosis to Mysis, mature water from the biofilter system is added to fill the larval rearing tank and exchange water back to the biofilter. Sometimes batches are lost or show poor survival, which could be due to bacterial infections. But since the aim is not to sell a lot of postlarvae, but to select the strongest animals, that is not an issue.

Selection process

All batches of postlarvae consist of 1, 2 or 3 individual spawns. They are transferred to a nursery tank at PL15-PL20 stage. The growth and survival are monitored on each batch of postlarvae.

Every six months, a new batch of broodstock is selected from the best performing grow-out tanks. Performance is a combination of survival and growth. The best growers from the best tank are selected to be prepared for maturation in a separate pre-maturation tank. This way, we can select, generation after generation, a breed that is adapted to intensive culture in a closed system.

In Table 1, it can be observed that growth was not improved, but the survival rate increased a lot, indicating the postlarvae produced on the farm are stronger and better adapted.

Biosecurity

This project shows that breeding can be controlled in a closed, biosecure facility. For more than two years, no external shrimp broodstock or PL were taken into the farm, which is the best guarantee for disease-free animals. Since the water source is rainwater (to compensate for evaporation), this potential contamination source is also avoided. A project like this can be built anywhere inland, preferably far from other farms or hatcheries, but still able to supply breeders locally and continuously to avoid expensive imports. Furthermore, the project could mimic the environmental conditions for the target farms in a controlled environment.

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Genetic selection and sustainable aquafeed in the farming of Malaysian giant freshwater prawn

Giva Kuppusamy, M. Janaranjani, Farahani Muhammad Azam, Wan Nur Hazwani,
GK Aqua Sdn Bhd

Figure 1. Female (A) and male (B) giant freshwater prawn. Phenotypically selected brood on right for A and B. Source: GK Aqua Sdn Bhd.

Towards the circular green economy of freshwater prawn farming

GK AQUA is an emerging biotechnology company formed to commercialize giant freshwater prawn (*Macrobrachium rosenbergii*) by implementing its pioneering technology to improve its farming. *M. rosenbergii*, commonly known as Malaysian giant freshwater prawn, is a native species in tropical regions in Southeast Asia and Northen Australia (Gallo *et al*., 2016).

This respective species has turned into a potential candidate in aquaculture due to its adaptability to tolerate a wide range of temperatures (14-35°C) and 0-25 ppt salinity (Cheng & Chen, 2000). The production of farmed *M. rosenbergii* has increased 13,900% since 2008 from its production of 3,000 tonnes 30 years ago (Banu & Christianus, 2016). Although the global culture of giant freshwater prawns faced tremendous growth, Malaysia faced major bottlenecks with inconsistent production and poor survival rate of the larvae. However, with a research methodology and established protocol, GK Aqua successfully overcame the bottlenecks with two approaches, genetic selection and nutrient manipulation.

Genetic selection

A crucial element to selectively choose specific or targeted traits in cultured species to enhance the phenotypic traits such as growth, body colors and disease resistance is genetic selection. Giant freshwater prawn is a unique species with the presence of five morphotypes of males, categorized as small claw (SM), orange claw (OC), blue claw (BC), old blue claw (OBC) and no claw (NC) (Sagi & Ra'anan, 1988).

Figure 2. Incorporation of Sesbania's nutrient into BSF meal. Source: GK Aqua Sdn Bhd.

Each type of male prawn possesses its own unique characteristics. However, among all, males with blue claws are generally the largest and have the fastest growth rate (Dinh & Nguyen, 2014) which would make the ideal candidate as an aquaculture species. Female prawns have a slower growth rate compared to males and abstain from varied morphotypes.

Considering the quality of offspring produced, GK Aqua specifically focuses on the brood prawn phenotypic selection (Fig. 1), screened for diseases before the prawn was transferred to mating tanks. Further, submission of a Bio-project (SAMN24815316) on *M. rosenbergii* genome in Genbank is our onward step to further this genetic selection research via genotypic level. We believe a complete analysis through both phenotypic and genotypic selection of broodstock will give promising results on the post-larvae obtained in the near future.

Broodstock feed improvement

Another significant approach to enhance the quality of broodstock prawns is through feed manipulation and nutrient retention. Massive studies have been reported on protein (Teshima *et al.*, 2006; Sarman *et al.*, 2011), lipid (Cavalli *et al.*, 2001; Sun *et al.*, 2020), herbs (Radhakrishnan *et al.*, 2014), insect meal (Feng *et al.*, 2019; Amiruddin *et al.*, 2021) and cereal and pulses (Bhavan*et al.*, 2010). However, there is still a paucity of knowledge of sustainable aquafeeds that meet the nutrient requirements of cultured prawns that are environmentally sustainable.

The preliminary research conducted by GK Aqua on putrefied sesbania (*Sesbania grandiflora*) fed black soldier fly (BSF) meal (Fig. 2) showed higher deposition of protein (43.5%), 2-fold lesser lipid (16.7%) than the BSF meal obtained from domestic/kitchen waste substrates and significant deposition of amino acids (Kuppusamy *et al.*, 2020). It was reported that the conventional method of BSF rearing with agricultural byproducts, domestic wastes and other organic wastes causes a higher accumulation of heavy metals, such as chromium, copper, lead, zinc, cadmium and mercury (Kuppusamy *et al.*, 2020; Elechi *et al.*, 2021). However, interestingly, the BSF meal reared on sesbania showed a substantial reduction in chromium (9-fold), selenium (3-fold) and mercury (undetected) content than those prepared conventionally. This could solve many foods

Figure 3. Berried giant freshwater prawn female.

security issues, where the source of origin and hygienic condition of cultured substrate is a major consideration in the deliberation of BSF meal as a possible alternative to other aquafeed ingredients (i.e, fishmeal), which will be used for animal feed in turn for human consumption. Sesbania is a plant from the family Fabaceae and is massively used in agriculture as green manure to enhance the production of food crops due to their unique characteristics of rapid growth, adaptation to survive in varied types of soil and tendency to increase soil fertility (Evans & Rotar, 2020). To date, Sesbania sp. has been evaluated for its extraordinary nutraceutical properties, as anti-inflammatory, anti-bacterial, anti-oxidant, anti-mutagenic and its capability to eliminate heavy metals (Goswami *et al*., 2016; Pinakin *et al*., 2020). Our interest in sesbania began when we brought up the consideration of the circular green economy, whereby we strictly hindered the usage of marine-based ingredients and instead produced potential aquafeed constituents that promoted economic and environmental sustainability. Utilization of sesbania via BSF will not only promote the growth and immune parameters of cultured prawn but also the plantation of sesbania positively regulates the carbon neutrality and nitrogen cycle in the environment which can be a solution to many global issues such as global warming and pollution. With this, GK Aqua believes, the exploitation of marine resources to produce aquafeed ingredients can be significantly reduced, environmental balance can be sustained and the quality and nutrient retention of brood prawns can be enhanced.

References available on request.

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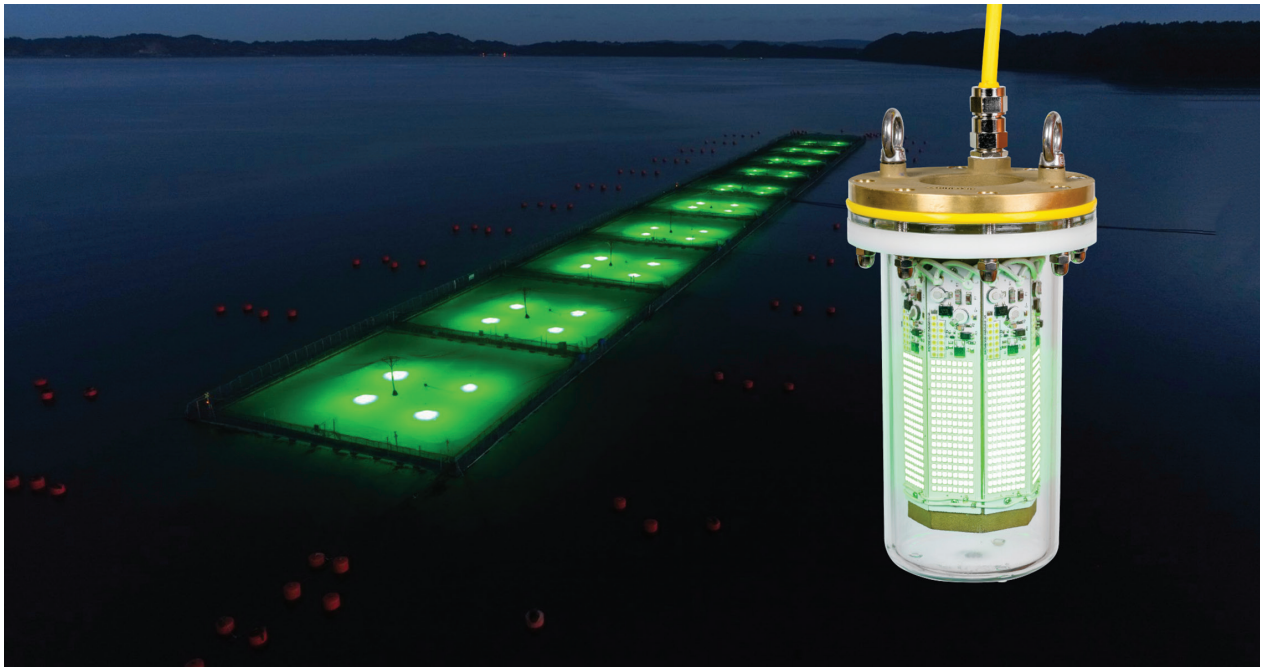
Photoperiod with artificial intelligence demonstrates improved salmon smoltification

Marco Galaz, Luxmeter

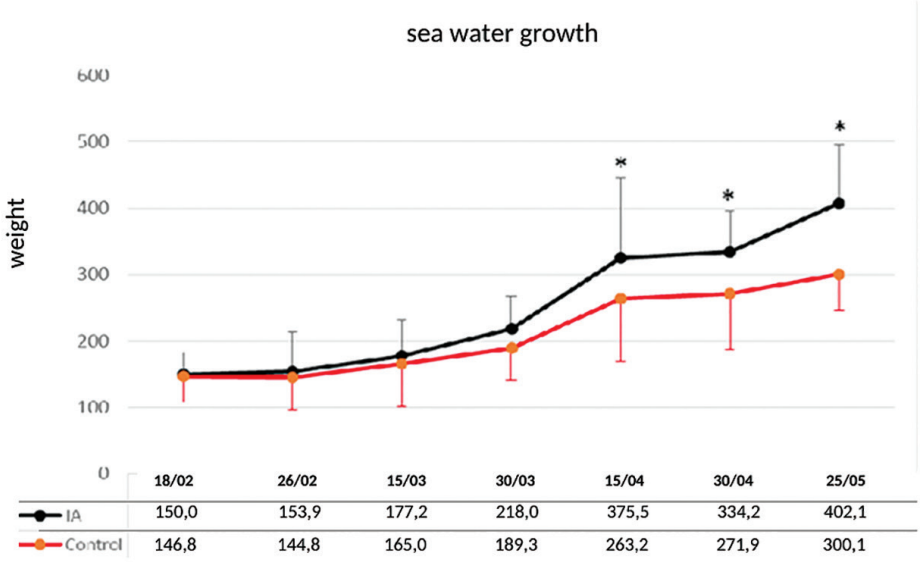
A study carried out by Luxmeter for more than eight months compared the growth of smolts using a new photoperiod with AI against the standard control system. Results showed 35.7% more fish growth, greater strength and maturity at the time of seawater transfer.



High enzyme levels and greater growth of Atlantic salmon were the conclusions of a study carried out by Luxmeter, a Chilean company with 20 years of experience in lighting, demonstrating that the use of its photoperiod system with Artificial Intelligence (AI) effectively stimulates the growth of smolts from their land-based phase to later consolidate in seawater.

The results of the study provided relevant information that accounts for a rise of more than 35.7% in the total growth of the smolts, which corresponded to 19.1% during the first four weeks in seawater, indicating more homogeneous conditions for the health of fish, and improving and stimulating their gill health from the fish farming stage.

"At Luxmeter, we are focused on taking a leap into the new era of photoperiod with artificial intelligence and the use of software that allows us to improve the control of different factors that affect the lighting conditions of fish. We have great potential at



	18/02	26/02	15/03	30/03	15/04	30/04	25/05
IA	150,0	153,9	177,2	218,0	375,5	334,2	402,1
Control	146,8	144,8	165,0	189,3	263,2	271,9	300,1

Figure 1. Seawater growth of salmon smolts after the exposure of AI and control photoperiod.

a corporate organization level, to provide expert advice based on reliable results," said Marco Galaz, Luxmeter's CEO.

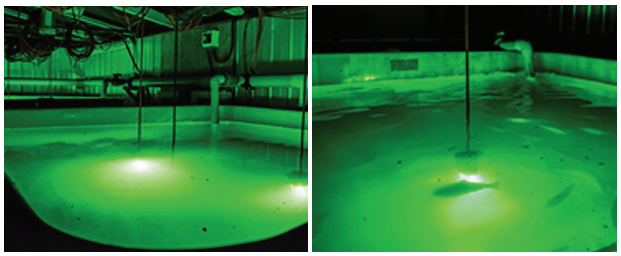
The study, carried out together with CORFO, the Chilean government agency for innovation and entrepreneurship, was performed during the smoltification phase. In addition, the levels of Na+, branchial K+ ATPase, melatonin and weight control of the fish were analyzed.

Through this type of experience, Luxmeter has expanded its range of services, and together with CORFO and Fundación Chile, have expanded the use of the photoperiod system to other types of species such as corvina (*Cilus gilberti*).

In addition, the positive results that this study showed can continue to achieve encouraging results due to the launch of the new Luxmeter 1,000-watt iLED submersible lamp with a copper body and lighting from the leading Japanese company Nichia Corporation that enables a 30% increase in the efficiency of the light and longer life with less maintenance, making this technology is increasingly profitable.

The proof

In a first stage, a team from Universidad de los Lagos, Chile evaluated at the Rupanco Lake Experimental Fish Farm (PELR) a system based on artificial intelligence to maintain constant light intensity to improve the process and quality of the smolt, comparing it with the standard control process.



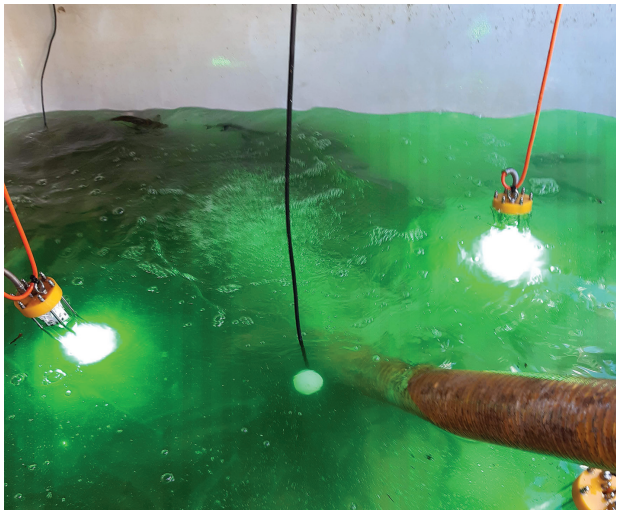
Smolt tank with AI photoperiod (left) and with control photoperiod (right).

For this phase, two 4 m^3 ponds were used with more than 1,000 salmon of a similar weight and size with 24 hours of constant light for 6 weeks, artificially creating a summer light condition. Subsequently, it was changed to a winter phase of 8 hours of light for 12 weeks. Through these conditions, a culture density higher than 35 kg/m^3 was reached and the initial strategy of 24 hours of light was resumed for 4 more weeks.

Increased growth in seawater stages

After this stage in freshwater, salmon were transferred to seawater conditions, showing that after 40 days in seawater, a higher growth was observed in the group with photoperiod based on AI, with 35.7% more growth compared to the control group. It's important to note that between the last samplings from April 30 to May 25, a growth increase of 19.1% was observed.

The greater growth of the AI group remained significantly different from the control group until the end of the experiment, with weights of 407.1 g (initial



weight ± 89.5 g) for the fish with AI and 300.1 g (initial weight ± 54.2 g) for the control ones (Fig. 1).

Branchial Na+ K+ ATPase analysis: Significant differences

During the first 5 weeks in seawater, although the ATPase curve of both groups showed the same trend and similar average values, it was observed that the enzymatic activity of the fish with photoperiod based on AI was much more homogeneous compared to the control group. This was reflected while analyzing the coefficient of variation (CV), which showed that in all the seawater samples, the group with AI always presented considerably lower CV values.

For 20 years, Luxmeter has developed a photoperiod system to raise production levels and has worked towards a modern aquaculture industry, focusing efforts on research and innovation for a comprehensive system that provides solid results. In 2022, Luxmeter is facing international growth, alongside corporate alliances and its innovative new iLed Cooper lamp, to strongly enter the Norwegian and Canadian markets.

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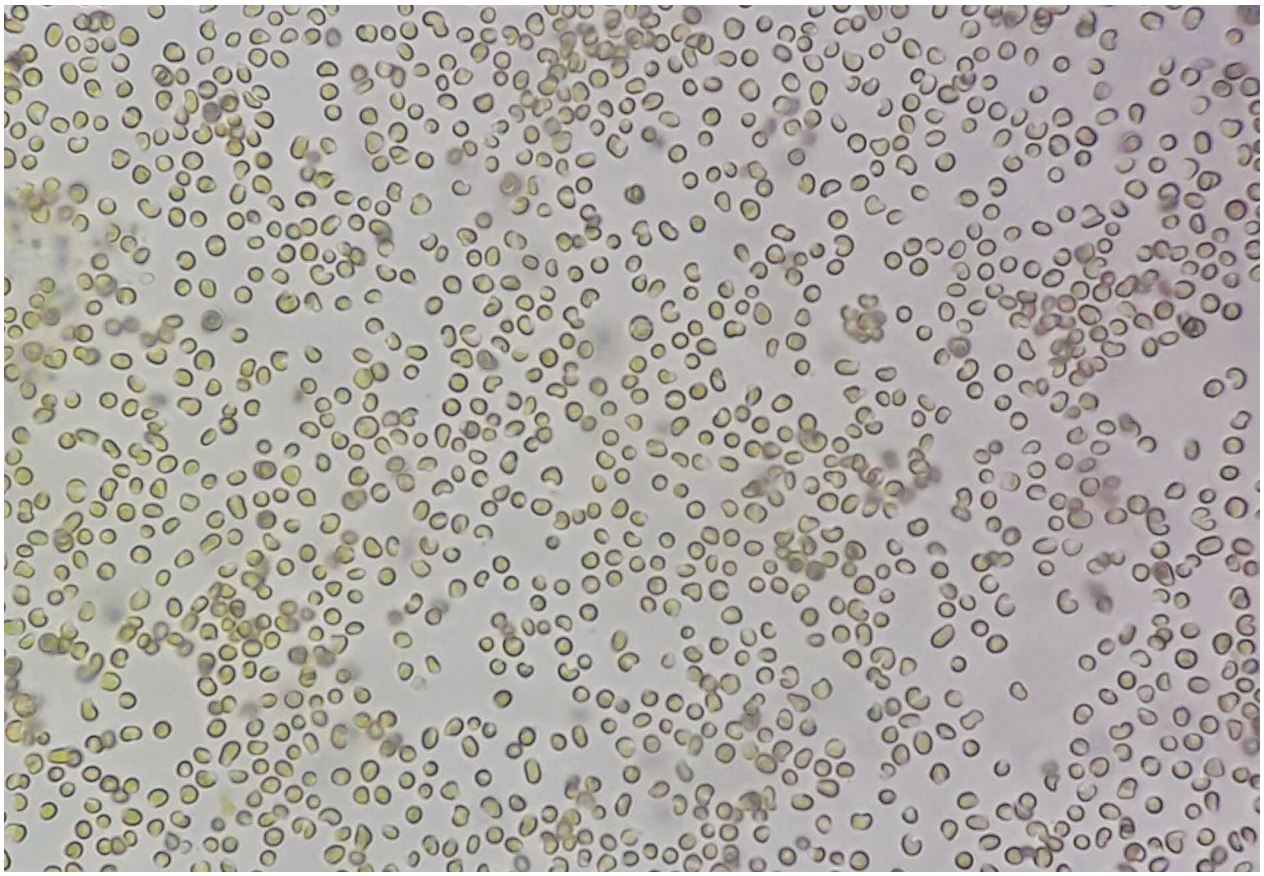
Photobioreactors to improve aquaculture hatcheries



Isochrysis galbana affinis Tahiti.

Synoxis algae designs and markets a wide range of automated high-density algae cultivation systems: photobioreactors, barrels, raceways. From microalgae to macroalgae, it provides different solutions for algae culture experts around the world from different sectors, such as food, cosmetics, research, green chemistry, and aquaculture.

As a spin-off of the plastics manufacturer Synoxis, existing since 1981, Synoxis algae has twenty years of experience in the field of aquaculture. In 2003, Synoxis participated in the thesis of Erell Olivo called “Design and study of a photobioreactor for the continuous production of microalgae in aquaculture hatcheries”, in partnership with Ifremer and the University of Nantes. This led to several years of research and development on algae cultivation systems and algal biotechnology.

Its range of microalgae photobioreactors is made up of three models: a small benchtop model NANO (3 L) for research laboratories, a nomadic intermediate model LUCY (16 L) for the production of inoculum and an industrial model JUMBO (285 L) for mass production.

Incorporating photobioreactors improve aquaculture hatcheries by dividing the volumes of fluid transferred and the number of containers by 15, dividing the working times by 10, dividing the area of the culture rooms by 5 and increasing biosecurity.

Today, Synoxis algae has 60 installations in France and abroad in all business sectors, especially in aquaculture.

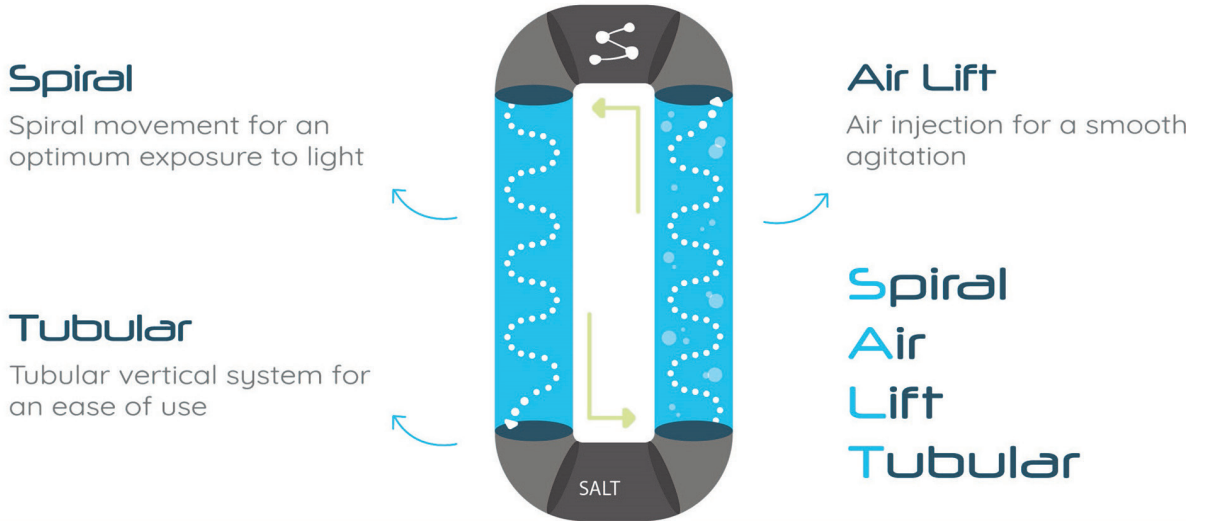


Figure 1. SALT technology.

"One and a half years ago, we acquired a LUCY photobioreactor for our shellfish hatchery to diversify our microalgae production techniques used to feed our animals. We were trained and adjusted the system to our premises and our way of working. We were able to count on the members of Synoxis Algae who were available and attentive to our needs, which allowed us to learn and work efficiently in close collaboration. Today, LUCY fully meets our expectations in terms of quality, performance and productivity and we are looking forward to continuing working with Synoxis Algae in the future," said Simon Ollier from Marinove in France.

Hight density culture

For microalgae culture, the company has developed a unique and patented technology (SALT) that reaches high cell density while avoiding biofilm formation. The system creates a vertical spiral movement with an airlift (by air injection) in a vertical tubular system. This agitation facilitates gas exchanges (carbon input and degassing in O_2) and favors light exposure while minimizing shear stress on the fragile microalgae cells strains.

For example, with *Isochrysis affinis galbana Tahiti*, the technology reaches 130 million cells per mL, compared to 5 million cells per mL in traditional systems.

Figure 2. **NANO** (left), **LUCY** (center) and **JUMBO** (right) photobioreactors.

This smooth homogenization allows the culture of a wide variety of microalgae, such as *Arthrospira platensis (Spirulina), Isochrysis galbana affinis Tahiti, Chlorella, Skeletonema costatum, Chaetoceros vulgaris, Rhodomonas salina, Chlorella autotrophica, Nannochloropsis gaditana, Cylindrotheca closterium, Odontella aurita, Phaeodactylum tricornutum, Porphyridium* and *Isochrysis sp.*

Continuous automated cultures

SALT technology is essential and works with an automated regulation system. All photobioreactors have with an identical control system to automate the essential needs. This automation reduces working times, optimizes consumption and maintains cultures in continuous production over several weeks or months. It also eliminates any problems while scaling up with Erlenmeyer and balloons.

Photobioreactor models

NANO is a 3 L benchtop photobioreactor used to test any culture conditions or strain identification. It has two separate chambers that allow the culture of several microalgae strains.

LUCY, with its 16 L and 1m² platform on wheels, is the easiest tool to start a small production or inoculate ponds.

JUMBO is designed for aquaculture. Depending on the hatchery needs, it can be a full-fledged production tool or an inoculum production tool. While comparing a JUMBO to a 300 L tank, both growing T-Isochrysis, JUMBO produces the equivalent of 20 tanks of 300 L, 20 barrels of 30 L, 20 balloons of 2 L and 20 Erlenmeyer of 250 mL.

The system is self-cleaning allowing users to stop and restart the photobioreactor easily and safely, without disassembly. The system secures the production of biomass throughout the year and the cultures can be carried out in semi-continuous or continuous, by bringing new medium.

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Industry Events

Send your meeting details to:
editor@hatcheryfm.com

2022

MARCH

25 - 28:	Aquaculture Africa, Egypt	www.was.org
28 - April 1:	XVI International Symposium on Aquaculture Nutrition, Online	sina.aema.mx

MAY

3 - 5:	Aquaculture UK	www.aquacultureuk.com
4 - 5:	AQUA EXPO Manabí, Ecuador	aquaexpo.com.ec
22 - 24:	Alltech ONE Conference, USA	one.alltech.com
24 - 27:	World Aquaculture 2021, Mexico	www.was.org
25 - 26:	Aquafarm, Italy	www.aquafarm.show

JUNE

5 - 9:	XX International Symposium on Fish Nutrition and Feeding, Italy	www.isfnf2022.org
8 - 10:	Aquaculture Symposium Guatemala	export.co.gt
8 - 10:	INFOFISH World Shrimp Trade Conference and Exhibition, Virtual	shrimp.infofish.org

JULY

19 - 21:	AQUA EXPO El Oro, Ecuador	aquaexpo.com.ec

AUGUST

3 - 5:	ILDEX Vietnam	www.ildex-vietnam.com
15 - 18:	Aquaculture Canada and WAS North America 2022	www.was.org
23 - 26:	Symposium on Diseases in Asian Aquaculture, Malaysia	www.daa11.org

SEPTEMBER

27 - 30:	Aquaculture Europe	www.aquaeas.org

OCTOBER

25 - 28:	AQUA EXPO Guayaquil, Ecuador	aquaexpo.com.ec

NOVEMBER

9 - 11:	ILDEX Indonesia	www.ildex-indonesia.com
29 - Dec 2:	World Aquaculture Singapore 2022	www.was.org

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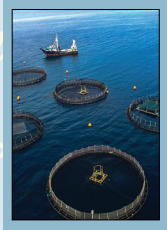

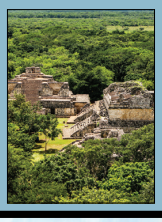

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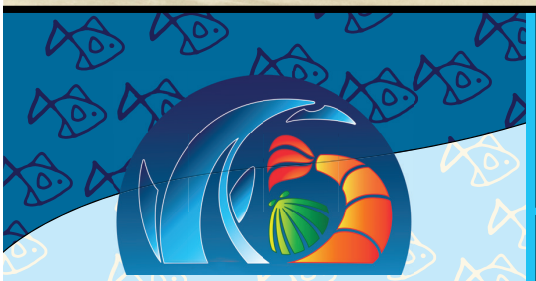

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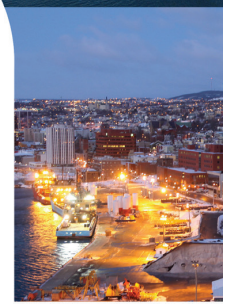

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