Recent advances in breeding and seed production of marine finfish

G. Gopakumar
Emeritus Scientist, Vizhinjam Research Centre of Central Marine Fisheries Research Institute,
Vizhinjam, Thiruvananthapuram, Kerala, India
E-mail: drggopakumar@gmail.com

Introduction

Mariculture produces many high value finfish, crustaceans and molluscs like oysters, mussels, clams, cockles and scallops. In 2012 mariculture has contributed around 24.7 million tonnes of food fish globally which formed about 35.7% of the aquaculture production. World aquaculture production was 90.4 million tonnes in 2012, which contributed 42.2% to the total fish production and supplied 9.4 kg of food fish per person. Molluscs dominated the global mariculture production (60.3%) followed by finfish (22.5%), crustaceans (15.9%) and others (1.3%). In recent years a rapid growth in marine finfish culture is noted which has shown an average annual growth rate of 9.3% from 1990 onwards. The major finfish groups which are maricultured include salmonids, amberjacks, sea breams, sea bass, croakers, groupers, drums, mullets, turbot, other flatfishes, snappers, cobia, pompano, cods, puffers and tunas. (FAO, 2012; 2014). The expansion of sea cage farming on a global basis can be attributed as a shot in the arm for the increased farming of marine finfish. Cage culture has made possible the large-scale production of commercial finfish in many parts of the world and can be considered as the most efficient and economical way of rising fish. The most vital prerequisite for the development of sea cage farming is the technology for breeding and seed production and the reliable supply of good quality hatchery produced seeds of suitable high value marine finfishes. The major species for which captive breeding and hatchery production methods have been established include: Atlantic salmon *Salmo salar*, yellowtail *Seriola quinqueradiata*, breams *Sparus aurata*, *Pagrus major*, *Acanthopagrus schlegeli,* European sea bass *Dicentrarchus labrax*, Asian sea bass *Lates calcarifer,* red snapper *Lutjanus argentimaculatus,* cobia *Rachycentron canadum*, turbot *Scophthalmus maximus*, halibut *Hippoglossus hippoglossus*, cod *Gadus morhua,* Japanese flounder

Paralichthys olivaceus, yellow croaker *Pseudosciaena crocea,* many species of groupers (*Epinephelus* spp.) and pompanos (*Trachinotus* spp.).

Many countries in the Asia-Pacific Region like Australia, China, Japan, Taiwan, Philippines, Indonesia, Thailand, Malaysia and Vietnam have made substantial progress in the development of commercial level seed production technologies of high value finfish for sea farming. A good deal of research and development activities is being pursued internationally in this direction and much advancement is emerging in this sector. The chief areas of research thrust include (i) reproductive biological parameters essential for broodstock development, (ii) broodstock development systems, (iii) broodstock conditioning (iv) broodstock nutrition, (v) hormonal manipulations (vi) live feed and larviculture technologies and (vii) biotechnological interventions.

(i) Reproductive parameters

Generally the broodstock of marine finfish is developed from pre adult fishes collected from the wild. Sound knowledge on the reproductive biology of candidate species is essential for broodstock development. The age and size at first maturity, fecundity, gonado-somatic index, the size of mature egg and the reproductive strategy (whether a gonochorist or hermaphrodite) are also of concern. If the concerned species is a hermaphrodite, then the type *viz.*protandrous/ protogynous, sequential/simultaneous has to be ascertained. Hermaphroditism in fishes is often misdiagnosed, leading to the need for more conclusive diagnostic criteria and clarified terminology (Mitcheson and Liu, 2008).

Knowledge on gametogenesis (spermatogenesis and oogenesis) is essential for determining the maturity stage of the gonad, which is vital for breeding a fish. Most species recruit oocytes from primary growth (PG) to secondary growth (SG) and complete the secondary growth of oocytes in less than a year. Reproductive timing strategies in marine fishes fall along a continous from semelparous total spawners to iteroparous batch spawners with extended spawning seasons and long reproductive life spans. Oocyte developmental patterns reflect these timing strategies in terms of oocyte recruitment from PG to SG and within secondary growth. At the lifetime scale, semelparous species, which participate in only one reproductive cycle and then die, recruit all of their oocytes into secondary growth. In contrast, iteroparous species, which are more common and have the potential to participate in multiple reproductive cycles, constantly maintain a reserve of PG oocytes. One of the major objectives of aquaculture industry is to produce a large number of viable eggs with high survival. Major achievements have been made in recent years in improving protocols for higher efficiency of egg production and viability of progeny. Understanding the mechanism underlying the processes of oocyte growth and development and how these processes are coordinated, is essential for perceiving the factors affecting egg quality and fertilization. Research efforts were directed to identify environmental influences on egg quality, including the difference in quality as a consequence of diet, especially lipids, protein and vitamin content, photoperiod and physiochemical properties of the water, and husbandry practices. Great advances were also made in revealing endocrine pathways regulating egg formation and functional aspects that contribute the proper development of the future embryo (Lubzens *et al*. 2010).

A recent study in the yellowtail kingfish *Seriola lalandi* showed that changes in the size distributions and proportions of oocyte stages during ovarian

development indicated multiple group synchronous oocyte development and the presence of all developmental stages of oocytes in mature ovaries revealed a capacity for multiple spawning within a reproductive season. Change in developmental stages of gametes during testicular maturation and the presence of all gamete stages in partially and fully spermiated males indicated multiple group synchronous gamete development in males. Another investigation on the reproductive maturation of wild caught female and male southern bluefin tuna *Thunnus maccoyii* (SBT) under captive conditions showed that sexually mature female SBT were observed from 101 kg in body weight and 155 cm in fork length, while male SBT were sexually mature at a smaller size (51 kg body weight and 128 cm fork length). Gonadosomatic and gonad index generally increased with increasing stages of maturation for female and male SBT and the fish sourced from the wild and held in the captive environment up to 9 years can achieve sexual maturity (Erin *et al.,* 2012).

(ii) Effective broodstock development systems

The vital requirement of broodstock development of marine finfish is a facility where the biosecure stocks can be maintained and controlled spawning can be obtained year round. The principles of broodstock management are recently reviewed by Duncan *et al.* (2013). The broodstock developed in sea cages are susceptible to mortality due to the changes in the water quality of the cage site, disease problems and impact of harmful algal blooms. Recirculating Aquaculture Systems (RAS) are tank-based systems in which fish can be grown at high density under controlled conditions. RAS use land based units to pump water in a closed loop through fish rearing tanks and consist of a series of sub-systems for water treatment which include equipment for solids removal, biological filtration, heating or cooling, dissolved gas control, water sterilization and photo-thermal control. Using RAS, sustainable production of biosecure seed of high value species all through the year employing photo-thermal conditioning is possible. Successful induced as well as volitional spawning of cobia and pompano were obtained in the recirculation aquaculture system established by CMFRI at Mandapam.

(iii) Broodstock Nutrition

The nutritional quality of the broodstock diet, the feed intake rate or the feeding period can all affect spawning, egg and larval quality and viability of any species. In the case of tropical fishes, ovarian development is often asynchronous. Oocytes in all stages of development are present at the same time and sometimes independent of season. The ovarian development starts with the formation of primary oocytes. Eventhough the oocyte may increase in size several fold during primary and early secondary growth, the most conspicuous size increase occurs during the last part of secondary growth, vitellogenesis. The fatty acid composition of yolk protein precursors, vitellogenins, synthesized in the liver and secreted into the blood can be affected by long term imbalances in the broodstock diet. Diet rich in vitamins, poly-unsaturated fatty acids (n- 3 PUFA) and micro-nutrients is essential for obtaining viable eggs and larvae. Dry pellets should include n-3 PUFA, in particular EPA (20:5 μ 3) and DHA (20:6 μ 3), which cannot be synthesized by metabolism. As in higher vertebrates, vitamin E deficiency affects reproductive performance, causing immature gonads and lower hatching rate and survival of offspring. For example, elevation of dietary a-tocopherol levels has been found to reduce the percentage of abnormal eggs and increase fecundity in the gilthead seabream *Sparus aurata*. Ascorbic acid has also been shown to play an important role in salmonid reproduction, where the dietary requirement of broodstock was higher than

that of juveniles. Among different feed ingredients, cuttlefish, squid and krill meals are recognized as valuable components of broodstock diets (Izquierdo *et al*., 2001).

(iv) Broodstock conditioning

A number of environmental factors have been implicated as possible cues including photoperiod, temperature, rainfall, food supplies and pheromones, responsible for reproduction in majority of fishes. Among these, photoperiod is the most critical and the principal determinant of maturation in the salmonids, bass, breams, mullet, flatfish, sciaenids and seriolids. A combined interaction of photoperiod and temperature also play a role in the gonadal maturation of many species. Photothermal conditioning has been successfully employed in controlled breeding in land based broodstock systems. In a recent study gametogenesis was monitored histologicaly in wild caught red snapper *Lutjanus campechanus* maintained in captivity under simulated natural photothermal conditions. The results indicated that captive red snappers can complete gametogenesis in photothermal controlled conditions (Bardon *et.al*., 2015). In a report on the spawning of tiger grouper *Epinephelus fuscoguttatus* and square tail coral grouper *Plectropomus areolatus* in sea cages and onshore tanks in Andaman-Nicobar Islands, India it was noted that higher water temperature exceeding the upper thermal inhibitory limit for both the grouper species inhibited spawning (Rimmer *et al*., 2013).

(v) Hormonal manipulations

The fish reproductive cycle is separated in the growth (gametogenesis) and maturation phase (oocyte maturation and spermiation), both controlled by the reproductive hormones of the brain, pituitary and gonad. Hormonal manipulations of reproductive function in cultured fishes have focused on the use of either exogenous luteinizing hormone (LH) preparations that act directly at the level of the gonad, or synthetic agonists of gonadotropin-releasing hormone (GnRHa) that act at the level of the pituitary to induce release of the endogenous LH stores, which, in turn act at the level of the gonad to induce steroidogenesis and the process of OM and spermiation. The main factors that may have significant consequences on gamete quality-mainly on eggs-and should be considered when choosing a spawning induction procedure include (a) the developmental stage of the gonads at the time the hormonal therapy is applied, (b) the type of hormonal therapy, (c) the possible stress induced by the manipulation necessary for the hormone administration and (d) in the case of artificial insemination, the latency period between hormonal stimulation and stripping for *in vitro* fertilization (Constantinos *et al*..2010).

a. Improvement of broodstock

The ability to manipulate growth rates through the introduction of additional growth hormone (GH) can be applied to develop better broodstock instead of the conventional selective breeding. Dramatic growth enhancement has been shown using the technique in salmonids (Du *et al*., 1992; Delvin *et al*., 1994).

b. Sex change

Hormonal therapies are also applied for sex reversal and improvement of broodstock. Simultaneous hermaphrodites function concurrently as both male and female and are capable of releasing viable eggs and sperms during the same spawning event. In contrast, sequential hermaphrodites function as a male in one life phase and as female in another. If the male phase develops first, with later sex change into a female, the fish is protandrous; if the female phase develops first, with later sex change into a male, the fish is protogynous. The ability to

change sex is present in at least 23 teleostean families (Helfman *et al.,* 1997) including over 350 species (Munday, 2001) of which most inhabit coral reefs.

A variety of experimental techniques have been developed to induce sex change, thus enabling measurements of hormone metabolism during the sexual transition period. Either a male in protogynous species or a female in protandrous species is removed from the social unit (*i.e.* sex change by release of suppressive dominance) or multiple numbers of the initial sex are recruited together in captivity in the absence of the terminal sex (*i.e.* sex change by induction). In both the situations, at least one individual of the initial sex is expected to undergo sexual transition (Shapiro, 1984; Munoz and Warner, 2003). These methods have been applied in the broodstock development of clown fishes and damselfishes which are highly valued coral reef fishes in the ornamental fish trade. The second method of manipulation of sex change is the administration of sex steroids (*e.g.* testosterone T), derivatives thereof (*e.g.* methyl testosterone: MT) or inhibitors of steroidogenic enzymes (*e.g.* fadrozole). These technologies have been instrumental in the successful development of broodstock of many commercially important marine finfishes such as seabass and groupers.

c. Controlled Gonadal maturation

Acquisition of seed stock from the wild during the seasonal spawning period of fish is unreliable and unpredictable and hence not suitable for commercialization of aquaculture. If reproduction can be controlled, a steady supply of seed can be produced by off-season spawning. But many fishes exhibit reproductive dysfunctions when reared in captivity due to the fact that the fish in captivity do not experience the conditions of spawning grounds and as a result there is a failure in release of maturational gonadotropin and luteinising hormone (LH). In many species hormonal treatments are the only means of controlling reproduction reliably. Over the years, a variety of hormonal techniques have been used successfully.

d. Induction of spawning

Most R & D efforts on the use of hormones to control finfish reproductive cycles in aquaculture have focused on the induction of FOM, ovulation, spermiation and spawning in fish that do not complete these processes in captivity. But, hormonal manipulations have important applications in commercial aquaculture, even for fishes that undergo FOM and spermiation spontaneously in captivity.

(vi) (a) Live feeds and Larviculture

Most marine finfishes have altricial larvae and when yolk sac is exhausted, they remain in an undeveloped state. The digestive system is rudimentary, lacking a stomach and much of the protein digestion takes place in the hind-gut epithelial cells. Altricial larvae cannot digest formulated feeds and hence live feed is vital for their survival. The movement of live feed in water stimulates larval feeding responses. Live feed organisms with a thin exoskeleton and high water content may be more palatable to the larvae when compared to the formulated diets (Stottrup and McEvoy, 2003). The hatchery production of juveniles of marine finfish is achieved globally by the use of green-water technique and the live feeds rotifer and *Artemia.*

(b) Microdiets as alternative for live feeds

Marine fish larvae fed on microdiets have not yet matched the growth and survival performances demonstrated by live feeds such as rotifers and *Artemia*. But there have been substantial achievements in reducing the reliance on live feeds especially the use of *Artemia* and weaning the larvae

earlier onto microdiets; microdiets still cannot completely replace live feeds for most species. The reasons for this are like; the feed particle needs to be attractive to the larvae. The micro particles should be available to the larvae at all time, while limiting fouling of the tank. After ingestion, easier to digest raw materials should be tested and adjusted to balance the amino acid requirements of specific species. This should be linked to diet manufacture methods that may increase or decrease the particle digestibility (Kolkovski, 2013).

(c) Larval feeding behaviour

Successful prey capture by the larvae is the key factor in larviculture and hence lot of advancements has been made on larval feeding behavior. It involves interaction of complex processes *viz.* searching, detection, attack, capture, ingestion, digestion and evacuation. The feeding strategy is related to the specific characteristics of each species. Availability of suitable prey is one of the most determinant biotic factors, but feeding mode and amount of food intake are also influenced by prevailing environmental conditions. Searching depends basically on swimming capacity, while detection depends largely by means of visual, chemical and mechanical stimuli. Most marine fish hatch with immature anatomical features. Olfaction allows for more remote detection of a stimulus. The olfactory organ appears early during embryonic development. The intra and extra oral taste buds develop or proliferate some days or weeks after the first feeding. Mechanical stimuli such as touching or water movements are detected by neuromasts and the lateral line system. The progressive development and completion of all these sensory organs increase the capacity for detection and recognition of potential prey. Basically fish larvae exhibit alternating periods of swimming ability and inactivity. At first feeding, even the smallest larvae have some primordial hunting habits, but the efficacy increases with development and growth, changing from passive feeding to an active prey searching capacity.

Capture success relies not only on development stage and concomitant hunting capacity but also on the availability and accessibility of prey. After mouth opening, fish larvae need to learn hunting and have to do it quickly. High prey availability and accessibility are crucial for successfully initiating feeding. Prey size and swimming ability are primary factors determining the efficacy with which the prey is caught. Mouth gape limits the dimensions of the prey that can be ingested. Searching for appropriate prey of adequate size has been a priority for rearing fish larvae. The established prey sequencing is based on rotifers of different sizes and *Artemia* nauplii and metanauplii. Overall, the current commonly used live feeds, *Brachionus* spp. and *Artemia* spp. meet well the feeding behavior of most larvae except very small larvae at mouth opening (Holt, 2011).

(d) Larval Nutrition

Essential fatty acids (EFAs) play a vital role in larval fish nutrition. They function as (i) source of metabolic energy (ii) structural components of phospholipids (PLs) of cellular membranes and (iii) precursors of bioactive molecules. On an average the requirements for n-3LCPUFAs in larvae of marine finfish are about 3% DW diet or live prey. Optimum DHA levels in larval feeds range from 0.5 to 2.5%. Reported requirements of EPA range from 0.7 to 1.6%. Dietary ARA requirements for marine larval fish range from 0.5 to 1.2%. The optimum EPA/ARA ratios range from 3.5 to 5, whereas optimum dietary DHA/EPA ratios range from 1.2 to 8 (Holt, 2011).

Factors affecting larviculture

Light is an important environmental parameter that is known to significantly affect growth, development and survival of marine fish larvae. Larval

fish rely heavily on visual cues for feeding and developmental success, and hence providing proper photoperiod, light intensity and wavelength of the light given to the larvae are essential to successful production. The light requirements are species specific and lighting characteristics are unique to a fish's environmental niche for optimal growth, survival and development. Feeding success can also be affected by varying the contrast in larval rearing tanks through different tank colours or the use of algal cells or inorganic particles to change the level of turbidity. Improved larval feeding can be attributed to improved vision in turbid waters because turbidity may provide greater contrast between the prey and the ambient background. Photoperiod also plays a vital role in larval rearing. Manipulation of photoperiod can have a major impact on larval growth and survival. Many species have shown improved growth rates when exposed to longer than natural photoperiods.

During the critical period, the density of the live feed and its nutritional qualities determine the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. When changing from smaller size live feed to larger size, co-feeding with both sizes of live feeds is needed for a few days. Weaning to formulated feed has to be done with great care. First feeding of the day can be done with appropriate size formulated feed. Feeding with live feed can be continued till all the larvae are weaned to formulated feed. Different sizes of formulated feeds need to be used as per the mouth size of the larvae. In addition, variety of other factors such as size of the tank, water temperature, water quality, etc., affects the larval survival and growth.

(vi) Biotechnological interventions

a. Hybridization

Hybridization is often used by aquaculturists in order to take advantage of potential desirable culture traits in offspring. Attempts to produce grouper hybrids have been difficult because of reproduction failures and low larval survival. However, in the case of *Epinephelus fuscogutatus* x *Epinephelus polyphekadion* hybrids, offspring have been shown to grow faster than either of the parental species. Successful hybridization of *E. coioides* x *E. lanceolatus* was achieved for the first time using cryopreserved sperm from giant grouper. There was no difference in percent fertilization and hatch between hybrid and non-hybrid orange-spotted grouper, but percent deformity of the hybrids (47%) was higher than non-hybrid (21%). Survival (22%) of the hybrid was lower than non-hybrid (51%) at 12 days post hatching (first feeding period). However, this difference was not significant at the end of 45-day study period (Anocha *et al*., 2011).

b. Cryopreservation

Cryopreservation of fish spermatozoa is a powerful technique for preserving the germplasm and is a prerequisite for establishing gene banks and can provide a year-round supply of fish semen, which brings great convenience for breeding and genetic studies. Most studies on gamete preservation are conducted on a laboratory scale. Not much practical progress of fish spermatozoa cryopreservation in large volume cryovials for the construction of cryobanks or commercial purposes has yet been achieved. Recently a technique for cryopreserving sea perch (*Lateolabrax japonicus*, Cuvier) semen in 1.8ml cryovials was developed. The fertilization rates of frozen semen cryopreserved for 3 days or 1 year in liquid nitrogen were not significantly different from that of fresh sperm. In fertilization trials of 230-ml eggs with frozen semen cryopreserved for 3days in liquid nitrogen, 84.8% fertilization rate and 70.1% hatching rate were obtained. (Ji *et al*., 2004)

c. Gynogenesis

Gynogenesis refers to a process of uniparental inheritance whereby the resulting offspring retain only maternal DNA. It has been used to identify sex-determining mechanisms in fish and to produce all-female populations for aquaculture. A protocol for the production of gynogenetic Atlantic halibut was developed. Various milt concentrations and UV doses were tested for providing genetically inactivated, yet motile, spermatozoa for the production of gynogenetic haploids. A population of gynogenetic diploids which comprised solely of females, was produced which makes possible the commercial culture of Atlantic halibut through gynogenesis (Harald *et al.*, 2006)

d. Surrogate broodstock

Large-scale release of hatchery-produced seeds has been conducted in order to restore worldwide fishery production;however, concerns exist regarding the genetic effects of hatchery stock on wild fish populations, due to the reduced genetic variation often associated with hatchery-reared fish. Therefore, it is important that fish seeds used in stock enhancement possess sufficient genetic diversity to mitigate their genetic impact on wildfish populations. To promote genetic diversity of artificial seed, seed production should be performed using a sufficiently large broodstock. In order to circumvent the need for these investments, a means of producing gametes that possess a large amount of genetic diversity using only a small number of surrogate-broodstock through spermatogonial transplantation was experimented. It was demonstrated that donor derived type A spermatogonia (ASGs) were capable of being colonised within the gonads of the recipients and can differentiate into either functional eggs or sperm depending on the sex of the recipient. ASGs obtained from cryopreserved whole testis could be incorporated into the recipient gonads. This would allow sufficient numbers of donor testes to be collected from local wild fish and stored using liquid nitrogen without the need to rear the donor individuals in captivity. Furthermore, if ASGs are isolated from several donor individuals and mixed prior to their transplantation into a single recipient, the resulting recipient would be expected to produce gametes genetically derived from several donor individuals. These results indicated that that the method of spermatogonial transplantation for production of surrogate broodstock could serve as a novel and efficient method of producing fish seeds with increased genetic diversity for use in aquaculture and stock enhancement (Yoji *et al.,* 2007).

e. Transgenic strain

Transgenic fish strains can be established by microinjection, but this powerful method has not been developed in marine fishes due to the difficulties associated with handling their small and fragile pelagic eggs and larvae. Recently the production of a transgenic strain of Nibe croaker *Nibea mitsukurii* (Sciaenidae), amarine fish that produces small, pelagic eggs was reported (Yoji *etal.*, 2011). Transgenic technique can also be employed to improve the broodstock. An 'all fish' gene construct consisting of ocean pout antifreeze protein (AFP) promoter fused to Chinook salmon GH cDNA was injected into salmonid embryos and due to the availability of transcription factors required for its activation, enhancement of growth in adult salmon to an average size of 3-5 times the size of non-transgenic controls , with some individuals , especially during the first few months of growth, reached as much as 10-30 times the size of controls (Du *et al*., 1992, Delvin *et al*., 1994). These fish generally appeared healthy, and some produced second and third generation offspring (Saunders *et al.,* 1998). The enhanced growth phenotype was inherited along with the genotypes.

The economic advantage of this type of manipulation is obvious and in comparison with selective breeding methods takes very little time for attaining similar success (Melamed *et al*., 2002). The establishment of genetic modification methods in marine aquaculture fishes would be a powerful tool for improving their commercially valuable traits by introducing genes that control a wide range of biological phenomena.

Summary

It is quite evident that a good deal of recent research advances had led to the success in development and standardization of broodstock and seed production of high valued marine food fishes. The knowledge on the endocrine mechanisms of sex reversal has contributed much for the development sex reversal techniques for many species. The biotechnological techniques like RIA and ELISA have played key roles in understanding the role of sex hormones and to develop techniques for inducing the final maturation and spawning of many species. Larviculture of many marine food fishes and ornamental fishes have been successful mainly due to the development of green-water technique and appropriate live feed and nutritional enrichment procedures. Efficient production of microalgae by fermentation techniques, better understanding of green-water technique and mass production of resting eggs of rotifers and copepods are vital areas which can improve larviculture success. Further advances in biotechnological interventions such as the development of better quality broodstock, hybridization techniques, cryopreservation, gynogenesis, transgenic strains and surrogate broodstock through spermatogonial transplantation can lead to the commercialisation of these technologies. In conclusion it can be said the all these research advancescan pave the way for more effective and economic seed production techniques of many high value species for the sustainable expansion marine finfish seed production and farming in the near future.

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