Winter School on Technological Advances in Mariculture for Production Enhancement and Sustainability



# **Marine Fish Hatchery**

Dr. Boby Ignatius Central Marine Fisheries Research Institute Post Box No. 1603, Ernakulam North P. O., Cochin, Kerala, India boby\_cmfri@yahoo.com

## Introduction

Availability of required quantities of high quality juveniles at the right time and at reasonable cost is the prime requirement for successful commercial fish farming. Any failure to obtain the right seed in quantity and quality at the right time, the production plans will not be attained. Thus, it has long been internationally recognized that a good source of juveniles is the most important prerequisite for fish farming. Most of the worlds fish aquaculture still depend on the fry almost comes exclusively from wild. Seed supply from the wild is often unpredictable and seasonal. Controlled hatchery production of seeds of economically important finfish ensures a steady supply of quality seeds for aquaculture operations.

For a successful hatchery production of marine fin fishes, number of factors have to be considered. In the following sections advances in fish hatchery management particularly in the areas of brood management, induced spawning and larval rearing will be reviewed.

# BROODSTOCK

An adequate supply of broodfish is essential for successful induced-breeding operations or artificial propagation, especially of the most important cultured species. There are two sources of finfish broodstock: wild-caught adults and those reared in ponds or cages. Most marine fish (groupers, seabass, snappers etc.) broodstock are obtained as wild adults. The disadvantages of using wild stock are uncertainty of capturing them, the relatively large expenditure needed for their capture and transport, and the limited opportunities of obtaining good quality eggs.

In certain cases, it is also difficult to obtain adults from the wild. Thus developing breeders in ponds/cages is another option. Fishes domesticated for few years attains sexual maturity in captive conditions. It is advantageous to use pond or cage-reared broodstock as they are already used to culture conditions and are thus easier to develop into broodfish. In general, fish selected for broodstock should be fast-growing, lively fish, among the largest and strongest members of their age group and free from parasites and diseases.

## Age at maturity

The age at maturity varies for different species of fishes. Knowledge about the age at which the species matures is useful in the selection of right sized brooders for breeding purpose. Rabbitfish begins sexual maturation



and spawning in one year of captivity. As Protandrous hermaphrodites, the seabass are mature males in the third year of captivity and became females on the following year. On the other hand, groupers, being protogynous hermaphrodites, are mature females after four years of its growth. It takes longer for them to be transformed to mature males. Both milkfish and snappers take 5 years to attain sexual maturity.

## Determination of sex and maturity of spawners

Two common aspects in the artificial propagation of finfish are the determination of sex and the maturity of spawners. Often, it is difficult to determine the sex of spawners through examining the external morphology of the fish. In some species, a gravid female exhibits a fuller profile than the ripe male; its abdomen is distended. Ripe males are easy to distinguish during the spawning season since milt oozes out from the urogenital pore as its abdomen is pressed. If the degree of maturity is right, the milt will be white and creamy; poor milt is watery and curdled. Milt which is not ripe will demand strong pressure and will be mixed with blood.

Assessment of gonadal maturation of broodstock is still a major difficulty in the artificial propagation of finfish. The commonly-used method to assess sexual development is through gonadal biopsy. Gametes are removed from either an anaesthetized or unanaesthetized fish by using a polyethylene cannula. The inner diameter of the cannula to be used varies with the size of eggs to be sampled. The cannula is inserted 4–15 cm into the ovary or testis and gametes are drawn into the cannula by aspiration as the cannula is slowly withdrawn. The distance to which the cannula is inserted varies with the length of the ovary or testis. Samples from the middle portion, especially of the ovary, are generally considered to be the most representative.

The eggs collected are removed from the cannula by blowing them into a Petridish. They are preserved in 1% formalin in 0.9% NaCl. The average egg diameter is determined from a batch of 50–10 by using a micrometer and their developmental stage is assessed under the microscope. Gonadal maturation is then expressed in terms of average egg diameter and the developmental stage of the eggs.

The milt collected is removed from the cannula by blowing it onto a clean dry Petridish. A small portion of this is mixed with a drop of seawater or brackishwater, depending upon the species, and examined immediately under the microscope. Sperm motility and vitality are then assessed.

## **Transporting spawners**

There are several ways of transporting spawners: from the most simple receptacle, such as plastic bags, to the most sophisticated like special transport vehicles. Containers vary with the size, species and number of fish to be transported and the location of the collecting grounds. A combination of pre-transport starvation, rapid anaesthetization at capture, cool transport water and anesthetization at transport were also used for long distance transport.

## Factors affecting gonad development

## Nutrition

Poor nutrition can result in poor or no reproductive performance and that lack of vitamin supplement could affect sperm quality. Mere reliance on natural food may lead to poor or variable reproductive performance. Fish broodstock diets are now formulated to include high levels of n-3 fatty acids which include enhanced levels of both decosahexaenoic acid and eicosapentaenoic acid. Eggs considered to be of better quality have higher content of these fatty acids. Furthermore, successful embryonic development in fish has been shown to be

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dependant on the balance of aminoacids present in the egg. Results of other studies indicate that reduced feed levels may adversely affect fecundity and composition of ova. Deficiency of Vitamin C in the diet results in eggs that show considerably higher mortalities than eggs. However broodstock fed on 'natural diet/s' often produce eggs of better quality than those on formulated commercial diets. Thus it appears that different fish species may have different dietary requirements and that diets of broodstock should be tailor made to ensure good egg quality.

# Environment

## Photoperiod

One of the factors considered being of great importance to the inducement of sexual maturation and spawning is photoperiod. Photoperiod manipulation is now being employed to alter the normal reproduction of a few cultured species, for example, mullet, rabbitfish, rainbow trout, tilapia, carp and catfish. The greatest advantage of altering the spawning time of the cultured species is the availability of fry for stocking in ponds, pens and cages throughout the year.

## Temperature

Water temperature is another important factor which influences the maturation and spawning of fish. In some species of fish functional maturity is directly controlled by temperature; in others, the time of spawning is regulated by the day-length cycle such that it occurs when the temperature is optimum for survival and the food supply is adequate.

## **Salinity**

Some species of fish, e.g., salmon, migrate from the marine to the freshwater environment in order to spawn, while other species, such as eels, migrate from freshwater to the marine environment to complete their reproductive cycle. This confirms that salinity is somehow related to maturation and spawning.

## Other environmental factors

In addition to photoperiod, temperature and salinity, there are other less obvious factors which may affect the maturation and spawning of broodstock. There is, however, paucity of information regarding the effects of these less obvious factors, which include rainfall, stress, sex ratios, stocking density, isolation from human disturbance, dissolved oxygen, social behaviour of fish, heavy metals, pesticides, and irradiation. Furthermore, the design of holding systems for broodstock such as ponds, tanks and cages is largely unknown.

# SPAWNING AND FERTILIZATION

## **Selection of spawners**

The selection of spawners from the broodstock should be done months before the beginning of natural spawning to allow ample time for the fish to be conditioned to environmental and diet controls. Spawners are normally selected based on the following criteria:

- fish should be active
- fins and scales should be complete
- fish should be free from disease and parasites



- fish should be free from injury or wounds
- males and females of similar size are preferred

# Spawning

Presently, two major techniques are employed in the mass production of fish seeds: artificial fertilization and induced spawning.

## I. Artificial Fertilization

Spawners are caught in natural spawning grounds near the mouth of the river or in saltwater lakes. Normally, the fishermen will net the fish during spring tide 2–3 days before the new moon or full moon, up to 5–6 days after the new moon or full moon.

The degree of maturity of the collected spawners should be immediately checked. If the female has ripe eggs and the milt of the male is at the running stage, stripping is done in the boat. The fertilized eggs can then be transported to the hatchery for subsequent hatching. In cases where only the male is caught, the milt is collected by stripping into a dry glass container and is then stored in an ice box or refrigerator. The milt can maintain its viability after a week in cold storage  $(5^{\circ}-15^{\circ}C)$ . The preserved milt should be made available for immediate use when a ripe female is caught.

The dry method of fertilization is normally used in this case. The eggs are stripped directly from the female into a dry and clean container where the milt is added. A feather is used in mixing the milt and eggs for about 5 min. Filtered seawater are added to the mixture while stirring and then allowed to stand undisturbed for 5 min.

# 2. Induced Spawning

All of the cultured species exhibit spontaneous spawning but this is seasonal and at times unpredictable. Thus induced spawning to ensure availability of eggs, to meet fry demand and as a supplement to natural spawning may be undertaken.

Manipulations of various environmental parameters, such as temperature, photoperiod, salinity, tank volume and depth, substrate vegetation, etc. can often improve the reliability of spawning. However, in some species hormonal treatments are the only means of controlling reproduction reliably. Over the years, a variety of hormonal approaches have been used successfully. These methods began with the crude use of ground pituitaries from mature fish containing gonadotropin (GtH. - which were injected into broodstock to induce spawning (Houssay, 1930. Today, various synthetic, highly potent agonists of the gonadotropin-releasing hormone (GnRHa) are available as well as sustained-release delivery systems for their controlled administration. These methods have contributed significantly to the development of more reliable, less species-specific methods for the control of reproduction of captive broodstocks.

# A. Hormonal induction of ovulation and spawning

Most research and development efforts on the use of hormones to control finfish reproductive cycles in aquaculture have focused on the induction of Final Oocyte Maturation (FOM), ovulation, spermiation and spawning in fish that do not complete these processes in captivity.

However, hormonal manipulations have important applications in commercial aquaculture, even for fish that do undergo FOM and spermiation spontaneously in captivity. In many fish hatcheries, ovulation is induced



with hormones in order to synchronize and optimize egg collection and fry production, thereby minimizing the handling and stress to the fish, and reducing labor requirements

## SPH - acetone-dried pituitary gland homogenate

Hypophysation, the use of ground pituitaries and pituitary extracts to induce spawning in fish, started in the late 1930s in Brazil. Collection of pituitaries for hypophysation was done from reproductively mature broodstock, either males or females (Fontenele, 1955). It was found that pituitaries collected during the spawning season were more efficacious in inducing spawning. Use of ground pituitaries, however, is associated with various drawbacks, the most important ones being (a) the great variability in pituitary LH content; (b) the administration of additional hormones present in the pituitary that may adversely affect the physiology of the treated fish, and the potential for transmission of diseases from donor fish to recipient broodstocks.

# Human chorionic gonadotropin (hCG)

Unlike LH preparations of piscine origin, hCG is often given in a single dose, which ranges between 100 and 4000 international units (IU) per kg body weight. There is one situation in which hCG be preferred over GnRHa. The advantage of hCG is that it acts directly at the level of the gonad and does not require the existence of LH stores or activation of the pituitary gonadotropes. hCG may be more appropriate because it acts much faster, via direct stimulation of the gonad, in inducing FOM, spermiation and spawning

# Use of gonadotropin-releasing hormone (GnRH) and agonists (GnRHa)

Studies in female broodstocks indicated that GnRH and GnRHa were effective in inducing ovarian development, FOM and ovulation in doses ranging from 1 to 15 mg GnRH <sup>kg-1</sup> or 1 to 100 mg GnRHa kg<sup>-1</sup>. The use of GnRH peptides for spawning induction therapies has important advantages over the use of GtH preparations. First, GnRH and its agonists are small decapeptides that do not trigger an immune response and can be used again in subsequent spawning seasons with no reduction in their efficacy. Second, by inducing the release of the endogenous LH, the GnRH repairs the endocrine disruption that results in the failure of captive fish to undergo FOM, ovulation and spawning. Also, GnRH acts at a higher level of the hypothalamus–pituitary–gonad axis. Consequently, GnRH can provide a more balanced stimulation of reproductive events and, presumably, a better integration of these events with other physiological functions, by directly or indirectly affecting the release of other hormones necessary for successful FOM, spermiation and spawning. A third advantage of GnRHa, is that it can be synthesized and obtained in pure form, and thus does not carry the risk of transmitting diseases. Finally, because of the structural similarity of the GnRHs among many fish species the use of GnRHs, unlike the use of gonadotropins, is generic and the same GnRHa has been successfully applied to a wide range of fish species.

# Sustained-release delivery systems for GnRHa

Almost from the first experiments using pituitary extracts for spawning induction, it was recognized that administration of the hormone in a sustained fashion would improve the efficacy of the procedure. The multiple treatments those are often necessary for a successful response present various problems to the hatchery manager. First, repetitive handling of broodstock requires substantial labor, time and monitoring. Especially in situations where the broodfish are kept outdoors, in ponds or cages, it is difficult, very time consuming, and labor intensive to crowd, capture, anaesthetize and inject the fish with hormones, frequently while hatchery personnel are exposed to the elements of nature. Secondly, repetitive handling is stressful to the fish and can often result in pre-spawning mortalities, or at the very least it can adversely affect the progression of FOM.



Over the last 20 years, a variety of GnRHa-delivery systems have been developed and tested in cultured fishes for the control of FOM, ovulation and spermiation. The first such delivery system was prepared using cholesterol and was tested in Atlantic salmon. Cholesterol implants are prepared as solid, cylindrical pellets (3 mm in diameter) and are implanted intramuscularly using an implanter or a scalpel. This GnRHa-delivery system is easy to fabricate and relatively inexpensive, but the GnRHa release from the pellets seems to be extremely variable probably because each implant is prepared individually. The next type of GnRHa-delivery system was fabricated in the form of microspheres (5–200 mm in diameter), using co-polymers of lactic acid and glycolic acid (LGA). The greatest advantage of biodegradable, microspheric delivery systems is that the same preparation can be used to treat fish varying in size from a few grams to many kg. This can be done because the microspheres are suspended in vehicle and are administered on a volume to weight basis. The last type of GnRHa-delivery system system used for spawning induction is prepared in the form of a solid, monolithic implant, using a non-degradable co-polymer of Ethylene and Vinyl Acetate (EVAc. Unlike the biodegradable microspheres and similar to the cholesterol pellets, EVAc delivery systems have a long shelf-life and can maintain their effectiveness for up to 3 years if stored desiccated at 20 °C.

Fishes having eggs with an average diameter equal to or greater than 0.65 mm are induced to spawn by injecting hormones intramuscularly a few centimetres below the dorsal fin. In the first injection the fish is given a combination of 10 mg SPH/kg body weight + 1 000–10 000 IU HCG/kg body weight. In the second injection, the fish is given a combination of 10 mg SPH/kg body weight + 2 000–20 000 IU HCG/kg body weight. Injections are administered intramuscularly a few centimetres below the dorsal fin after which the fish is completely anaesthetized by immersing it in seawater containing 100 ppm 2-phenoxyethanol. The time interval between injections is 24 hours for most marine fish. This interval was selected to ensure that final maturation of eggs is completed before the fish dies or before the eyes of the breeders are completely covered with a white opaque substance.

Milkfish can be induced to spawn when females possess oocytes of 0.67mm in average diameter. The females and the males with milt are injected with either 1000IU of Human Chorionic Gonadotropin (HCG) or  $100\mu$ g of LHRHa per kg of body weight. Similar spaning agent and dosage was used for snapper, but he minimum oocyte diameter is only 0.42mm as spawned eggs of snapper (0.80mm) was smaller than that of milkfish (1.20mm) the effectiveness of LHRHa, administered by injection or pellet implantation has been demonstrated to induce spawning of seabass. A single injection of  $100\mu$ g/kg BW induced spawning of seabass with an initial oocyte diameter of 0.40mm and above. A single injection of hCG ( 2IU/g BW) or implantation of LHRHa can induce spawning of the rabbitifsh.

Usually, only two injections are needed to induce both captive and wild adult fish to spawn as long as the dosage and time-interval mentioned above are followed; however, badly injured fish may need a third injection. In such cases, the dosage of the third injection is that of the second injection. When a third injection is necessary, usually the fertilization and hatching rates are very low.

#### **B.** Induced Spawning by Environmental Manipulation

The method involves the simulation of the natural spawning environment in which temperature, artificial rainfall and tidal fluctuation are manipulated.

At the beginning of the new moon or full moon, the water temperature in the spawning tank is manipulated



by reducing the water level in the tank to 30cm deep at noon and exposing to the sun for 2–3 hours. This procedure increases water temperature in the spawning tank to  $31^{\circ}-32^{\circ}$ C. Filtered seawater is then rapidly added to the tank to stimulate the rising tide. In effect, the water temperature is drastically decreased to  $27^{\circ}-28^{\circ}$ C.

The fish spawn immediately the night after manipulation (18.00-20.00 h) or, if no spawning occurs, manipulation is repeated for 2–3 more days until spawning is achieved.

Whether the fish are induced to spawn by hormone treatment or environmental manipulation, they would continue to spawn for 3–5 days after the first spawning provided the environmental factors that stimulate spawning are present, e.g., new or full moon, changes in salinity and temperature, etc.

# FERTILIZATION AND INCUBATION

The fish that are induced to spawn by hormone injection will be ready to spawn within 9–12 hours after the final injection. The schedule of injections for subsequent spawning must be synchronized with the natural spawning time of the fish which occurs in late evening between 18.00 and 24.00 h. On the other hand, in the stripping method, it is still necessary to sample the eggs from gonads by cannulation and examine them under the microscope. The fish has spawned only if at least 40% of the eggs are transparent.

# Determination of egg and larval quality

Several parameters are used to assess fish egg and larval quality. These include the rates of egg viability, hatching and normal larvae. Chemical composition of eggs are also analysed and of the egg chemical constituents, fatty acids, amino acids, ascorbic acid, yolk protein and DNA and RNA have been reported to have an influence on egg and larval quality.

# LARVAL-REARING

The rearing tanks are usually made of plastic, fibreglass or concrete. The shape of the tanks can be retangular or circular. Volume ranges from 1 to 10m<sup>3</sup>. The tanks are usually protected from sunshine and heavy rain.

Five hours before hatching, the developing eggs are transferred to larvae-rearing tanks. The tanks are provided with mild aeration. The larvae start to hatch 16–25 h after fertilization depending on temperature and species. The usual stocking density of developing eggs is 100–200 eggs/l.

# Factors affecting mass-rearing of marine finfish larvae

- Type of food
- Food density
- Water quality
- Environmental factors

The most important environmental factors affecting larval growth and survival are: (1) light, (2) temperature, and (3) salinity.

(1) Light: The effect of light intensity and photoperiod on the growth and survival of larvae has received little attention in the past. Generally, fish larvae are reared either under continuous light or under day and night conditions.



Light is of primary importance since most marine fish larvae are visual feeders. Nevertheless, the larval eye at first feeding is very simple, with no capabilities of distinguishing between different illuminations. High light intensities of about 1000–2000 lx at the water surface are commonly used in hatcheries. (Naas, K., Huse), I. and Iglesias, J., 1996). Illumination in first feeding tanks for marine fish larvae. *Aquacult. Eng.* 15:4, pp. 291–300 Article | PDF (634 K) | View Record in Scopus | Cited By in Scopus (27). The reflections from surfaces in a tank are very important for the light distribution in the water body. Black tanks are best suited to reproduce natural illumination conditions. White-walled tanks should be avoided since they would be a perfect wall trap due to the phototaxis of the larvae. Green water and dark walled tanks seems to be beneficial, as growth, survival and nutritional condition are usually enhanced.

(2) **Temperature.** Temperature can be either beneficial or detrimental to fish larvae. Temperature regimes outside the tolerance limits of a particular species will cause mortality of larvae while temperature regimes within the range that give good survival may be used to accelerate or even maximize growth of the larvae. High temperatures will shorten the time from hatching to metamorphosis, and consequently, mortality may be reduced.

The effects of temperature on the growth and survival of fish larvae must be determined for each species. Apparently, the eggs and larvae of tropical and subtropical species are generally stenothermal.

(3) **Salinity.** The effect of salinity on the growth and survival of fish larvae is primarily on larval osmoregulation. Survival of larvae of many species may be better at low salinities than higher salinities since low salinities are osmotic to body fluids.

# **REARING ENVIRONMENT**

Good quality seawater at 30–31 ppt is required for larvae rearing. Water temperature is also important and should range from 26° to 28 °C to promote fast growth of larvae.

Larval tanks are prepared one to two days prior to the transfer of newly-hatched larvae. Filtered seawater is added to the tanks and very mild aeration is provided. After stocking, unicellular algae (*Tetraselmis* sp. or *Chlorella* sp.) are added to the tank and maintained at a density of  $8-10 \times 10$  or  $3-4 \times 10$  per ml for *Tetraselmis* sp. and *Chlorella* sp., respectively. These algae serve a dual purpose: as a direct food to the larvae and rotifer and as a water conditioner in the rearing tank.

## Green water and clear water

Microalgae affect the microbiology, nutrition, feeding and behaviour of larvae. The addition of microalgae to the tanks during early rearing of the larvae may affect rearing performance. Microalgae addition rapidly affects the biochemical composition of the rotifers in the larval tanks. Larvae from green water tanks showed higher survival and growth, and less gut contents than larvae reared in clear water. In the former, the ingested rotifers had higher energy and protein content, suggesting that these variables are important for achieving high growth and survival in the larvae.

The growth and survival of fish larvae can also be affected by the type of microalgae used. Interactions between algae and bacteria in the larval tanks might be more important than the nutritional value of the algae. Dead or dying algae would increase the bacterial substrate.



Fish larvae can be reared under stagnant or open-system conditions. Generally, partial water changes are provided and microalgae are supplied to the rearing tanks during the initial stages of culture. Low exchange rates of water may affect the retention time of prey in the larval tanks and changes may occur in the biochemical composition of the prey before being consumed by the larvae. Algal addition is advantageous since the prey can continue feeding. Consequently, in clear water systems, there is a progressive decrease with time in prey quality. This loss of prey quality can be partially avoided by reduction of the prey residence time through an adequate adjustment of the prey density and the prey/larvae ratio.

The day following stocking, the bottom of the larvae-rearing tank should be cleaned and every day thereafter. This is done by siphoning off unfertilized eggs, faeces, dead larvae and uneaten food accumulating on the bottom of the tank. About 20% of the tank water is changed daily for the first 25 days of the rearing period, then increased to 40–60% per day for the remaining culture period. Since seabass can also be cultured in freshwater, it is recommended to reduce the salinity of rearing water when the larvae are still in the hatchery, before transfer to a freshwater environment. Beginning from the twentieth day, salinity can be gradually lowered until freshwater condition is reached on the twenty-fifth day.

# FEED AND FEEDING

# **Prey size**

Prey size may affect the prey ingestion by early fish larvae. It has been reported that the use of small sized rotifers significantly improves the initial feeding performance of fish larvae at the earlier developmental stages. The effect on feeding of using small sized rotifers is mainly due to an increase in feeding incidence rather than in ingestion rates. Therefore, small rotifer supply would improve the incorporation of the larvae to the exogenous feeding from mouth opening. In spite of this, only large rotifers are commonly used in hatcheries for some species. Small sized nauplii of various copepod species were found to very useful for the larval rearing of marine finfishes especially for the species with small larval mouth openings.

# **Prey density**

Maintenance of appropriate feed density in the larval tanks is most important. Since the marine finfish larvae are visual feeders, availability of the prey in the vicinity increases the chances of feeding and saves energy of larvae used for searching the prey.

# LARVAL DIETS

Most species of marine fish that have been cultured are reared on a sequential diet of rotifers, brine shrimp nauplii and dry supplemental diets.

Microalgae are the customary food given to zooplankton that will be fed to larval fish. The type of culture, temperature, nutrients, other conditions and growth phase all can affect the nutritional value of microalgae to zooplankton and to the fish larvae eating them.

# Rotifers

The rotifers are considered as an important live feed in hatchery operation due to their planktonic nature, tolerance to a wide range of environmental conditions, high reproduction rate (0.7-1.4 offspring/female/day), small size and slow swimming nature. More over the filter-feeding nature of the rotifers facilitates the inclusion



of specific nutrients essential for the larval predators through bioencapsulation into their body tissues. As a result it became a suitable prey for fish larvae that have just resorbed their yolk sac. The availability of large quantities of this live food source has contributed to the successful hatchery production of more than 60 marine finfish species and 18 species of crustaceans worldwide.

Two main species of rotifer have been used are *Brachionus plicatilis* (large size) and *Brachionus rotundiformis* (small size).

Health and nutritional quality of rotifers depends on several culture factors: type of culture, water quality, temperature, foods, rotifer density and age of culture. Rotifers are also cultured in many species of algae. These algae should contain significant amounts of DHA and EPA because one or both of theses are essential fatty acids in the diet of marine fish. The ability of rotifers to synthesize these fatty acids is limited and their diet must include a generous portion of these if the requirements of marine fish larvae eating the rotifers are to be met.

The rotifer diet has little effect on the rotifer size and the use of different strains/species of rotifers is required to provide optimal prey size to the larvae.

## Artemia

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp Artemia constitute the most widely used food item. the unique property of the small branchiopod crustacean Artemia to form dormant embryos, so-called 'cysts', may account to a great extent to the designation of a convenient, suitable, or excellent larval food source that it has been credited with. In marine finfish larval rearing, artemia feeding is done when larvae is big enough to capture larger preys. Artemia is usually given after 5-10 days of initial rotifer feeding.

Artemia nauplii are maintained in the larval culture tank at densities of 0.5 to 2 per ml for most species of finfish. To estimate the amount of Artemia required one must consider both the volume of the tank and the expected number of Artemia the larvae will consume. Based on the stage or the age of the larvae, estimate a daily Artemia requirement per ml. The total requirement is calculated by multiplying the predicted requirement per ml by the total volume of the rearing tanks. Each gram of cysts contains approximately 200,000 to 300,000 cysts. Artemia generally have at least a 50 percent hatch.

#### Copepods

Copepods were found to be best alternative and most appropriate for marine fish larvae in which rotifers are an unsuitable first feed. Copepod nauplii are a common natural feed for marine fish larvae species. Small size of copepod nauplii make them suitable for small marie fish larvae at first feeding. Copepods has been used in successful production of marine fish larvae of groupers, snappers, etc. However, the ability to produce copepod nauplii on a large scale has yet to be accomplished as successfully as it has been for rotifers.

Trocophores of bivalves have been found to be a good supplement starter food if given with small rotifers and then replaced with rotifers as soon as the fish are ready. Wild planktons can collected with various nets and traps and can be used for feeding larvae. Nutritional quality is likely to be very high but the appreciable chance of introducing pathogens or pests into the system. Another alternative is extensive culture of zooplankton in ponds and impoundments.



# Feed quality

Enormous efforts has been done on improving the quality of both live foods and formulated diets for larval fish by better understanding of the nutrient requirements of larval fish. Enrichment of live foods has been a major area of emphasis. Artemia can be low in several fatty acids and various products and protocols have been investigated to improve artemia nutrient quality. Rotifers, a commonly given first food, are often enriched in an attempt to improve their nutrient quality. There are a number of commercial products are now available for fatty acid enrichment of live foods. The appropriate concentration of a specific fatty acid and how it interacts with other fatty acids is to explored for a better management of feed quality.

Amino acids are another important component of the diet of rapidly developing larvae providing the building blocks for protein synthesis, and are important energy substrates.

# **Enrichment of live feeds**

To reduce uncertainty concerning lipid quality of zooplankton, they can be enriched. Some marine oils are reliable sources of EPA and DHA, and mixtures of purified oils are also used. Because rotifers cultured in baker's yeast alone are deficient in DHA and EPA, they can be enriched upto 48 hrs with yeast enriched with oil and sometimes vitamin or with other materials. Addition of cuttle liver oil to bakers yeast fed to rotifers increased both EPA nad DHA levels. Brine shrimp nauplii can also be enriched with emulsified marine oil or a micronutrient-fortified marine oil emulsion. A variety of commercial enrichment media for rotifers and artemia are available to improve the nutritional quality of these organisms.

# **Compound larval feeds**

The three main types are microencapsulated, microbound and microcoated diets. early marine fish larvae have difficulty in accepting and digesting microcapsules and microparticulates. Early weaning was the orinal goal of supplementing with compounded feed, but co feeding of compounded feeds with live feeds can at least reduce the live food requirement. Microencapulsted eeds provide ad alternative way to administer vaccines and therapeutic agents to larvae. During early stages, larvae have difficulty in recognizing inert particles as feed. Typically, early marine larvae probably depend on to a greater degree on small colloidal proteins in zooplankton because they donot have the enzymes necessary for digesting and absorbing larger protein molecules. Older larvae have greater capabilities to make more kinds of enzymes and to adjust enzyme production according to the type of food.

As greater understanding of the nutrient requirements of larval fish are gained and weaning protocols are improved, formulated diets will become more widely used and used earlier in the life history for many fish.

# Feed management

Newly hatched larvae are usually not given food on the first day because they derived their nourishment from the yolk and the eyes and mouth are still non functional. During the initial days the larvae were given enriched rotifers at a density of 5-20 rotifers/ml depnding upon the species and age of the larvae. As the larvae grows bigger, freshly hatched brine shrimp nauplii at a density of 1-10 linduviduals /ml depending upon the species and age of the larvae. As the feeding of brine shrimp progress the rotifer density is slowly decreased and finally stopped. As the larvae grow bigger, compounded feeds were given to larvae at a rate of 1-4g/t.



#### Water management

Siphoning of the tank bottom to remove dirt, dead larvae, wastes and decaying uneaten food should be done everyday starting from the second day of rearing. Daily water exchange from as high as 70% of the tank volume to as low as 30% is undertaken prior to feeding. The percentage of water exchange is dependent on the age of the larvae.

## Fry harvest/packing /transport

At the end of larviculture, fry can be harvested and transported to fish farms. Transport is usually done in cool periods of the day. Fishes are transported in oxygenated bags places inside carton boxes lined with thermocole sheets. The transport densities depend upon the size of the fish, species of the fish, distance to be traveled etc. Reducing the temperature and salinity during transport help to improve the survival.

## Conclusion

The hatchery phase is one of the bottlenecks for aquaculture expansion. Broodstock development and induced spawning techniques have improved drastically over years for a number of species by administering gonadotropin releasing hormones via injection or implantation. Advances have been made in broodstock diets, specifically in the use of fatty acids to improve egg quality and quantity to equal that of brooders given natural diets. Advances in the larval rearing systems, better understanding of rearing environment has improved the growth and survival of larvae in captivity. Better understanding of nutritional requirements and by improving the larval feed quality made the hatchery production marine finfishes more successful. Improvements in formulated diets for larval fish have reduced the dependence on live foods at earlier and earlier stages in the life history. Co-feeding during the larval stages helps to reduce the need for live foods and facilitates the transition to formulated diets.

Recent advances in hatchery management have resulted in a much better control of critical life stages of fish. These advances will continue until the science of aquaculture is on a level with that of the other animal sciences.