Seed Production of Marine Fishes

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Introduction

It has long been recognized that a good source of juveniles is the most important prerequisite for fish farming. Non availability of the seed for stocking in quantity and quality at the right time, will affect the production plans. 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. Hatchery production of seeds of economically important finfish ensures a steady supply of quality seeds for aquaculture operations.

The successful hatchery production of marine fin fishes, depends on various factors like proper maintenance of broodstock, efficient live feed production systems, larval rearing protocols including water quality management, feed management and nursery rearing systems.

BROODSTOCK

Availability of adequate number of healthy broodstock is of prime importance in 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. It is advantageous to use cultured broodstock as they are acclimatized to captive conditions, free from exogenous pathogens and diseases. 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. The selection bloodstock should based on the following criteria: fish movement should be active, fins and scales should be complete, and free from diseases and parasites. Upon arrival at hatchery, the fishes should be treated with approved antibiotics.

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 on 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

Determination of sex and the maturity of spawners are very important in the artificial propagation of marine finfishes. Determination the sex of spawners through examining the external morphology of the fish is often difficult and unreliable. Ripe males are easy to distinguish during the spawning season since milt oozes out from the urogenital l pore as its abdomen is pressed. If fishes are fully matured, 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.

The commonly-used method to assess gonadal maturation of broodstock 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 gonad through urogenital pore 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 gonads. Samples from the middle portion, especially of the ovary, are generally considered to be the most representative.

The eggs collected through cannula are observed for its eggs diameter and 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 Petri dish. 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.

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 docosahexaenoic 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 dependent on the balance of aminoacids present in the egg. 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. 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

Salinity is related to maturation and spawning especially for the spawning which shows spawning migrations.

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. These less obvious factors, which include rainfall, stress, sex ratios, stocking density, isolation from human disturbance, dissolved oxygen, social behaviour of fish, presence of heavy metals and pesticides also influence maturation in fishes.

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

After the selection of spawners, the fishes are transferred to spawning tanks. The ratio of male : female in spawning tanks 1:2. Water in spawning tanks should be clean and in good condition.

Two major techniques are used in the spawning of finfishes namely natural spawning by environmental manipulations and hormonal induction of spawning.

Natural 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 30 cm deep at noon and exposing to the sun for 2–3 hours. This procedure increases water temperature in the spawning tank to 31°–32°C. Filtered seawater is then rapidly

added to the tank to simulate the rising tide. In effect, the water temperature is drastically decreased to 27°–28°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.

Hormonal induction of 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 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.

Agents used for induced spawning in fishes

SPH - acetone-dried pituitary gland homogenate

It was found that pituitaries collected during the spawning season were more effective in inducing spawning.

Human chorionic gonadotropin (hCG)

Unlike pituitary extract, Human chorionic gonadotropin (hCG) is often given in a single dose, which ranges between 100 and 4000 international units (IU) per kg body weight.

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 kg^{-1}or 1 to 100 mg GnRHa kg^{-1}. GnRH and its agonists can be used again in subsequent spawning seasons with no reduction in their efficacy. GnRH acts at a higher level of the hypothalamus–pituitary–gonad axis. Consequently, GnRH can provide a more balanced stimulation of reproductive events by directly or indirectly affecting the release of other hormones necessary for successful FOM, spermiation and spawning.

Sustained-release delivery systems for GnRHa

Repeated handling of broodstock requires substantial labor, time and monitoring. A variety of GnRHa-delivery systems have been developed and tested for the sustained release of hormones.

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.

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.

LARVAE-REARING

The rearing tanks are usually made of plastic, fiberglass or concrete. The shape of the tanks can be rectangular or circular. Volume ranges from 1 to 10m^3. 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.

- 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. **15**The reflections from surfaces in a tank are very important for the light distribution in the . 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 isosmotic 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* spp.) are added to the tank and maintained at a density of 8–10 × 10 or 3–4 × 10 per ml for *Tetraselmis* sp. and *Chlorella* spp., 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. The growth and survival of fish larvae

can also be affected by the type of microalgae used. Dead or dying would increase the 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 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).

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.

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 marine fish larvae at first feeding.

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.

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. Microencapulsted feeds provide an alternative way to administer vaccines and therapeutic agents to larvae. During early stages, larvae have difficulty in recognizing inert particles as feed

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 depending upon the species and age of the larvae. As the larvae grows bigger, freshly hatched brine shrimp nauplii at a density of 1-10 1induviduals /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 every day 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 thermocol 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.