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An overview of mariculture techniques

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Introduction

It is widely accepted that the catch and catch rates of many marine fishery resources are declining mainly due to overcapitalization and overexploitation.. In this context, it is very much relevant to resort to resource augmentation methods through mariculture and allied techniques to enhance the seafood production. Mariculture is the farming and husbandry of marine plants and animals in marine environments. On a global basis, aquaculture is the fastest growing animal food production sector with per capita supply increasing from 0.7 kg in 1970 to 7.8 kg in 2006 with an average annual growth rate of 6.9%. Most of the global aquaculture production of fish, crustaceans and molluscs continues to come from inland waters (61% by quantity and 53% by value). Mariculture contributes 34% of the total aquaculture production and 36% of the value. While much of the marine production is contributed by high value finfish, relatively low priced mussels and oysters are also widely farmed. While the overall share of farmed fish in marine finfish production has stayed much low, for the species that are farmed, cultured fish dominates the market. This is the case of Asian seabass, gilthead sea bream, red drum, bastard halibut and cobia. It is also a fact that for such species, the quantities now produced by aquaculture are often substantially higher than the past highest catch recorded by capture fisheries. In the last decade, salmonids have overtaken shrimp as the top aquaculture group in Latin America and the Caribbean as a result of outbreaks of disease in the major shrimp producing areas (FAO,2009).

Aquaculture in the Asia-Pacific region has been growing steadily over the last few decades and to satisfy the demand of the local and export markets, many countries are expanding their aquaculture activities in the sea, including offshore areas where competition is less. Mariculture in this region is exceptionally biodiverse and relies on many species and hence the nature of mariculture is rapidly changing in this area (Rimmer, 2008). Some of the countries like China, Vietnam, Australia, Indonesia, and Japan. Korea DPR, Korea Rep, Malaysia, Phillippines, Thailand are much ahead in mariculture in this region and agencies like NACA should take intergovernmental regional programmes so as to develop mariculture in the region as a whole.

Mariculture -Indian scenario

The dwindling catch rates in capture fisheries and rampant unemployment in the coastal region focus towards the development of mariculture and coastal aquaculture as a remunerative alternate

occupation. Recent estimates quantify the per capita fish consumption in India around 8-10kg per year and is likely to grow to 16.7kg by 2015. Although about 1.2 million hectares are suitable for land based saline aquaculture in India, currently only 13 % is utilized. Farmed shrimp contributes about 60% by volume and 82% by value of India's total shrimp export. Share of cultured shrimp export is 82,600 tonnes. The farming of shrimp is largely dependant on small holdings of less than 2 hectares, as these farms account for over 90% of the total area utilized for shrimp culture. Coastal aquaculture is mainly concentrated in the states of Andhra Pradesh, Tamil Nadu, Orissa and West Bengal. In recent years, the demand for mussels, clams, edible oysters, crabs, lobsters, sea weeds and a few marine finfishes is continuously increasing and brings premium price in the international market. The long coastline of 8129 km along with the adjacent landward coastal agro climatic zone and the sea-ward inshore waters with large number of calm bays and lagoons offer good scope to develop mariculture in the country.

In this context, the Central marine Fisheries Research Institute (CMFRI) is the pioneering institution in the country which has initiated mariculture research and has been developing appropriate mariculture technologies in India (Devaraj *et al*, 1999, ICAR, 2000, Pillai and Menon, 2000, Pillai *et al*, 2003, Mohan Joseph 2004 Modayil *et al.* 2008, Gopakumar *et al* 2007). In India till date mariculture activities are confined only to coastal brackish water aquaculture, chiefly shrimp farming. The other coastal aquaculture activities are green mussel farming which is confined to Malabar Coast in Kerala producing about 10,000 tonnes and seaweed farming along Ramanathapuram and Tuticorin coasts of Tamilnadu producing 5000 tonnes annually.

The potentially cultivable candidate species in India include about 20 species of finfishes, 29 crustaceans, 17 molluscs, 7 seaweeds and many other species of ornamental and therapeutic value. Many mariculture technologies are very simple, eco-friendly and use only locally available infrastructure facilities for construction of farm, feed and seed and hence the entire farming can be practiced by traditional fishermen. Another advantage is that most of our brackish and coastal areas are free from pollution and suited for aquaculture. But hardly 10% of the potential cultivable area is presently used for aquaculture in spite of growing demand for cultured shrimp, bivalves, crabs, and lobsters etc., all of which are in high demand in the export market. In addition a fast growing trade of marine ornamental fishes and other tropical marines has also emerged in the recent years which open up the possibility of culture and trade of these organisms. . The policies pertaining to advent of alternative avocations to fishers by providing the awareness, training and initial resource capabilities can do better in the way of providing flexibility to other sectors. Employment in aquaculture (inland and marine) has been increasing and is now estimated to account for about 25 percent of the total. (Govt. of India, 2001).

Coastal aquaculture is a significant contributor to marine fish production, constituting mainly the shrimps like *Penaeus monodon* and *P. indicus.* However, vast water bodies highly suitable for aquaculture and the varied biodiversity that has the potential to capture new markets with a wide range of seafood products, have prompted consideration of other candidate species like oysters, mussels, crabs, lobsters, scampi, sea bass, groupers, sea cucumber, ornamental fishes and sea weeds in the new aquaculture scenario in the country. Hatchery and rearing techniques have also been standardised for many of these organisms. (ICAR, 2000)

Existing major mariculture practices

Shrimp seed production and culture

Shrimps being a highly valued export commodity, shrimp farming is considered a lucrative industry. Production-wise *Penaeus monodon* contributed 75% and *F. indicus* 20%. Depending on the area of the pond; inputs like seed, feed and management measures like predator control, water exchange through tidal effects or pumping, etc., farming systems have been classified into four groups: extensive, modified extensive, semi-intensive and intensive. Currently, 80 per cent of the shrimp production comes from small and marginal holdings, with farms of less than 2 ha constituting 49.2 per cent of the total area under culture, between 2-5 ha (15.8 per cent), 5-10 ha (13 per cent) and the rest >10 ha. The farming community has now become more responsive to the concepts of environment-friendliness and sustainable aquaculture. Disease problems are being overcome through adoption of closed system of farming (recirculation system, zero water exchange) in grow outs, application of probiotics, secondary aquaculture of selected fishes like mullets, milkfish, molluscs and seaweeds in reservoirs and drain canals, adoption of indigenous, good quality seed and feed and reduction in stocking density.

Lobster farming and Fattening

Increasing demand for live lobsters in the export market led the farmers and entrepreneurs to collect juvenile lobsters from the wild and grow to marketable size in ponds and tanks by feeding trash fishes and other discards. In many maritime states juvenile lobsters of *Panulirus homarus, P. ornatus* and *P. poyphagus* are grown in captivity and the eyestalk ablated lobsters attained 180 – 200 g in 5 – 6 months period. This type of lobster fattening at a stocking density of 10 – 15 young ones per square meter yielded appreciable growth rates with a profit margin of Rs.50, 000/- from a pond of 70 m^2. Fattening and grow out trials with artificial pellet feeds has been successfully completed. Cage farming of spiny lobsters was successfully demonstrated by CMFRI at Vizhinjam, Mandapam and Veravel. Recently major breakthrough in breeding and hatchery production of two species of scyllarid lobsters, *Thenus orientalis* and *Petrarctus rugosus* was achieved. Successful hatchery production of seeds of *T. orientalis* and its compatibility with *F. indicus* at high density race way culture with very high production rates of 3-5kg/sq.m is highly promising..

Crab farming / fattening

Live mud crabs (*Scylla serrata*, *S. tranquebarica)* being a much sought after export commodity, mud crab fattening is considered the best alternative. Seed stock consists of freshly moulted crabs (water crabs) of 550 g which are stocked in small brackishwater ponds at a stocking density of 1/sq. m or in individual cages for a period of 3-4 weeks while being fed thrice daily with low value fish @ 5-10 per cent of their biomass. Selective harvesting is done according to size, growth and demand and the venture is profitable because of low operating costs and fast turnover. Monoculture (with single size and multiple sizes stocking) and polyculture with milkfish and mullets are being carried out on a small scale, as the seed supply is still mainly from the wild. Hatchery technology for breeding and seed production of the blue swimming crab, *Portunus pelagicus*, has also been developed and four generations of crabs have been produced by domestication. Fattening and grow out trials with artificial pellet feeds has been successfully completed. The hatchery seed is being mainly utilized for stock enhancement programmes along the east coast.

Edible Oyster Farming

CMFRI has developed methods for edible oyster (*Crassostrea madrasensis*) culture and has produced a complete package of technology, which is presently being widely adopted by small scale farmers in shallow estuaries, bays and backwaters all along the coast. In the adopted rack and ren method, a series of vertical poles are driven into the bottom in rows, on top of which horizontal bars are placed. Spat collection is done either from the wild or produced in hatcheries, on suitable cultch materials. Spat collectors consist of clean oyster shells (5-6 Nos.) suspended on a 3 mm nylon rope at spaced intervals of 15-20 cm and suspended from racks, close to natural oyster beds. Spat collection and further rearing is carried out at the same farm site and harvestable size of 80 mm is reached in 8-10 months. Harvesting is done manually with a production rate of 8-10 tonnes/ha. Oyster shells are also in demand by local cement and lime industry and culture production has increased to 800 tonnes in the year 2000.

Mussel Farming

The Institute has developed technologies for culture of bivalves like raft method (in bays, inshore waters), rack method (in brackishwater, estuaries) or long line method (open sea) are commonly adopted for mussel farming (*Perna indica* and *P. viridis*). Mussel seeds of 15-25 mm size collected from intertidal and sub tidal beds are attached to coir/nylon ropes of 1-6 m length and enveloped by mosquito or cotton netting. Seeds get attached to rope within a few days while the netting disintegrates. The seeded ropes are hung from rafts, racks or longlines. A harvestable size of 70-80 mm is reached in 5-7 months and production of 12-14 kg mussel (shell on) per metre of rope can be obtained. Attempts to demonstrate the economic feasibility of mussel culture has led to the development of group farming activities in the coastal communities (especially rural women groups) with active support from local administration and developmental agencies like Brackishwater Fish Farmers Development Agency (BFFDA) and State Fisheries Department. Cultured mussel production has increased from 20 tonnes (1996) to 18,000tonnes (2009) mainly through the rack culture system in estuarine area.

Pearl Oyster Farming and Pearl Production

In India, the marine pearls are obtained from the pearl oyster, *Pinctada fucata*. Success in the production of cultured pearls was achieved for the first time in 1973 by CMFRI Raft culture and rack culture in nearshore areas are the two methods commonly adopted for rearing pearl oysters and recently attempts have been made to develop onshore culture methods. Shell bead nucleus (3-8 mm) implantation is done in the gonads of the oyster through surgical incision while graft tissues are prepared from donor oysters of the same size and age group. Implanted oysters are kept under observation for 3-4 days in the labs, under flow through system and then shifted to the farm in suitable cages for rearing. Periodic monitoring is done and harvest is carried out after 3-12 months. Pearls are categorized into A, B and C types depending on colour, luster and iridescence. 25 per cent pearl production has been successfully demonstrated in a series of farm trials at various locations along the Indian coast. Research is also directed towards development of a technology for *in vitro* pearl production using mantle tissue culture of pearl oyster. The technology for mass production of pearl oyster seed and pearl production has paved the way for its emergence as a profitable coastal aquaculture activity at certain selected centres along the coast. Village level pearl oyster farming

and pearl production, through direct involvement of small scale fishermen have been carried out successfully as part of technology transfer programme along the Valinokkam Bay on the east coast. Recently success has been obtained in the production of Mabe pearls and tissue culture of pearls. Success was achieved in the organ culture of mantle of pearl oyster and abalone. A breakthrough has been achieved by developing a tissue culture technology for marine pearl production using the pearl oyster *Pinctada fucata* and abalone *Haliotis varia* for the first time in the world. This technology can be easily extended to other pearl producing molluscs and gives ample scope for manipulation of pearl quality and also increased pearl production. Mabe pearl production was standardised for production of base images with ten different types of moulds. Technology for production of jewellery from Mabe pearl was also standardised.

Clam Culture

Package of clam culture practices has been developed for the blood clam *Anadara granosa* and *Paphia malabarica*, where production of 40 tonnes/ ha/6 months and 15-25 tonnes/ha/4-5 months have been achieved in field trials. Induced spawning and larval rearing to setting of spat has been perfected for clams like *P. malabarica*, *Meretrix meretrix* and *Marcia opima*.

Abalone Culture

Abalones are marine gastropods of the genus Haliotis. They are known for the production of gem quality pearls and also for their succulent meat. *Haliotis varia* is the commercially important species along the Indian coast. CMFRI has developed methods for the seed production and culture of this species.

Marine Finfish Culture

In the area of marine fish seed production and culture, the country is still in the experimental phase only..Seed production technology is available only for the Asian seabass *Lates calcarifer*. The Central Institute of Brackishwater Aquaculture (CIBA) has developed an indigenous hatchery technology for Asian seabass. The Rajiv Gandhi Centre for Aquaculture (RGCA) has also been propagating the seed production and farming techniques in the country. Recently CMFRI has successfully demonstrated the cage farming of sea bass at different parts of the coast. The broodstock development and spawning of the grouper *Epinephelus tauvina* was achieved at CMFRI. Attempts are being made to develop suitable hatchery and farming technology for cobia, mullets, pearl spot, rabbitfish, groupers, snappers, breams and pompano. The broodstock development of cobia in cages and induced spawning and fingerling production was achieved for the first time in India at Mandapam Regional Centre of CMFRI. The standardisation of fingerling production of cobia can lead to the development of cobia aquaculture in the country.

Ornamental Fish Culture

On a global basis a lucrative marine ornamental fish trade has emerged in recent years which have become a low volume high value industry. There are a wide variety of ornamental fishes in the vast water bodies and coral reef ecosystems along the Indian coast, which if judiciously used, can earn a sizeable foreign exchange. A long term sustainable trade of marine ornamental fishes could be developed only through hatchery produced fish.

The Central Marine Fisheries Research Institute has intensified its research on breeding, seed production and culture of marine ornamental fishes. One of the milestones in this programme is the recent success in the hatchery production technology of clown fish (Gopakumar *et al.,* 2001a, Ignatius *et al.,* 2001, Madhu 2002, Madhu 2006). Success was also obtained on the broodstock development, larval rearing and seed production of 7 species of damsel fishes (Gopakumar *et al,* 2001b, Gopakumar, 2005). The marine ornamental fishes for which breeding and seed production technologies were developed by CMFRI are the following.

1) *Amphiprion sebae*
2) *Amphiprion percula*
3) *Amphiprion ocellaris*
4) *Premnas biaculeatus*
5) *Pomacentrus pavo*
6) *Neopomacentrus filamentosus*
7) *Neopomacentrus nemurus*
8) *Dascyllus aruanus*
9) *Dascyllus trimaculatus*
10) *Chromis viridis*
11) *Pomacentrus caeruleus*
12) *Chrysiptera cyanea*

The technologies developed have to scale up and demonstrated for commercial level production. Hatchery production and culture of marine tropical ornamental fish can prove to be more economically feasible than that of marine food fish culture, due to the high price per unit of ornamental fish. The clown fishes and damselfishes of the family Pomacentridae offer immediate scope for hatchery production due to the availability of seed production methodologies. .

Seaweed Culture

Around 60 species of commercially important seaweeds with a standing crop of one lakh tonne occur along the Indian coast from which, nearly 880 tonnes dry agarophytes and 3,600 tonnes dry alginophytes are exploited annually. Seaweed products like agar, algin, carrageenan and liquid fertilizer are in demand in global markets and some economically viable seaweed cultivation technologies have been developed in India by CMFRI and Central Salt and Marine Chemical Research Institute (CSMCRI). CMFRI has developed technology to culture seaweeds by either vegetative propagation using fragments of seaweeds collected from natural beds or spores (tetraspores/ carpospores). It has the potential to develop in large productive coastal belts and also in onshore culture tanks, ponds and raceways. The rate of production of *Gelidiella acerosa* from culture amounts to 5 tonnes dry weight per hectare, while *Gracilaria edulis* and *Hypnea* production is about 15 tonnes dry weight per hectare. Recently the culture of the carageenan yielding sea weed *Kappaphycus alvarezii* has become very popular due to its fast growth and less susceptibility to grazing by fishes and is being cultivated extensively along the Ramanathapuram and Tuticorin coasts of Tamil Nadu Commercial level cultivation of *K. alvarezi* has been practised along different parts of Tamil Nadu coast contributing nearly 5000 t dry weight annually.

Open sea cage culture

For the first time in India a marine cage was successfully launched and operated at Visakhapatnam, in the east coast of India by the Central Marine Fisheries Research Institute. Asian seabass (*Lates calcarifer*) was stocked during the first stocking as a trial. Successful harvesting was done after four months. A few demonstration cages are deployed in different parts of our coast with fishermen participation and successful harvests could be made at some places. The standardisation sea cage farming methods along with the commercial level production of fish seed can augment the mariculture production in the country.

Frontier areas of Biotechnological interventions in Mariculture

Broodstock Development

It is well understood that the first step towards seed production technology is the development of best quality 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). 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 was achieved. 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).

Sex Change

Sex change is common among certain groups of fishes of aquaculture importance like groupers and sea bass and hence knowledge of the mechanisms involved is essential for endocrinological manipulations to induce sex reversal for broodstock development. Simultaneous hermaphrodites function concurrently as both male and female and are capable of releasing viable eggs and sperms during the same spawning event (Helfman *et al*. 1997). In contrast, sequential hermaphrodites function as a male in one life phase and as female in another (Warner, 1988). 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. Changing the sex serves to increase the fish's lifetime reproductive success. 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 (Reinboth, 1988).

The families of fish renowned for sequential hermaphroditism include the Sparidae, Serranidae, Pomacentridae, Scaridae and Labridae. In many of the reef dwelling species, such as the protogynous saddleback wrasse *Thalassoma duperrey*, and the protandrous anemonefish *Amphiprion melanopus*, individuals form discrete units of social organisation (Nakamura *et al.,* 1989; Godwin and Thomoas,

1993). Within these units, intraspecific social interactions mediate sex change. Fishes with recognizable social groups are therefore particularly useful models for investigating sexual regulation in fishes so that manipulation techniques for sex reversal can be developed. Most investigations on sex change endocrinology have been done in species which are important to aquaculture such as sparids and serranids. The development of sex change technology is instrumental for improving the efficiency of broodstock development by overcoming shortages of either male or female broodstock (eg. male grouper) which are rare or difficult to catch.

For over 30 years biologists have hypothesized about the involvement of steroid hormones in sex change (Frisch, 2004). However recent biotechnological applications like radio immunoassay (RIA) techniques and enzyme-linked immunosorbent assay (ELISA) have enabled the rapid and accurate determination of steroid concentrations. A variety of experimental techniques have been developed to induce sex change, thus enabling measurements of hormone metabolism during the sexual transition period. The discovery that the sex change in certain fishes is controlled by social interaction (Robertson, 1972) has enabled researchers to stimulate sex change by manipulation of fish's social environment (Godwin and Thomas, 1993; Ohta *et al*., 2003). Either a member of the terminal sex (i.e. 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 by introducing multiple numbers of the initial sex together in captivity can bring about sex change (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 clownfishes 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), derivatives thereof (e.g. methyl testosterone) or inhibitors of steroidogenic enzymes (eg. fadrozole). These technologies have been instrumental in the successful development of broodstock of many commercially important marine finfishes such as seabass and groupers.

Endocrine Manipulations of Spawning

Acquisition of seed stock from the wild (larvae or fry or gametes from gravid broodstock) 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 (Bromage and Roberts, 1995) and genetic manipulations can be employed to enhance their growth, survival and meat quality (Thorgaard, 1995). But many fishes exhibit reproductive dysfunctions when reared in captivity. These dysfunctions are 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 of the pituitary to release the maturational gonadotropin, luteinising hormone (LH). Most commonly, females fail to undergo final oocyte maturation (FOM) and thus ovulation and spawning (Zohar, 1988, 1989a, b; Peter *et al*. 1993), while males produce small volumes of milt or milt of low quality (Billard, 1986, 1989). 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.

Most research and development 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. For example in many salmonid 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 labour requirements. Development of genetic selection programmes often requires artificial fertilization and hormonal manipulations can be used to enable proper maturation and timely collection of gametes. Hence hormonal manipulations for the induction of ovulation, spermiation and spawning will continue to play an important role in commercial broodstock management of marine finfishes(Zohar and Mylonas, 2001).

The earliest techniques employed freshly ground pituitaries collected from reproductively mature fish, which contained gonadotropins (mainly LH). Eventually purified gonadotropins became available, both of piscine and mammalian origin (eg. carp or salmon gonadotropin and human chorionic gonadotropin). In the 1970s, spawning induction methods began employing the newly discovered gonadotropin releasing hormone (GnRH) which induces the secretion of fish's own gonadotropin from the pituitary. Development of highly potent, synthetic agonists of GnRH (GnRHa) constituted the next generation of hormonal manipulation therapies and created a surge in the use of hormones to control reproductive processes in aquaculture. The most recent development is the incorporation of GnRHa into polymeric sustained –release delivery systems, which release the hormone over a period of two weeks. These delivery systems alleviate the need for multiple treatments and induce long term elevation in sperm production and multiple spawning in fish with asynchronous or multiple –batch group-synchronous ovarian physiology. Based on the recent discovery of GnRH multiplicity in fish and the increasing understanding of its functional significance, new GnRH agonists can be designed for more potent, affordable and physiologically compatible spawning induction therapies. These methods have contributed significantly to the development of more reliable and less species-specific methods for the control of reproduction of captive broodstock. Future strategies for improved spawning manipulations will be based on understanding the captivity-induced alterations in the GnRH system, and on new approaches for their repair at the level of GnRH gene expression and release.

Live feed research

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 hindgut epithelial cells. Altricial larvae cannot digest formulated feeds and hence live feed is vital for their survival. Live feeds are able to swim in water column and are thus constantly available to the larvae. 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 hard formulated diets (Stottrup and Mc Evoy, 2003).

The hatchery production of juveniles of marine finfish is achieved globally by the use of 'greenwater technique' and the live feeds like rotifers and copepods.

(i) Greenwater technique

Microalgae are used in the 'greenwater technique' employed for marine finfish larviculture and play a critical role in the larviculture of marine finfishes. Microalgae are generally free living, pelagic and in the nannoplankton range (2-20µm). Batch cultures are generally run according to production cycles of 3-7 days. The cultures obtained in hatcheries seldom exceed a density of 6 x 10^6 cells ml^1

at the end of 5 days. In Industrial facilities specialized in the production of microalgae in controlled conditions such as photobioreactors, the cost of production can be reduced considerably. The productivity of microalgal systems used in aquaculture hatcheries is 10 – fold lower than that of photobioreactors, which is in turn 10 fold lower than that of fermentation techniques. But aquaculture operators are reluctant to take up these technologies mainly because of the significant investment involved. It is likely that microalgae for fish aquaculture will be produced in the near future by specialized companies implementing high technology.

Microalgae have been shown to play a significant role in larviculture of marine finfish. When phytoplankton was included in larval rearing tanks, the survival, growth and food conversion index of many marine finfish species were better than in clear water condition. The green water technique (larviculture in an endogenous bloom of phytoplankton and rotifers) and the 'pseudo green water technique' (larviculture in a tank supplemented daily with exogenous phytoplankton and rotifers) have much commercial application in marine finfish larviculture (Divanach and Kentouri, 2000). Micro algae can also influence live feed and larval microbiology. It has been found that exudates of some algal species can either enhance or inhibit the feeding activity of copepods in cultures (Van Alstyne, 1986). These substances are also involved in the settlement of micro flora required in the gut of fish larvae to prevent intestinal opportunistic bacteria from causing disease. Bacteria associated with live feed can be transmitted to larval fish during feeding (Benavente and Gatesoupe, 1988). As live prey actively ingest bacteria, it is possible to introduce favourable bacteria as probiotic. In the 'green water technique' of larviculture micro algae contribute to maintaining the nutritional quality of live food and also positively influence on the settlement of a healthy intestinal micro flora in fish larvae (Skjermo and Vadstein 1993). Micro algae can also possibly influence the endotrophic stages (egg and pre–larvae) and early exotrophic stages. Micro algal background has an important effect on the timing and intensity of first zooplanktonic feeding. Micro algae also play a role in intestinal transit and gut repletion. Improvement in the survival at first feeding is the main result of larviculture with micro algae. Improvement in growth efficiency during rotifer period is another result of micro algal background in larval tanks. Early enhancement of digestive and assimilative functions improves the survival and growth of fish larvae and favours the transition to exotrophy. The use of micro algae in tanks increases the production of pancreatic and intestinal digestive enzymes and improves the quality of gut flora. Even after the endo-exotrophic phase, micro algae have a positive effect on larviculture and may increase the resistance of larvae to further stressing or adaptive conditions. The indirect effects of micro algae on larvae are mainly related to water quality, luminosity, the bacteriology of water and the quality and accessibility of rotifers. It is thus evident that strategic use of micro algae in hatcheries during the very early life of marine fish improves the success of first feeding, a prerequisite for efficient survival, growth and quality in fish larviculture. A lot of research focus is needed in future on microalgal biotechnology for larviculture.

(ii) Rotifers

Rotifers have been used as live feed for cultured marine fish, since four decades. It is well known that a continuous, stable and reliable supply of nutritionally adequate rotifers is the key to the larviculture of marine finfish. Rotifers of the species *Brachiounus rotundiformis* and *B. plicatilis* are almost indispensable for larval rearing of most marine finfish (Gopakumar and Jayaprakas, 2001; 2003; 2004).

The success of rotifer cultivation is dependent on selecting the most suitable rotifer species or strain for local culture conditions, maintaining water quality in culture tanks and choosing the most appropriate culture technique. Size, the type of reproduction and reproductive rates are species or strain specific. Culture temperatures, salinities, type of food and its quality - all influence the type of reproduction and its rates. Mass production of rotifers is achieved by encouraging rotifers to reproduce asexually, since sexual reproduction results in males and resting eggs. New high density culture technologies for rotifers, such as closed recirculation systems are offering new possibilities for continuous supplies of high quality rotifers at ten times higher than in batch cultures. Evaluating the physiological state of rotifer culture is very important in hatcheries since larval production depends on a predictable and reliable supply of rotifers. Six parameters *viz* egg ratio, swimming velocity, ingestion rate, viscosity, enzyme activity and diseases are employed for assessing the state of health of rotifer cultures.

The nutritional quality of rotifers is improved by enrichment, in which rotifers are collected or harvested from culture tanks into containers where they are kept at very high densities and incubated for 8 – 20 hours with enrichment dietary components like HUFA. In addition to nutritional enrichment, rotifers can be enriched with antibiotics (Verpraet *et al*, 1992) or with probiotic bacteria (Markridis *et al*, 1999, 2000). The nutritional value of rotifers depends on their dry weight, caloric value and chemical composition (Lubzens *et al*, 1989).

Various methods of storing rotifers have been studied. Frozen rotifers are not usually adequate as feed because of leaching of nutrients. Live *B .plicatilis* can be stored at 4^0C at relatively high densities for at least one month (Lubzens *et al*, 1990). Rotifers can be kept at -1^0 C without feeding or water exchange for about 2 weeks (Lubzens *et al*, 1995). *B. rotundiformis* strains are less tolerant to 4^0 C than *B .plicatilis* rotifer strains and the strains known as SS type are most susceptible and showed lowest survival. Amictic eggs of rotifers can be preserved by cryopreservation in liquid nitrogen after they have been impregnated with cryoprotective agents like dimethyl sulfoxide (DMSO) (Hadani *et al*, 1992). This method ensures full preservation of genetic traits of importance to aquaculture. Cryopreservation is not a suitable method for preservation of large numbers of rotifers for direct use as feed.

Artificially produced rotifer eggs have been tried as an alternative to daily production of rotifers. The production of these eggs can be manipulated by environmental factors, such as salinity, food quality and quantity, rotifer culture density, exchange of culture media and temperature and varies between *B. plicatilis* and *B. rotundiformis*. The cost of producing resting eggs is very high and therefore not yet been extensively adapted in hatcheries.

It is evident that rotifer cultures will continue to be indispensable in marine finfish hatcheries. Current methodologies of producing and enriching rotifers are meeting the requirements of the industry. The need to have very small sized rotifers is difficult to achieve, although several super small strains have been found and cultured (Hagiwara *et al*, 2001). Improved methods for predicting the health of cultured rotifers may be useful in preventing culture crashes. Using preserved rotifers may eliminate the dependence on daily production of rotifers. Cheaper methods of resting egg production and high density culture techniques are the major areas which requires research attention in future.

(iii) Copepods

The rapid expansion of hatchery production of seeds for farming of many marine food fishes and the hatchery rearing of marine ornamental fishes to replace wild caught fishes in the trade, could not be met by conventional live feeds such as rotifers and *Artemia*. Thus interest in copepods has been generated and the use of copepods as live feeds in finfish hatcheries is gaining impetus. Copepods are employed mainly because they are the only acceptably sized prey for small larvae of many species of marine finfish and ornamental species. Copepods have a larger size range from first nauplii to adult copepodites and offer good size ranges for the entire hatchery phases for certain species of finfish. When compared to rotifers and *Artemia* nauplii, copepods can improve the larval growth, survival and the ratio of normally pigmented juveniles when fed either alone or in combination with conventional live feeds.

It is well understood that the mass culture of copepods has several limitations especially due to its low multiplication rate when compared to rotifers. The species that are mass cultured fall under three orders – Calanoida, Harpacticoida and Cyclopoida. In calanoids, species belonging to the genera *Acartia*, *Centropages* and *Eutemora* are in most widespread use in mono and mixed cultures. Among harpacticoids, species belonging to the genera *Euterpina*, *Tigriopus* and *Tisbe* have been widely used. Under cyclopoids, *Oithona spp*. and *Apocyclops spp*. are recognized as suitable for marine finfish larvae.

Improved growth, survival and /or rates of normal pigmentation have been documented for several marine fish species fed copepods alone or as supplement to the traditional diets of rotifers or *Artemia* nauplii compared with traditional diets alone(Kraul 1983; Heath& Moore 1997;Mc Evoy *et al*. 1998; Naess & Lie 1998; Nanton and Castell 1999). In many hatcheries, malpigmentation of the reared juveniles constitutes a major problem .Flatfish larvae fed natural or laboratory reared zooplankton exhibit higher rates of normal pigmentation than larvae fed *Artemia* nauplii (Seikai *et al*. 1987, Naess *et al.* 1995, Mc Evoy *et. al*. 1998). Larval nutrition is suggested to be the major factor determining pigmentation patterns. The documented improvements in larval growth, survival and rates of normal pigmentation are generally attributed to levels of DHA, EPA and/or arachidonic acid (ARA) in the diet (Castell *et. al.,* 1994; Reitan *et.al*.,1994; Zheng *et. al.,*1996; Sargent *et al.* 1997) and in particular to the DHA : EPA ratio in the diet (Bell *et al.* 1995b; Sargent *et al*. 1997: Nanton and Castell 1998) and EPA : ARA ratio (Bell *et al.,* 1995a; Sargent *et al.,* 1997; Estevez *et al* 1999). DHA can be synthesized from shorter chain precursors in some marine fish larvae, but at rates insufficient to meet requirements for their normal growth and survival. A minimum of 0.5 to 1.0% of dry weight as n-3 HUFA is required for juvenile marine fish and higher amounts are required for rapidly growing fish larvae. Marine copepods, the principal diet for most marine fish larvae in nature, contain high levels of DHA and other PUFA, either obtained through their phytoplankton diet or accumulated despite low PUFA levels in the diet. DHA levels in wild copepods can be more than 10 times higher than in enriched *Artemia* (Mc Evoy *et*. *al.,*1998).DHA is important in maintaining structural and functional integrity in fish cell membranes, in neural development and function, and especially in retinal development and vision (Bell & Tocher 1989; Bell & sergent 1996). It is suggested to play an important role in the development of normal pigmentation when provided in sufficient quantities at particular times during the larval stage(Reitan *et al*., 1994). EPA cannot be synthesized by most marine fish and it is therefore essential in the diet of the fish. EPA gives rise to less biologically active

eicosanoids than those produced from ARA. Since it competes metabolically for the same enzyme systems required for ARA derived eicosanoid production, EPA is very important in modulating the production of these highly biologically active eicosanoids. This metabolic interaction necessitates an optimal EPA : ARA ratio in the diet. Eicosanoids of n-6 origin are important for the normal function of vital organs such as kidney, gill, intestine and ovaries of marine fish. Levels of ARA in copepods are high in both calanoids and harpacticoids. Apart from the superior fatty acid composition in copepods, they contain high amounts of polar lipids (Fraser *et al*. 1989). Polar lipids are more easily digested by larvae and may also facilitate digestion of other lipids in the undeveloped gut of marine fish larvae. Varying concentrations of the carotenoid astaxanthin were found in the various copepods and its possible value for fish is as a precursor to Vitamin A. Copepods are also an important source of exogenous digestive enzymes and are thought to play an important role in fish larval digestion (Munilla –Moran *et al.,* 1990).

The apparent inability to be cultured in high densities is the major constraint for the commercial use of copepods as live feed in hatcheries. Copepod cultures rarely exceed 2 per ml for adults and ten per ml for nauplii (Stottrup *et al*. 1986; Mc Kinnon *et al*. 2003). Although much experience has been gained in culturing different calanoid species a lot of research is further needed on achieving stable cultures and finding the optimal conditions for maximum production. This includes the optimal feeding regime for cultures, the optimal quality (size and nutrition) and how to ensure the food availability of copepods to maximize production. A method that includes all these features in the most efficient manner and ensuring stability would be a big leap in calanoid culture. Work towards improving the quality of cold stored non-diapause eggs is also needed to increase the benefits of cold storage. Harpacticoids can be cultured in higher densities than calanoids. The densities may reach more than 100 per ml (Fleeger 2005). It should be possible to develop semi automated systems that would minimize labour and make culturing of harpacticoids more efficient. Reliable rearing systems for mass production of small sized copepods that can meet the needs of marine finfish larviculture is the key area of research to be focused in the immediate future for commercializing the seed production of many high value finfishes with altricial type of larvae. Eventhough many potential species of copepods for culture have been studied, the possibility of an ideal species which can produce higher densities of nauplii per ml can be another important aspect of future research. Production of resting eggs for sale on a commercial scale can revolutionize the seed production of many high value finfishes for mariculture.

Recirculating Aquaculture System (RAS)

A recirculating Aquaculture System (RAS) can be defined as an aquaculture system that incorporates the treatment and reuse of water with less than 10% total water volume replaced per day. The concept of RAS is to reuse a volume of water through continual treatment and delivery to the organisms being cultured. Water treatment components used in RAS need to accommodate the input of high amounts of feed required to sustain high rates of growth and stocking densities. Generally RAS consists of mechanical and biological filtration components, pumps and holding tanks and may include a number of additional water treatment elements that improve water quality and provide disease control in the system.

Conclusion

Research and development on commercial level seed production technologies of high value finfish and shellfish, popularisation of sea cage farming and evolving suitable policies for sea farming are the key areas to be focused urgently to make mariculture as a significant seafood production sector in India. In this context, biotechnological interventions in controlled reproduction, induction of spawning, live feed technology and recirculating aquaculture systems can go a long way in the improvement of mariculture technologies. Mariculture and allied post harvest technologies if scaled up with spatial and seasonal variations with community participation the coastal productivity and economy can prosper.

References

Bell, M.V. & Sargent, J.R. 1996. Lipid nutrition and fish recruitment. *Mar. Ecol. Prog. Ser.,* 134 :315 – 316.

Bell, M.V., Batty, R.S., Dick, J.R. Fretwell, K., Navarro, J.C. & Sargent, J.R. 1995a. Dietary deficiency of docosah-exaenoic acid impairs vision at low light intensities in juveniles herring (*Clupea harengus* L.). *Lipids*, 30 : 443-449.

Bell, M.V. & Tocher, D.R. 1989. Molecular species composition of the major phospholipids in brain and retina from rainbow trout (*Salmo gairdneri)*. Occurrence of high levels of di-(n-3) polyunsaturated fatty acid species. *Biochem. J.,* 264 :909-915.

Benavente, P.G. and Gatesoupe, F.J., 1988. Bacteria associated with cultured rotifers and artemia are detrimental to larval turbot, *Scophthalmus maximus*. *Aqcult.Eng.*, 7: 289-293.

Billard, R.1986.Spermatogenesis and spermatology of some teleost fish species. *Reprod.Nutr.Dev*.26 : 877-920.

Billard, R. 1989. Endocrinology and fish culture. *Fish Physiol. Biochem..* 7 : 49-58.

Bromage, N.R. and Roberts, R.J.1995. Broodstock management and egg and larval quality. Blackwell, Oxford, 424pp.

Castell, J.D., Bell, J.G., Tocher, D.R. & Sargent, J.R. 1994. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty and composition of juvenile turbot *(Scophthalmus maximus). Aquaculture,* 128 :315 – 333.

Devaraj, M., V.K. Pillai, K.K. Appukuttan, C. Suseelan, V.S.R. Murty, P. Kaladharan, G. Sudhakara Rao, N.G.K. Pillai, N.N. Pillai, K. Balan, V. Chandrika, K.C. George and K.S. Sobhana, 1999. Packages of practices for sustainable, ecofriendly mariculture (land- based saline aquaculture and seafarming). *In: Aquaculture and the Environment*, (Mohan Joseph Modayil, ed.) Asian Fisheries Society, Indian Branch: 33-69.

Devin, R.H., Yesake, T.Y., Biagi, C.A., Donaldson, E.M., Swanson, P., Chan, W.K. 1994. Extraordinary salmon growth. Nature 371, 209-210

Divanach, P. and Kentouri, M., 2000. Hatchery techniques for specific diversification in Mediterranean finfish larviculture. *CIHEM – FAO publication* 47: 75 – 87.

Du, S.J.,Gong, Z., Fletcher, G.L, Shears, M.A., King, M.J, Idler, D.R., Hew, C.L. 1992. Growth enhancementin transgenic Atlantic salmon by the use of an 'all fish' chimeric growth hormone gene construct. *Bio/Technology* 10 : 176-180.

Estevez, A., McEvoy, L.A., Bell, J.G. & Sargent, J.R. 1999. Growth, survival, lipid composition and pigmentation of turbot *(Scophthalmus maximus)* larvae fed live-prey enriched in arachidomic and eicosapentaenoic acids. *Aquaculture,* 180 : 21-343.

FAO/NACA.2008. The future of Mariculture : A Regional approach for responsible development in the Asia-Pacific Region. FAO Fisheries Proceedings 11, FAPO, Rome, 325pp

FAO, 2009. State of World Fisheries and Aquaculture. FAO Rome, 174pp.

Fleager, W. John. 2005. The potential to mass culture harpacticoid copepods for use as food for larval fish. In: Lee et al (Eds). *Copepods in Aquaculture*, Blackwell Publishing Professional, USA: 11 – 24.

Fraser,A.J., Sargent J.R. & Gamble, J.C. 1989. Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis* Muller from a nutrient enriched seawater enclosure. *J. Exp. Mar. Biol. Ecol.,* 130 : 81-92.

Frisch, A. 2004. Sex change and gonadal steroids in sequentially-hermaphroditic Teleost fish. *Reviews in Fish Biology and Fisheries,* 14: 481-499.

Godwin, J.R. and Thomas, P. 1993. Sex change and steroid profiles in the Protandrous anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). *Gen.Comp.Endocrinol.* 132: 223-230.

Gopakumar, G., G. Sreeraj, T.T. Ajithkumar, T.N. Sukumaran, B.Raju, C. Unnikrishnan, P. Hillary and V.P. Benziger, 2001a. Breeding and larval rearings of three species of damselfishes (Family Pomacentridae). *Mar.Fish. Infor.Serv.,* 171:3-5

Gopakumar, G. and Jayaprakas, V. 2001a. Rotifers as live feeds for larviculture of marine fishes – a research review. In: N.G. Menon and P.P. Pillai (Eds.) 2001. *Perspectives in Mariculture*, the Marine Biological Association of India, Cochin: 35 – 66.

Gopakumar, G., Rani Mary George, and S. Jasmine, 2001b. Hatchery production of clown fish *Amphiprion chrysogaster.* In: N.G. Menon and P.P. Pillai (Eds) 2001, *Perspective in Mariculture,* The Marine Biological Association of India, Cochin; 305-310.

Gopakumar, G. and Jayaprakas, V. (2003). Community structure and succession of brackish water rotifers in relation to ecological parameters. *J.Mar.Biol.Assn. Indi,* 45 (1): 20-30.

Gopakumar, G. and Jayaprakas, V. 2004. Life table parameters of *Brachionus plicatilis* and *B.rotiundiformis* in relation to salinity and temperature. *J.Mar.Biol.Assn. India,*46(1) : 21- 31.

Gopakumar, G. 2005. Marine Ornamental Fish Culture – Status, Constraints and Potential. In: *Proceedings of Ocean Life Food & Medicine,* Aquaculture Foundation of India, Chennai. pp 347-359.

Gopakumar,G., K.R.Manmadhan Nair and V.Kripa. 2007. Mariculture research in India – status, constraints and prospects.In: Mohan Joseph Modayil and N.G.K.Pillai (Eds.). 2007. Status and Perspectives in Marine Fisheries Research in India, CMFRI, Kochi. 404pp.

Govt. of India, 2001. *Report of the Working Group on Fisheries for the Tenth Five Year Plan,* TFYP. Working Group Sr. No 16/2001

Hadani, A., Beddig,S. and Lubzens,E.1992. Factors affecting survival of cryopreserved rotifers (*Brachionus plicatilis* O.F. Muller). In: *Progress in Aquaculture Research* (Ed. by B.Moav, V.Hilge & H.Rosenthal), pp.253-267.

Hagiwara,A., Gallardo,W.G., Assavaaree,M., Koyani,T. and De Araujo, A.B. 2001. Live food production in Japan: recent progress and future aspects. *Aquaculture,*200: 111-127.

Heath, P.L. & Moore, C.G. 1997. Rearing Dover sole larvae on *Tisbe* and *Artemia* diets. *Aquacult. Int.,* 5 : 29-39.

Helfman, G.S., Collette, B.B. and Facey, D.E. 1997. The diversity of fishes. Blackwell Science, MaldenA. 544pp

ICAR, 2000. Technologies from ICAR (for industrialisation) Indian Council of Agricultural Research, New Delhi, 350 pp.

Ignatius, B, G. Rathore, I. Kandasami, D., and A.C.C Victor, 2001. Spawning and larval rearing technique for tropical clownfish *Amphiprion sebae* under captive conditions. *J Aqua. Trop;* 16 (3):653-662.

Kraul, S. 1983. Results and hypotheses for the propagation of the grey muller, *Mugil Cephalus* L. *Aquaculture,* 30 : 273-284.

Lubzens, E., Kolodny,G., Perry, B., Galai,N., Sheshinski,R. and Wax, Y. 1990. Factors affecting survival of rotifers (*Brachionus plicatilis*) culture. *Aquaculture*, 133: 295 – 309.

Lubzens, E., Rankevich, D., Kolodny, G., Gibson,O., Cohen,A. and Khayat, M. 1995. Physiological adaptations in the survival of rotifers (*Brachionus plicatilis* O.F. Muller) at low temperatures. *Hydrobiologia*, 313/314: 175 – 183.

Lubzens,E., Tandler, A. and Minkoff,G.1989. Rotifers as food in aquaculture. *Hydrobiologia,* 86/187: 387-400

Madhu, K., and Rema Madhu, 2002. Successful breeding of common clown fish under captive conditions in Andaman and Nicobar Islands. *Fishing Chimes* 22(9): 16-17.

Madhu, K., Rema Madhu, L.Krishnan, C.S. Sasidharan and K.M. Venugopalan. 2006. Spawning and larval rearing of Amphiprion ocellaris under captive conditions. *Mar.Fish.Infor.Serv*. No.188: 1-5.

Markridis,P., Fiellheim,A.J., Skjermo,J. and Vadstein,O.2000. Control of bacteria flora of *Brachionus plicatilis* and *Artemia franciscana* by incubation in bacterial suspensions. *Aquaculture*, 185: 207-218.

Markridis,P., Bergh,O., Fiellheim,A.J., Skjermo,J. and Vadstein,O.1999. Microbial control of live food cultures. In: *Towards Predictable Quality. Aquaculture Europe 99* (Ed. By L. Laird and H. Reinertsen), pp. 155 – 157.European Aquaculture Society, Special Publication No.27, Ostend.

McEvoy, L., Naess, T., Bell, J.G. & Lie, O. 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. *Aquaculture,* 163, 235-248.

McEvoy, L., Naess, T., Bell, J.G. & Lie, O. 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. *Aquaculture,* 163, 235-248.

McKinnon, S. Duggan, P.D. Nichols, M.A. Rimmer, G. Semmens and B. Robino. 2003. The potential of tropical paracalanid copepods as live feeds in aquaculture. *Aquaculture.* 223: 89 – 106.

Melamed, P., Zhiyuan Gong, Garth Fletcher and Choy L.Hew. 2002. The potential impact of modern biology on fish aquaculture. *Aquaculture* 204: 255-269.

Moav, V. Hilge and H. Rosenthal), pp.253-267. European Aquaculture Society, Special Publication No.17, Ostend.

Mohan Joseph Modayil 2004. Prospects for expansion of Mariculture in India. Current scenario and future needs of Indian Fisheries, Decennial Publication of FOFP-2004, Forum of Fisheries Professionals, Visakhapatnam.

Mohan Joseph Modayil, R.Sathiadhas and G.Gopakumar. 2008. Country experiences –India. In. FAO/NACA Regional workshop: The future of mariculture : a regional approach for responsible development in Asia-Pacific Region: 145-171.

Munoz, R.C and Warner, R.R. 2003. Alternative contexts of sex change with social control in the bucktooth parrotfish, *Sparisoma radians*. *Environ. Biol.Fish.* 68 : 307-319.

Munday, P.L. 2001. Changing sex. Nature Australia, September 2001 : 51-59.

Munilla-Moran, R., Stark, J.R. & Barbour, A. 1990. The role of exogenous enzymes I digestion in cultured turbot larvae *(Scophthalmus maximus* L.) *Aquaculture,* 88: 337-350.

Nakamura, M., Hourigan, T.F.,Yamauchi, K., Nagahama, Y and Grau, E.G. 1989. Histological and ultrastructural evidence for the role of gonadal steroid hormones in sex change in the protogynous wrasse *Thalassoma duperrey*. *Environmental Biol. Fish.* 24: 117- 136.

Nanton, D.A. & Castell, J.D. 1999. The effects of temperature and dietary fatty acids on the fatty acid composition of harpacticoid copepods, for use as a live food marine fish larvae. *Aquaculture,* 175, 167-181

Naess, T, & Lie, O. 1998. A sensitive period during first feeding for the determination of pigmentation pattern in Atlantic halibut, *Hippoglossus hippoglossus* L., juveniles: the role of diet. *Aquacult*. *Res.,* 29, 925 – 934.

Naess,T., Germain-Henry, M. & Nass, K.E. 1995. First feeding of Atlantic halibut (*Hippoglossus hippoglossus)* using different combinations of *Artemia* and wild zooplankton. *Aquaculture,* 130, 235 – 250.

Nanton, D.A. & Castell, J.D. 1998. The effects of dietary fatty acids on the fatty acid composition of the harpacticoid copepod, *Tisbe sp.,* for use as a live food for marine fish larvae. *Aquaculture,* 163, 251-261.

Ohta, K., Mine, T., Yamaguchi, A., Takeda, T and Matsuyama, M.2003. Bi-directional sex change and its steroidogenesis in the wrasse *Psudolabrus sieboldi*. *Fish Physiol. Biochem.* 28: 173-174.

Peter, R.E., Lin, H.R, van der Kraak, G., Little, M. 1993..Releasing hormones,dopamine antagonists and induced spawning . *In*: Muir, J.F., Roberts, R.J. (Eds.) *Recent advances in Aquaculture*. Blackwell Scientific, Oxford: 25-30.

Pillai, V.N. and N.G. Menon (Eds).2000. Marine Fisheries Research and Management, Central Marine Fisheries Research Institute, Cochin. 914pp.

Pillai, N.G.K., M. J. Modayil and U. Ganga. 2003. Marine Fishing Practices and Coastal Aquaculture Technologies in India. *In*: Anjani Kumar, Pradeep K Katiha and P.K. Joshi (Eds) A profile of people, technologies and policies in Fisheries sector in India: Proceedings Series 10 National Centre for Agricultural Economics and Policy Research, New Delhi. 83-121.

Reinboth, R. 1988. Physiological problems of Teleost ambisexuality. *Environ.Biol.Fish.*22 : 249-259.

Reitan, K.I., Rainuzzo, J.R. & Olsen, Y.1994. Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. *Aquacult. Int.,* 2, 33-48.

Rimmer, Michael, A. 2008. Regional review on existing mariculture species and farming technologies. In. FAO/NACA Regional workshop: The future of mariculture : a regional approach for responsible development in Asia-Pacific Region: 105-125.

Roberson, D.R. 1972. Social control of sex reversal in a coral reef fish. *Science*, 177: 1007- 1009.

Sargent, J.R., McEvoy, L.A., & Bell, J.G. 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture,* 155, 117 – 127.

Saunders, R.L., Fletcher, G.L. and Hew, C.L.1998. Smolt development in growth hormone transgenic Atlantic salmon. *Aquaculture* 168, 177-193.

Seikai, T., Watanabe, T. & Shimozaki, M. 1987. Influence of three geographically different strains of *Artemia* nauplii on occurrence of albinism in hatchery-reared founder *Paralichthys olivaceus. Bull. Jap. Soc. Sci. Fish.,* 53, 195 – 200.

Shapiro, D.Y. 1984. Sex reversal and sociodemographic processes in coral reef fishes. In: Potts, G.W and Wootton, R.J. (Eds.) *Fish reproduction: Strategies and Tactics.* Academic Press, London, 113-118.

Skjermo, J. and Vadstein, O. 1993. The effect of microalgae on skin and gut microbial flora halibut larvae. In: By H. Reinerstsen, L.A. Dalhe, L. Jorgensen & K. Tvinnerein (Eds.) *'Proceedings from International Conference on Fish farming Technology,* Trondheim, August , pp.61 – 67

Stottrup, J.G. (2003) Production and nutritional value of copepods. *In*: Stottrup, J.G. and, McEvoy, L.A. . (Eds.), *Live feeds in marine aquaculture*. pp.145-205.

StØttrup, J.G., Richardson, K., Kirkegaard. E. & Pihl, N. J. 1986. The cultivation of *Acartia tonsa* Dana for use as a live food for marine fish larvae. *Aquaculture,* 52, 87 – 96.

Thorgaard, G.H.1995. Biotechnological approaches to broodstock management. *In*: Bromage, N.R. and Roberts, R.J.1995.Broodstock management and egg and larval quality. Blackwell, Oxford : 76-93.

Van Alstyne, K.L. 1986. Effects of phytoplankton taste and smell on feeding behavior of the copepod *Centropages hamatus*. *Mar.Ecol.Prog.Ser.,* 34: 187 – 190.

Verpraet, R., Chair, M., Leger, P., Nelis, H., Sorgeloos, P. and DeLenheer, A. 1992. Live food mediated drug delivery as a tool for disease treatment in larviculture - The enrichment of therapeutics in rotifers and artemia nauplii. *Aquacult.Eng.*, 11: 133-139.

Warner, R.R. 1988b. Sex change in fishes: hypothesis, evidence and objections. *Environ. Biol.Fish.* 22: 81-90.

Zheng, F., Takeuchi, T., Yoseda, K., Kobayashi, M. Hirokawa, J. & Watanabe, T. 1996. Requirement of larval cod for arachidomic acid, eicosapentaenoic acid and docosahexaenoi acid using enriched *Artemia* nauplii. *Bull. Jap. Soc. Sci. Fish,* 62, 669 – 676.

Zohar, Y.1988. Gonadotropin releasing hormone in spawning induction in teleosts. *In*: Zohar, Y., Breton, B. (Eds.)Reproduction in fish: basic and applied aspects in endocrinology and genetics.. INRA Press, Paris : 47-62.

Zohar, Y. 1989a. Fish reproduction: its physiology and artificial manipulation.In: Shilo, M.,Sarig. S.(Eds.) Fish culture in warm water systems: problems and trends, CRC Press, Boca Raton: 65-119.

Zohar, Y. 1989b. Endocrinology and Fish Farming: aspects in reproduction, growth and smoltification. *Fish Physiol. Biochem* 7: 395-405.

Zohar, Y and Constantinos C.Mylonas. 2001. Endocrine manipulations of spawning in cultured fish. *Aquaculture* 197: 99-136.