HATCHERY TECHNOLOGY OF MARINE FISH

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Introduction

The full cycle aquaculture starts at the hatchery, and encompasses broodstock maintenance and conditioning to spawn, incubation of eggs, live feeds production, water quality and microbial management, larval rearing, and fingerling production through the nursery stages. All these phases are of crucial importance. Despite a number of challenges, technology continues to improve steadfastly, allowing the aquaculture industry to farther explore opportunities and new species.

Some of the important tropical and subtropical marine fish species whose aquaculture technology is available nowadays are cobia (*Rachycentron canadum*), hamachi/kampachi (*Seriola rivoliana, S. lalandi/S. dorsalis*), pompanos (*Trachinotus carolinus*), Pacific red snapper (*Lutjanus peru*), pink snapper (*Lutjanus guttatus*), red snapper (*L. campechunus*), mahi-mahi or dolphinfish (*Coryphaena hippurus*), totoaba (*Totoaba macdonaldi*), red drum (*Sciaenops ocellatus*), groupers (*Epinephelus spp*), snooks (*Centropomidae*), among others. Full cycle aquaculture techniques of temperate species such as the sea bream (*Sparus aurata*), seabass (*Dicentrachus labrax*), hirame or Japanese flounder (*Paralichthys olivaceus*), and turbot (*Scophtalmus maximus*) have been mastered. There have also been efforts to develop technology to close the cycle of bluefin tuna (*Thunnus thynnus*), yellowfin tuna (*T. albacares*) and blackfin tuna (*T. atlanticus*).

Arguably, the fundamental methodologies of semi-intensive and intensive systems employed for raising marine fish larvae have not changed considerably in the last decades. To a certain extent, the experiences and knowledge gained from traditional practices in Asian and European countries have been efficiently adapted to hatcheries in the Americas. However, new tropical and subtropical species exhibit their own idiosyncrasies, and it has been necessary to develop, and implement new hatchery technologies to successfully raise difficult, delicate marine fish species from egg to market.

In some Asian countries, extensive methods of marine fish larval rearing in ponds bloomed with plankton are still being used for some species with little or no significant changes in the last decades. Basic methods for raising marine fish larvae in semi-intensive systems (good water quality and systems management, siphoning the bottom of the tanks and skimming the surface of the water, and using microalgae, rotifers and artemia and often times copepods as live feeds) remain similar. However, technology has evolved at an enormous pace in intensive larval rearing systems.

In this report, we point out important technology advances and identify the most important challenges faced by modern marine fish hatcheries.

The Role of Technology

It all starts with biosecurity and animal welfare. Biosecurity is key to prevent outbreaks of diseases and maintain a healthy hatchery environment free of pathogens. Fish welfare has become a key pillar to sustainability and commercial viability of aquaculture production, affecting final consumers perceptions, acceptance and price of the product in the marketplace.

Albeit constantly evolving, basic larval rearing methods being used today were developed and have been used for several decades. Many Asian countries rely on semi-intensive, mesocosms systems for successfully produce marine fish juveniles. These systems work traditionally very well for a number of species (e.g. several grouper species and hybrids of the Family Serranidae).

However, mesocosms have no or very low water exchange and rely primarily on the natural productivity of the systems, offering limited level of control of temperature and other biotic and abiotic factors, resulting in highly variable survival rates – often times, compromising reliability and productivity (see Appendix 1).

Conversely, modern hatcheries around the world are increasingly adapting to using sophisticated intensive systems with flow-through, highly filtered and sterilized water (U.V., ozone). Many hatcheries are using Recirculating Aquaculture Systems (RAS) technology, with high levels of automation. Self-cleaning tanks and 24-light exposure with continuous feeding of enriched live feeds controlled by timers and peristaltic pumps are being successfully used. Some hatcheries even resort to machine learning and artificial intelligence tools to control water quality, feeding levels, aeration, dissolved oxygen, ammonia, temperature, etc. In intensive systems, larva and juveniles are raised with high water exchange rates, aeration, pure oxygen, constant feeding (often 24 hours a day), and sophisticated filtration. – with some even resorting to machine learning and artificial intelligence tools to control water quality, feeding levels, aeration levels, dissolved oxygen and aeration levels, etc. In intensive systems, larval and juveniles fish are raised with high water exchange rates, aeration, pure oxygen, constant feeding (often 24 hours a day), and sophisticated filtration.

At high stocking densities, any human flaws, system failures, mistakes or accidents are unforgiving. These systems are more complex and expensive to implement and run. A comparison between mesocosms (semi-intensive) and intensive systems is provided in Table 1.

Regardless of the methods being used, when it comes to marine fish hatchery technology, it is well known that small details are the ones posing major challenges to dependable, on-demand production of seedstock, for many commercially valuable tropical and sub-tropical species. Successful larviculture production of difficult, delicate species depends on a number of factors –starting with genetics and nutrition and our ability to control the environment and the larval microbiome. Assuming that optimal genetics and excellent nutrition are observed, microbiological control seems to be key to success. Yet, we still have limited knowledge and a restricted array of proven options to control and manage the environment and the microbiota of the organisms. The microbiome are all the microbes in a given community. A healthy microbes community is essential to colonize and form the gut microbiota of the early developmental larvae. Arguably, it is at this point that the fate of the organisms, and the success of the production, may be determined.

Some hatcheries are using copepods for first feeding of marine fish larvae. This strategy can improve first feeding success in some species whose mouth gapes are small at first feeding stage. Unless they're naturally bloomed in mesocosms (which offer little control), raising and maintaining pure, isolated copepods cultures in large scale represent in itself a major challenge that needs to be considered. We have been successfully using small-strain, properly enriched rotifers (*Brachionus rotundiformis*) for first-feeding of delicate marine fish larvae and recommend this strategy.

Much of the progress achieved with marine hatchery technologies worldwide has been due to our collective efforts of diversifying and targeting a larger number of new species over the years. We have succeeded with some species and have failed with others. Success has certainly been species-specific and limited to a relatively small number of new species, hence demonstrating the paramount importance of selecting the right species. Species selection is key: make it or break it!

In European countries, hatchery technologies that succeed in consistently mass-producing

fingerlings of temperate species, such as sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), have not been effective when applied to tropical species of snappers, for example. Likewise, the same methods used for raising various groupers (*Epinephelus spp*) and snapper (*Lutjanus spp*) in Asian countries yield highly variable survival rates with some species and very low in others. Inconsistent results have been an impediment to mass culture of many species. Variable, unpredictable survival rates hinder planning, production and profitability. What has made a huge impact is that we have been continuously trying, and often times succeeding, in raising new species. Some species are difficult to raise, while others respond to the methods we use and grow and survive beyond our original expectations. The yellowtail or kingfsh (*Seriola*), some species of groupers and snappers and cobia are some examples of it.

Finally, intensive systems offer advantages with regards to product traceability and quality control – which are very important at all levels -, and technology has been driving advances in these areas as well. For example, Near Field Communications (NFC) technology has been used to track down seafood products to their origin, from farm to market.

Improved Nutrition and Health

Most, if not all problems leading to low survival and crashes of marine fish larviculture, are likely caused by opportunistic bacteria, mostly *Vibrios*. These turn pathogenic when reaching threshold numbers, favored by the open flow-through systems that we use. It is likely that the low survival rate attained with certain species is related to the capacity that each species has to cope with the extremely high bacterial loads of the live cultures we provide as feeds to the larvae. All exogenous, abiotic parameters being the same, the observed differences in survival rates during early developmental stages among difficult to raise marine fish species may be related to how they evolved their reproductive strategies over time. Even the best technologies and management cannot control evolution and reproductive strategies with respect to the microbiological environment and larval microbiome. This is one of the main reasons why selecting a hardy species is so important.

In marine fish hatchery research, microbiome manipulation and microbiological control of the environment point the way forward. The importance of broodstock nutrition cannot be underestimated. Beyond the nutritional value, what the broodstock animals are fed with will be vertically transferred to their eggs and larvae, which are key determinants of their quality. Likewise, once the larvae open their mouth and drink water for the first time before they even start feeding, the microorganisms present in the water immediately colonize their guts, establishing the microbiome and perhaps even their ultimate fate. Using appropriate, effective disinfecting methods for gaining control over the bacterial loads in the live feed cultures fed to the larvae (microalgae, rotifers and artemia) is of paramount importance but is often overlooked.

Hatchery operators everywhere face the same issues: relatively low survivals, various inconsistencies and high standard deviations. Besides low survivals, variability is such that it is almost like that larval rearing production runs are still like a historical hit-and-miss for many species. Water filtration and sterilization are not enough for certain species whose larvae did not evolve to cope with abnormally high bacterial loads in a hatchery environment. We must continually try to address, understand and resolve why large mortalities and standard deviations are experienced during larviculture in marine fish hatcheries.

To that end, continuous advances in science and technology are key to understanding the nutritional requirements and nutrient digestibility of the different species commercially cultured, particularly at their

early life stages. This has allowed for improved enrichment medias for live feeds and optimized formulations of starting (weaning) and juveniles stages –leading to better survivals and larval quality, increasing hatchery productivity at lower ecological and economic costs.

At the University of Miami, our Aquaculture Nutrition Program is dedicated to addressing and resolving marine fish nutrition challenges and have been working towards developing formulations that consider the nutritional requirements of different species at different life stages as well as the digestibility of ingredients by targeted species (Suarez et al., 2013).

Looking Forward

The key ingredients in a recipe for improved hatchery technology are:

1. Selective breeding - genetics
2. Right species selected – must have good market and be hardy
3. Water quality – right site selected
4. Technology – improved mesocosm(semi-intensive) and intensive, automated systems
5. Biosecurity – key control points to be rigorously followed
6. Gut microbiome – microbiological control of the environment and the microbiota
7. Fish welfare – outstanding nutrition and health
8. Social responsibility – job creation and community role
9. Skilled trained personnel –it's all about the people. The success of the operation depends on those who run it.
10. Diligent work – reiterating the importance of dedication and qualified personnel.

To improve successful culture of challenging and valuable species of marine fish on a commercially viable basis, we must focus on fish welfare, which essentially encompasses genetics, nutrition, health and microbiological control. Under these broad "umbrellas", a vast array of "ammunition" is becoming available to assist hatchery managers and producers to improve results. In this context, nutrigenomics and functional feeds are used to enhance immune systems and improve performance. Microbiome control is key.

Modern hatcheries are beginning to rely on bacteriophages, probiotics, prebiotics, eubiotics, organic acids, essential oils, beta-glucans, monoglycerides and nucleotides to gain control over the systems and the organisms. We still have much to learn and apply. Marine fish larvae are unforgiven and when it comes to hatchery management, excellence is entry level. Species, water quality, nutrition, health, and above all well trained and qualified staff at all levels are basic requirements to succeed.

Appendix 1

A "Mesocosm Recipe" For Semi-Intensive Larval Husbandry of Marine Finfish.

A protocol for using mesocosm systems bloomed with phytoplankton and zooplankton for semi-intensive larval husbandry of marine finfish entails a series of simple steps:

We recommend tanks no smaller than 5-tons or larger than 50-tons for ease of management. Small tanks are difficult circular tank measuring 4.5 m diameter x 1.2 m fitted with an internal central standpipe wrapped in 300 µm mesh (a normal volume and size used for production in hatcheries). One week prior to stocking, the tank should be were washed and scrubbed using muriatic acid (10%) before being sterilized with chlorine solution at 50 ppm for 24 hours and neutralized with sodium thiosulphate. As an option, the tank can be fitted with artificial substrates such as Aquamats (a surface media used to reduce levels of NH4 by attached bacteria that acts as a natural habitat for larvae and organisms within the mesocosm). If using artificial substrates, we recommend place sections measuring 10 ft. (3.3 m) in a cross formation within the tank and weighted with sand-filled PVC pipes to stay 2 inches off the bottom to

allow proper water circulation. Aeration should be kept at moderate levels at all times and can be provided by 4 air stones evenly distributed around the tank. Optionally, an air powered protein skimmer can also be used to control organics and fine suspended solids. Fertilizers (1 liter of Fritz F1 and F2 or similar, corresponding nutrient media) are added to the water prior to inoculating with microalgae. A combination of *Nannochloropsis sp* and Isochrysis (1:1) (other microalgae such as *Tetraselmis sp* may be used as well) along with other naturally occurring phytoplankton (primarily diatoms) should be also allowed to bloom in the tanks. Probiotics of various types, primarily yeast-based containing a blend of *Bacillus* and *Lactobacillus spp* are routinely used to attempt to exercise some level of control over the microbiology of the water system (primarily at the bacterial level) –which will ultimately colonize the larvae guts and establish their microbiome. The composition, base, strength (Colony Forming Units - CFUs) and blends of probiotics available in the market as well as the manufacturers' recommendations vary according to the product. We advise to follow the manufacturer's recommendations (e.g., depending on the CFU/s, adding 10-20 ppm to the systems before stocking the larvae and periodically thereafter). Ae do. Not recommend to stock eggs. Once the system is "mature" (it may take between one and two weeks), we recommend stocking newly-hatched larvae of the selected species at relatively low densities (4-12 larvae/liter). For example, a 20-ton tank would be stocked with of 80,000-200,000 yolk-sac larvae. At this stage, a highly diverse assortment of zooplanktonic organisms -–consisting mostly of copepods and other phytoplankton and zooplankton organisms– are available to the larvae. The blooms will likely initially occur in small pockets lining the perimeter of the tank and tend to spread throughout the entire system at concentrations >10 organisms/ml before tapering off 2 weeks after the initial introduction of copepods. Besides copepods, other zooplankton bloomed within the mesocosm systems, including jellyfish and other potentially dangerous species. Rotifers and Artemia can be added at various concentrations as needed to supplement the natural productivity of the system.

In our experience with a number of species, while survival rates remain low, larval rates of growth and development are faster in outdoors mesocosm semi-intensive systems than in intensive systems indoors. With this method, plankton blooms can be routinely maintained for several weeks through subtle manipulation of shading, water flow, aeration, and the addition of microalgae. Secchi disc readings averaging 50 cm seem ideal but can be highly variable. While results can be satisfactory, the method offers limited control and production results are highly variable. Larval growth and development in these systems are faster than in intensive systems, but survival rates can range from 0-20%.

What follows is a step by step guide to mesocosm preparation, maintenance and control.

Mesocosm Protocol

Preparation

1. Wash and clean tank with 10% muriatic/hydrochloric acid. Rinse thoroughly.
2. Chlorinate tank, air lines, air stones with 50 ppm chlorine for 24 hours.
3. Neutralize chlorine with sodium thiosulfate or if not available, aerate the water vigorously for 6 hours, drain and rinse tank. Refill tank and aerate again for 6 hours to remove traces of residual chlorine.
4. Fit tank with airlines, air stones, and standpipe with screen (50 to 300 micron). A 50 micron screen will retain the nauplii stages of most copepod species but can create problems with clogging. A larger size screen reduces clogging but increases the chance of losing eggs and smaller organisms. Ultimately, the choice of screen

size is determined by the size of zooplankton used in the mesocosm.

5. Fill tank with either filtered seawater or raw seawater. The use of filtered water provides greater biosecurity but means that the mesocosm will have to be seeded with the desired species of plankton. The use of raw water seeds the mesocosm with naturally occurring species but can also lead to the introduction of unwanted pathogens, parasites, and/or predators.
6. Optionally, the mesocosm can utilize artificial substrates and probiotics. Artificial substrates can provide habitat for organisms within the mesocosm and can provide additional surface area for beneficial bacteria species. The use of probiotics (10 ppm) may reduce the proliferation of pathogenic bacteria and help maintain water quality in the mesocosm.
7. Fertilize the mesocosm using available products such as Fritz's F1/F2 or similar mixture of nutrients. The composition of the fertilizer will be determined by the choice of species in the mesocosm (i.e., diatoms will require a source of silica). The mixtures described below are used for rapid, mass production of microalgae. For a mesocosm, it is desirable to have the microalgae "bloom" at a slower more controllable rate. In this case, the amount of fertilizer can be reduced to 50, 25, or 10 percent of the amount listed below.

Composition (g/1000L)	(F1)	(F2)	(F3)
Ammonium sulfate	100	80	100
Super phosphate	15	15	10
Clewat 32	15	4	2.5
Urea			5
Sodium silicate (used for Chaetoceros, diatoms)	45		

Mix with distilled water (1L)

8. Monitor the system for a bloom of naturally occurring plankton (if raw seawater used), or seed the mesocosm with the desired species of phytoplankton such as *Nannochloropsis, Isochrysis, Tetraselmis, Chaetoceros, Thallasiosira*, etc.

Operation and Maintenance of the System

1. Monitor water quality parameters such as oxygen, pH, salinity, temperature, and total ammonia on a daily basis. Water quality parameters should be stable before addition of zooplankton to the system.
2. After the microalgae has bloomed, seed the mesocosm (if necessary) with the desired species of zooplankton, ie copepods, rotifers, etc. Zooplankton collected from the wild should be treated for parasites before introduction to the mesocosm. Treatment with 0.3 ppm copper sulfate for 1 hour is effective against parasites.
3. Monitor plankton density. Count microalgae cells and zooplankton organisms per/mL to establish plankton density and the population dynamics of the system. Recommended ranges for algal densities vary from 200,000 cells/mL to 1,000,000 cells/mL but must be high enough to support zooplankton grazing. It is important to determine the egg ratio for zooplankton ([total # eggs/ total # organisms] x100) to estimate the fertility and productivity of zooplankton species in the system.
4. Once zooplankton have reached the desired composition and density, zooplankton can be routinely harvested for use in intensive larval rearing, or newly hatched larvae can be stocked directly (5 to 10 larvae/L) into the mesocosm for semi-intensive larval culture. For larval culture in the mesocosm, favorable densities of the desired zooplankton are from 5 to 10 organisms per mL (ie nauplii, rotifers).
5. Sample daily to check larvae for growth and development as well as health (finfold integrity for bacteria, skin parasites, gill parasites after 2 weeks). Use formalin treatments as needed.

Additional Considerations

The mesocosm requires daily attention to maintain satisfactory water quality and to achieve stability and productivity in the system. Seeding the mesocosm with a mixture of several species of microalgae is recommended in order to provide a broad nutritional profile for the different species and stages of zooplankton present in the system. When using copepods in the mesocosm it is not recommended to add rotifers in the beginning. The rapid life cycle and high fecundity of rotifers allows them to outcompete copepods and quickly dominate the system. Microalgae and zooplankton may be added to the system as necessary to achieve required density. The system should be equipped with multiple sources of aeration distributed equally throughout the system with aeration provided at low to moderate levels. Oxygen supply is also recommended so as to maintain DO levels close to saturation (6.5 mg/L@ 26 C). Water exchange and volume changes can be used to maintain water quality or adjust plankton density. However, changes should be implemented gradually and water exchanges performed slowly. In the beginning, recommended water exchange rates are approximately 5-10% per day and can be increased to up to 1000% 3 to 4 weeks after larval stocking.

Table 1. Basic characteristics and differences between mesocosms and intensive systems used in marine fish hatcheries

Inputs/Systems	Mesocosm - Semi-Intensive	Intensive
Stocking densities	Low (≤ 5-20 larvae/L)	High (≤ 50-100 larvae/L)
Water exchange rates	Low (0-20%/day)	High (100-2000%/day)
Fertilization	Yes (inorganic nutrients mix)	No
Microalgae/Phytoplankton or microalgae paste	Live 100,000 cells/ml to bloom	Live or paste, "green" water (200,000+ cells/ml)
Rotifers/Artemia/Copepods	Initially, to maintain at 3-10/ml	Yes, daily, to keep 10-20/ml
Aeration	Low to moderate	Moderate to high
Pure oxygen	No	Yes, to maintain 7-10 mg/L
Reliance on natural productivity	Yes	No
Level of Control	Little control	High level of control
Running costs	Low	High
Production (fingerlings/L)	Low to medium (0.1-1/L)	Medium to high (2-20/L)
Level of management	Low to medium	Very high
Consistency and reliability	Low consistency, variable results	High consistency, reliable results
Automatic feeding	No	Yes
Photoperiod	Natural	Artificial, variable
Self-cleaning tanks	No	Sometimes

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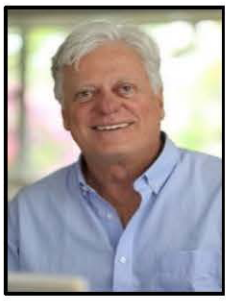

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He has published over 140 articles in aquaculture science, technology and production, has been a consultant for the private and government sectors and is internationally recognized for his contributions to modern aquaculture. He has extensive experience with the industry and has been a consultant for the government and private sectors in Latin America, U.S., Europe, Asia, Caribbean, Africa, Australia and the Middle East. He collaborates with researchers and institutions the world over. Some of the countries he has consulted or is currently consulting for are: US, Chile, Peru, Ecuador, Colombia, Panama, Costa Rica, Mexico, Brazil, the Bahamas, Turkey, Kuwait, Kingdom of Saudi Arabia, Thailand, Japan, and China. Benetti has been a member of the SABs at a number of committees such as FAO, WWF, MBA Seafood Watch, the Pew Oceans Commission, among others. He currently chairs the prestigious BAP Hatchery Committee.

Benetti directs a very productive academic and research program and currently advises 18 graduate students (PhD, MS and MPS). He specializes in technology transfer and planning and has experience in obtaining funding, planning, designing, implementing and running marine fish hatcheries and growout aquaculture operations, including training of scientific, technical and managerial staff. His work is centered on innovative approaches to ensure that seafood production through mariculture is science based, wholesome, environmentally sustainable and economic viable.

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Soy In Aquaculture Program

This technical paper was created through the USSEC Soy In Aquaculture (SIA) program and the USSEC Southeast Asian Regional Program. USSEC works with target audiences in Southeast Asia and globally to show the utility and benefits of using United States soybean products in aquaculture diets.

The SIA program replaces the Managed Aquaculture Marketing and Research Program (the AquaSoy Initiative, funded and supported by the United Soybean Board and American Soybean Association) which was designed to remove the barrier to soybean meal use in diets fed to aquaculture species.

The objective of the SIA is to optimize soy product use in aquaculture diets and to create a preference for U.S. soy products in particular, including but not limited to U.S. soybean meal, soybean oil, soybean lecithin, and "advanced soy proteins" such as fermented soy and soybean protein concentrate.

This paper follows the tradition of USSEC to provide useful technical materials to target audiences in the aquaculture industry.

For more information on soybean use in aquaculture and to view additional technical papers, please visit the Soy-In-Aquaculture website at www.soyaqua.org.

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