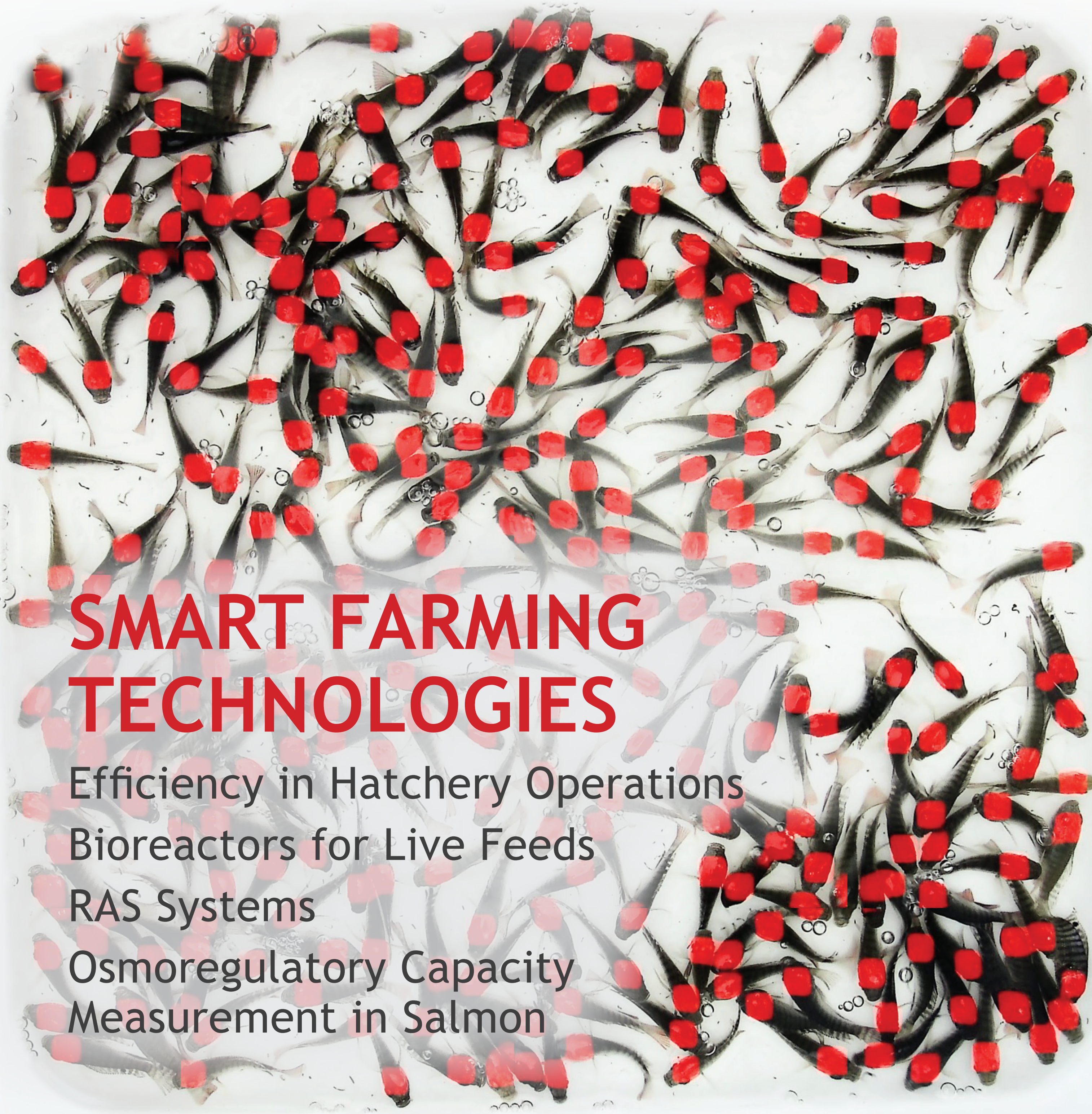
Vol 8 Issue 1 2020

HATCHERY
FEED & MANAGEMENT



SMART FARMING TECHNOLOGIES

Efficiency in Hatchery Operations

Bioreactors for Live Feeds

RAS Systems

Osmoregulatory Capacity Measurement in Salmon

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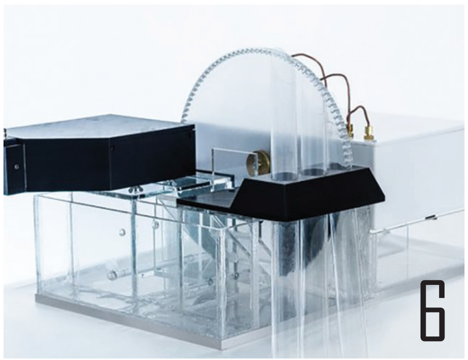

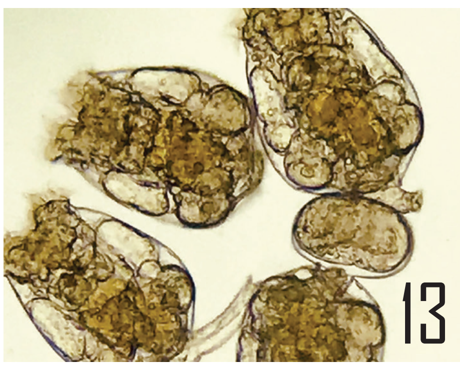

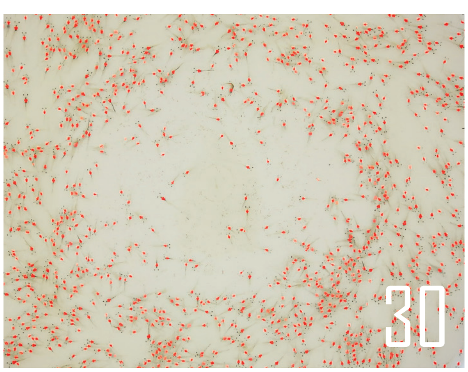

FROM THE PUBLISHER

Stavros Paraskevopoulos

We are excited to bring you the first issue of Hatchery Feed & Management, the successor to Hatcheryfeed magazine. As you see, HFM moves beyond feed to cover the wider issues of interest to aquaculture hatchery managers.

So, why did we do it? In conversation with many of you, our readers, we were told that what you wanted was a truly international, reliable, high quality source of information on all aspects of running a successful hatchery. Not just feed, but hatchery management and technology as well.

We are fortunate that our editor, Lucia, has many years of experience managing hatcheries, and she is looking forward to developing the magazine in the coming issues. Further strengthening our expertise, we are delighted to welcome Stavros Paraskevopoulos on board as a partner. Stavros is the CEO and technical director of StavRAS Aquatic Solutions. Stavros will support the technical team of HFM to promote and generate technical articles with an emphasis on technological advances in aquaculture. The StavRAS team will also be handling our advertising sales.

At the same time, our new website (www.HatcheryFM.com) is evolving, and already packed with manuals, reports, updated news and other resources. We encourage you to drop by often and make full use of the wealth of information available.

Suzi Dominy, Publisher

We are grateful to the following companies for sponsoring this issue of the magazine. Their support allows us to make our publications available without charge. We thank them for partnering with us to support the development of our industry through education and information.

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NEWS REVIEW

Highlights of recent news from Hatcheryfm.com

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

Alltech Coppens to provide next-generation starter feeds

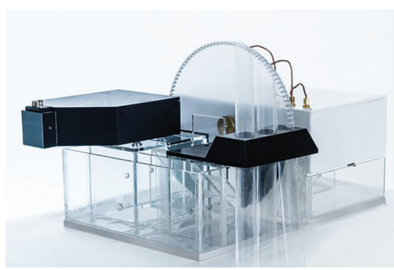
Alltech Coppens launched an improved range of innovative starter feeds for trout. Backed by extensive research at Alltech Coppens Aqua Centre (ACAC), TOP fry feed is shown to provide key nutrients while improving water quality. Understanding the importance of early nutrition, the company advanced its range of starter feeds to support trout farmers in raising high-quality products for consumers. Research at ACAC shows that Alltech Coppens TOP fry feed has an optimized ratio between digestible protein and digestible energy (DP:DE). The result is better performance, higher protein utilization and lower ammonia excretion, leading to improved feed efficiency and better water quality. Two of the largest sizes of TOP feed are now available as micro-pellets that combine a slow sink rate paired with high water stability. This allows trout fry more time to eat while the water is kept cleaner.

New rapid response range of genetic services

Since 2012, Xelect has been working with major producers globally to manage their breeding programs and provide critical support services. In response to the high demand from the industry, a new rapid response range of genetic services, Xelect Express, is now available. It offers quick, cost effective access to the company's genetics expertise for genotyping, pedigree assignment, gene expression, ploidy and sex determination.

"Our breeding program customers have always had access to our genetic testing services, but recently we've seen how the demand increased. Whether it's impartially checking the ploidy of eggs or conducting spot checks to avoid in-breeding in broodstock, people want to have certainty over the quality of their supplies and the long-term health of their stocks," CEO, Ian Johnston, said.

Automated egg sorter identifies fast-growing fish before feeding



GenetiRate, Inc. unveiled an automated system that sorts 125,000 eyed eggs per hour employing a patented test that identifies fast-growth fish. This sorting occurs at the same time as eggs are sorted to remove dead individuals, fitting easily into the egg handling schedule. Benjamin Renquist, GenetiRate's co-founder, said that "because we GenetiRate salmonids at the eyed egg stage, the GenetiRate test is the first to identify fast-growing fish prior to feeding. Accordingly, GenetiRate's measure of growth is independent of food intake."

Although the automation is currently only applicable to salmonids, the next focus is towards developing a sorter that can be applied in non-salmonid species.

New innovative genotyping tool for whiteleg shrimp

The Center for Aquaculture Technologies (CAT) launched the AQUAarray HD [vannamei], a universally applicable tool incorporating the latest advancements in genetic marker information for whiteleg shrimp.

Developed in collaboration with Neogen and available now from The Center for Aquaculture Technologies, the AQUAarray HD [vannamei] is the first product to be released in the AQUAarray line and can be used for a variety of applications including parentage assignment, assessment of genetic diversity, traceability, genomic selection, and more.

Transformational fish vaccination robot to increase hatcheries productivity

With support from Agri-EPI, Aqualife has won £250,000 funding from the Seafood Innovation Fund, awarded by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), to develop and launch a "transformational" fish vaccinating robot by the end of 2021.

The robot, named Incubot 2, will be able to vaccinate fish at sizes below 20 grams, as opposed to the common weight of between 30 and 120 grams, allowing producers to increase productivity by growing their fish out of hatcheries far sooner. It will be capable of vaccinating most species of farmed fish, in large numbers.

Incubot 2 will be a mobile platform allowing Aqualife to offer automated vaccination to smaller fish farms which cannot afford to invest in large scale immobile systems. The robot will also help to improve fish quality using artificial intelligence and "deep learning" algorithms to increase vaccination accuracy and improve fish grading.

Premium barramundi to be produced in Singapore

Barramundi Asia, one of the world largest barramundi producers, acquired the Temasek startup, Allegro Aqua, that commercializes a "premium" barramundi broodstock with specific genetic traits. The start-up was established in 2018 to scale up the production of a higher quality strain of Asian sea bass after scientists from the Temasek Life Sciences Laboratory (TLL) succeeded in breeding superior fingerlings with the support of the former Agri-food and Veterinary Authority of Singapore and the National Research Foundation.

The merger of the two companies is expected to not only help Barramundi Asia produce more fish but also reduce the mortality rate of its stocks using the technology developed by Allegro Aqua.

MSD Animal Health acquires Vaki from Pentair

The announcement further positions MSD Animal Health as a global leader in broadening its aquaculture portfolio by expanding into complementary fish farming and conservation areas to generate outcomes with precision farming and fish welfare solutions, which complement its existing portfolio of vaccines and pharmaceuticals. Vaki will be a leading brand under the Biomark business within MSD Animal Health, focused on a range of equipment, products and technology for fish counting and size estimation from freshwater to saltwater rearing, while c ollecting data and analytics for each stage of fish production.

Genetic resistance to lethal virus found in tilapia

Scientists found resistance to Tilapia Lake Virus (TiLV) which was mainly due to differences in genes between tilapia families. The finding could help to protect stocks of tilapia, which is an important food source in Africa, Asia and South America and worth nearly $10 billion to the global economy.

Researchers from University of Edinburgh's Roslin Institute and WorldFish analyzed the genes of 1,821 Genetically Improved Farmed Tilapia (GIFT), which were tagged and placed in a pond that had an outbreak of TiLV. The fish used in this experiment were members of 124 families, and the team discovered that there was a large variation in family survival. Some family groups had no deaths, whereas others found to have a 100% mortality rate.

Statistical models showed that resistance to the virus was very heritable, and this means that selective breeding to produce more resistant tilapia strains is likely to be effective. The variation in TiLV resistance were found to be independent of genetic variation in growth, meaning that any future breeding programs for GIFT that produce fish resistant to TiLV will not adversely affect the growth of the fish, and will benefit farmers' yields.

The GIFT strain was been selectively bred to be fast growing and adaptable to a wide range of environments. The strain is produced in at least 14 countries, helping to reduce poverty and hunger.

New Artemia Nauplii Center in Ecuador

Luk Van Nieuwenhove and **Frank Indigne,** I&V BIO

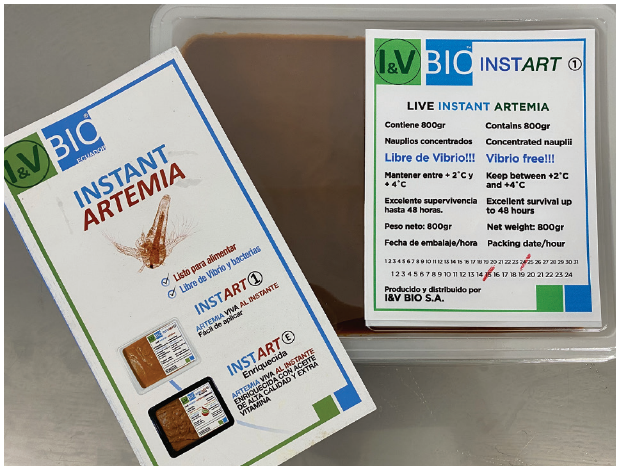
Since the early beginning of shrimp aquaculture, Artemia has been recognized as a good nutritional feed for small shrimp and fish larvae. The fact that the Artemia cysts could be dried and stored for a long time made it convenient for the aquaculture sector in general. Ever since the early 1980s, the industrial fish and shrimp hatcheries have been buying and using Artemia cysts in the same way. The Artemia cyst suppliers offer dry cysts in a can, but the shrimp hatchery actually wants Artemia nauplii to feed to their shrimp.

Uncertainties of traditional Artemia culture

Producing Artemia nauplii from cysts seems like an easy task, but there are important obstacles on the way to a clean Artemia nauplii, such as good quality Artemia cysts, clean seawater at the right temperature, sufficient light and oxygen and right harvest timing.

The biggest concerns for the shrimp hatchery managers are:

- Will I have enough Artemia nauplii for today?
- Will these Artemia nauplii be contaminated with *Vibrio*?
- Will I be able to separate the Artemia nauplii from the empty and full (non-hatched) cysts?
- Will I be able to separate the cysts shells without damaging the Artemia nauplii?
- How can I know how much Artemia nauplii I am feeding to each shrimp tank?



All these risks result in frustration for the shrimp hatchery owners and managers. Actually, these people want to focus on shrimp cultivation rather than putting so much time and effort in hatching Artemia cysts. It is very disappointing to be confronted with hatching results not matching the promises of the cyst-can supplier and the unknown consequences of feeding Artemia nauplii loaded with bacterial contamination.

Artemia Nauplii Centers

As the shrimp industry is rapidly evolving to more professional and controlled production from broodstock to market size shrimp, there is a task for the feed industry to keep the same pace. During the last decade, a lot has been achieved through improved feed formulation, development of probiotics, improved broodstock and more ecological awareness. Over all these years, one thing has remained the same which is the daily hatching of Artemia. Neither the packaging nor the instructions for use were ever changed.

Eight years ago, I&V-BIO started working on a revolutionary solution which finally took the Artemia burden away from the shrimp hatcheries by creating Artemia hatching facilities where all the know-how combined with new ideas could produce Artemia nauplii in a professional and industrial way. The result was a pure Artemia nauplii (Instar1) free of shell and other impurities that was undamaged thanks to a patented new technology and free of *Vibrio*.

The Artemia nauplii are disinfected and brought into suspended animation and de-watered until a consistent paste is achieved. It is then packed into 800 g trays and delivered daily to the shrimp farms.

One tray of 800 g is the equivalent in biomass of a GSL cyst can with 70% hatching rate. The Artemia nauplii are ready to use and can be scooped from the tray directly into the shrimp tank.

From a can, it is not sure if 70% would hatch. It all depend on the quality and the hatching conditions. It is also not sure if all of the Artemia nauplii could be recovered during manual separation with the risk of damaged nauplii and *Vibrio* contamination.

New facility in Ecuador

I&V-BIO Ecuador S.A., a joint venture between the CODEMET Group and I&V BIO Group, opened a new, state-of-the-art Artemia Nauplii Centre in Ecuador. This is the first Artemia nauplii center in Latin America and the fifth world-wide.

Ecuador is the biggest producer/exporter of shrimp and has a great future. Today the Artemia consumption in hatcheries is relatively low compared to other shrimp producing countries. One of the reasons is the uncertainty of the bacterial load of the traditionally hatched Artemia cyst. With clean and *Vibrio*-free nauplii from I&V-BIO at an attractive price, the company expects hatcheries will use more nauplii as it is proven that feeding 100% Artemia nauplii from Mysis until PL5 produces bigger and healthier PLs compared to any other feeding regime.

Besides Ecuador, I&V-BIO has similar Artemia Nauplii Centers in Thailand, India, Indonesia and Vietnam that are all operational and successful. By August 2020, I&V-BIO Bangladesh will be operational, and two more locations are under negotiation.

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Efficiency in tropical marine finfish hatcheries: Enhancing new species development and boosting fully commercial operations for incremental survival and superior fry quality

Marcell Boaventura, Filipe Pereira and **Cuong Huynh Tran,** ADM animal nutrition

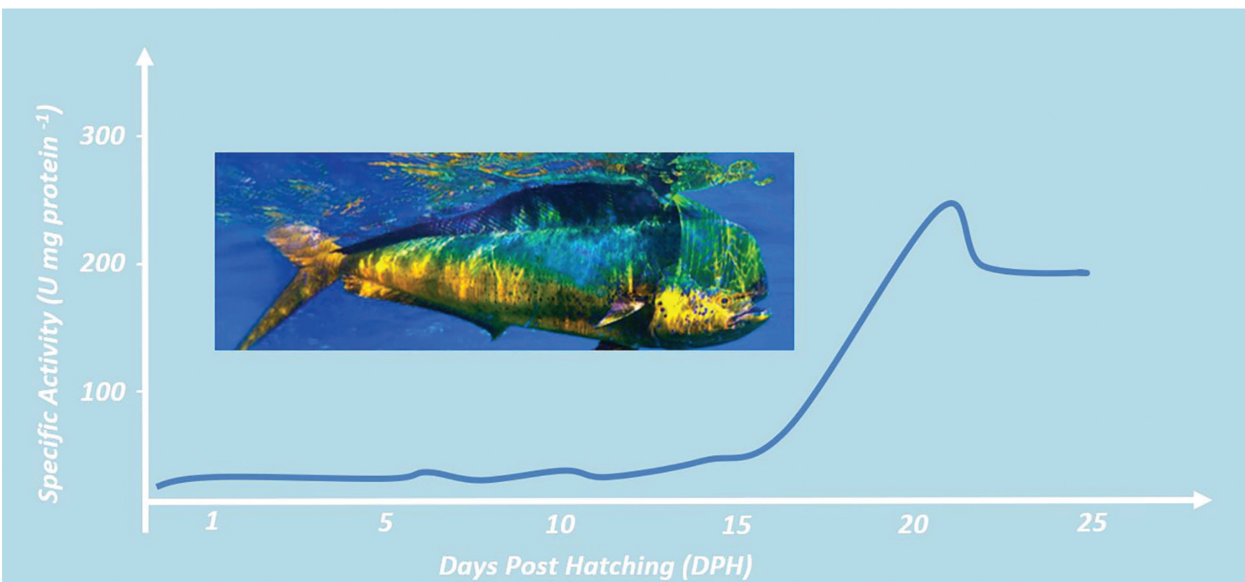

Figure 1. Acid digestive protease (Pepsin) activity during early ontogeny of Mahi-mahi (*Coryphaena hippurus*) (Stieglitz *et al.*, 2020). Mahi-mahi image courtesy of the University of Miami.

The progress of the global aquaculture industry depends on the availability of quality seeds; in commercial numbers, being able to sustain the increasing demand for high quality seafood. Make no mistake, this is no "light-bulb" moment where it all just came together for many professionals in aquaculture. However, the more we engage with hatcheries around the globe, the more we can see the true value and payback obtained from elevating hatchery standards with practices connected to species and attention to details such as early trophic ontogeny, quality of live preys and early fry behavior. In this article, we will cover a few factors influencing efficiency in some of the best marine finfish hatcheries around the globe, and provide a practical outlook for implementation from a scientific point of view.

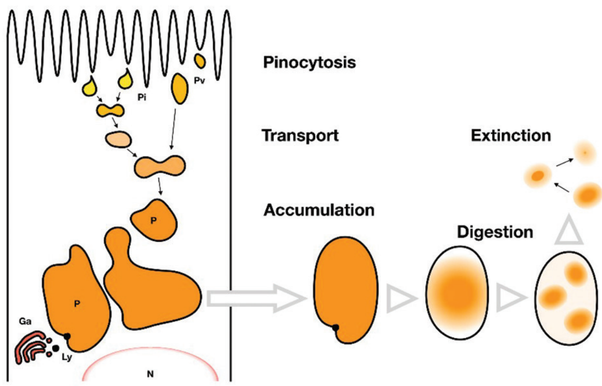

Figure 2. Successive, five stages of protein absorption and intracellular digestion by the hindgut epithelial cells of fish larvae. Organelles include pinocytotic invagination (Pi), pinocytotic vesicles (Pv), protein inclusion bodies (P), Golgi apparatus (Ga), lysosomes (Ly), and nucleus (N) (Govoni *et al.*, 1986).

Some aspects within the trophic ontogeny of marine finfish larvae

What has been entirely unambiguous to this point is the fact that species have distinct requirements. However, most species prosper through ontogeny in a similar way, giving managers important cues for advanced technical management. In terms of trophic ontogeny, at the very beginning, the larvae possess a simple and undifferentiated alimentary canal. This primordial structure evolves and by the end of metamorphosis, it comprises a complex functional segmentation, and a digestive architecture is maintained until adult fish. Before the distinct digestive organs appears, most fish larvae still lack digestive enzymes. In practical terms, without digestive enzymes, especially pepsin, proteins cannot be deconstructed into amino acids by the larvae, the "building blocks of life." So how can we ensure fish larvae are taking in most of the nutrients in feeds during such a critical stage in the hatchery? Ensuring the nutritional quality and cleanliness of live feeds and selecting feeds rich in free-amino acids and small peptides.

What makes a copepod nutritionally superior to a rotifer is the content of total and free amino acids (Dabrowski & Rusiecki, 1983). With the challenges in producing copepods, the commercial focus must be on making other live feeds nutritionally comparable to copepods. The nutritional profile of live feeds can be managed and increasing content of free amino acids is linked to the use of certain algal mediums (Aragão *et al.*, 2004).

Live feeds nutrition and cleanliness

The passage of nutrient particles through the digestive tract of a rotifer is fast, especially at tropical temperatures. When microalgae are available (e.g. when rotifers are placed in larval tanks with thick green water), enrichment is eliminated and particles excreted are still rich in absorbable nutrients (Lindemann & Kleinow, 2000). This characteristic can become an advantage if nutrients are incorporated to the rotifer vitellarium. That happens when rotifers are fed certain single cell microalgae.

Rich vitellarium eliminates the dependency on the gut content for larvae nutrition which is usually

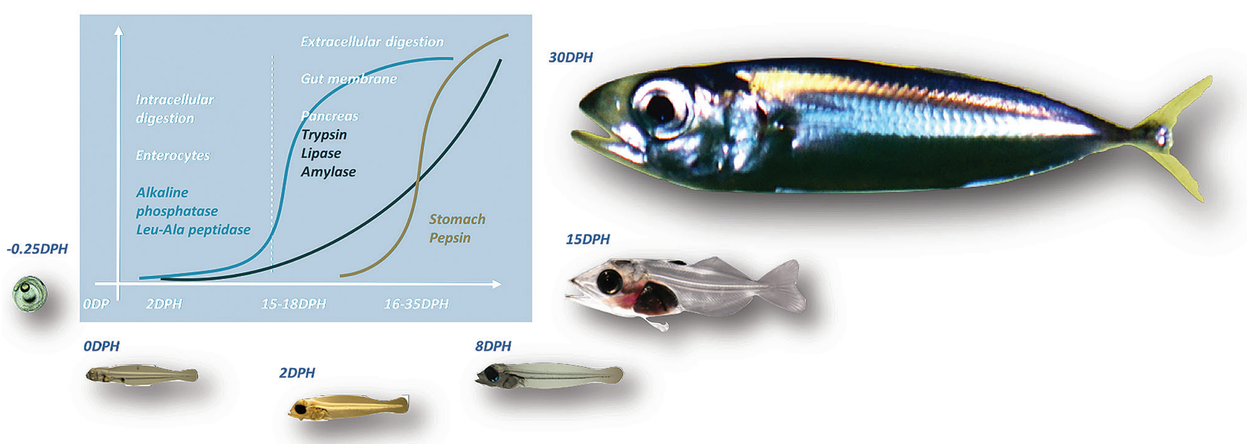

Figure 3. Progression of assimilation and intra-cellular digestion into extracellular digestion post-metamorphosis illustrated with Southern bluefin tuna (*Thunnus maccoyii*) larvae and juveniles.

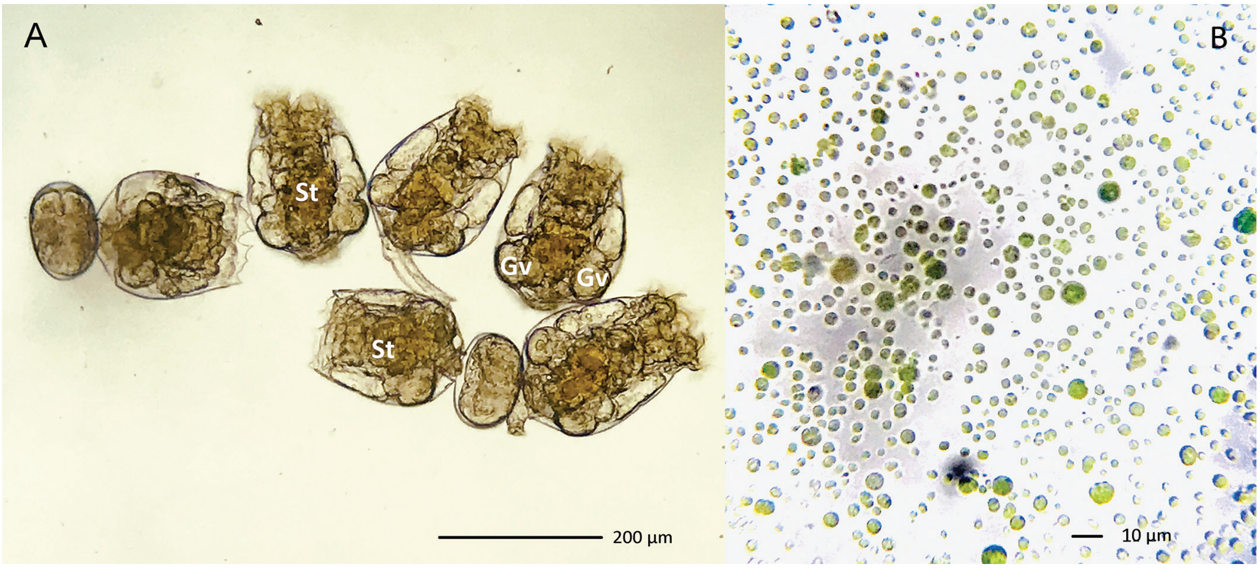

Figure 4. (A) Rotifers simultaneously cultivated and DHA/EPA enriched with ω3 Algae from Bernaqua, (St) Stomach and Germo-Vitellarium. A dry algal medium, based on *Chlorella vulgaris*, enriched with essential fatty acids. (B) Hydrated ω3 Algae, single cell separation.

contaminated with *Vibrio* spp. and other undesired microorganisms. The gut content can be substituted by probiotic before feeding the larval tanks. This process, called "bioencapsulation", allows the engineering of the microbiological profile of rotifers with no loss to its nutritional value. Bioencapsulation can also be applied during Artemia enrichment and has been demonstrated in commercial hatcheries with increments in early survival.

Another factor to control when managing quality in live feeds is the availability in larval rearing tanks. Long exposure of live feed residuals in the larval rearing tanks can become an issue. As filter feeders, live feeds continue to accumulate in their gut system any opportunistic bacteria which are fast growing in the larval rearing tanks. In water, these bacteria are usually harmless to the larvae, but when assimilated by the live feeds, the potential to harm the larvae increases. Fish larvae are non-selective hunters as far as quality and cleanliness, hence when residuals are excessive, increasing a cyclic daily water exchange is usually effective.

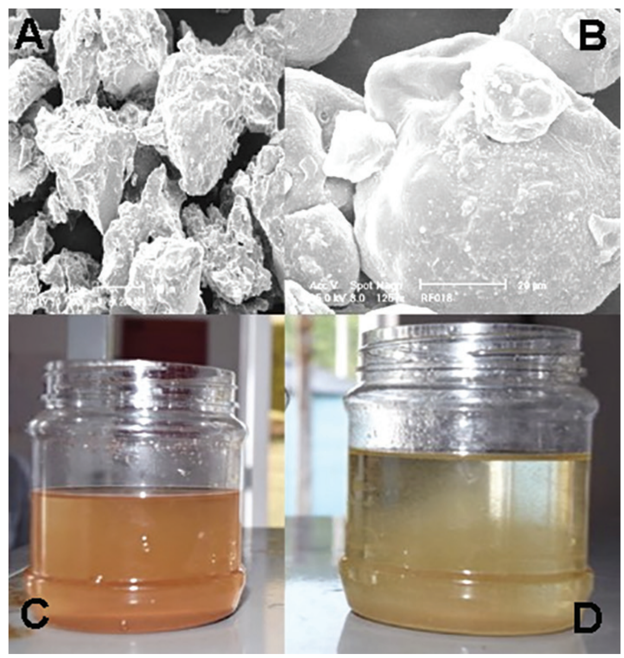

Figure 5. Feeds produced by different technologies, its appearance on the electronic microscope and its behavior in water. (A) Microparticulate diet. (B) Micro-encapsulated diet, "Caviar" by Bernaqua. (C) The behavior of a micro-particulate diet in water (soaking, leaching and sinking). (D) Behavior of a micro-encapsulated diet in water (clear water as no leaching occurs and the maintenance of particles in suspension).

Efficiency during co-feeding and weaning

Last but not least, we will discuss a final few points.

- The efficient introduction of dry feeds, followed by the intensive "training" of fry on diet recognition and feeding. The co-feeding stage is a crucial step for survival and fry quality. During co-feeding, interest to inert feed is only "investigative", hence frequent exposure of larvae to diets are key. The problem lies in the fact that most co-feeding diets are microparticulate and slow sinking. These diets leach and sink to the bottom,

Figure 7. High quality and colorful Asian seabass (*Lates calcarifer*) sampled during high density weaning in Cam Ranh, Vietnam. Image courtesy of ADM team Vietnam.

damaging the water quality and increasing bacterial contamination in tanks that contaminates the live feeds.

- Micro-encapsulated diets represent an important advantage for fish hatcheries. During the co-feeding stage, micro-encapsulated diets stay in suspension long enough to increase the exposure of larvae to the feed with minimal leaching. Co-feeding ration can be reduced by at least 30% for a positive impact on water cleanliness.
- Delaying the introduction of Artemia by 2-3 days. This factor promotes batch homogeneity. When Artemia is offered too early, only a small portion of larvae may feed on Artemia leading to size disparity and very soon increased cannibalism. Delayed offerings of Artemia allow most of the population to feed on Artemia at first introduction. A complete elimination of Artemia has also been reported when quality and cleanliness of rotifers are combined.
- During weaning, attentiveness to behavioral cues of each species is pivotal. With higher enzymatic functions after the metamorphosis, hunting and cannibalism peak. In contrast, many species are prompted to "schooling", an innate reflex observed both in nature and captivity. To the advantage of hatchery managers, schooling promotes imitation making weaning, which basically is training the fry to eat artificial diets, an efficient effort.

It is important to note that schooling promotes a cooperative defensive behavior which has been confirmed by a number of fish behaviorists (Palov & Kasumyan, 2000). In commercial hatcheries, we have observed and replicated key triggers for schooling leading to sensible benefits in survival, early weaning and fry quality. In short, those triggers include density, light and hydrodynamics. We also note schooling behavior can be disrupted when fry size within a school differs by more than 50% of the average body length (Shaw, 1962), and the cooperative defensive behavior can't be promoted at low fry densities.

In Asian seabass (*Lates calcarifer*) for instance, we have experienced improvements in fry survival and robustness when weaning happened under schooling. Fry are crowded with 100-150 fry per liter under certain conditions of tank hydrodynamic, water exchange, light and feeding regime. Schooling and cooperative defensive behavior is triggered along with imitation behavior, which lead to enhanced feeding and mass early weaning at 22-23 DPH.

In conclusion, it is important to reinforce that hatchery efficiency and performance is a multi-factorial equation, which changes from species to species and in most cases is also site-specific. We believe that close observation and generous attention to details by competent hatchery managers are a couple of the factors responsible for value creation in hatchery businesses.

References available on request.

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Automated bioreactors for live feeds

Robert Roulston and **Jennifer Long,** Industrial Plankton

Figure 1. PBR 1250L.

Reliably producing enough live feed is the bottleneck for many shellfish, shrimp and marine finfish hatcheries. The feed has to be of good quality with both high nutrition levels and low pathogen levels. Without enough feed, animals starve or are underfed. When operating a hatchery, operators have probably calculated the cost of this loss and weighed it against the live feed's production cost.

The true cost of poor-quality live feeds is often given less attention, since it's usually impossible to calculate the impact on the entire value chain. Live feeds are commonly contaminated with pathogens that increase the mortality of hatchery animals, or even kill the entire cohort. Animals that do survive by fighting off early infections often have less efficient feed conversion ratios and lower growth rates, often resulting in a lower yield in the grow-out phase.

To help solve these problems with reliable production and feed quality, Industrial Plankton has developed live feed equipment for producing algae and zooplankton with a focus on maintaining a stable biosecure culture environment.

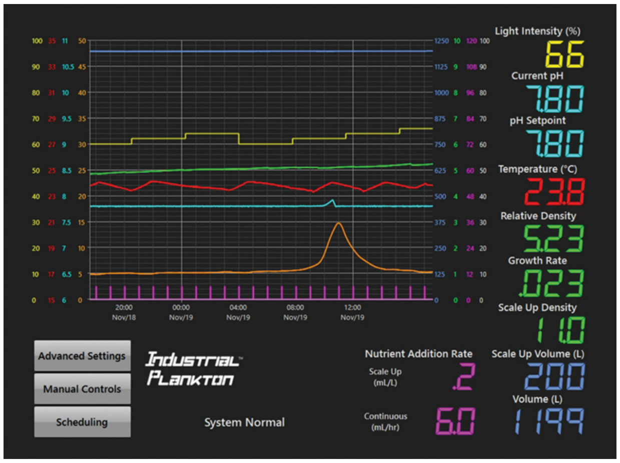

Figure 2. *Nannochloropsis oculata* culture in continuous harvest. Here, the CO_2 supply was shut off. The resulting pH spike was easy to spot and fix before the pH got too high.

Algae

Consistently producing algae on site can be time consuming and unreliable. By maintaining a clean, stable culture environment and automating many routine functions, Industrial Plankton photobioreactors (Fig. 1) make it easy to consistently produce algae to meet hatchery needs.

Biosecurity

A big challenge to producing clean algae outside a lab is biosecurity. A single grazer or pathogenic bacteria can invade a culture and establish a population, causing reduced productivity or larval mortality. To establish a clean culture environment, the PBR 1250L uses an automated clean-in-place (CIP) system to clean and disinfect the tank before starting a new culture. Additionally, the bioreactor has micron filters to maintain biosecurity while adding the water, nutrients, air and CO_2.

To start a new culture, the inoculum is connected to the PBR with sterile disconnects, then pumped in with a biosecure pump. By maintaining biosecurity, a PBR can continuously produce high quality clean algae for months.

Reliability through automation

After inoculation, the next step is providing the right temperature and the amount of nutrients, CO_2, and light needed to sustain production and prevent culture crashes. To minimize time and effort, the PBR 1250L automates these functions. Nutrients are stirred and added automatically and closed loop pH control adds CO_2 as the algae grows, maintaining constant pH and preventing carbon limitation. Harvesting can be done with the push of a button, scheduled to harvest automatically, or set to harvest continuously. Temperature is controlled using a chiller and an internal heat exchanger. As long as the PBR has a supply of water, CO_2 and nutrients, it can produce algae automatically.

A touchscreen control makes it easy to adjust settings. The controls also log and graph the sensor data, simplifying troubleshooting (Fig. 2).

Cleaning

One of the most unreliable and labor-intensive tasks required for culturing algae is cleaning tanks. By providing a self-cleaning biosecure tank, algae cultures can grow productively for longer. When a tank needs to be cleaned, the PBR 1250L's automatic cleaning system removes the need for manual cleaning, working like a dishwasher to pressure wash the inside of the tank.

Cell density

When culturing algae, it's important to quantify algae biomass. However, counting algae cells on a microscope is time consuming and requires technical skills. To reduce the need for manual cell counts, the PBR 1250L uses a density sensor to output a linearized 0-10 value (Fig. 2, in green). This real-time density feedback helps operators maximize productivity by seeing the effect of changing light, nutrient additions, harvest rate, etc.

Rotifers

Rotifers are a critical first food for many larval fish but are labor intensive to produce, and are a common source of *Vibrio* and other pathogens.

Biosecurity

Most rotifer production is still done in batch systems. The problem with batch production is lack of control over the bacterial population. *Vibro* is often present in rotifer cultures or introduced with incoming water, so operators are often scaling up a batch of *Vibrio* along with the batch of rotifers.

Figure 3. Industrial Plankton rotifer system.

In a stable environment without excess organic waste, pathogenic bacteria can be kept in check. If system parameters are unstable, the fluctuating organic load can allow faster growing pathogenic bacteria to rapidly increase in numbers. In a stable system, the bacterial population becomes dominated by beneficial heterotrophic bacteria.

The constant removal of organic waste from the culture is also critical, since high levels of organic waste generally encourages growth of opportunistic bacteria.

Reliability through automation

To reduce levels of pathogens in the culture, the Industrial Plankton Rotifer System (Fig. 3) operates as a continuously recirculating system with an attached biofilter. This provides a stable culture environment rich in beneficial bacteria, preventing opportunistic pathogenic bacteria from proliferating.

The rotifer system uses a touchscreen interface to display and log data, and controls vital parameters such as temperature, pH, and dissolved oxygen. To maintain good water quality, organic waste is removed using a self-cleaning mechanical filter, a foam fractionator, and finally a biofilter to detoxify ammonia.

Conclusion

Live feeds produced from Industrial Plankton's automated equipment are a perfect solution to the bottleneck experienced by many shellfish, shrimp and marine finfish hatcheries. The reliable production of high-quality live feed in a stable biosecure culture environment allows for improved nutrition and lower chances of early infections in animals, resulting in higher hatchery survival and growth rates. The equipment is currently operating in 18 countries to support the hatchery production of oysters, mussels, clams, vannamei shrimp, monodon shrimp and marine finfish.

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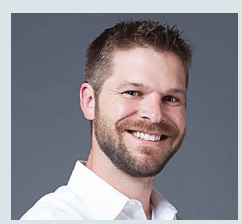

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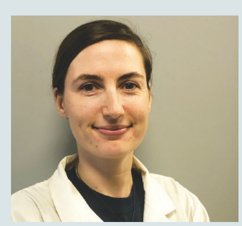

Developments in aquaculture from Aquaculture Ghent University

Prof. Dr. Gilbert Van Stappen, Laboratory of Aquaculture & Artemia Reference Center

Microbes rule the world – the sequel

Previous contributions to this column have highlighted the importance of microorganisms in larviculture practice and in performance of fish and shellfish larvae in the hatchery and in aquaculture in general. So far, "microorganisms" within an aquaculture context were always understood as, firstly, microalgae (see also the green water technique) and, more recently, as bacteria.

It is well-known that all living organisms can be subdivided in two big groups (let's keep viruses aside for the sake of this exercise), the so-called "domains". The first ones are the Eukaryotes or – translated from Greek - organisms with "a proper nucleus", either unicellular (such as microalgae) or multicellular, consisting of cell(s) characterized by, summarizing, a membrane-bound nucleus with the genetic material within, and several intracellular organelles. The second domain are Bacteria, basically, organisms consisting of a single cell lacking (among other differences as compared to Eukaryotes), the typical nucleus. However, only recently it was found that life also presents itself under a third "format" or "Domain": these are the "Archaea", a specific group of single-cell organisms. A mouthful of ancient Greek always comes in handy: their name means "ancient beings", as the first representatives of this group ever studied were methane producing microorganisms. It was assumed that their metabolism reflected the Earth's primitive atmosphere and that in evolution they thus colonized our planet before the more "modern" bacteria and Eukaryote organisms. However, as new habitats were studied, more of these archaeic organisms were discovered, sometimes living in conditions very hostile for other life forms, such as environments characterized by high salinity or extreme temperatures, where they survive thanks to very specific physiological adaptations. Although indeed Archaea are often the only form of life (as we know it) found to survive in extreme environments today (hence their qualification as extremophile), they are known to be a large and diverse group of organisms abundantly distributed throughout nature, including even the animal (and human!) gut.

Let's look at another extremophile: the brine shrimp Artemia, which we all know as a common live food organism in larviculture, thanks to its storable cysts and its nauplii hatching from them. Brine shrimp survive in the very saline conditions of salt ponds and salt lakes where virtually no other multicellular ("higher") organism or metazoan survives. Although there are just few field studies on this, here Artemia is supposed to live side by side with Archaea that must thus make up for a substantial part of its "associated" microbial environment. The role of microalgae, on the contrary, as food for Artemia, has been investigated in detail, both in the field and in the laboratory. More recently, also the nutritional contribution of bacteria to the Artemia diet has been well documented.

This detour, via the world of extremophiles, brings us back to the role of microorganisms in aquaculture, and to the so-called "microbial management": in essence, steering the microbial background of aquaculture operations to the advantage of bacteria-that-we-want-to-be-present-and-active, and to the disadvantage of those-that-we-want-to-stay-out (or at least: keeping low profile). If the Archaea are indeed ubiquitous and occur in any aquatic ecosystem, as we know now, is it too far-going to assume that just as any other microorganism, they may interact in various ways with higher organisms, just as microalgae and bacteria do? Can they be food? Can they promote health? (No pathogenic Archaea have been identified until now. So far so good!) Given the fact that Archaea contain a variety of compounds, and have a variety of processes in place to protect them against their extreme/stressful environment, one can indeed wonder to what extent they can have a beneficial role when they are eaten by a higher organism – such as Artemia.

The first question to be answered, however, is: can Archaea be taken up at all by higher organisms? The question is not as futile as it seems. One of the characteristics that makes Archaea quite different from the other two domains of life is the chemical structure of their cell membrane lipids, which is quite unique. It is, for example, not known if these very special archaeal cell membranes can be digested at all by the digestive enzymes of Artemia (or other filter feeding organisms), and if archaeal lipids may provide the critical fatty acids that most metazoans need for survival. In fact, it was not before 2012 that one of the very first studies documented the uptake of Archaea by a higher organism, so-called "archivory": a specific marine polychaete worm species, living in marine methane seeps, seems to be able to assimilate Archaea.

To study interactions between Archaea and higher organisms in more depth, Artemia comes in the picture again: it is an excellent model in studying bacteria-host interactions within an aquaculture context, and the results of these studies have proven highly instrumental in developing techniques and protocols for microbial management. Recent studies at the Laboratory of Aquaculture & Artemia Reference Center of Ghent University, Belgium, have demonstrated that Artemia is able to survive and grow for at least a few days in a laboratory environment in the exclusive presence of Archaea, thus ruling out interference of microalgae, bacteria, or any other food source. Thus, although the "scoop" of documenting archivory has gone to the marine polychaete, Artemia is an honorable second. Moreover, assimilation of archaeal biomass into Artemia biomass was studied in great detail, using the technique of stable isotope (SI) labelling and tracing, a tool frequently used in nutritional studies to study the uptake of organisms by other organisms, tracing the fate of specific microbial dietary components, when taken up by a higher organism. This was the first case ever of using SI labelling for nutritional studies with Archaea: the Archaea labelled with SI, could indeed be traced back in the so-called isotopic "signature" of the Artemia fed with them, proving without any doubt their assimilation into Artemia biomass.

What does all this mean for larviculture and aquaculture in general? We do not operate in extreme conditions, do we? That's right, but remember that - just like Bacteria - Archaea seem to be everywhere, even within the gut of the reader of this text. Now that the way has been paved (yes, Archaea can be taken up by higher organisms! Yes, we have tools in place to follow their assimilation!) the road is open for further work on microbial management that includes not just microalgae and bacteria, but also Archaea. With these assumedly ancient forms of life, and their contemporary descendants, the "art" of microbial management in aquaculture has acquired a fascinating new dimension, to be further explored in the future.

Dovetailing AquaMaof's technology and biology: Sustainable Atlantic salmon smolt production

Andrew C. Preston, AquaMaof Aquaculture Technologies Ltd

Figure 1. 50-200m³ tanks used during production.

The industry's demand for Atlantic salmon smolt is increasing globally with production. The Norwegian Directorate of Fisheries indicate smolt numbers have increased from 151 million in 2005 to approximately 349 million in 2018 (www.fiskeridir.no). Likewise, in Scotland, smolt numbers increased from 36 million in 2005 to approximately 47 million in 2018 (www.gov.scot/marine-and-fisheries/). This trend appears to be similar for other salmon producing countries including Chile and Canada.

Traditional *vs* modern smolt production

The "traditional" production of smolt was synonymous with seawater ready smolts <120 grams in body weight. More recently, the industry has shifted production towards larger smolts or post-smolts in closed systems. This change in practice aims at avoiding sea lice and mitigates against other health challenges during marine culture.

At AquaMaof Aquaculture Technologies, there is an understanding that the "traditional smolt" is becoming a fish of the past and one of the key strategies has been to deliver the new requirements within the company's systems. Over the past number of years, there has been a focus on the production of large smolt and learning how to dovetail the systems with the biology of the Atlantic salmon. To achieve this goal, the company embarked on a commercial R&D project as a proof of concept. At the land-based facility north of Warsaw in Poland, the highest quality salmon eggs have been arriving every two months for the past three years. The eggs undergo the highest biosecurity procedures prior to arrival. On site, the tightest biosecurity and veterinary health and welfare plan have been implemented using internal and external resources.

In addition, standard operating procedures have been implemented to facilitate training and refinement of processes. These "live" documents are updated regularly to reflect subtle changes to husbandry, system management and other critical processes. Duplication of critical system components and redundancies ensure the welfare of salmon. Four distinct production areas hold fish of increasing size. Each rearing area is independent from the other and arranged into completely biosecure rearing units. Within each biosecure unit, transfer of fish into and out of these areas can only be conducted when fish have undergone rigorous health screening. By refinement of production processes and attention to detail, ground is being broken exploring the potential of salmon performance in the company's systems.

Larger smolts in less time

AquaMaof land-based technology has proven that modest smolt grow larger with "jumbo smolts" capable of >1200 grams, or larger, in less time to produce a traditional S1 smolt. It provides a larger, more robust salmon to sea and reduces time in the seawater. Clearly, this performance is only achievable using elevated water temperature and constant illumination compared to traditional farming. The company is aware of how important production figures are to customers and the feed conversion ratio (FCR), which would rival the most efficient smolt producer. The latest batches of salmon appear to supersede all previous batches and the collective package of genetics, optimal feed efficiency and nutrition combined with solid husbandry is propelling a direction that is certainly raising eyebrows. AquaMaof has been challenging much of the published data to determine "company limits and thresholds" for many important technical parameters that impact growth and welfare. This benchmarking of biology and system performance has allowed us to take the knowledge and data generated and transcribe to many of the other projects.

Figure 2. Highest welfare standards promote health and low mortality.

Less complexity, higher efficiency

These systems feature as few moving parts as possible to reduce operating costs. Interestingly, in walking around the facility, not one drum filter is utilized; instead several settlers and biofilters are used. The AquaMaof system is not overly complicated and what it lacks in over complexity it makes up through efficiency (Fig. 1). The systems use only the main circulation pumps and lack any mechanical filters/drums to lower operating costs (OPEX). Tank design has been engineered to promote the hydrocyclone action and maximize the removal of solids. Robust ergonomically designed settlers maximize sedimentation and removal of sludge. This is complemented with advanced technology to optimize flow across the biofilter maximizing stripping capacity and minimizing fowling. In addition, laminar flow ensures optimal fish welfare as a core value.

Improving fish health

Clearly, complete control of the water certainly allows improvements in fish health. The fact that no antibiotics and/or any other veterinary treatments apart from the odd salt bath since inception is a credit to biosecurity procedures. In addition, this demonstrates the commitment and dedication of staff to the highest sanitation levels. The health benefits associated with a well-managed, land-based system cannot be denied with mortality remaining consistently very low (Fig. 2). Fin quality is rigorously monitored as a welfare indicator and is always something a company is proud to show off in smolts. In addition, environmental monitoring

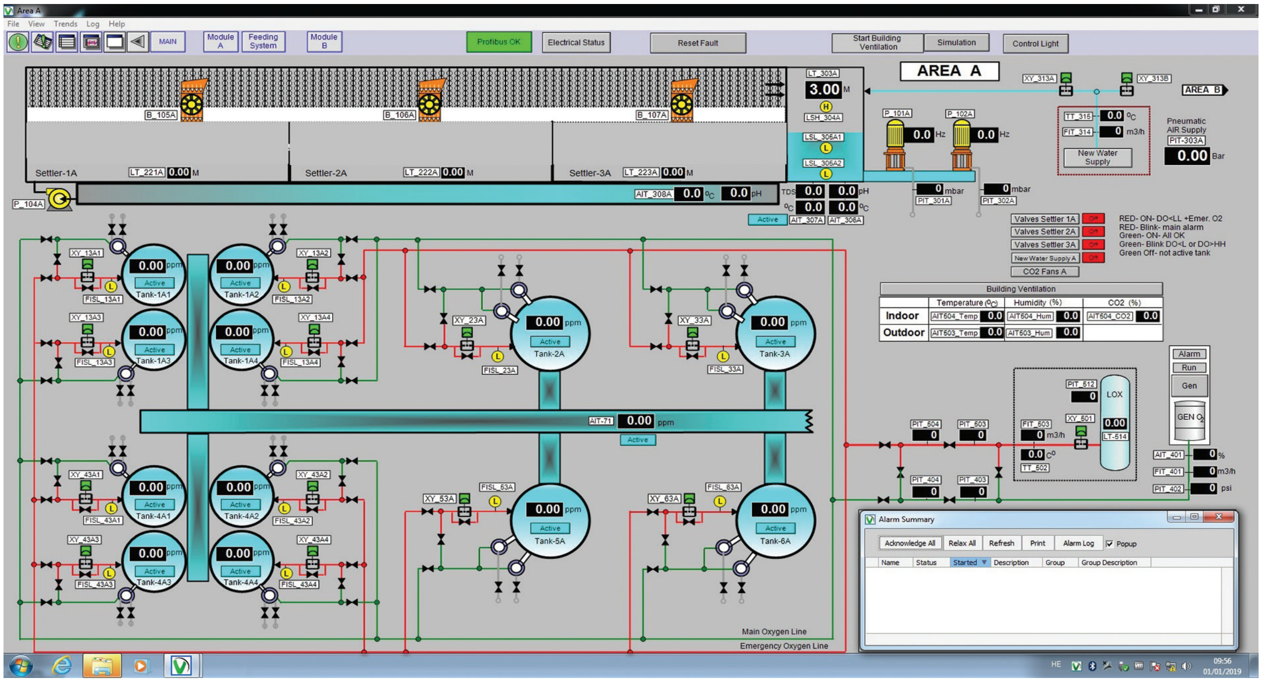

Figure 3. HMI provides 24/7 monitoring of critical system components and redundancies to ensure the welfare of salmon.

and access to reliable water chemistry data is vital for daily management. On site, a well-equipped laboratory is utilized for the monitoring of key physiochemical parameters. Real time in situ sampling is conducted and linked to the human machine interface (HMI) providing 24/7 monitoring remotely (Fig. 3).

Sustainability

The focus of the company's work is to grow smolts in an environmentally sustainable nature. To achieve this, AquaMaof has recently received Aquaculture Stewardship Certification (ASC), one of only very few land-based facilities to do so globally. Sustainability and welfare are core values of the company. In addition, to be successful in this more advanced form of aquaculture requires training and education. Recently, the site experienced a strategic change to Pure Salmon Poland and the AquaMaof research and development (R&D) and training facility. The purpose is to provide a platform for continuing commercial R&D and to educate the future managers (Fig. 4).

The sustainable development of land-based aquaculture is a pillar of the company's philosophy. The ongoing collaboration with certifiers will promote a specific land-based certification with the goal of ensuring the highest welfare standards within land-based smolt farming. Collectively, there is a need to promote the highest welfare standards in smolt farming to ensure positive exposure within the plethora of modern media.

Figure 4. Growth performance during smolt production has been promoted by facilitating training, development of standard operating procedures, optimal feeding and system management.

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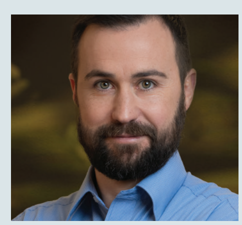

Make it modular, make it standard, make it nanoRAS

Maddi Badiola, Alpha Aqua

R&D: the core of any company/industry

Research & Development (R&D) plays a very important role in the success of a business, contributing to its sustainability. R&D is the core of any primary, secondary and/or tertiary industry. In today's competitive scenario, where customers are hankering after new products and new technologies, the R&D department plays an important role, being relevant and essential. Thus, the firm that can successfully leverage its R&D efforts building new products will find itself ahead of its competitors. R&D (in its development role) can act as a catalyst for speeding up the growth of organizations by way of introducing breakthrough products in the market.

Why replicate in R&D?

Replication is the key to the support of any worthwhile theory and involves the process of repeating a study using the same methods, different subjects and different experimenters. That is why replications and robustness checks are key elements of the scientific method and a staple in many disciplines. Motivations to replicate include assurance that results are valid and reliable, determination of generalizability or the role of extraneous variables, application of results to real world situations, and inspiration of new research combining previous findings from related studies.

The nanoRAS TM solution: replicability of aquaculture in 2.5 m^2

The Alpha Aqua way of thinking states “challenging what we know today is the only way to continue moving forward in the aquaculture world.” And that is what the highly experienced, knowledgable group of experts compromising Alpha Aqua team has achieved with the nanoRAS TM solution. This repicable, scalable, upgradable, updatable and environmentally consious PP food grade module focuses on having the most compact RAS without compromising the best water quality (i.e. low total suspended solids (TSS), high nitrification rates and excellent gas balance). Despite its little dimensions, this nano solution is a full water treatment unit with the maximum flexibility (movable with a pallet lifter), hidden and protected valves and inner pipes to ensure safety and biosecurity by design.

The nanoRAS TM solution is mounted over a flat concrete surface, with no dedicated concrete constructions. It has full flexibility and adaptability of the technical solutions to satisfy the client production needs while maintaining the highest degree of sustainability and preferred materials as food-grade PP. Moreover, water management is fully controlled.

What is within the nanoRAS TM solution?

The nanoRAS TM solution is built in recyclable food-grade PP making it completely corrosion free. The standard solution includes a drumfilter with 40-micron screen and self-cleaning nozzles (the option on modifying screen size is available upon demand (10 micron to 100 micron), a drumfilter dedicated backwash pump for self-cleaning pattern, a gas balancing filter, an integrated fixed/moving bed biofilter cluster (adaptation to go from moving bed biofilter to fixed bed biofilter is included), an integrated microparticle filter, (down to 4-10 microns of mechanical filter equivalent), a pump sump with cooling coil for cooling control and electrical heating element for heat control, a UV in PP with counter and click-in bulb system, a lift pump located in dedicated pump room inside the nanoRAS TM solution, level and temperature sensors and, an integrated MCC with HMI touch screen. Apart from those components the ozone, skimmer, heating/chilling, heat recovery, sludge treatment, oxygen cone and oxygenation and country specific power connection, among others, are options available upon demand.

All components mentioned are comprehended in 1.3 by 1.9 meters, with a comprehensive access and are easy to clean and operate. Total water volume is 1,5 m^3, flow is 30 m^3 /h, weight is 260 kg and 1,760 kg with water. This solution is capable of running as a full RAS and it has been designed and built with big consideration for the environment, user-friendliness and robustness. It is made in food grade quality PP and FAT tested on our premises and it is resistant to aggressive environments that have high salinity and temperatures. It can be used for R&D purposes as well as be key for water quality control in hatchery and first feeding operations as well as grow-out operations of limited tonnage. For example, approximately, 7.5 MT of annual production of yellowtail kingfish (2 kg harvest size), 8 MT of rainbow trout (350 g), salmon (2 kg) or barramundi (500 g) and 10 MT of African catfish (800 g) can be achieved in a setup of four tanks (1.6 m diameter and 1.3 m height) with a total footprint of 28 m^2 per system.

More than R&D: local food production is possible

As described, the nanoRAS TM solution is the most compact aquaculture unit designed, ideal for local food production. Together with fish tanks and grow boxes, it grows fish and vegetables sustainably and organically. It also reduces the global carbon foot print as it can be located near to the end customer. With this sytem any species can be grown anywhere in the planet, helping anyone from local communities to big fish producers in their businesses.

It is a full RAS solution with plug’n’play integrations (i.e. CE plug, wireless data and pipe couplings) that include all the needed equipment of RAS in the most compact and efficient design.

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The future of fish grading is here

Albert Ingi Haraldsson, Vaki

Grading fish in aquaculture is extremely important and its advantages are numerous. An ungraded batch of fish typically displays a normal distribution. The normal distribution, sometimes referred to as the bell curve, has two main characteristics - the mean, or expectation of the distribution, and the standard deviation. The coefficient of variation (CV) is defined as the ratio of the standard deviation to the mean. It shows the extent of variability in relation to the mean of the population. It's a common practice to grade when the CV has reached 30%.

One of the objectives of grading fish is to split the fish up by size to get a certain percentage or biomass in each grade in order to utilize the farm to its fullest. To achieve this, the grader needs constant fine-tuning based on results from the counter. This can be difficult and time consuming and often results in an unsatisfactory output. VAKI has been working on making the grading process in hatchery simpler, more practical and more automatic. For this purpose, a system was created called SmartFlow.

Grading challenges

Traditional industrial grading consists of a pump, a grader and a counter. The pump transports the fish from a tank to a grader. The grader divides the fish based on body thickness. Next, the fish flows though a counter which counts the number of fish in each grade separately.

The system falls under the category of a multi input, multi output system (MIMO). The system is sensitive to any changes. A small change to the input can cause a dramatic change in the final output and it's not uncommon that hundreds of thousands of fish are graded in one session and the size range varies from a few grams to a few hundred grams. The process of grading fish also falls under the category of a nonlinear system, which in layman's terms means the output does not react the same to changes in the input throughout the process.

All these factors contribute to making the grading process fairly complicated and hard to tune.

Here is where SmartFlow comes in.

Figure 1. Openings of each grading category (in mm) for manual and SmartFlow grading (Graph 1, 2). Average size of the fish (in g) when x many fish have gone through the counter (Graph 3).

Artificial Neural Networks

An Artificial Neural Network (ANN) is a network consisting of simple processing elements called neurons which can exhibit complex global behavior, vaguely inspired by the biological neural networks that constitutes as a biological processor, or brain. The structure of which the neurons are connected, is termed a computational model or a network. The model itself is not an algorithm, but rather a framework.

ANN is an adaptive system that changes its structure based on external and internal information that flows through the network. In more practical terms, a neural network is a non-linear statistical data modeling and decision-making tool. They can be used to model complex relationships between inputs and outputs or to find patterns in data. Such systems “learn” to perform tasks by considering examples, generally without being programmed with any specific guidelines beforehand. For a network to “learn” the relationship between a set of inputs and outputs, it will need to be “trained” on an associated subset of data.

SmartFlow

SmartFlow connects all the devices used for grading fish together in a hatchery for an automatic grading. It is a control system that optimizes the grading process by controlling the entire process. All aspects of the grading can be controlled. Enter in the desired output and the system takes care of the rest. It also offers many additional tools to assist with monitoring and quality control, like VAKI Cloud, VAKI Web and Modbus services.

Neural nets are specifically suited when control problems are nonlinear in nature. VAKI trained an artificial deep neural network (ANN/DNN) to control the grading process. The system gathers information from the equipment and uses the data to dynamically adjust the grader. SmartFlow processes the data and passes it to the DNN which, in return, outputs a decision, for instance increase/decrease size of grade 1, etc.

Data collection, augmentation and training

Hundreds of counting records were analyzed with the aim of generating data for training. The moving average of the fish width was plotted and the curves were used to generate multiple augmentation (“fake”) records, where the number of fish varied from 50,000 to 500,000, the average weight varied from 5 g to 1 kg and the CV varied from 10% to 100%.

The best values for the grader gaps satisfying the loss function, was found for each record in order to generate the inputs associated with the best possible outputs. The records were then simulated, where the starting positions of the grader gaps were sent to a random position. The simulation carried out like a normal grading session, but instead of using a controller, the actuators were always sent to the best values and the inputs and outputs were logged.

Data for 20,000 records was generated. The model was trained on 18,000 records and 2,000 were used for validation. The training was stopped when the validation error converged with an accuracy over 99%.

Tests

A prototype was running on a farm which in return supplied the grading recorded. Thus, a grading session was carried out by a team of experienced farmers. The grading can be emulated by using the record, in other words, we can make the AI grade the same for each batch of fish. Therefore, the controllers can be compared with human performance. The AI was set to the same starting positions and given the same criteria. The first two graphs on Figure 1 show the openings of each grading category (in mm) and the third one shows the average size of the fish (in grams) when x many fish have gone through the counter. The top one is from the manual grade, which was carried out by experienced fish farmers, and the middle one is the result from the AI. The dotted lines are the optimal positions.

Conclusions

The AI was able to learn how to grade and was explicitly good at predicting if the size of the fish would change over the grading session. Thus, the network has learned to anticipate that the average weight will increase over time. This anticipation process, that is acting before exhibiting is hard to achieve with typical control algorithms. VAKI's new SmartGrader and pumps are all fully compatible with SmartFlow. As more SmartFlow systems go up, we will run more tests and further develop the system. VAKI truly believes that eventually most, if not all, grades will be conducted bysome sort of AI.

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Artificial intelligent device for counting small aquatic animals

Kunn Kangvansaichol, Bordyn Cheevatanakornkul, Chetnuwat Danlaphon, Algaeba

Shrimp and fish hatcheries in many developing countries still rely on manual and volumetric counting to enumerate the postlarvae and fry for sale to customers. This counting ensures that the customers receive what they pay for.

The counting process requires time and patience and is usually conducted by skilled operators. Typically, for *Penaeus vannamei* postlarvae (PL or PLs), 1,500-2,000 PLs can be counted by one operator within 15-20 minutes. To speed this up, hatcheries deploy more than one operator to count PLs. The PLs sold may be counted by hand again at the site of customers, i.e. shrimp farms, or fish farms. In addition, for Nile tilapia fry, up to 500 for >5.5mm graded, and 1,000 fries for 4 mm graded are usually counted in one bag, of which takes 5-10 minutes per count.

In the past decade, a few efforts were made to develop a device to count these small aquatic animals

P.vannamei PLs (number)	800	1500	2000
Number of tested images	26	15	21
Mean absolute error (%)	3.01	2.78	4.69
CV (%)	2.89	2.26	1.38
Undercounted rate (%)	81	100	100

Table 1. Counting results of *P. vannamei* PLs with SeaThru counter.

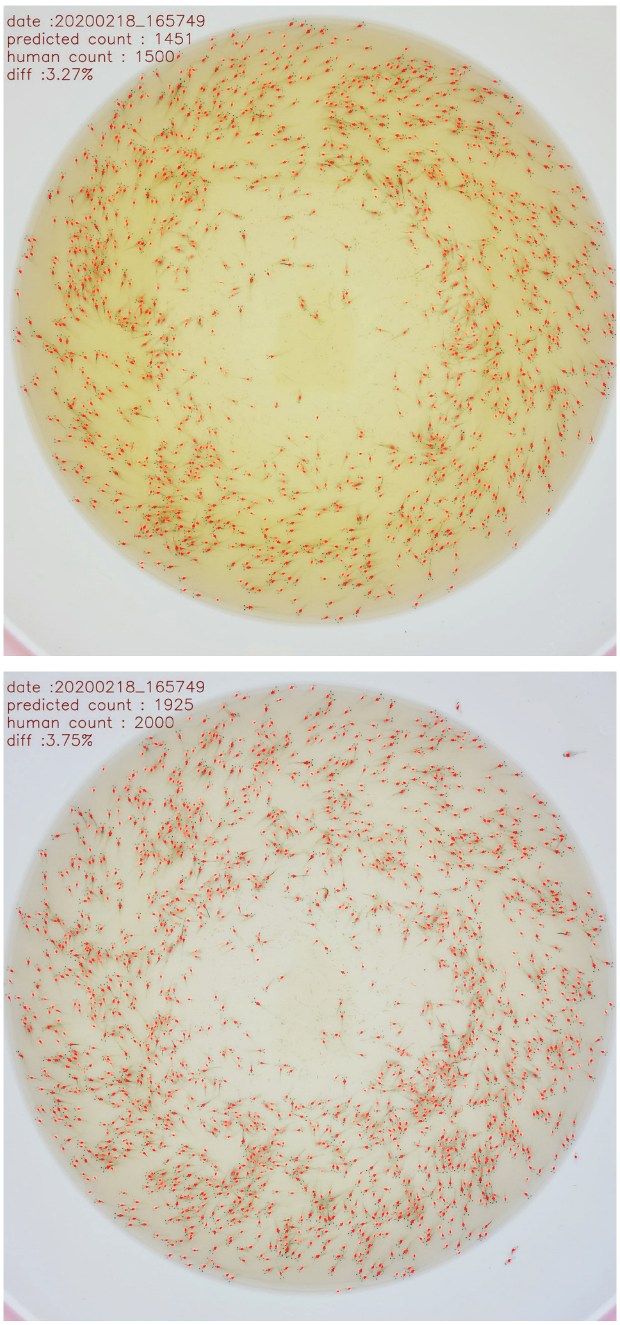

Figure 1. Images taken from SeaThru counter for *P. vannamei* PLs. Colored water (up).

with high accuracy, but they are still considered expensive to most of the hatcheries, especially those in developing countries like Thailand, India, Indonesia, etc.

This article shows the implementation of artificial intelligence technology with computer vision to solve the counting problem into a solution device called SeaThru COUNTER. Some of the recent results in *Penaeus vannamei* and Nile tilapia are shown.

Penaeus vannamei

Photos of PLs of several stages (PL8-PL15) and quantities (100-2,000) were taken through the SeaThru COUNTER at 16MP (mega pixels) and the system was trained with a framework based on convolutional neural network (CNN). The result model was used to test the image set.

The mean absolute error of the counting was agreed with hatchery managers not to exceed 5%, the number per count is 800 for large PLs, and a maximum of 2,000 for PL8. All these counter features fit the hatcheries' expectations.

The count obtained should be less or equal to the real number. Undercounted rate (%) was calculated

Figure 2. Images taken from SeaThru Counter for Nile tilapia fry.

as the number of results that are less than the real number divided by the total number of results. The higher undercounted rate, the better for the hatchery. For shrimp PLs, all counts obtained were over 80% (Table 1).

This technology can withstand colored water, a technique that adds colorants to water that help shrimp and crab keep calm (Fig. 1). Moreover, PLs batches with different sizes can be counted with less than 5% of mean average error.

Nile tilapia (number)	100	200	300	400
Number of tested images	88	40	40	20
Mean absolute error (%)	2.60	2.72	1.09	1.79
CV (%)	1.88	1.91	1.05	1.48
Undercounted rate (%)	98	100	65	90

Table 2. Counting results of Nile tilapia fry with SeaThru counter.

Nile tilapia

Photos of tilapia fry at different stages (4 mm-5.5 mm, S to L size) and different quantities (100-400) were taken with SeaThru COUNTER at 16MP (mega pixels) and the system was trained with a framework based on convolutional neural network (CNN). The result model was used to test the image set.

The counter identifies the head of each fry with a red mark (Fig. 2). The mean absolute error of the counting was lower than 5% (Table 2). The undercounted issue also happens with tilapia fry. The undercounted rate was higher than 65%.

This technology can withstand dirty water. The dirt can come from small feed or debris. In both cases, only fry are counted. The system can also count different fry sizes with less than 3% error.

Since hatcheries require up to 1,000 fries per count, the counter is being modified to count all the fry at the same time.

Conclusion

Artificial intelligence (AI) will play an important role in the future of the aquaculture industry. The advantage is that the results can be traced and farmers can always check the results by repeating manual counting. The technology creates transparency and trust among all stakeholders, including hatcheries, transporters and farmers. This technology will also prevent overstocking, reduce loss and feed used and increase farmers profit.

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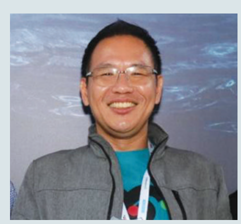
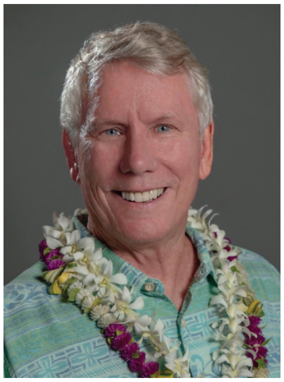


SPF shrimp technology: past, present & future

Jim Wyban, Marine Genetics LLC, Hawaii, USA

SPF shrimp technology present

This is the second of a series of articles titled "SPF Shrimp Technology, Past, Present and Future." This article will describe the present, including some overview of the current broodstock suppliers, exports from Hawaii and some recent developments in the industry.



SPF broodstock

Hawaii's SPF program is based on developing shrimp that are certifiably free of "listed pathogens" which are disease-causing microbes that can be diagnosed and physically excluded from a facility. The listed pathogens used in the original SPF certification were based on histopathology since PCR had not yet been developed. The current listed pathogens for SPF certification are listed in Table 1.

What is an SPF shrimp? SPF is short for Specific Pathogen Free. An SPF shrimp is a shrimp that is certified pathogen free. In Hawaii, SPF certification is under the auspices of the State Aquatic Veterinarian. To get certified, a company requests the State Veterinarian to collect samples of the farm's shrimp.

Status	Pathogen Type	Pathogen acronym Name
OIE Listed	Virus	WSSV – White Spot
		YHV – Yellow Head
		TSV – Taura Syndrome
		IHHNV, IMNV, NHP
		HPV, BP, LSNV, MoV
		MBV, SHIV, CMNV
OIE Listed	Bacteria	AHPND
OIE Listed	Parasite	EHP

Table 1. SPF shrimp pathogens excluded from Hawaii SPF broodstock in 2020.

These are submitted to the shrimp disease diagnostic lab at the University of Arizona in Tucson. The shrimp samples are tested with a collection of PCR tests which are specific for individual shrimp pathogens (disease causing microbes). If the routine samples are all negative for shrimp disease, then the farm enters the SPF process. The farm then gets tested every six months. After two years of testing, if all samples test negative for disease, the farm is elevated to the status of certified SPF. With an SPF status, the farmer can get an SPF certificate from the State Veterinarian stating that their shrimp are SPF. Such a certificate is required by foreign governments in order for a broodstock supplier to supply that country.

Hawaii SPF broodstock industry

Because of Hawaii's unique remote island location and as the home of SPF technology development, Hawaii has emerged as a shrimp breeding capital. The shrimp broodstock companies in Hawaii are listed in Table 2.

Broodstock sales from Hawaii increased rapidly between 2009 and 2015 and peaked at 800,000 pieces in 2015 (Fig. 1). Since 2015, Hawaii broodstock supply has declined and by 2019 was about half (400,000) of the previous peak. At an average price of $50/pc, the Hawaii broodstock market is worth $20 -$30 million per year. Cumulative exports of Hawaii SPF broodstock since 2003 are more than five million pieces worth more than $250 million. Based on knowledge of the industry, global demand for SPF broodstock has not declined but Hawaii broodstock suppliers have lost market shares to other locations. One alternate SPF broodstock supplier is discussed below.

Location (island)	Company	Species
Hawaii Island	Shrimp Improvement Systems (SIS)	*P. vannamei*
Hawaii Island	Shrimp Improvement Systems (SIS)	*P. stylirostris*
Hawaii Island	Shrimp Improvement Systems (SIS)	*P. monodon*
Hawaii Island	Moana Technologies	*P. monodon*
Oahu	Oceanic Institute	*P. vannamei*
Molokai	Molokai Seafarms	*P. vannamei*
Kauai	Kona Bay/Hendrix Genetics	*P. vannamei*
Kauai	Kona Bay/Hendrix Genetics	*P. monodon*

Table 2. Shrimp broodstock companies.

Figure 2 shows the annual broodstock purchases by country in 2015-2019. China was the leading buyer in 2015 and purchased 300,000 broodstock from Hawaii. Since 2015 though, China's purchase of Hawaii broodstock dropped precipitously. In 2015, Indonesia was the second leading buyer country but like China, Indonesia's purchases of Hawaii broodstock have dropped steadily. India and Vietnam show a different pattern. Both countries were buying increasing amounts of broodstock in 2015-2018. This data is consistent with the rapid growth in shrimp production in their industries during those years. In both countries, Hawaii broodstock purchases declined in 2019.

New SPF germplasm

Ecuador is the leading shrimp farming country in the Americas. They follow a distinctly different model for shrimp farming that uses hatchery-produced seed (PL) stocked at low densities (10-25 pcs/m^2) in large (2-20 ha) ponds that are mostly not aerated. Most of Ecuador's production is produced by large integrated companies. These approaches to shrimp farming have evolved over more than 40 years. In 1999, white spot virus mortalities struck Ecuador and total output plummeted.

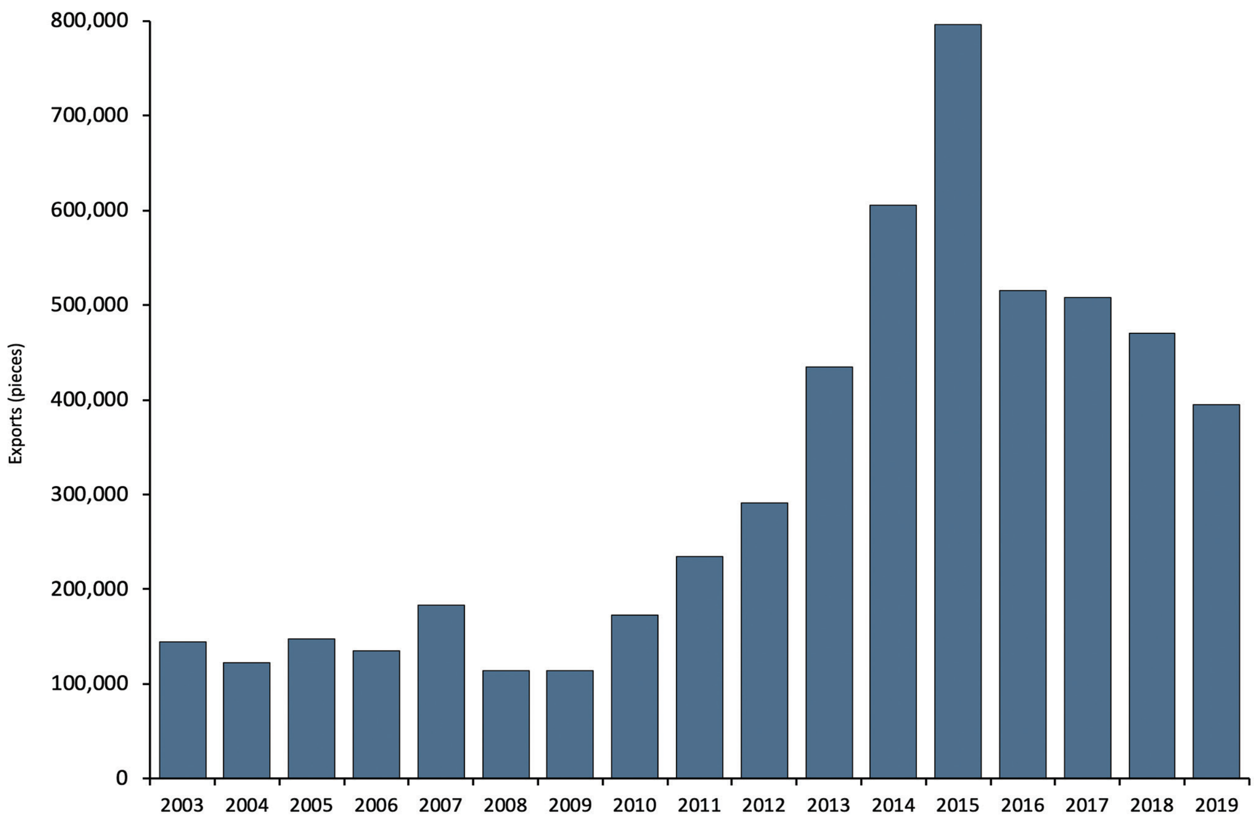


Figure 1. Hawaii SPF broodstock exports.

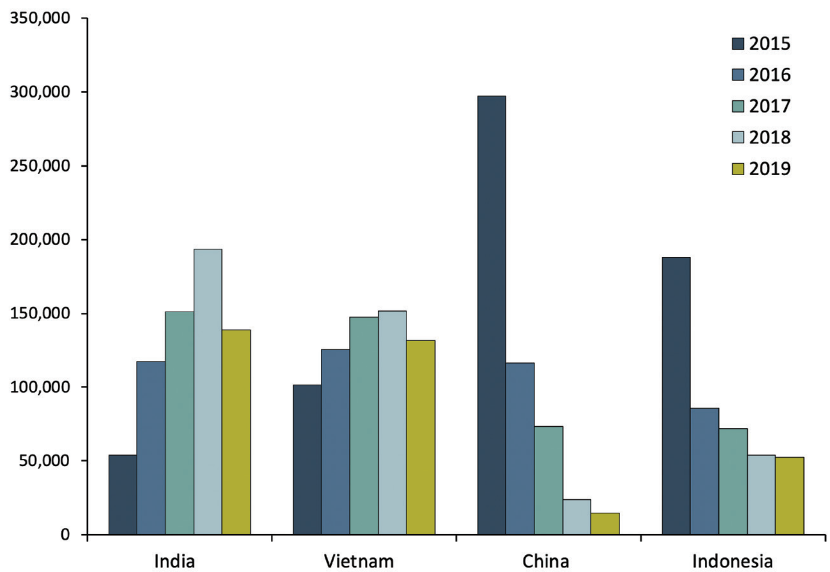


Figure 2. SPF broodstock buyers by country by year.

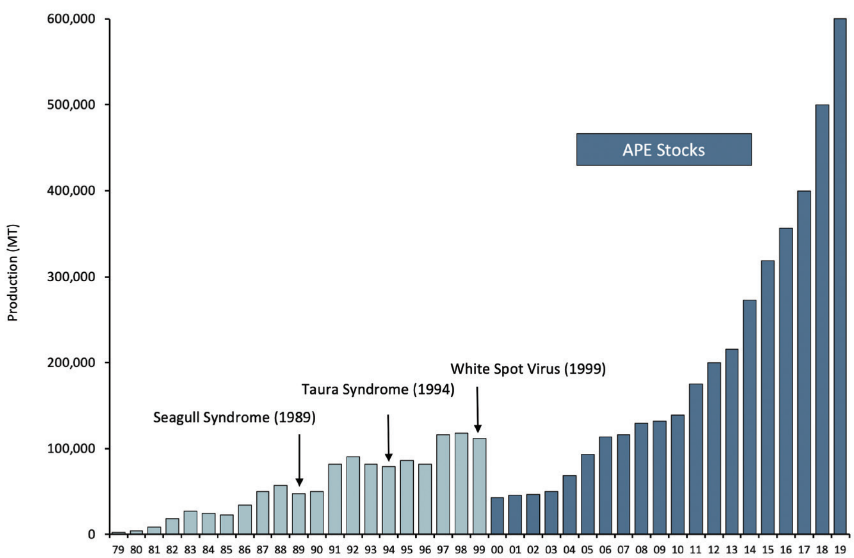


Figure 3. Ecuador shrimp farming annual production.

In response to this disease crisis, Ecuadorian companies began selectively breeding pond survivors following growout cycles to become breeding stock for the next generation. They incorporated DNA markers into their selection program to select animals of maximum genetic distance in their broodstock lines. By selecting survivors, they were mass selecting for resistance or tolerance to WS. They call the animals produced from this approach APE or All Pathogen Exposed. There is limited screening for IHHNV to limit that virus from the breeding stock, but otherwise, there has been no attempt to clean up their stocks to make them SPF and there has been no attempt at biosecurity or pathogen exclusion. This approach has proven extremely successful with steady growth of the industry. Year after year, survivals steadily increased and industry output steadily improved. The benefits of this approach are clearly illustrated in Ecuador's steady increase in production reaching 625,000 MT by 2019 (Fig. 3).

While the Ecuadorian approach does not use SPF technology, the APE germplasm represents a valuable new disease resistant germplasm that multiple breeding companies around the world have obtained and are working to incorporate into their SPF breeding programs. Recent developments by two different commercial shrimp projects illustrate the value of the new APE germplasm particularly when it has been "cleaned up" and made SPF. In the U.S., an intensive shrimp farming company in Florida called American Penaeid (API) has developed an SPF stock of *P. vannamei* that was derived from the Ecuador APE shrimp. The company acquired a non-SPF Ecuadorian APE stock and passed them through a quarantine and testing process to make them SPF. API is now supplying the resultant broodstock (SPF/APE) to China and results have been impressive. As a result, API has captured a significant share of the China broodstock market which likely came from Hawaii's lost share. While shrimp growth rates by the SPF/APE stock are slower than other SPF stocks, survivals are much higher, and farmers prefer these animals. API is actively breeding their stock called "High-Vigor" to improve growth performance.

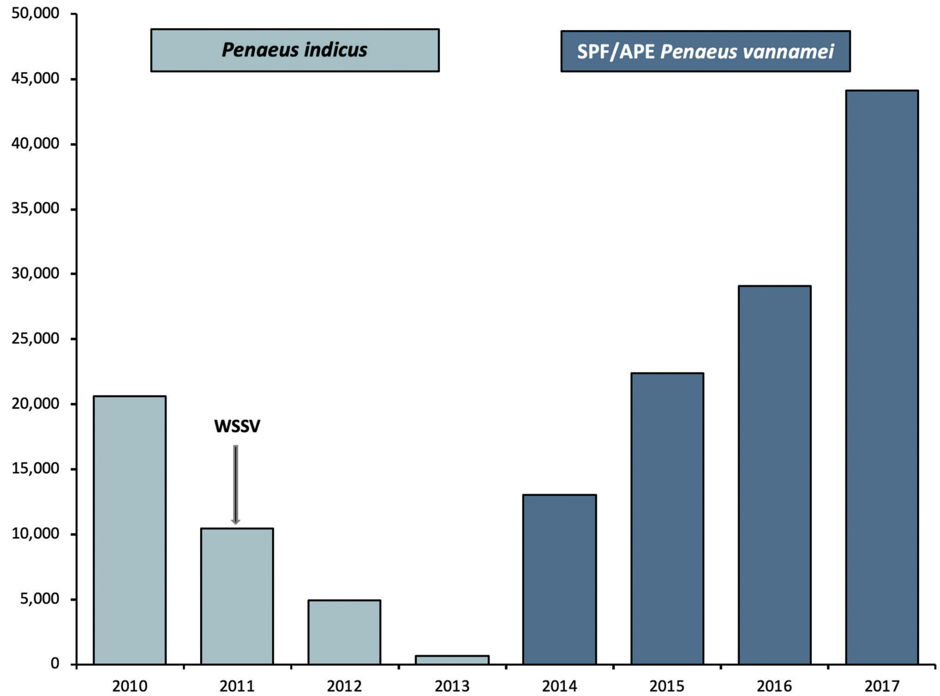


Figure 4. Shrimp farming production by NAQUA in Saudi Arabia by domesticated *P. vannamei* (Alday-Sanz *et al.*, 2018).

A similar strategy was followed by a large shrimp company in Central America called Pescanova. They acquired non-SPF APE stock from Ecuador and cleaned them up to make them SPF. A subset of these SPF/APE stock were transferred in 2014 to National Aquaculture Group (NAQUA) in Saudi Arabia. Results to date at NAQUA using the SPF/APE shrimp have been impressive (Fig. 4).

Each of the three approaches to shrimp breeding described here have both benefits and problems. Going forward, shrimp industries will try various combinations of these to try to optimize production economics. It is clear that SPF technology and shrimp breeding are extremely valuable technologies that can make or break a national industry.

Economic impact

Widespread adoption of SPF *P. vannamei* in Asia significantly improved the economics and reliability of shrimp farming. The driving force in Asia's switch to *P. vannamei* was based on the higher profit achieved with *P. vannamei* compared to Black Tigers.

Domestication, breeding and globalization of *P. vannamei* added tremendous value to the world shrimp industry. In the mid-90s, global farmed shrimp production was 700,000 MT per year with a total crop value of about $3.5 billion. Current crop value is more than $25 billion with production at 4.1 MMT. This six-fold increase in industry production and value resulted from the domestication, breeding and widespread use of *P. vannamei*. The cumulative value added to the shrimp industry crop value from the introduction of SPF *P. vannamei* to Asia is about $225 billion. This industry transformation was driven by SPF *P. vannamei's* lower production costs and reduced disease risks which derive from their disease-free status, advancing domestication and *P. vannamei's* natural growth traits. The biggest opportunity to lower costs in shrimp farming is through continual advanced breeding SPF *P. vannamei* for improved performance and providing farmers with top quality, disease free (SPF) postlarvae with high performance genetics cultured under optimum, controlled conditions to maximize their growth potential.

The next article in this series will discuss the future situation in SPF technology.
The complete manuscript was presented as the keynote address by Dr. Wyban at Aquaculture America 2020.

Superior Southern trout ova

Tijan Oberholzer, Dunkeld Trout Hatcheries

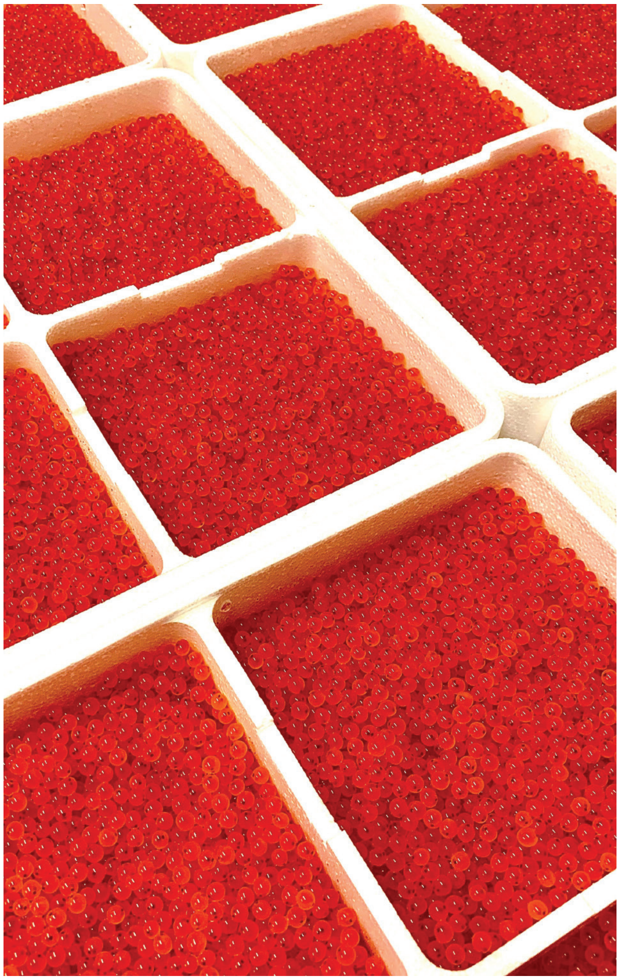
Due to a market shortage of high-quality trout ova in the Northern Hemisphere during their summer months, a niche market was identified by producing a high-quality product from naturally spawning stock in order to supply the Northern Hemisphere as trout are a naturally winter-spawning species.

To facilitate the high demand of production to supply this demand, Dunkeld Trout Hatcheries (DTH) built a state-of-the-art RAS hatchery to produce off-season trout eggs at competitive prices. Since its inception in 2016, DTH has established themselves as the largest certified, disease free rainbow trout breeder in the Southern Hemisphere. The facility boasts a broodstock inventory of 40,000 fish that can produce in excess of 60 million ova a year. Supplying a product that is high in quality called for an excessive amount of research, groundwork and genetic selection.

Disease-free ova and quality

DTH recorded its first full year of production in 2017. Disease testing and routine surveillance takes place twice per year and it took two years before freedom from all diseases could be claimed. Only then could the product be exported and customers assured of the status. DTH's confidence in the disease-free certification is equally important to availability, quality and performance.

The hatchery is certified as free from all recognized trout pathogens. The certification meets the highest standards as required by OIE, DEFRA (UK) and the European Union. The virology, bacteriology and parasitology samples are sent to and tested by the Onderstepoort Veterinary Institute (also supported and approved by OIE).



In addition to its many health certifications, the company is accredited by Global G.A.P since April 2019, the first in Africa, and is currently in the process of obtaining an ASC and RSPCA accreditation.

The company has formed an alliance with Troutex in Demark. This will ensure customers can obtain the reliability and proven product all year round. In addition, the company is in the process of entering the table trout market by developing a RAS facility as South Africa remain a net importer of trout and they aim to contribute sustainably toward this.

Where the industry standard guarantees a 90% hatch rate, DTH guarantees an additional 5% over others. However, this statistic may vary from country to country. Most farmers have achieved a 99% hatch rate. To assist customers in achieving their potential and the best possible results, DTH offers to guide them through the process and supply complimentary technical support.

South Africa challenges

The biggest challenge that Dunkeld Trout Hatcheries faced in its' beginning stages was the procurement of technically advanced equipment. In terms of RAS systems, South Africa was far behind the industry requirements and state-of-the-art equipment had to be purchased abroad. To add to this challenge were the volatile exchange rates of a rather weak currency.

Additional challenges included the import of high-quality feed as products supplied in South Africa were not designed for recirculating systems. As the owner of the largest freshwater recirculated aquaculture establishment in South Africa, the company overcame this challenge and now has a reliable feed partner, namely Alltech Coppens, by its side together with the correct permits to import feed of all sizes.

The facility

Nestled in the Mpumalanga Highlands, Dullstroom is the trout capital of Africa. The facility boasts a 46,100,000 liter capacity supplying the broodstock and juvenile tanks, nursery and hatchery. The buildings are entirely insulated and represent three epidemiological units where each system has its own filtration, biological filtration and water supply. 72,000 m³ is recirculated, sterilized, degassed and oxygenated daily.

Situated in a high rainfall catchment area, the location directly obtains clear and pure underground water for the RAS system. This water is certified by the OIE as a protected water source.

Ninety-five percent of the produced trout ova are exported to Iran, Russia, Georgia, Romania, Turkey, UK, Peru and Denmark as these countries are considered large markets. The company is working on getting a foot hold in the Canadian market amongst others and pride themselves on personally visiting their customers around the world.

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Validation of a new biological indicator to quantify the osmoregulatory capacity of salmonid species

Marco Rozas-Serri, Jorge Vásquez, Rodolfo Correa, Andrea Peña, Ariel Muñoz, Lucerina Maldonado, Pathovet

Smoltification is one of the most critical and important stages of the salmon production process. It consists of a series of biochemical, physiological, morphological and behavioral changes that allow a juvenile salmon to continue its productive cycle in seawater. It is an irreversible process, so there is a limited period of time for fish to be ready for ocean entry (smolt window).

Currently, smolt producers measure Na+/K+ ATPase pump activity as a routine indicator to determine if the smolts are ready to be transferred to the sea. However, this indicator shows a low time variability and a low predictive value.

The objective of this study was to validate Smoltmeter® as a laboratory service to predict the optimal osmoregulatory capacity of Atlantic salmon smolts (*Salmo salar*) and minimize losses. Smoltmeter® is based on multiple RT-qPCR that evaluates the relative expression of three genes that show a high predictive level of the optimal transfer time of animals to the sea, specifically, a freshwater subunit gene (SAD), a seawater subunit gene (SAM) and a cotransporter gene (COT), in addition to the respective associations between the expression of each of them.

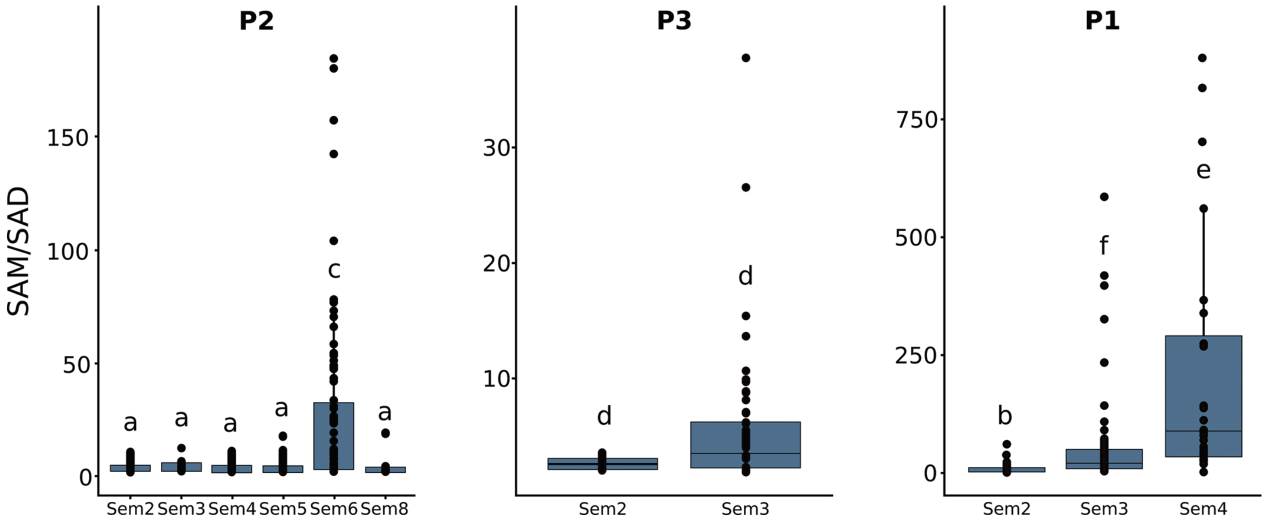

Figure 1. SAM/SAD ratio during smolt photoperiod weeks in each farm (P1, P2, P3).[abcdef] show significant differences between the group, p<0.05.

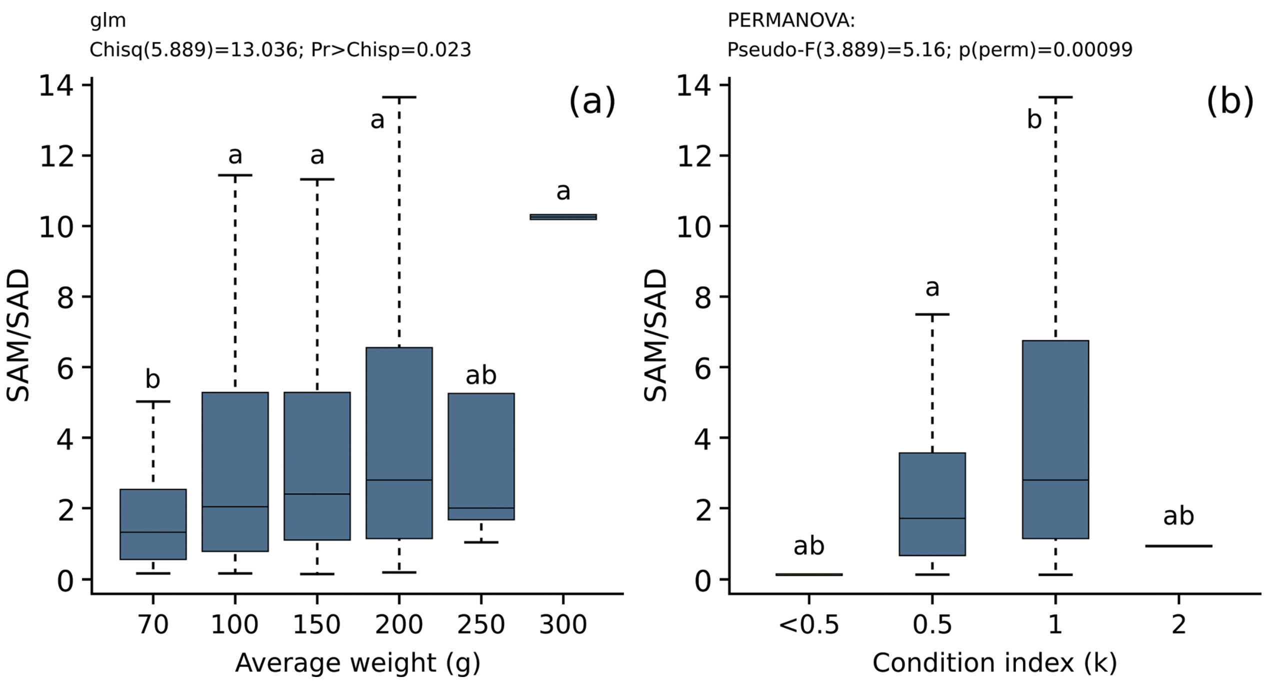

Figure 2. SAM/SAD ratio between the average weight (a) and the condition index (b).

Methods

Fish from three farms (P1, P2, P3) were transferred to a cage farm (CM1). Duplicate samples were collected from the second gill arch of each fish for RT-qPCR analysis (Smoltmeter®) and to measure the activity of the enzyme Na+/K+ ATPase. Data on weight, length, condition factor, body color and fins on each stage were recorded.

The density function for the SAM/SAD ratio was estimated during the summer photoperiod weeks and changes between farms. Weight and condition factors were evaluated. The relationship between the SAM/SAD ratio and the activity of the Na+/K+ ATPase enzyme in failed smolts was also analyzed.

Summer photoperiod weeks

The SAM/SAD ratio showed significant changes during smolt photoperiod. The first change took place on weeks two and three, followed by a significant increase on week five and a reduction on week eight. All SAM/

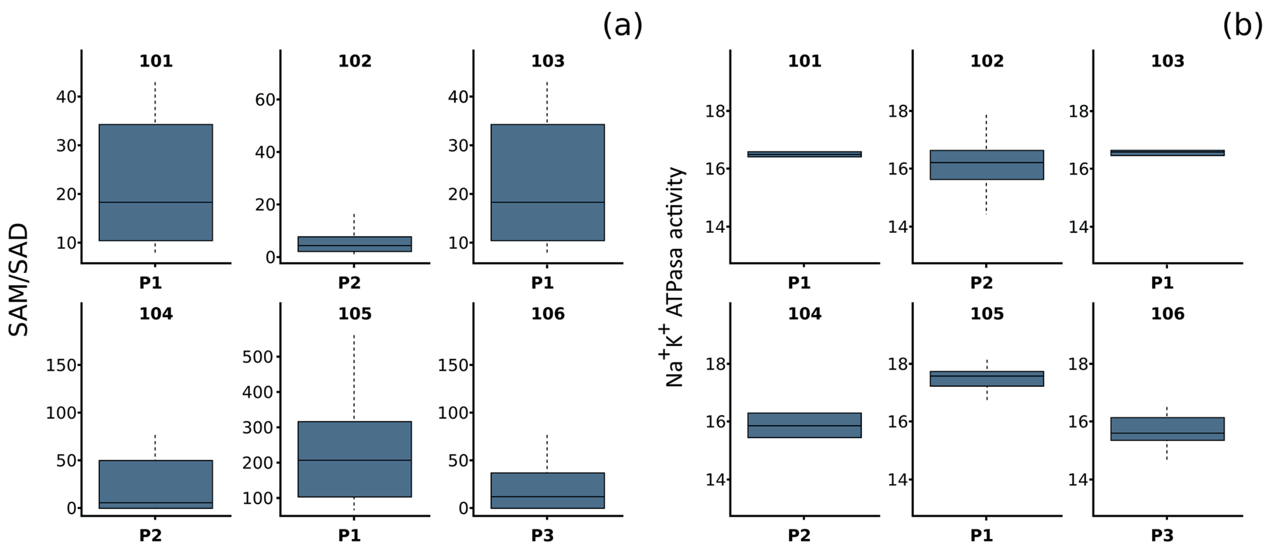

Figure 3. Differences in SAM/SAD ratio (a) and Na+/K+ ATPasa activity (b) in fish depending on its farm origin and cage destiny.

SAD levels were greater than 1.8 (Fig. 1). Different levels of SAM/SAD ratio between fish farms were observed, but P1 had the highest values (Fig. 1).

Additionally, significant differences were observed between the average weight and the condition index depending on the SAM/SAD ratio (Fig. 2). A significant increase in SAM/SAD was observed in fish bigger than 100 g (Fig. 2a) and with a higher condition index (Fig. 2b).

Marine center

One hundred percent of the smolts from P1 were smoltified while the rate of the fish from P2 and P3 was about 60%. Na+/K+ ATPase enzyme activity showed low variability and a value close to 16 in all fish farms (Fig. 3b). However, the proportion of failed smolts in CM1 was higher in fish from P2 and P3, a situation that could not have been predicted by the enzyme. The SAM/SAD ratio was lower in the center P2 and P3 fish (Fig. 3a), which correlated with higher mortality due to mismatches in CM1.

A negative correlation between SAM/SAD and failed smolts at 90 days was observed. Therefore, SAM/SAD can be a good predictor of failed smolts mortality. Mortality began after the sixth week after admission. Cage 104 had the highest mortality rate and cages 101 and 103 with fish from P1 farm cages of P1 has the lowest.

Failed smolts mortality is negatively related when the SAM/SAD ratio is higher than 1.8. The cages with a smaller proportion of fish with a SAM/SAD ratio higher than 1.8 had higher mortality rates. This indicates that there is a significant relationship between failed smolts mortality and a high proportion of SAM/SAD fish> 1.8.

Conclusions

According to the present field study, it is concluded that the higher the proportion of smolts, the lower the failed smolts mortality. The higher the proportion of fish with SAM/SAD ratio> 1.8 in each cage, the lower the risk of failed smolts mortality.

Failed smolts mortality depends on the amount of non-failed smolts (SAM/SAD ratio> 1.8), rather than on farms of origin. To minimize mortality, a homogeneous population is required, where 100% of the cage fish population has SAM/SAD> 1.8.

SAM/SAD> 1.8 shows a better predictive value of failed smolts mortality at the destination center than the activity of the enzyme Na+/K+ ATPase.

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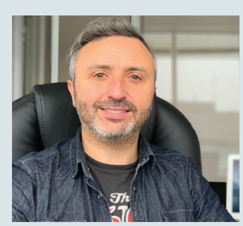

Industry Events

Send your meeting details to:
editor@hatcheryfm.com

MARCH

16 - 17:	International Conference on Aquaculture, Bangkok, Thailand	aemconferences.com/aqua/
16 - 18:	Engineering, Management & Operation of Extrusion Plant Course, Potchefstroom, South Africa	www.fie.com.au
17 - 19:	AFIA Purchasing & Ingredient Suppliers, Seattle, USA	www.afia.org
March 30 - April 1:	Aquafeed Extrusion Technology Course, Temuco, Chile	www.fie.com.au

APRIL

2 - 3:	Food and Feed Drying Technology Course, Temuco, Chile	www.fie.com.au
20 - 21:	International Conference on Aquaculture and Marine Biology, Tokyo, Japan	www.conferencemind.com
22 - 24:	International Genomics in Aquaculture Symposium, Granada, Spain	www.gia2020.es
23:	Biofloc Technology 2020, Singapore	www.aquaculturesg.org

MAY

7 - 9:	Guatemala Aquaculture Symposium, Antigua Guatemala, Guatemala	acuiculturaypescaenguatemala.com
17 - 19:	ONE: The Alltech Ideas Conference, Lexington, KY, USA	one.alltech.com
19 - 21:	Aquaculture UK, Scotland, UK	aquacultureuk.com
20 - 21:	World Aquaculture and Fisheries Conference, Tokyo, Japan	worldaquacultureconference.com
27 - 28:	US Microalgae Industry Summit, Orlando, USA	www.wplgroup.com
31 - June 5:	International Symposium on Fish Nutrition and Feeding, Busan, South Korea	worldaquacultureconference.com

JUNE

2 - 6:	Insects to Feed the World, Quebec, Canada	www.ifw2020.org
3 - 5:	XXIX FEFAC Congress, Antwerp, Belgium	www.fefac.eu
8 - 12:	World Aquaculture 2020, Singapore	was.org
17 - 18:	EnhanceMicroAlgae Conference, Manchester, UK	lfc44.uconn.edu
21 - 26:	44th Larval Fish Conference, Mystic, CT, USA	enhancemicroalgae.eu