Marine Fish Hatchery: Developments and Future Trends

Clarissa L. Marte[a] *and Joebert D. Toledo*[b*]

[a] Integrated Services for the Development of Aquaculture and Fisheries (ISDA)
[b] Marine Finfish Seed Production Specialist, Feedmix Specialists Inc II
* jdtoledo52@gmail.com

Abstract

The basic procedures for producing marine fish fry in hatcheries developed for milkfish fry production nearly 3 decades ago are the basis of fry production systems for all other marine fish species that are now reared in hatcheries in the Philippines and other Southeast Asian countries. These include large-scale microalgae production in outdoor tanks, feeding of appropriate sized rotifer grown on microalgae such as *Nannochlorum* during the first feeding phase, and shifting to larger prey such as *Artemia* towards the latter stages of production.

In recent years, the increasing demand for high-value species such as groupers, sea bass, red snapper, and pompano in both local and export markets has encouraged a number of hatcheries to produce fry to supply the requirements of fish cage farmers. Techniques are modified using information from research institutions and multi-national firms active in developing products and equipment to improve commercial production of these species. Larval feeds of appropriate sizes, forms and presentation for various larval stages incorporating essential nutrients, micronutrients, and feed stimulants are now available in the market. Diseases in marine fish hatcheries have become common occurrences such that various chemotherapeutants, vaccines, and immunostimulants are now available and increasingly being applied in fish hatcheries. Technological developments in hatchery systems, such as the use of recirculating systems, water pretreatment protocols (ozonation, mircrofiltration, UV light treatment) are also increasingly being adopted by commercial establishments.

A critical link between fry production and production of marketable fish is fingerling/juvenile production in nurseries. Fry are commonly grown in brackishwater fishponds to appropriate size for stocking in fish cages. Methods to improve growth through proper feeding and nutrition, eliminate or reduce disease occurrence and parasite infestation, reduce cannibalism in cannibalistic species such as sea bass, grouper and snappers are active areas of research. Nursery production is integrated with fry production in large commercial facilities but is also done by small-scale fish farmers who have access to fry either from the wild or hatcheries. Commercial hatcheries adopt fingerling production from well-studied species in developed countries. Small-scale farmers however still rely on zooplanktons collected from the wild such as copepods, *Moina*, mysids, and trash fish as feed. Production is dependent on availability of feed sources and susceptibility to pathogens and parasites that come with the feed. It can also be erratic since small-scale farms are vulnerable to changes in climate and weather conditions.

Further technological advancement in marine fish hatcheries will increasingly be led by commercial establishments and industries developing equipment like photobioreactor for microalgae to produce algal paste, or methods to develop intensive systems for rotifer culture. Research institutions will however need to support the needs of the small-scale farmers and

hatchery operators who may not be able to apply costly products from these companies by developing innovative simple techniques that can improve culture systems such as producing fry and fingerlings in mesocosm pond system, appropriate use of probiotics as water stabilizer, and production of zooplankton in ponds.

Keywords: marine fish, hatchery, larval rearing, nursery, broodstock

Introduction

Fish farming has been practiced for centuries in Southeast Asia with production coming mainly from freshwater culture. Brackishwater culture of milkfish however was a major activity in the Philippines, Indonesia and Taiwan, with milkfish contributing a sizable percentage of the food fish consumed by the population. Milkfish culture has been and continues to be the main aquaculture enterprise in the Philippines with fry traditionally sourced from the sea. Milkfish is the staple food fish in the Philippines, and contributes the largest share in fish produced from aquaculture in the Philippines and Indonesia. However, since more than three decades ago, fry supply had been difficult to procure for some months of the year because of seasonal changes, adverse climatic conditions and actual decrease in volume caught by fry gatherers even during peak months. To assure continuous and reliable fry supply, milkfish breeding research was initiated at the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC AQD)in the 1970s. In collaboration with other international research institutions, the research effort led to the development of broodstock management technologies including induced spawning (Liao *et al.*, 1979), spontaneous maturation and spawning in floating cages (Marte and Lacanilao, 1986) and tanks (Emata and Marte, 1994), and larval rearing technologies (Juario *et al.*, 1984; Gapasin and Marte, 1990). Through the years, these technologies were continuously improved and refined with research on nutrition, physiology, behaviour, disease prevention and management. The broodstock and hatchery technology developed for milkfish was subsequently modified and applied in developing breeding andlarval rearing methods for other marine fish that have high commercial value such as sea bass, grouper, snapper, pompano and rabbitfish (Marte, 2003).

Fry production is the first stage in the fish farming cycle that ends in the production of marketable fish. A necessary and crucial stage however is the production of fingerlings to supply the requirements of fishponds and marine and freshwater cages. While pond culture of fingerlings for stocking in grow-out farms is traditionally practiced by milkfish farmers as part of the farming cycle, recent innovations in nursery rearing has improved production. The nursery subsector of the milkfish industry is now emerging as a lucrative business enterprise.

The basic techniques in larval rearing developed for milkfish, modifications adopted for carnivorous species and those with long larval gestation phases, technologies developed by multinational companies and the private sector to

improve production in the hatchery, and recent innovations in nursery production are described in the following sections.

Broodstock Development and Management

Aquaculture had been dependent on wild-caught fry and juveniles for stocking in fishponds or cages. The practice was unsustainable particularly for species such as groupers that are often caught using destructive methods such as the use of cyanide. Even for species such as milkfish whose fry is traditionally caught along the shoreline using fine-meshed nets, the numerous other fry species caught together with milkfish that are discarded contribute to the depletion of important species that are part of the marine food chain making the capture method ecologically unsound. The development of marine fish broodstock and establishment of commercial hatcheries has long been recognized as a primary means of reducing pressure on wild juvenile stocks and supply the demand for seedstock of fish farmers.

Source of breeders: farmed or wild

Fish broodstock may be caught as adults from the wild and brought to the broodstock/hatchery facility for spawning if these are reproductively ripe. Spawning techniques developed in research facilities such as injection of human chorionic gonadotropin (HCG) or luteinizing hormone releasing hormone (LHRHa) is applied at the appropriate dose and the fish are either strip-spawned or allowed to spawn naturally. Young adults are reared for several years and acclimated to captive conditions of the facility until they show signs of reproductive readiness. As with ripe adults, breeders are checked for spawning readiness and induced to spawn with hormones or allowed to spawn naturally. Facilities for rearing and maintaining marine fish broodstock are either cages located in clean, safe environments such as marine coves, or in land-based canvas or concrete tanks. For practical and economic considerations, young adults are first reared in cages or ponds to reduce maintenance cost and later transferred to land-based facilities when the fish are ready for spawning. Milkfish farmers often leave juveniles and young adults in brackish or marine ponds for 2-4 years before these are transferred to either cages or tanks.

For many marine fish, most of the nutritional requirements of the broodstock have been determined or are currently being refined by nutritionists in research institutions. Commercial feed companies or broodstock operators use the information in formulating appropriate broodstock feeds. Nutritionists determine basic protein, lipid and energy requirements of broodstock and focus on some of the essential nutrients such as highly unsaturated fatty acids (HUFAs) and vitamins that directly affect egg production and quality.

Marine fish broodstock spawn during their natural breeding season although they may be induced to spawn at other times of the year using hormonal and/or environmental triggers. Sea bass for instance may spawn outside their natural breeding season when maintained at 30-35 ppt, 29-30°C and day light regime of 13 hours. Temperature is reduced to 23-24°C for 8-10 weeks a year to simulate cold months and to allow gamete development (Fielder, pers. comm.). Changes in climate patterns appear to have an effect on

spawning and egg production as observed recently for milkfish that have been spawning almost year round.

Marine fish Larval Rearing- then and now

The specifications and requirements for a small-scale marine fish hatchery are detailed in Sim, et. al (2005). Figure 1 illustrates the basic design of a hatchery suitable for rearing various marine fish such as milkfish, sea bass, grouper, rabbitfish, pompano and others. Various modifications are made by hatchery operators, depending on their projected production targets, availability of construction materials, financing, market, etc. Site requirements and availability of support services will be the same for small-scale and large commercial hatcheries.

Hatchery production technologies for marine fish in the Philippines started with the development of breeding methods for milkfish in the 1980s. With the successful hatchery production of milkfish fry, research efforts to develop technologies for other marine fish such as sea bass, rabbitfish (Ayson *et al.*, 2014), snapper (Duray *et al.*, 1996), grouper (Sugama *et al.*, 2012) and pompano (Reyes *et al.*, 2014) were done resulting in the production of fry in commercial hatcheries.

The basic milkfish larval rearing scheme is shown in Figure 2. Newly hatched larvae are stocked at 10-20 larvae per liter in concrete or canvas tanks filled with seawater that has been seeded with the microalgae *Nannochlorum*. Rotifers are added on the second or third day, initially at 2-3 rotifers per ml, and then gradually increased to 10-20 individuals per ml as the larvae grow. Water management involves replacing 10-20% of the rearing water with fresh seawater during the first week of larval rearing and increasing the volume to about 50% towards the later phase of rearing. Microalgae density is maintained at 1-3 x 10^5 cells per ml during the entire rearing period. With information on nutritional requirements of larvae, microparticulate diets have been developed and these are given at 0.5-2g/ton/day as supplemental feed for larvae as early as the 8^{th} day of rearing until harvest. Milkfish larvae have a short gestation period and fry are harvested on the 18^{th} to 20^{th} day (Figure 2).

Modifications based on the larval rearing scheme developed for milkfish were adopted for the rearing of seabass, rabbitfish, grouper, snapper and pompano larvae. Larval rearing of these species takes from 50 to 60 days that may be divided into two phases: 1) an early rearing phase lasting 20 days and following the procedure used for milkfish larval rearing; and 2) an extended second phase lasting until the 60^{th} day of rearing where larger plankton prey such as *Artemia nauplii*, on-grown *Artemia*, copepods, or mysids are added as live food for the increasingly cannibalistic larvae (Toledo *et al.*, 1999). Artificial diets are also provided, at increasing amounts of up to 3-5g/ton/day. Similar water management methods, such as siphoning of tank bottom to remove debris and excess feeds from the 5^{th} day onwards and water change from 20-30% until the 20^{th} day, increasing to 50-70% until the 35-40^{th} day, are employed. Continuous flow-through water exchange is done from the 40^{th} day until harvest (Figure 3).

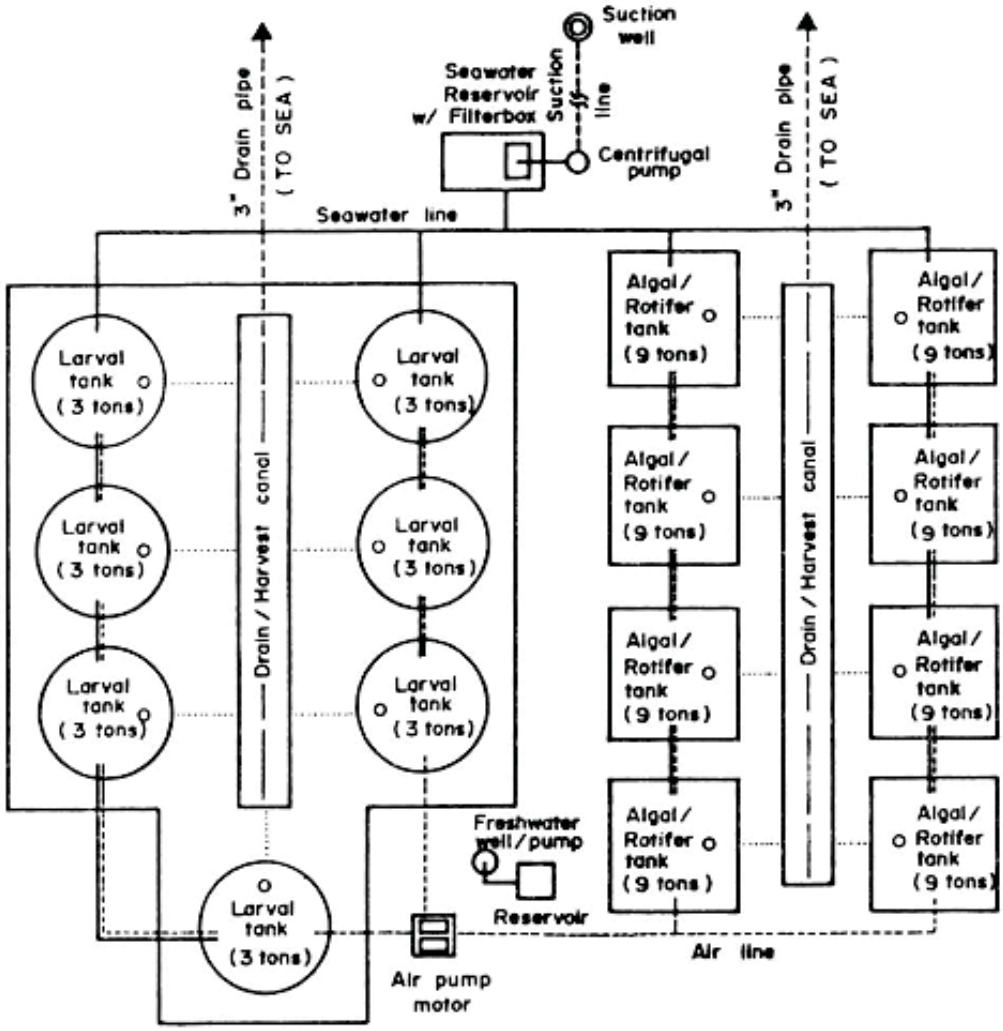

Figure 1. Layout of a typical small-scale milkfish hatchery (Gapasin and Marte, 1990).

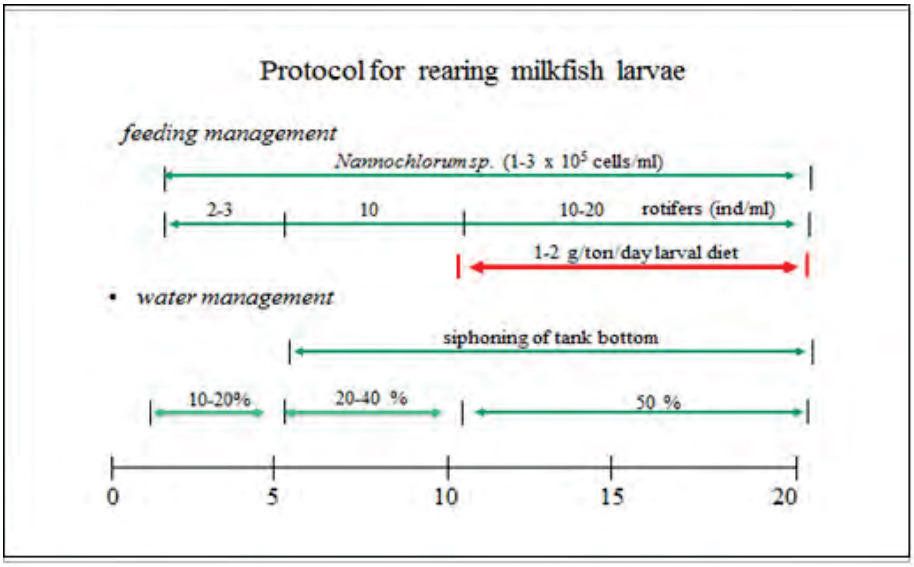

Figure 2. Larval rearing scheme for milkfish.

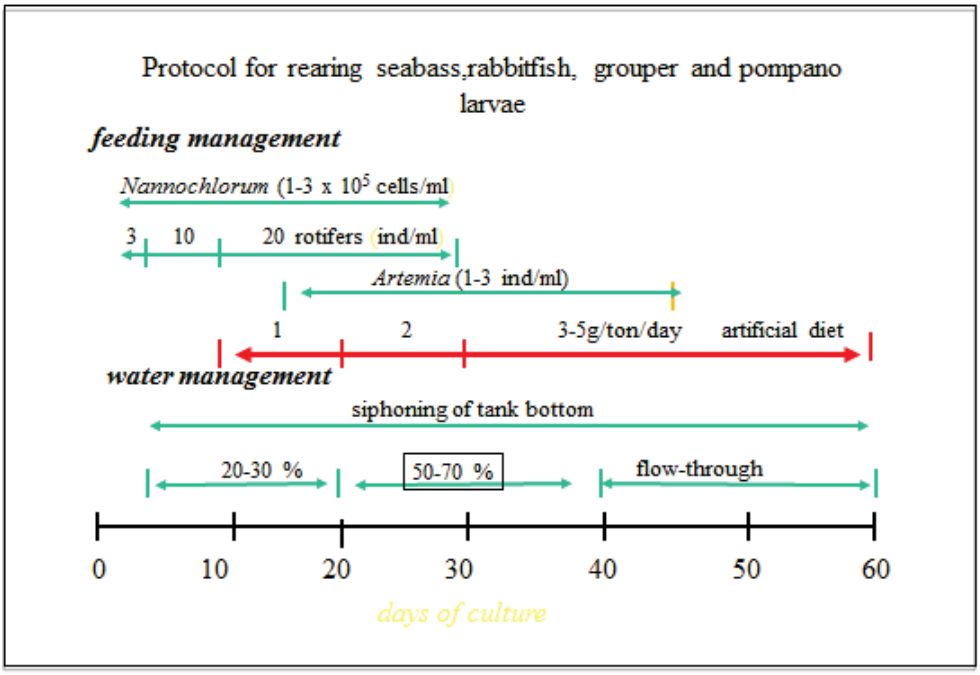

Figure 3. Larval rearing scheme for high value fish (sea bass, grouper, rabbitfish, snapper and pompano).

Recent Developments in Fish Hatchery and Nursery Technologies

Microalgae and rotifers are essential first food for marine fish larvae and culture techniques continue to be improved. Since microalgae and rotifer production requires more than half of the tank facilities of a hatchery, ways to increase cell densities, and methods to produce and to preserve concentrated microalgal paste or slurry are active areas of research although some products from commercial companies are already available to big hatchery operators. Aside from the conventional method of rotifer culture in outdoor tanks using microalgae as sole food, rotifers may now be grown using a combination of Baker's yeast, commercially available *Nannochlorum* or *Chlorella* paste, and live microalgae. These innovations led to the development of super intensive rotifer system with a production efficiency of more than 50 times the conventional system. Because intensive rotifer systems may reach a density as high as 10,000 ind/ml, a system is used to remove solid wastes, neutralize ammonia levels and maintain dissolved oxygen levels higher than 4ppm (for review, see Dhert *et al.*, 2001). A high-density continuous recirculating system using sodium hydroxymethanesulfonate to neutralize ammonia was recently reported to produce large quantities of rotifers on a daily basis without the use of a biofilter and with a lower production cost than a batch culture system (Bentley *et al.*, 2008).

Rotifers and *Artemia* are deficient in highly unsaturated fatty acids (HUFA) that are essential for normal growth and survival of marine fish fry (Ogata *et al.*, 2006). Methods to enrich rotifers and *Artemia* have been developed and products for boosting fatty acid levels are now available. Various formulations containing docosahexaenoic

acid (DHA), eicosapentaenoic acid (EPA) arachidonic acid (ARA) and Vitamin C can be used to enrich rotifers and *Artemia*. Enriched rotifers and *Artemia* fed to milkfish and other marine fish larvae result in improved fry survival rates and reduced morphological deformities (Gapasin and Duray, 2001). Microorganisms that produce high levels of HUFA such as *Thraustocrytrids* have been shown to improve survival rate of milkfish fry and was comparable to commercial products when used to supplement larval food (Estudillo-del Castillo *et al.,* 2009). To date, there are a number of commercial enrichment products available but these are costly.

Growing microalgae and rotifers to feed to marine fish larvae is labor-intensive. Natural food production is also unpredictable and affected by changing weather patterns especially for small-scale hatcheries that have little or no effective protection against unfavorable weather. With the development of larval diets based on the known nutritional requirements of larval and juvenile stages, microbound, and microencapsulated feeds are fed to the larvae midway during the rotifer feeding period and in most cases may completely replace live food during the latter phases of rearing. Artificial diets should be of appropriate size for the stage of the larvae, attractive to the larvae, digestible and contain nutrients needed by the larvae. In addition, the physical properties of the larval diet is critical in ensuring efficient utilization of the nutrients it contains.

Hatchery facilities range from low-cost canvas tanks of backyard hatcheries to large industrial type integrated broodstock and hatcheries. Support facilities for backyard hatcheries consist mainly of at least two seawater pumps, an aeration system, and a power generator as a back-up. Integrated broodstock and hatchery support facilities may, in addition, include systems to filter incoming water, UV facilities, or other water sterilization equipment such as ozonators to disinfect seawater. These additional equipment are usually included in recirculating systems to control entry of predators, and pathogens.

Mesocosm Systems

These are culture systems for fish larvae with water volume ranging from 1 to 10,000 m^3 where a pelagic ecosystem is developed consisting of multi-species, natural food chain of phytoplankton and zooplankton for fish larvae. Most common systems used are the pond and tank mesocosm. Cement 50-100 m^3 tanks or 300-1000 m^2 earthen ponds are cleaned and sun dried for 3-4 days and filled with filtered seawater rich in phyto- and zooplankton. The tanks are then fertilized with commercial sources of nitrogen and phosphorus. Fish larvae, just before complete yolk absorption, are introduced into the system when the abundance of the plankton is enough to support the population. It is important to have proper timing of the availability of larvae for stocking and the available quantity and quality of zooplankton population. Stocking densities vary from 0.1 to 1.0 larva per liter. In a pond mesocosm system in Taiwan, a 500 m^2 pond is stocked with 500,000 larvae of the giant grouper *Epinephelus lanceolatus*. The pond is provided with moderate aeration during the first 2 weeks using a single propulsion-type aerator from the 3rd week until harvest. Harvest is done by seine between days 30-35 when total length (TL) is about 1.8-2.5 cm. Additional zooplankton (rotifer, copepod, or mysids)

are supplied when needed. Separate ponds may also be prepared to culture these food organisms. Formulated feeds are given in increasing amounts from third week after stocking. Probiotics are widely used to maintain the desired water quality. About 50,000 giant grouper fry may be harvested from this system depending on the quality of the larvae stocked, abundance of natural food and weather conditions (Toledo, personal observation).

Nursery Phase

Larval rearing ends after the larvae achieve full metamorphosis. Fry harvested from larval tanks or mesocosm system are often not large nor strong enough for stocking directly in grow-out farms. Milkfish fry are usually stocked in nursery ponds until they reach a size of about 2-3cm. Nursery ponds are prepared by complete drying to eliminate predators and application of appropriate fertilization to promote growth of natural food. Once the natural food are depleted, the "hatirin" are harvested and transferred to a prepared pond to grow further to 10-15 cm for stocking in grow-out farms. Formulated feed is introduced when the natural food in the pond is almost consumed.

Other high value marine species achieve complete metamorphosis at various age and size. Seabass and snub-nose pompano metamorphose between days 21-25 at a size of 1.6 to 2.2 cm TL. Mangrove snapper and tiger and green groupers metamorphose into juveniles from 2.0-2.5 cm TL at days 35-45. Newly-metamorphosed groupers and pompano are usually reared in cement tanks of about 2-5 tons in a flow-through system. Net cages in brackishwater ponds or coastal waters may alternately be used for nursery of metamorphosed snapper and seabass fry. The fry at this stage are weaned to formulated feeds from live food such as copepods, mysids or on-grown *Artemia*. There is an increasing trend in using probiotics in nursery tanks to improve the water quality and reduce water consumption for flow-through system. Size grading is done at least once a week to control cannibalism and to check for parasite and bacterial infection.

A major disease problem encountered in marine fish hatcheries and nurseries is infestations from the dinoflagellate *Amyloodinium* that occur during certain months. Small-scale hatchery operators are mainly affected by the infestation because of lack of filtration facilities, improperly located hatchery (close to rivers and other polluting establishments) and perhaps poor water management. If uncontrolled, this can cause large mortalities in larvae and considerable loss in harvestable fry (Cruz-Lacierda *et al.*, 2004). *Amyloodinium* affects milkfish, seabass, grouper and pompano larvae as early as a week after hatching and may cause massive mortalities if not controlled. Overnight bath at the larval stage in 0.50 ppm copper sulphate may eliminate the free swimming dinospore stage of the parasite but may not eradicate the trophonts attached to larvae nor the reproductive cysts (Toledo, personal observation). The parasite can be controlled at the fry stage by application of low concentration of formalin or hydrogen peroxide and freshwater bath.

Fish with long gestation periods such as seabass, grouper, rabbitfish, pompano and snapper are susceptible to Viral Nervous Necrosis (IVNN), a viral disease that is transmitted from broodstock, plankton, or infected food organisms. The disease was

first reported in 2002 in 35 day-old orange spotted grouper (Maeno *et al.*, 2002) and in 14 day-old seabass larvae (Maeno *et al.*, 2004). VNN is now a major disease problem occurring in most fish reared in the hatchery, nursery and grow-out culture. Ways to prevent and control the disease has been a continuing research effort. Since VNN is transmitted from broodstock to eggs and larvae, steps to prevent VNN infection in the hatchery starts with screening broodstock using molecular tools (RT-PCR) and selection of VNN negative fish as spawners. Further screening of eggs, feeding of broodstock with artificial diets, and periodic sampling of larvae for the presence of the virus, need to be done to ensure prevention of the disease (de la Peña, 2010). Husbandry procedures such as thorough cleaning and disinfection of tanks and hatchery paraphernalia, discarding dead fish and reducing stressors to broodstock and larvae also need to be followed. A promising method to prevent viral infection in hatcheries is by enhancing the immune response of broodstock resulting in virus-resistant breeders that produce VNN-free eggs (Pakingking *et al.*, 2010). An annual vaccination regimen using a formalin inactivated virus applied to seabass enhanced neutralizing antibody titers against VNN in broodstock and antibodies transmitted to spawned eggs (Pakingking *et al.*, 2012). Development and maintenance of VNN-free broodstock as source of spawned eggs will be an essential step to ensure disease free larvae in the hatchery. Commercial vaccines currently being developed and tested by a number of pharmaceutical companies (e.g. AquaVac) are expensive and only large-scale hatchery and farm operators may be able to afford these once these become available in the market.

Future directions

Farming of marine fish will increasingly rely on hatchery-produced seeds with new species added to the roster of available species that are being cultured. Research institutions and commercial establishments will actively pursue various avenues to improve production of marine fish fry. These will include improvement of the design of hatchery facilities by developing more cost-efficient filtration and sterilization facilities, improved biosecurity measures, and nutritionally superior broodstock, larval and nursery feeds. For species that are currently being produced in hatcheries, future directions will include undertaking breeding programs to improve growth rates, improve feed quality for breeders to increase egg production and adopt broodstock management procedures that will promote extension of the spawning season of seasonal breeders. Many carnivorous fish are still fed fish by-catch, hence, good quality artificial diets that are attractive to mature breeders still need to be developed. Studies to enhance resistance to diseases, identify new disease agents, and prevent vertical transmission of disease agents from breeders to fry are active research areas. However, cost-effective vaccines and vaccination procedures especially for fry and fingerlings still need to be developed to make these available to small-scale hatchery operators. There are numerous feed supplements currently available but these are costly and may not be affordable to small-scale operators. Natural food candidates that are nutritionally superior to the currently available food items, and natural sources of feed additives can reduce production cost. Efforts to identify, isolate, and develop culture techniques for these

organisms need to be pursued. There are already available concentrated preserved microalgal products that are mainly used for the production of biofuels or food supplements for animals but there are very few microalgal species used in marine fish larval rearing that are amenable to preservation. Methods to concentrate, preserve and extend the shelf life of these preserved microalgae for hatcheries will need to be developed. These technologies will considerably reduce requirement for tank facilities, increase fry production potential by utilizing tanks intended for microalgae production but these need to be cost-effective and made available to commercial and small-scale operators.

References

Ayson FG, Reyes OS and de Jesus-Ayson EG. 2014. Seed production of rabbitfish *Siganus guttatus*. Aquaculture Extension Manual No. 59. SEAFDEC Aquaculture Department. Tigbauan, Iloilo, Philippines. 18 pp.

Bentley CD, Carroll PM, Watanabe WO and Riedel AM. 2008. Intensive rotifer production in a pilot scale continuous culture recirculating system using nonviable microalgae and an ammonia neutralizer. Journal of the World Aquaculture Society 39: 625-635.

Cruz-Lacierda E, Maeno Y, Pineda AJT and Matey VE. 2004. Mass mortality of hatchery-reared milkfish (*Chanos chanos*) and mangrove red snapper (*Lutjanus argentimaculatus*) caused by *Amyloodinium nocellatum* (Dinoflagellida). Aquaculture 236: 85-94.

de la Peña L. 2010. Prevention and control measures against viral nervous necrosis (VNN) in marine fish hatcheries. Aquaculture Extension Manual No. 44. SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines 26 pp.

Dhert P, Rombaut G, Suantika G and Sorgeloos P. 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200: 129–146.

Duray MN, Alpasan LG and Estudillo CB. 1996. Improved hatchery rearing of mangrove red snapper, *Lutjanus argentimaculatus*, in large tanks with small rotifer (*Brachionus plicatilis*) and *Artemia*. Israeli Journal Of Aquaculture - Bamidgeh 48: 123-132.

Emata AC and Marte CL. 1994. Natural spawning, egg and fry production of milkfish *Chanos chanos* (Forsskal), broodstock reared in concrete tanks. Journal of Applied Ichthyology 10(1): 10-16.

Estudillo-Del Castillo C, Gapasin RSJ and Leaño EM. 2009. Enrichment potential of HUFA-rich thraustochytrid *Schizochytrium mangrovei* for the rotifer *Brachionus plicatilis*. Aquaculture 293: 57-61.

Gapasin RSJ and Marte CL. 1990. Milkfish Hatchery Operations. Aquaculture Extension Manual No.17. SEAFDEC Aquaculture Department. Tigbauan, Iloilo, Philippines. 24 pp.

Gapasin RSJ and Duray MN. 2001. Effects of DHA-enriched live food on growth, survival and incidence of opercular deformities in milkfish (*Chanos chanos*). Aquaculture 193: 49-63.

Juario JV, Duray MN, Nacario JF and Almendras JM. 1984. Induced breeding and larval rearing experiments with milkfish *Chanos chanos* (Forsskal) in the Philippines. Aquaculture 36: 61-70.

Liao IC, Juario JV, Kumagai S, Nakajima H, Natividad M and Buri P. 1979. On the induced spawning and larval rearing of milkfish, *Chanos chanos* (Forsskal). Aquaculture 18: 75-93.

Maeno Y, de la Peña LD and Cruz-Lacierda ER. 2002. Nodavirus infection in hatchery-reared orange-spotted grouper *Epinephelus coiodes*: First record of viral nervous necrosis in the Philippines. Fish Pathology 37: 87-89.

Maeno Y, de la Peña LD and Cruz-Lacierda ER. 2004. Mass mortalities associated with viral nervous necrosis in hatchery-reared sea bass *Lates calcarifer* in the Philippines. Japan Agricultural Research Quarterly 38(1): 69-73.

Marte CL. 2003. Larviculture of marine species in Southeast Asia: current research and industry prospects. Aquaculture 227(1-4): 293-304.

Marte CL and Lacanilao FJ. 1986. Spontaneous maturation and spawning of milkfish in floating net cages. Aquaculture 53: 115-132.

Ogata HY, Chavez DR, Garibay ES, Furuita H and Suloma A. 2006. Hatchery-produced milkfish (*Chanos chanos*) fry should be fed docosahexaenoic acid-enriched live food: A case of the difficulty in the transfer of improved aquaculture technology in the Philippines. Japan Agricultural Research Quarterly 40(4): 393-402.

Pakingking Jr. R, Bautista NB, de Jesus-Ayson EG and Reyes O. 2010. Protective immunity against viral nervous necrosis (VNN) in brown-marbled grouper (*Epinephelus fuscoguttatus*) following vaccination with inactivated betanodavirus. Fish and Shellfish Immunology 28: 525-533.

Pakingking Jr. R, Reyes O and de Jesus-Ayson EG. 2012. Establishment of an immunization regimen for the prevention of viral nervous necrosis (VNN) in high value marine broodfish. Annual Report Government of Japan Trust Fund Projects SEAFDEC Aquaculture Department, Tigbauan, Iloilo 5021, Philippines.

Reyes OS, de Jesus-Ayson EG, Pedroso F and Cabanilla MI. 2014. Hatchery production of snubnose pompano *Trachinotus blochii* Lacepede. Aquaculture Extension Manual No. 56. SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines. 25 pp.

Sim SY, Rimmer MA, Toledo JD, Sugama K, Rumengan I, Williams K and Phillips M. 2005. A Guide to Small-scale Finfish Hatchery Technology. NACA, Bangkok Thailand 17 pp.

Sugama K, Rimmer MA, Ismi S, Koesharyani I, Suwirya K, Giri NA and Alava VR. 2012. Hatchery management of tiger grouper (*Epinephelus fuscoguttatus*): A best-practice manual. Monograph No. 149. Australian Centre for International Agricultural Research: Canberra. 66 pp.

Toledo JD, Golez MS, Doi M and Ohno A. 1999. Use of copepod nauplii during early feeding stage of grouper *Epinephelus coiodes*. Fisheries Science 65: 390-397.

Suggested Readings

Gapasin RSJ, Bombeo R, Lavens RP, Sorgeloos P and Nelis H. 1998. Enrichment of live food with essential fatty acids and vitamin C: Effects on milkfish (*Chanos chanos*) larval performance. Aquaculture 162: 269-286.