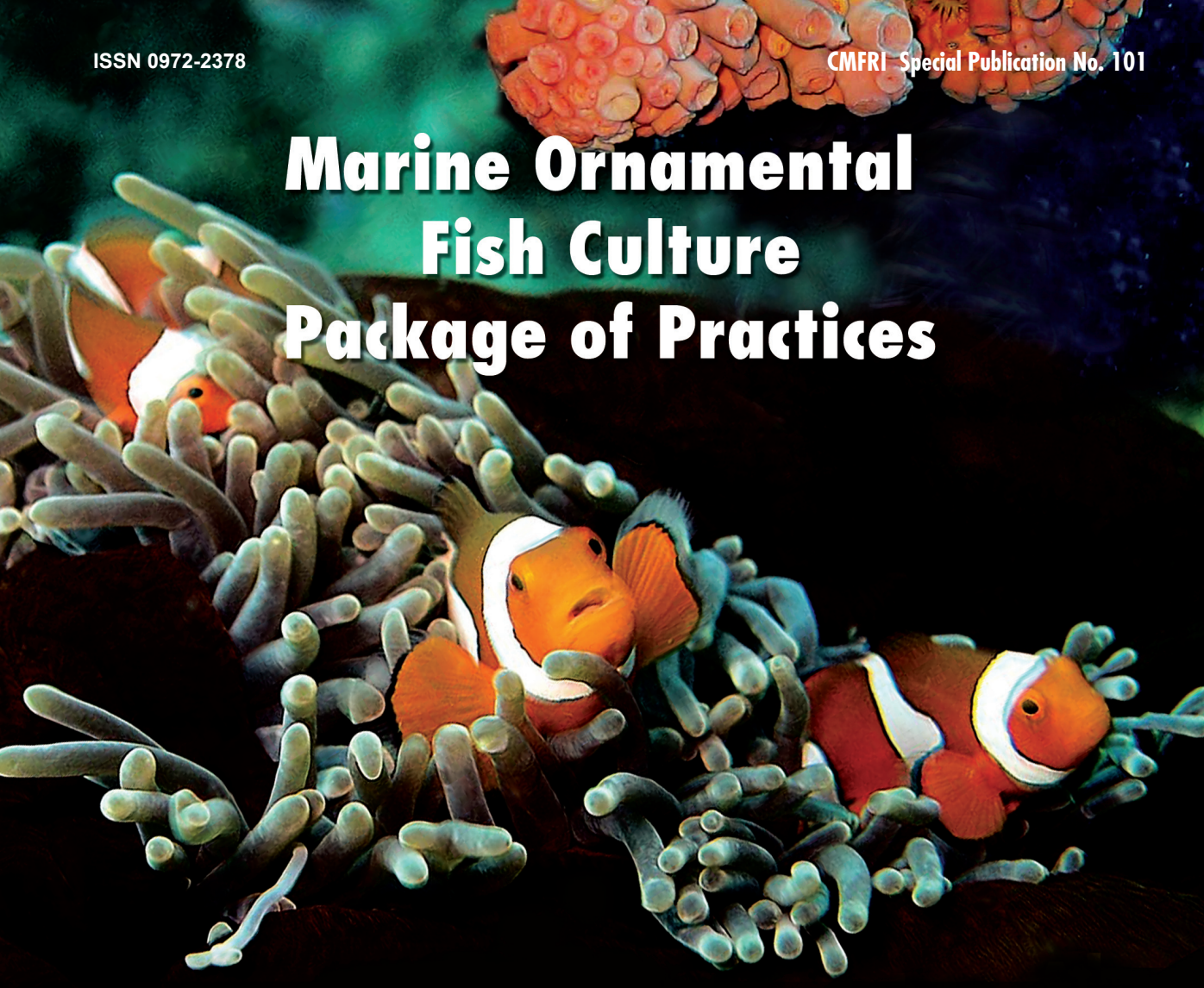
ISSN 0972-2378

CMFRI Special Publication No. 101

Marine Ornamental Fish Culture Package of Practices



G. Gopakumar
K. Madhu
Rema Madhu
M. K. Anil
Boby Ignatius

भाकृ अनुप
ICAR

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
Post Box 1603, Ernakulam North P.O.
Kochi – 682 018, Kerala, India

कडलमीन™
cadalmin

www.cmfri.org.in

CMFRI Special Publication No. 101

Marine Ornamental Fish Culture – Package of Practices

G. Gopakumar
K. Madhu
Rema Madhu
M. K. Anil
Boby Ignatius

Central Marine Fisheries Research Institute
(Indian Council of Agricultural Research)
Post Box No. 1603, Ernakulam North P.O., Kochi - 682 018, Kerala, India
www.cmfri.org.in

Marine Ornamental Fish Culture – Package of Practices

Published by

Dr. G. Syda Rao
Director
Central Marine Fisheries Research Institute
P. O. Box 1603, Kochi - 682 018, Kerala, India
Phone : +91-484-2394867
Fax : +91-484-2394909
E-Mail : mdcmfri@md2.vsnl.net.in
www.cmfri.org.in

Authors

G. Gopakumar
K. Madhu
Rema Madhu
M. K. Anil
Boby Ignatius

ISSN 0972-2378
CMFRI Special Publication No. 101
March 2011

Front Cover : Adult pairs of *Amphiprion ocellaris* with sea anemone *Heteractis magnifica*
Back Cover : *Top :* Hatchery produced juveniles of *Amphiprion ocellaris*
Middle : Microscopic view of eggs of *Amphiprion ocellaris*
Bottom : Hatchery produced juveniles of *Chrysiptera cyanae*

Printed at :

Niseema Printers and Publishers, Kochi - 682 018, Ph : 2403760

PREFACE

The trade of marine ornamentals has been expanding in recent years and has grown into a multimillion dollar enterprise mainly due to the emergence of modern aquarium gadgets and technologies for setting and maintenance of miniature reef aquaria. The global marine ornamental trade is estimated at US$ 200-330 million. The marine ornamental trade is operated throughout the tropics. India is endowed with a vast resource potential of marine ornamentals distributed in the coral seas and rocky coasts with patchy coral formations. In the context of the expanding global scenario and the increasing demand in the domestic trade, it appears very much lucrative for India to venture into this industry. But it is a multi-stakeholder industry ranging from specimen collectors, culturists, wholesalers, transhippers, retailers, hobbyists, researchers, government resource managers and conservators and hence involves a series of issues to be addressed and policies to be formulated for developing and expanding a sustainable trade.

In recent years it has been reported that nearly 1500 species of marine ornamental fishes are traded globally and most of these are associated with coral reefs. Nearly 98% of the marine ornamental fishes marketed are wild collected from coral reefs of tropical countries such as Philippines, Indonesia, Solomon Islands, Sri Lanka, Australia, Fiji, Maldives and Palau. This has been threatening the long term sustainability of the trade due to indiscriminate exploitation of coral reef areas. The three key words in the development of marine ornamental fish trade are – collection, culture and conservation. The development of technologies for hatchery production of selected marine ornamental fishes is the only option for evolving a long term sustainable trade without damaging the coral reef ecosystem. Even at an international level, the technologies for hatchery production of ornamental fishes are limited to a few species. The Central Marine Fisheries Research Institute (CMFRI) has been focusing on this vital aspect for the past few years. The Institute was able to develop and standardise hatchery production methods for a dozen species of ornamental fishes which are in high demand in the domestic as well as international trade.

The marine ornamental fish trade is low volume and high value industry and hence it is very lucrative to initiate a hatchery produced trade. Eventhough many publications on marine ornamental fish breeding and related subjects by the Institute are available, they are scattered in different sources. The need for a compilation of the same is felt essential so that all the relevant informations in the form of package of practices are readily available from one source. I wish to congratulate the authors for the compilation of information in all the relevant topics such as broodstock development, breeding, live feed culture, larviculture protocols, growout methods, aquarium technology, diseases, packing and transportation and setting up of a small-scale hatchery with details of economics. I hope that the publication will be of much utility to fisheries developmental agencies interested in developing marine ornamental fish trade, entrepreneurs, students and researchers in this area.

G. Syda Rao
Director

CONTENTS

MARINE ORNAMENTAL FISH CULTURE – PACKAGE OF PRACTICES

G. Gopakumar, K. Madhu, Rema Madhu, M. K. Anil and Boby Ignatius
Central Marine Fisheries Research Institute, Kochi

1. Introduction

In recent years, the trade of marine ornamentals has been expanding and it is estimated that 1.5 – 2.0 million people worldwide keep marine aquaria and the value of annual marine ornamental trade is estimated to range between US$ 200 – 330 million. Almost the entire trade is contributed by collections from coral reef habitats which raises doubts regarding its sustainability. The damaging techniques such as use of sodium cyanide are non selective methods used to capture fish and they adversely affect the health of the fish and kill the non target organisms. The over harvesting of target organisms is another aspect of concern. In addition there are high levels of mortality associated with insensitive shipping and poor husbandry practices.

The ultimate answer to a long term sustainable trade of marine ornamentals can be achieved only through the development of culture technologies. It is well accepted as an environmentally sound way to increase the supply of marine ornamentals by reducing the pressure on wild population and producing juvenile and market sized fish of wide variety year round. In addition, hatchery produced fish are hardier and fair better in captivity and survive longer. The list of marine ornamental fishes reared in captivity today contains more than 100 species. The maximum number of species reared are from the family Pomacentridae. Attempts for spawning and rearing in closed systems have proved technically challenging for most species. Breeding and hatchery technologies for most tropical fishes are yet to be developed and standardized on a commercial scale. At present there has been only a few developments in the breeding and rearing of marine ornamental fishes and currently it has gained momentum in many countries and improved technologies are emerging in this direction. The Central Marine Fisheries Research Institute (CMFRI) has been intensifying research for the past one decade on the seed production technologies and has successfully developed and standardised the package of practices for a dozen species which are in high demand in the marine ornamental fish trade.

2. Broodstock development, breeding and larval rearing – General aspects

The absence of sexual dimorphism, the complex patterns of sex change in certain groups and the problems of larval rearing can be considered as the major reasons for the slow progress in the culture of marine ornamental fishes. The most important aspect is the lack of understanding on reproductive strategies and the difficulties of creating the pelagic environment essential for larval survival. The concepts of breeding and larval rearing of freshwater ornamental fishes are mostly not applicable in the case of marine ornamental fishes. Marine fish in general do not care their young ones after they have hatched. Since most fish spend their larval period as part of the plankton, plankton feeding fish quickly eat their own larvae, if they happened to drift nearby. During their time in the plankton, larval fish are totally dependent on planktonic microorganisms for food and their major movement is dependent on ocean currents during their early larval life.

Reproduction of marine fish can be categorized in 4 basic patterns.

1. The most common is release of tiny, transparent, free-floating eggs with complete absence of parental care. Angel fishes, butterfly fishes, tangs, groupers, snappers, wrasses and parrot fishes are among those with this type of reproductive style.
2. The second most common mode is attachment of the eggs to a secure substrate, usually near the bottom, with nesting behaviour. These are termed demersal eggs and the resulting larvae may be large as in the case of clownfish or quite small as with damsel fish. Gobies, blennies, damselfish and clownfish are the common nest building marine tropical fishes.
3. Some groups exhibit oral incubation of eggs or mouth brooding. Instead of attaching the eggs to the bottom, the male retains them in his oral cavity during the period of incubation. Cardinal fishes are examples of this category.
4. A very few marine species such as sea horses give birth to well developed young ones.

Mouth brooders and those fish that lay demersal eggs produce far fewer eggs than fish that spawn pelagic eggs. These species aerate and protect their eggs from predators during early development. Nesting and mouth brooding species incubate their eggs from 3-10 days depending on the species, and the larvae are hatched with a small residual yolk sac, fully developed eyes and mouth parts and the ability to swim with purpose and direction. The spawn of species that protect their eggs varies in number from a low of 50-100 to a high of 10,000-15,000, whereas those species that spawn pelagic eggs can produce more than 2,00,000 eggs or more per spawn and may spawn almost daily

during the spawning season. Some species such as Pygmy angel fish spawn only fewer eggs, (300-500 per spawn) on daily basis. The successful rearing of marine fish larvae requires consistent care, daily observation, basic knowledge and a good deal of experience and dedication.

Two key bottlenecks currently limit the expansion of marine ornamental fish aquaculture. First is the control of maturation and spawning and the second is the identification of appropriate live food items for larval first feeding.

Reproduction

One of the cornerstones to success in marine ornamental fish culture is the controlled reproduction of broodstock animals to ensure a constant supply of seed stock throughout the year. Food fish farmers need to concentrate on one or a few species, whereas farmers engaged in ornamental aquaculture must maintain a variety of species to provide the product diversity corresponding to market demands. Currently, about 800 species are actively marketed in the marine ornamental fish trade, of which only about 30 species (less than 5%) are bred in captivity. The resulting challenge faced by the marine ornamental farmer is to determine the appropriate methods to control reproductive processes in a wide variety of targeted species to allow sufficient market penetration. The challenge is complicated by the fact that many reef species have complex reproductive strategies that include the formation of social hierarchies, hermaphroditism and sex change. Consequently factors that regulate social structure and sex of the broodstock must be considered carefully when setting up broodstock population.

Reproductive development can be divided into two distinct sequential processes:

1. Gonadal growth and development.
2. Final maturation and spawning.

Many species undergo relatively normal gonadal development in captivity when provided with suitable husbandry and appropriate environmental parameters. Rearing tank size and shape are important elements to reproductive success. Tanks that have proven adequate for growth may be inadequate for captive reproduction. Many ornamental reef species such as Pigmy angel fish and wrasses display complex and ritualized reproductive behaviour that require deeper tanks and specific reef substrates. Considerable success in spawning of ornamental fishes is reported through the introduction of live rocks and other natural substrates in the broodstock tank. But, once introduced, live rock cannot be easily cleaned and could carry with it a variety of pathogens that can seriously affect broodstock health. Similar success can be achieved with simple artificial structures, that can be easily removed and cleaned. It is reported

that PVC pipes were used to provide spawning dens for the Orchid dottyback. The flame angel fish and Potter's angel fish were successfully spawned by using a PVC scaffolding to mimic natural coral heads.

Broodstock water quality and nutrition are also typical parameters. The extra energy and nutritional requirements for reproduction over normal growth and activity should be met through appropriate broodstock feeds. Only filtered or sterilized natural sea water should be supplied to broodstock tanks for avoiding introduction of pathogens. The next aspect is to provide the correct environmental stimuli to induce reproductive activity. Most marine fishes studied have seasonal reproductive cycles in which gonad development and spawning are controlled either by photoperiod and / temperature. Manipulation of environmental parameters such as temperature and photoperiod can be used to accelerate or delay gonadal development. Ovarian biopsy procedures routinely used for monitoring gonadal development in food fish can be employed for marine ornamentals also. The hormonal induction of final maturation and spawning has not been used extensively for marine ornamental fishes. Attempts to obtain natural spawning and induced spawning through hormone administration in the diet may be more appropriate for inducing breeding in ornamental fishes.

3. Live feed culture and feeding of Ornamental fish larvae

Ornamental fish breeders often complain about the mass mortality of larvae within two or three days after their hatching. The cause of this problem can be traced to the inadequate feeding practice of the newly hatched larvae. The initial nourishment to the developing fish larvae is obtained from the egg yolk. When the yolk reserves have been completely utilized, the larval feeding capabilities are developed and hence at this stage the larval survival is entirely dependent on the availability and quality of food in sufficient quantities. The phase when yolk has just been depleted and the larvae turn to exogenous feeding for further development is the most critical stage. Few fish larvae possess big yolk sacs which provide nutrients for the first few weeks of their larval growth. However, many fish larvae have very limited yolk reserves and have to resort to exogenous feeding eventhough they have small mouths and primitive digestive systems. The gape of the mouth opening controls the size of the food that can be accepted by the larvae. As the larva grows, the mouth gape increases and larger food can be consumed. The nutritional requirement of the fish larvae at this stage is expected to match the composition of yolk that caters the needs of the pre-feeding fish. As the larva initiates exogenous feeding , the spurt in activity demands a great deal of energy and hence the larval nutrition is of vital significance. An artificial feed catering to the nutritional requirements at this stage of the larvae is yet to be formulated and research over the past few decades have revealed that live feeds can be successfully employed

for the rearing of the larvae during their critical stage from endogenous to exogenous feeding.

One area of continuing concern is the need for a wide assortment of suitable live feed organisms, particularly for first feeding larvae. Most marine species with smaller larvae are unable to thrive on conventional rotifer and *Artemia* based feeding practice, either because these organisms are too large for consumption for first feeding larvae or because they do not meet their nutritional needs. Smaller prey items including young and super small strains of rotifers, oyster trochophores, tintinnids, diatoms, phytoplankton and copepod nauplii have been used successfully as first feed items for groupers, which have comparable larvae in size to ornamental species. The most suitable live feed organisms to be considered as candidates for mass culture, for marine ornamental aquaculture would be those types and sizes found in the gut of wild larvae. It has been reported that the diet of first feeding reef fish in the wild includes mollusc veligers, barnacle nauplii, other larval forms, tintinnids and dinoflagellates. Copepod nauplii are another small and abundant natural food source being used successfully to culture a number of marine species. Other small live food organisms that have been considered are the marine protozoan *Euplotes* and sea urchin larvae. Most first foods considered for culture of marine ornamental fish should range in size from 50 to 100 µm. In many cases the inability of the larvae to eat certain live food organisms may not be linked with size, but with swimming or other behaviour patterns of the live feed organisms. It is reported that the larvae of marine angel fishes of the genus *Pomacanthus* simply refuse to eat rotifers eventhough the larvae are large enough to capture them. Contrast between live feed items and rearing tank background colours also has an effect on feeding efficiency and overall survival of some species.

The common approach in marine fish hatchery is to find an appropriate mix of food organisms, develop methods to mass culture them and device a feeding schedule. Until these methods are developed for marine ornamentals, alternative means may be required. Wild zooplankton sieved to appropriate size has been used successfully to culture several marine ornamental species. The disadvantages are inconsistency of supply and possibility of introducing planktonic predators and parasites.

One recent emerging area of interest is the role of microbial communities within the larval rearing tanks, and importance of conditioning, maturing or aging water to establish beneficial populations of bacteria prior to introduction of egg or larvae. These beneficial bacteria may influence the health of larvae either directly as a source of nutrition or antigen stimulation or indirectly by out-competing and thus preventing establishment of more harmful strains. The role of bacteria in culture systems may be critically important in early life stages of marine ornamental fish larvae. The use of

formulated micro-feeds to replace live feeds for marine ornamental fish culture appears challenging, especially for first feeding larvae, because of the very small particle sizes that would be required. Hence it appears that formulated feeds will have a limited value in the early life stages of cultured ornamental marine fish larvae, although they may be useful in later or post larval stages, particularly in combination with live food items.

The live feeds that are used on a world wide scale are different species of micro algae (2 – 100 µm), the rotifers *Brachionus plicatilis* and *B.rotundiformis* (110 –200 µm), the cladocerans like *Daphnia* and *Moina* (600 – 700 µm) and the brine shrimp *Artemia* (420 -8000µm) and copepods (nauplii, copepodites and adults of different size ranges).

Microalgae

These are floating microscopic plants which constitute the base of food chain in an aquatic ecosystem. Micro algae form an indispensable food source in the commercial rearing of many cultivated species of fish. They are used to produce mass quantities of rotifers, copepods, cladocerans and brine shrimp which serve as food for the larval stages of many ornamental fishes. In the 'green water technique' used for rearing marine fish larvae, algae are used directly within the larval rearing tanks where they serve as water conditioner by stabilizing the water quality, nutrition of the larvae and microbial control.

More than 40 species of micro algae isolated from different parts of the world are cultured as pure strains in intensive systems. They range in size from 2 micrometers to more than 100 micrometers. Some of the most commonly used genera in aquaculture include *Skeletonema*, *Chaetoceros*, *Isochrysis*, *Tetraselmis, Chlorella* and *Nannochloropsis*.

Growth Dynamics

A basic understanding of the algal growth dynamics is necessary to carry out their mass culture. An algal culture goes through the following phases

1. Lag or induction phase in which there is no increase in cell numbers
2. Exponential phase in which cell multiplication is rapid.
3. Stationary phase in which the culture will be stationary without any further cell division for a few days. In the stationary phase if the cells get a new environment, they may start further growth and reproduction.
4. Declining phase in which the growth and multiplication of cells will be arrested and slowly the cells show the symptom of decline.
5. Death phase in which the cells will lose its viability and start dying. At this stage the culture will become unsuitable either for reculturing or for feeding.

Culture Methods

The following are the steps involved in micro algal culture

(i) Preparation of culture media
(ii) Identification and isolation of the required species
(iii) Stock and working culture maintenance
(iv) Mass culture

Preparation of Media: Culture media mostly consist of nitrates and phosphates in the ratio 10 : 1 (N : P) besides trace metals and vitamins. Silicate is essential for culturing diatoms, as they have siliceous cell walls. The composition of the two commonly used media *viz.* Miquel's medium and Conway or Walne's medium is given below:

Miquel's Medium

A.	Potassium nitrate	-	20.2 g
	Distilled water	-	100ml
B.	Sodium orthophosphate	-	4 g
	Calcium chloride	-	2 g
	Ferric chloride	-	2 g
	Hydrochloric acid	-	2 ml
	Distilled water	-	100 ml

0.55 ml of A and 0.50ml of B are added to one litre of filtered and sterilized seawater.

Conway or Walne's Medium

A.	Potassium nitrate	-	100 g
	Sodium orthophosphate	-	20g
	EDTA (Na)	-	45 g
	Boric acid	-	33.4 g
	Ferric Chloride	-	1.3 g
	Manganese chloride	-	0.36 g
	Distilled water	-	1 litre
B.	Zinc chloride	-	4.2 g
	Cobalt chloride	-	4 g
	Copper sulphate	-	4 g
	Ammonium molybdate	-	1.8 g
	Distilled water	-	1 litre
	C. Vitamin B1 (Thiamin)	-	200 mg in 100 ml distilled water
	Vitamin B12 (Cyanocobalamine)	-	10 mg in 100 ml distilled water

Prepare A , B and C in different reagent bottles. Add 1ml of A , 0.5ml of B and 0.1ml of C to one litre of filtered, sterilized and cooled sea water.

Equipments and Glasswares: For identification of microalgae as well as for the determination of cell concentration of the culture, a powerful microscope is necessary. For stock culture maintenance the glasswares required are micropipettes, droppers, reagent bottles, culture tubes, conical flasks, Haufkin culture flasks, haemocytometer etc. For mass culture 10 litre polythene bags, 20 litre glass carboys, 100 litre Perspex tanks and 250 litre cylindrical transparent FRP tanks are used for the indoor culture while 250 litre , 500 litre and one tonne fiberglass tanks and 5 tonne concrete tanks can be used as per the requirement for the outdoor culture of micro algae.

Isolation of algal species: Twenty litres of water is collected from the water body and enriched with nutrients and left under light until algal bloom occurs. The nutrient added for enrichment should be appropriate to the species required to be isolated. The isolation of a single algal cell from the bloom can be accomplished by any one of the following methods:

1. Simple capillary pipette isolation Method: The mixed plankton sample is kept in a petridish under a binocular microscope. The desired species is isolated using a capillary pipette and transferred to culture tubes having suitable sterile culture medium.

2. Centrifuging method: By repeated centrifuging the water samples and then by inoculating the deposits, we can isolate several microalgae.

3. Serial dilution Method: This method is used mainly for the isolation of phytoflagellates(i.e. motile species). This involves systematic dilution of the inoculum in five stages (1, $10^{-1,}$ 10^{-2}, 10^{-3}, 10^{-4} or 4 steps 0.001, 0.01, 0.1 and 1ml) so that the subjected species is well separated from any contaminant. The species thus isolated is transferred to the culture tubes.

4. Agar plating Method: Agar medium is prepared by adding 1.5 gm of agar to one litre of suitable culture medium. This agar medium is sterilized in an autoclave for fifteen minutes under 120 lbs pressure and 100ºC temperature. Now the medium is poured in sterilized 15 cm petri dishes and kept for 24 hrs. The required species can be picked by platinum needle or loop under microscope and streaked on the surface of agar plate. After inoculation, these petridishes are placed in an incubation chamber for 7-8 days providing light (1000 lux) and constant temperature (25ºC). Within this time , the required species , if it has grown into a colony is removed by platinum loop under microscope and transferred to culture tubes. Further by chaning from the culture tubes to small conical flasks and larger flasks, the algae can be grown on a mass scale.

Stock culture Maintenance: The pure culture (0.1ml) isolated from the mixed culture is inoculated into 20 ml culture tubes or 50 ml culture flasks filled with enriched water and incubated in light intensity of 1000 lux (2 tube lights) with photoperiod of 12 hours to produce one million cells/ml. This forms the stock or starter culture for mass culture and thus can be maintained for 15 days. The above procedure should be repeated every 15 days in order to maintain the vigour of the culture.

Working culture maintenance: Some of the 50 ml flasks containing the starter culture are used for inoculating 250 ml flasks. After two days, culture in 250 ml flasks are transferred to 2 litre flasks with enriched water and incubated in light (1000 lux) with aeration for two days to get a density of three million cells/ml. This again is inoculated into 20 litre carboys with enriched water to get three million cells/ml density.

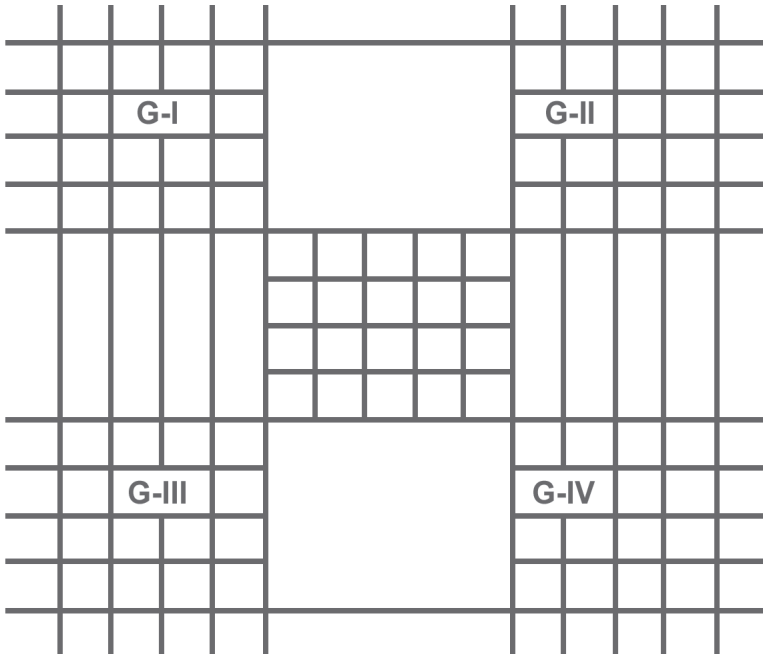
Mass culture: Large scale outdoor culture of microalgae required for hatcheries can be carried out economically by enriching with the following ingredients :

Ground nut oil cake	-	250 gm/tonne
Urea	-	10 gm/tonne
Superphospahate	-	5 gm/tonne

Soak the groundnut oil cake in water, then thoroughly smash the same to obtain a milky suspension which can be filtered through a cloth to remove larger sediments. The milky filtered suspension along with the inorgainic nutrients (urea and superphospahate) is added to enrich the water. The required inoculum for mass culture is added and kept under sunlight. The two methods of mass culture commonly employed are – batch culture and semicontinuous culture.

Batch culture: In this method the entire culture is harvested when the cell density reaches the desired level. The culture tank is filled with enriched water and the required inoculum is added. When the cell density reaches the desired level the entire culture is harvested. Batch culture method is the most reliable method, but it is labour intensive.

Semicontinuous culture: Here the microalgae are allowed to grow until a certain cell density is reached. Then it is partially harvested and fresh medium is added. The growth and harvest procedures are repeated several times before the tank be drained and cleaned. It involves less labour but is a less reliable method.



Surface view of haemocytometer showing grid areas (G=Grid)

Counting of Micro algal cell density: The apparatus used for counting cells is a haemocytometer with an improved Neubauer ruling. Before counting, both the cover slip and chamber must be rinsed clean and dried. The face of the counting chamber is composed of two gridded surfaces separated by canals. The cover slip is placed on the support bars along the canals and a drop of homogeneously mixed algae suspension is delivered from a Pasteur pipette by touching the pipette tip to the edge of the cover slip where it hangs over the V-shaped loading port. Slight pressure will cause the algal suspension to flow evenly across the surface, but not into the canals or on top of the cover slip.

A small drop of 5 to 10% formalin mixed into the sample is sufficient to immobilize cells for counting. Each half of the haemocytometer contains nine large grids. Only those algal cells which fall within the four large corner grids are counted. Each large corner grid is further subdivided into 16 small squares. Moving systematically back and forth across the squares, a minimum of 200 algal cells are counted in as many grids as necessary . To determine the algal cell density (number of algal cells per milli litre) in the suspension, the number of algal cells counted is divided by the large corner grid area covered and multiplied by 10,000. For example, if 300 algal cells were counted in 1.5 large corner grids (or 24 small squares), the cell density is 300 algal cells/1.5 corner grids x 10,000 = 2 x 10^6 cells per ml.

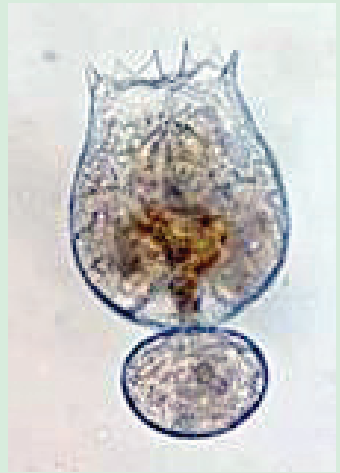
Infusoria

The term infusoria includes minute microscopic organisms like *Paramecium* and *Stylonychia*. They are the best starter feed for majority of ornamental fish larvae. Infusoria culture can be raised very easily. Raw potato/ cabbage is cut into pieces of ¼ inch squares and about sixty such squares are added to five litres of water. After a day's exposure to air and sunlight millions of infusoria are formed in the water which can be fed directly to the larvae.

Rotifers

Rotifers are microscopic animals larger than infusoria. They form excellent live feed for many newly hatched marine and freshwater fish larvae due to their (i) small size (ii) slow swimming speed (iii) habbit of staying suspended in the water column and (iv) ability to be cultured in high densities and (v) high reproductive rate. There are many species of rotifers but the most commonly used species are *Brachionus plicatilis* and *B.rotundiformis*

Brachionus plicatilis



Brachionus rotundiformis

Strain selection, collection, isolation and developing stock culture

Strain selection: *B.plicatilis* and *B.rotundiformis* are widely distributed in brackishwater ponds and lakes. There are lots of strain variations. The reproductive rate, size, optimum culture conditions including salinity and temperature can vary among strains and hence strain selection is very important.

Collection and Isolation: Zooplankton samples can be collected from suitable brackishwater ponds or lakes by employing zooplankton net with mesh size ranging

from 50 – 100 µm. Live samples can be observed under a stereo zoom microscope . Using a micropipette isolate a few egg bearing rotifers into a cavity block containing filtered brackishwater from the site of collection. Serially transfer the isolated individuals through several cavity blocks to eliminate any associated organisms.

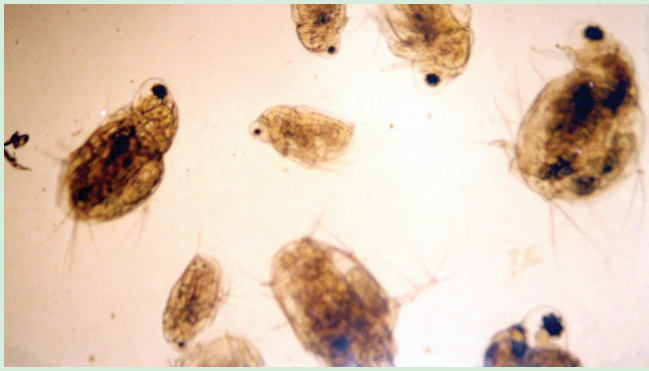
Development of Stock culture: The initial step towards the development of a stock culture is the culture of microalgae of appropriate size range 2 -20µm such as *Chlorella*. The isolated rotifers are transferred to the algal medium with sufficient cell density and within a few days, stock culture can be obtained. Alternately, an initial suitable strain of rotifers could be obtained from other hatcheries for mass culture.

Mass culture: Enrich filtered sea water/ brackishwater in one tonne capacity tank with fertilizers such as groundnut oil cake (250 g), urea (10 g) and superphosphate (5 g). This medium is inoculated with *Chlorella* on the same day at the rate of 5 – 10 litres per tonne. On the second or third day when the algal cell density has reached the appropriate level, rotifers are inoculated at the rate of 10 to 100 numbers per ml. Rotifers multiply rapidly by feeding on the algal cells and within a few days (5 –7 days) the culture attains maximum concentration of 400 – 500 Nos. per ml. Harvesting of rotifers is done with a handnet of 50 µm mesh size. The two methods employed for microalgal culture namely batch culture and semicontinuous culture can be followed for rotifer culture also.

Cladocerans

They are larger than rotifers and can be used as food for larvae of comparatively bigger fish such as angelfish, goldfish etc. Among the cladocerans *Moina micrura* is very common in freshwater ponds and lakes and they can be collected by using a fine plankton net during early morning hours.

Daphnia



Moina Culture

Stock culture

The egg bearing females are isolated from the plankton samples under a stereozoom microscope by a fine dropper and introduced into petri dish containing filtered well water. The isolated organisms are transferred to a 100 ml beaker with *Chlorella* water of appropriate cell density. Gradually the volume is increased to 500 ml or one litre by adding *Chlorella* water and within a few days the stock culture will be ready.

Mass culture

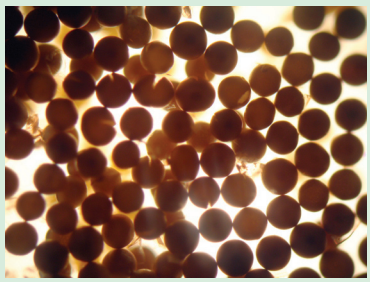
The culture tank (1 tonne) is filled with chlorine free water (tap or well water) and fertilized with groundnut oil cake (300 g) extract, urea (6 g) and superphosphate (3 g). This medium is inoculated with a culture of *Chlorella* (5 to 10 litres). On the second day when the water becomes greenish, a pure stock culture of *Moina* is introduced at a stocking rate of 5-10 animals per litre. *Moina* multiplies by feeding on *Chlorella* and within 5-7 days a concentration of 20,000 to 30,000 numbers per litre is obtained. At this stage, if semicontinuous method is followed 1/3 volume can be harvested every day and replaced by *Chlorella* water.

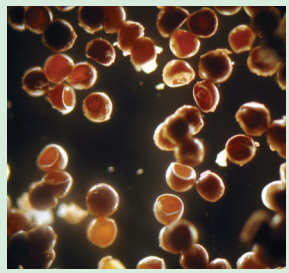
Other cladocerans of the genera like *Daphnia, Moinodaphnia* etc can be used for culture of larvae. These can be cultured in a similar manner as that of *Moina micrura.*

Artemia

Artemia nauplii are the most widely used live feed. Since the cysts are commercially available and can be stored, it is a ready source of food. While purchasing , cysts with

minimum impurities, high hatching synchrony (13 to 16 hrs + 8 hrs.) and cysts packed with moisture content of less than 5% should be selected. The naupliar size varies between 0.4 to 0.5mm.


Cysts - magnified


Hatching

Hatching Method

1. Best hatching in seawater in containers with a conical bottom
2. The aeration intensity should be sufficient to maintain oxygen levels above 2 mg/litre, preferably 5mg/litre
3. Temperature range 25 to 28 ºC , pH around 8
4. Cyst densities 2 to 5 g per litre
5. *Artemia* shells may be loaded with bacteria and fungi. For disinfection soak the cysts for 30 minutes in a 200ppm hypochlorite solution prior to incubation for hatching.
6. Strong illumination (about 200 lux) at water surface is essential during the first hours after complete hydration to trigger hatching mechanism


Artemia nauplii

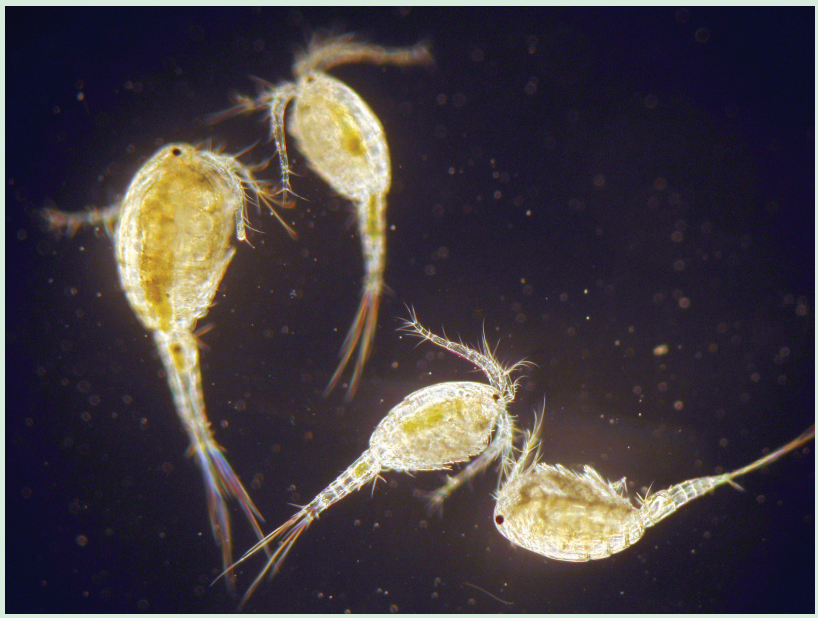

Adult Artemia

Harvesting and feeding

1. Switch off aeration, nauplii will concentrate at bottom
2. Nauplii should be separated from empty shells and unhatched cysts
3. Nauplii are phototactic – concentration can be improved by shading the upper part of the hatching tank and focusing a light source at the bottom
4. Unhatched cysts and other debris are drained off
5. Nauplii are collected by a fine mesh (less than 150µm) and rinsed thoroughly in tap water

Copepods

Copepods are a major component of the natural diet of marine fish larvae including ornamental fish larvae. The advantages of copepods over rotifers are that copepods have wide range of body sizes both within and between species. The early stage nauplii and copepodites can be extremely useful as initial prey for species that have very small larvae with small mouth gape at first feeding.



The calanoid *Pseudodiaptomus serricaudatus*

In extensive methods copepods are collected from nature and inoculated into outdoor tanks for mass production for use in fish larval rearing. The larvae are then transferred to these tanks. Additional copepods may be added during the larval rearing when necessary to maintain prey densities in the range of 200 – 500 l^{-1}. Disadvantages of this system include the inability to control production and thus food levels and predators. Lack of food results in differential growth in fish larvae.

Outdoor production of copepods in ponds or large tanks of 350 – 5000m^3 is carried out in Europe and Asia for cod, grouper and flatfish. Filtered seawater by using filters of around 20 – 40 µm is generally used in these systems. Phytoplankton bloom can be induced by application of commercial fertilizers. Filtering devices that allow for selective sieving are used to collect primarily nauplii (80 – 250 µm) and copepodite stages (80 – 600 µm) to inoculate the rearing tanks.

In many Asian countries, a mesh size of 400 – 600 µm was used to inoculate outdoor tanks for grouper rearing with copepodites and adult stages 3 days before stocking the newly hatched fish larvae at densities of 5 m^{-3}. Regular monitoring of densities of live prey in these outdoor systems is important for the successful rearing of marine fish larvae. An advantage of outdoor ponds over the extensive systems that rely on the local production of zooplankton is the possibility of culturing zooplankton over one generation before using them as food. Moreover, feeding wild plankton directly to the fish increases the risk of infections.

Several attempts for the mass culture of copepods in intensive systems have been undertaken with varying success. Species with relatively short generation at ambient temperatures are best suited for aquaculture purposes. Species inhabiting coastal environments are normally more tolerant to variations in salinity and temperature and have a wider thermal and salinity tolerance.

The most frequently cultured calanoid species belong to the genera found in coastal waters, such as *Acartia, Centropages, Eurytemora* and *Temora***.** These copepods are small, with relatively short generation time and a wide thermal and salinity tolerance and are easily adaptable to laboratory conditions. Most calanoids require phytoplankton although it has been demonstrated that it was possible to culture *Acartia tonsa* on rice bran.

Aeration is required to maintain phytoplankton in suspension and to create small turbulence which helps to distribute copepods within the culture tanks. Most calanoids require large volumes and the adult density rarely exceeds 100 per litre. Successful batch culture of the calanoid *Acartia* sp. was achieved in 1000 litre polyethylene tanks, 1.3m in diameter with a conical base. The tanks are emptied after the 8 day hatch cycle

and cleaned and a new batch culture was started. Contamination of copepod culture by bacterial blooms, ciliate infection, other copepods or rotifers may pose a problem. In commercial facilities, contamination by rotifers is most likely to cause the collapse of copepod culture, since the rotifers with their higher reproductive rate would quickly out compete the copepods. Hence these cultures should be strictly kept apart.

Ciliates are utilized by copepods and in periods of low phytoplankton concentration, they constitute the major dietary source. Ciliates are often an indication of overfeeding and if ciliates are noted in cultures it is advisable to empty the culture using a 60 or 80 µm mesh, which retains the adult copepods, but allows the ciliates to be washed out.

Harpacticoid copepods have several advantages for culturing.They include (i)High tolerance to a wide range of environmental conditions, (ii) Ability to feed on a wide range of live or inert diets, (iii)High reproduction capacity, (iv) Relatively short life cycles, (v)Ability to be cultured in high densities, (vi) Requirement for surface area rather than volume, (vii) Planktonic naupliar stages, (viii) Can be used as tank cleaners in rotifer cultures, other copepod culture or larval tanks.

Filtered seawater can be used for harpacticoid culture and most feeds are acceptable to many harpacticoid species. Algae which quickly sediment are also good feed because bacteria colonize these cells, and the mixture of algae and bacteria form a good dietary combination for harpacticoids. Photoperiod is known to influence the offspring production and sex ratio. A photoperiod of 12 L / 12 D was shown to be the most favourable parameter for offspring production. Many harpacticoids have wide thermal and salinity tolerances. Ciliates and rotifers in the culture tanks compete for food and may lead to crash of copepod culture.

Improved growth, survival and / or rates of normal pigmentation have been documented for several marine fish species fed copepods alone or as a supplement to other traditional live feeds. The improvements in larval growth, survival and normal pigmentation are generally attributed to the levels of DHA, EPA and / or arachidonic acid (ARA) in the diet and in particular to the DHA: EPA ratio in the diet. Copepods which constitute the major diet for marine fish larvae in nature contain high levels of DHA and other PUFA. DHA levels in wild copepods can be more than 10 times higher than in enriched *Artemia*.

The interest in copepod culture as live feed is gaining momentum in recent years for the rearing of altricial type of larvae which are very small and with very little yolk content at hatching. A few of the culture methods developed to date can be adapted in commercial hatcheries. However there is a need to evolve intensive culture methods for copepods in future. It is felt that the future expansion of mariculture especially of marine finfish depends largely on the development of production techniques of resting eggs of copepods on commercial scale.

Wild zooplankton

Hatcheries which are located close to the sea or large water bodies can make use of this natural live feed by collecting the same with a zooplankton net. The heterogeneous size composition of wild collected zooplankton makes it suitable for all target species. However, the risk of bringing parasites into the hatchery system, restricted availability and poor survival and storage possibilities are the disadvantages of wild zooplankton.

Bloodworms

They are larvae of *Chironomus* midges. They are blood red in colour and are common in ditches and ponds with decaying organic matter. When cement tanks containing manured soil and water is kept open, the adult flies get attracted and a number of eggs are laid. These hatch out in about three to four days into blood red worms. These can be used as excellent feed for freshwater ornamental fishes.

Bloodworms

Earthworms

Earthworms can be collected from the soil and used for feeding freshwater aquarium fishes.

***Tubifex* worms**

They form the most popular aquarium feed, commonly found in large numbers in sewage polluted waters. It can be kept alive by placing it under a gentle flow of water from a tap. It can also be cultured in a mixture of pig or sheep manure and sand. A gentle flow of water must be provided. The worms are inoculated into the medium after 2-5 days and in about 15 – 30 days a thick mass or *Tubifex* worms develop. The worms have to be thoroughly washed before feeding to fishes.

Feeding protocol

An appropriate feeding protocol should be developed for larval feeding. The live feed should be available in the medium at a proper concentration to enable the larvae to feed without wasting much energy for searching for feed. It is also risky to overfeed the larvae. As the larvae grow the size of the live feed supplied should be progressively larger. For instance, clownfish larvae can be fed entirely with *B.rotundiformis* for about

ten days. Thereafter it is necessary to feed them with large sized live feeds like freshly hatched *Artemia* nauplii.

4. Hatchery production of Clownfish

The list of marine ornamental fishes reared in captivity today, the world over contains more than 100 species. The maximum number of species reared are from the family Pomacentridae. The tropical marine anemonefishes (Pomacentridae) are important in the trade and are popular subjects of research. Over the last twenty years, mariculture centers and scientific laboratories have started rearing these fishes in large quantities.

Clownfishes continue to be the most demanded marine tropical fish and the technologies available at present on marine ornamental fish breeding are mainly centered around clownfishes. There are 28 known clownfish species. They are distinguished and taxonomically separated from other damsel fish by their dependence on anemones for protection. They are further distinguished from other damsels by their large capsule-shaped eggs and large larvae at hatch. Their swimming pattern consists of exaggerated lateral flexures and alternating paddling of their pectoral fins. Clownfishes are distributed throughout the Indo-West Pacific region. This area contains 10 known species. Species with widely spread regions are *Amphiprion akallopisos* (Yellow skunk), *A. bicinctus, A. clarkii, A. chrysopterus* (orange anemonefish), *A. frenatus* (tomato clownfish), *A. melanopus* (fire clownfish), *A. ocellaris*, *A. perideraion* (Pink skunk), *A. rubrocinctus* (red anemonefish) and *A. sebae* (sebae clown), *A. percula* (True percula) and *Premnas biaculeatus* (spine cheek anemone fish). Anemonefish as their name defines, live in a mutualistic relationship with anemones. In nature, selection of preferred anemones is species specific. Primary benefits to clownfish from anemone association are protection of the pair, their nests and a portion of their progeny from predation. The fish achieves protection from stinging of anemones by means of the development of a special external mucus layer. Clownfish appear to be monogamous, pairing for life. There is also a possibility that some species may be polygamous.

In India, till recently much attention was not focused on the culture of marine ornamental fishes. During the past few years the Central Marine Fisheries Research Institute has intensified its research on breeding, seed production and culture technologies for marine ornamental fishes. One of the milestones in this programme is the recent success in the hatchery production technologies of clownfishes and damselfishes. The Institute was able to develop hatchery production methods of the following twelve species of ornamental fishes (clownfishes and damselfishes) which are in high demand in the international trade.

1.	*Amphiprion percula*	-	Orange clown
2.	*A. ocellaris*	-	False clown
3.	*Amphiprion sebae*	-	Sebae Clown
4.	*Premnas biaculeatus*	-	Maroon clown(spine cheek anemone fish)
5.	*Pomacentrus caeruleus*	-	Blue damsel
6.	*Pomacentrus pavo*	-	Peacock damsel
7.	*Dascyllus trimaculatus*	-	Three spot damsel
8.	*Dascyllus aruanus*	-	Humbug damsel
9.	*Chromis viridis*	-	Bluegreen damsel
10.	*Neopomacentrus nemurus*	-	Yellowtail damsel
11.	*Neopomacentrus cyanomos*	-	Filamentous tail damsel
12.	*Chrysiptera cyanea*	-	Sapphiredevil damsel

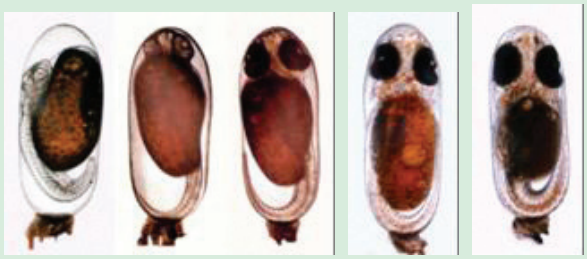
Success was obtained in the seed production of four species of clownfishes *viz., Amphiprion sebae, A. ocellaris, A. percula and Premnas biaculeatus* which are in good demand in the international trade of marine ornamental fishes.

Broodstock development of *A. sebae* was obtained by introducing 4-5 sub-adults of the species along with a single host anemone in a 500 litre FRP tank fitted with a biological filter. It is better to have a light intensity of 2500 to 3000 lux as the sea anemone requires sunlight for better survival in the hatchery. Boiled and chopped mussel/clam meat and fish roe can be fed *ad libitum* twice a day. Live feeds like *Artemia* nauplii, adult *Artemia* and *Moina micrura* can also be supplemented. The range of environmental parameters suited for broodstock development are temperature 26–29^0 C, salinity 33 – 36 ppt, dissolved oxygen 4.5 to 6 ml/lit, pH 8 - 8.5. All clown fishes are protandrous sequential hermaphrodites. Generally the clown fish start as males and change into females as per the social requirements. Male and female form a monogamous pair that lasts until one member of the pair dies. If the female dies first, the largest male changes into a female and the second largest individual becomes the active male. In a broodstock tank with 4-5 sub-adults after a period of three to four months, one pair grows ahead of others and becomes the spawning pair. Once pair formation has taken place they can be transferred to a separate breeding tank. Depending on the production capacity and seed demand several pairs can be maintained for the commercial hatcheries. It is essential to provide suitable substratum preferably tiles or earthen pots or PVC pipes for egg deposition, and which will also be helpful for transferring the eggs to the hatching tanks. On the day of spawning the parents select the suitable site for laying eggs and clean the area to remove algae and debris. Spawning occurs during day time

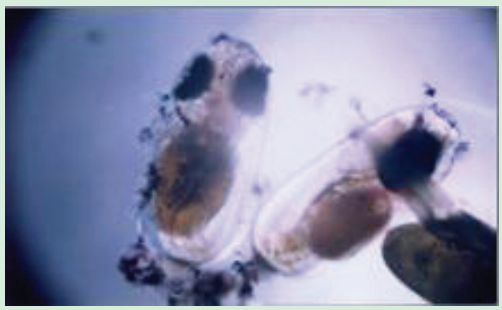
and it lasts for about one to one and half hours. Each female lays 300 to 1000 capsule shaped eggs at every twelve to fifteen days interval. Generally the egg size ranges between 1.5 to 3mm in length and 0.8 to 1.8 mm in width. Each egg is attached to the substratum by a stalk. During the incubation period both the parents carefully look after the eggs by fanning the eggs by their fins and removing the dead and infected eggs by mouth. Newly spawned eggs are bright orange in colour and these turned to

Amphiprion sebae

A pair of *A. sabae* depositing eggs on a granite inside broodstock tank



Development of *A. sebae* eggs



Hatching of *A. sebae* larvae

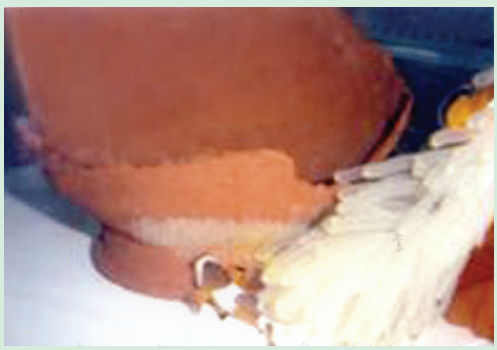
Hatchery produced clownfish (*A. sebae*) juveniles on sea anemone

Sub adults

Adults

black on 3-6th day and later to silvery colour with the eyes of the larvae prominent on the seventh day. The eggs hatch on the seventh day shortly after sunset at a water temperature range of 27 – 29 ^{0}C . On the expected day of hatching, 2 hours before sunset the eggs along with the substratum are transferred to hatching tanks. The larvae break their capsules and hatchlings emerge soon after sunset and peak hatching takes place between 1900 – 2000 hrs in darkness. The newly hatched larvae measured 3-4 mm in length and each has transparent body, large eyes, visible mouth and a small yolk sac. Soon after hatching the larvae are free swimming. The larval rearing was carried out in green water and feeding with rotifers initially from 1-8 day post hatch (dph) and subsequently during 9 – 20 dph with newly hatched *Artemia* nauplii. A minimum 8-10 nos of rotifers per ml is required during rotifer feeding period and 2-3 nos nauplii per ml during *Artemia* feeding stage. The larvae metamorphose between 15-20 days. After metamorphosis the larvae can be transferred to grow out tanks with sea anemone. Mild

Amphiprion ocellaris



Newly spawned eggs of *A.ocellaris* attached on earthen pot

Male and female *A.ocellaris* guarding the silvery eggs attached to earthen pot

15 days old juveniles settling in the sea anemone *Heteractis magnifica*

aeration can be provided during larval rearing. The larviculture period from 3-8 dph is critical due to the change in feeding from endogenous to exogenous. After 8 dph there will not be any further mortality if proper feeding and water quality parameters are maintained. The range of environmental parameters required are pH 8-8.2, temperature 26- 30^{0}C, DO 5 – 7.5 ml/lit, salinity 33-36 ppt, ammonia and nitrite at zero levels. The tank bottom should be cleaned daily. A minimum 25% water has to be exchanged. Sufficient greenwater should be added daily.

Captive spawning of the false clown *Amphiprion ocellaris* was achieved and methods of hatchery production of juveniles were standardised. The spawning time was during early morning hours and the frequency of spawning ranged from 12 to 15 days. The clutch size per spawning ranged from 300 to 1000 eggs. Hatching was on the evening of 7th day of incubation and the newly hatched larvae measured from 3.2 to 4.0mm in length. The larviculture protocols were developed by employing greenwater technique and feeding with rotifers and *Artemia* nauplii and during 15th to 17th day of hatching the larvae metamorphosed into juveniles.

Amphiprion percula

Adult of *A. percula*

Spawning of *A. percula*

Hatchery produced juveniles of *A.percula*

Spawning of the orange clown *Amphiprion percula* was obtained and methods of hatchery production were standardised. The spawning was during day time (0600 - 1530 hrs) and the spawning interval ranged from 14 to 18 days. The clutch size per spawning ranged from 112-557 eggs. The hatching was on the evening of the 7th day of incubation and the length of the newly hatched larvae ranged from 1.91 to 2.02mm.The larviculture protocols were similar to those employed for *A.ocellaris* and during the 19th -20th day of hatching, the larvae metamorphosed into juveniles.

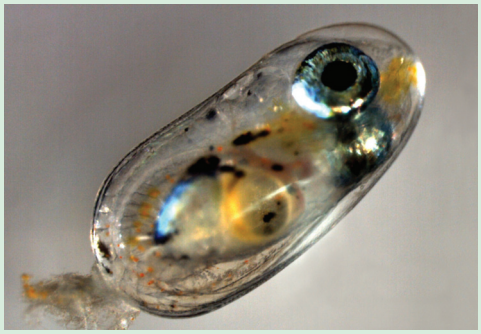
Captive breeding and seed production of the maroon clown (spine-cheek anemone fish) *Premnas biaculeatus* was also achieved. The broodstock was developed in 500 litre FRP tanks fitted with biological filtration and by providing special broodstock feeds. The spawning was during day time. The number of eggs per spawning ranged from 150 to 1000 numbers and the spawning interval was 15 to 20 days. Hatching occurred on the evening of the 6th day of incubation. The newly hatched larvae measured from 2.5 to 3.6 mm. Greenwater technique was employed for larval rearing and feeding

Premnas biaculeatus

Pair of *P. biaculeatus* with freshly laid eggs on tile

Microscopic view of capsule shaped eggs after 24 hrs of fertilization

Silvery eggs on final day of incubation



Embryo occupies the entire space in egg capsule on final day of incubation

protocols with enriched rotifers and newly hatched *Artemia* nauplii were developed. At 15 to 20th day of post hatch, the larvae metamorphosed and the size of the juveniles ranged from 12 to 16mm.

5. General aspects of clownfish hatchery technology

Age of the fish is the most important factor determining sexual maturity. Sexually matured adult clownfish are usually 9-18 months old. While selecting or establishing a pair it is not advisable to purchase or use full grown adult fish. Firstly adult clownfish in good condition will be costly. Secondly the fragile characteristics of newly captured adult clownfish make them at high risk. While selecting possible pairs or purchasing fish for pairing, it is best to buy sub-adults. Sub-adults do not form immediate pairs or fully display adult colouration, but they will quickly adjust and mature in good environmental conditions. Eventhough they are younger than adult pairs, they will only take about 3-6 months for initial spawning. A distinct advantage when pairing clownfish is their ability to change sex. The best and easiest approach in pairing clownfish is purchasing 3 or 4 fish of size 2.5 to 5cm in total length. Put all the fish in one established aquarium with no other fish. Since sex reversal is prevalent in clownfish, they simply decide which will become the male and which will become the female. Eventually, two fish will tend to stay together, chase others from specific areas, and attempt to keep away others. Sometimes a pair will accept and allow a few smaller individuals to remain in a reserve situation. Utilization of reserve fish is a unique adaptation in clownfish. When the female of a pair dies, the original male will become female and one of the reserve fishes will become the new male. Colouration can be used for sexing many species of clownfish. *A. polymnus* females have bright yellow coloured face, whereas most males have a dull brownish coloured face. *A. melanopus* females have a dull whitish red face, while the males have bright reddish face and fins. *A. ephippium* females have an overall dull red body colour and whitish red faces, while the males have bright red bodies and faces. *A. akallopisos* females are usually bright yellow orange than the males. *A. perideraion* males have a narrow orange trim on the edge of the soft dorsal and upper and lower margin of the caudal fin. Colour differences are found only on sexually matured adults.

Size can be another criterion determining males and females of many species of clownfish. In general females are largest individuals. Use of size criteria for pairing wild Tomato and Sebae complexes, *A. frenatus, A. ephippium* and *A. clarkii* is not of much value. In older and more established pairs, size differences between a male and female are not very conspicuous.. This is due to the fact that, as the fishes get older, the growth rates slow proportionally to their age and eventually they are about equal in size. Utilization of size characteristics can be deceiving when trying to select a pair

from the wild stock. Since they are collected throughout the Indo-Pacific, regional differences between isolated populations may be significant enough to make pairing difficult. Age/growth ratios may be completely different from region to region. Under aquarium conditions, these fishes are maintained under ideal environmental parameters, receive food *ad libitum* and grow at about equal pace. As they reach sexual maturity, tank-raised individuals normally show more dimorphic size differences.

Behaviour is another criterion for selecting males and females from adults or sub-adult fish. The first way to use behaviour is by introducing one adult after another to the tank. With net in hand, lower the new introductions slowly into the aquarium and let them go into the aquarium water. Stand back and observe for about 15 minutes. They will either accept each other or start to fight each other. A good possible pair will act basically gentle. Eventhough you have established a possible pair, there is a strong possibility of rejection later in the day or at night. Hence when using the introduction pairing technique make it start early in the morning and make continuous routine observations throughout the day. Established, dominant individuals often seem to respond instinctively and without provocation, and often injure the newly introduced fish. Hence it is better to put both fish in new environment. Placing both in a new environment greatly reduces dominance of established individuals and puts both fish on a more equal basis. Placing both fish on an equal basis often helps to slow down the initial instinctive aggressive behaviour. Two females will often accept each other and hence it is confusing to find out whether we have a pair or not. Test the possibility of two females within a pair, split the suspicious pair, and introduce a known male. Usually within a very short preiod of association, they start spawning. Since there is no way that two females can reverse their sex and form a productive pair, it is better to stop trying to get the biggest fish for making a breeding pair which may be probably two females.

Conditioning the fish is a prerequisite for spawning any fish. Conditioning is a term used to describe the utilization and manipulation of a combination of environmental factors to induce gonadal maturation and spawning. The factors may include light intensity, light duration and possibly wave length, temperature, water current, water quality, nitrogen, phosphate, ammonia, pH, type of food, tank size and shape, aeration and habitat. All fish do not respond to the same environmental cues which trigger spawning. Finding what works best can be a challenging task. Accumulating observations and data about the species you are working with, and applying this information will enable you to induce spawning. Eventhough there are hundreds of documented accounts on spawning of fish, the prior conditions for inducing spawning are usually poorly documented or understood. In order to develop a

consistent degree of control for continued routine spawning, a regimented set of conditioning parameters and records must be kept. Use of a log or diary should be mandatory by aquarists and aquaculturists. Proper continuous recorded documentation is essential for continued success. Fish are sensitive to every day changes within their environment which includes primarily water temperature, light period, light intensity and water quality. Seasonal changes within their environment have evolved into routine physiological and behavioural patters called biorhythms. Light and temperature are usually the triggers that spark gonadal development in temperate regions, but in tropics, wet and dry seasons may play more significant role. Conditioning of captive fish out of their normal spawning season requires temperature and light controlled rooms or aquaria equipped with timers, heaters, coolers and lights. Forcing gonadal development during their off season requires condensing a year into a specific period of time. Usually the procedure consists of lowering temperature and light to a point where fish gonads enter into a resting state. This sets the physiological clock at zero. During this period, the fish is maintained on a moderate diet. After 2-3 weeks, light, temperature and feeding are increased. Internally, fish starts to store excess fats within their body tissues which will later be incorporated in the developing eggs. Further increases in the photo-thermal regime induces maturing egg development to a point where all parameters are optimal for spawning. In the case of clownfish, light duration and intensity from the sun play minor roles in triggering spawning. Under natural conditions, wild clownfish spawn most of the year, but usually not more than one spawn per month. Under controlled conditioning regimes and proper feeding, they can be induced to spawn an average 2.2 spawns per month. The difference is primarily due to 3 factors – constant water temperatures, water quality and quality of diets. Clownfish will spawn within a fairly wide range of temperatures from 21^0C to 32^0C. Although spawning occurs within this wide temperature range, quality of nests and hatch is usually affected. Quality of the eggs and larvae produced in the extremes of this range will be low. In lower temperatures, metabolic rate is lowered and hatch is extended 2-4 days longer than optimal. Extended hatch usually results in weaker larvae and sometimes a lower hatch rate. At elevated temperatures, metabolic rate is accelerated above optimum, larvae may hatch early but are often inferior quality. Ideal incubation temperatures for clownfish eggs are 26-28 ^{0}C. Diurnal daily temperature fluctuations +/- 1^0C do not really affect the results, but may indeed enhance quality. Eventhough the tropics have a 12 : 12 light and night period, a 14 : 10 light / dark period is ideal for clownfish spawning. Lights should not be located directly on top of the tanks but should be elevated 12 inch or more above the tank. The salinity of around 28ppt is better while conditioning the fish. Lower salinity basically helps to reduce osmotic stress and may reduce disease problems associated with parasites that demand higher salinities to

survive. It also allows a large variance in salinity due to evaporation of tank water in the hatchery. A nitrate level of 20-30ppm, nitrite and ammonia level of less than 0.1ppm, pH around 8-8.3 are ideal in conditioning tanks. Normally the clownfish utilize the live anemone as their protective habitat and the hard surface beneath the anemone as their spawning substrate. By creating an artificial environment in which a spawning pair are comfortable and feel protected, the anemone can be easily eliminated. Clownfish are territorial and will not accept interference from other clownfish or most other fish and invertebrates. For best results, the pair should be kept in individual tanks with opaque sides. A moderate shade of aqua-blue colouration on the opaque sides is useful. Lighting should be moderate and located above the tank. Clownfish are basically bottom dwellers preferring some sort of habitat to hide in. A suitable multi-functional spawning substrate like clay pot is required. They provide security for each individual and a desirable spawning substrate. In addition, they can be easily removed for hatching and cleaning and replaced quickly. Substrates used should be large enough to house one or two adults at a time, large enough to allow the two adults to spawn inside and easy to remove and replace without disrupting the tank or upsetting the pair. Cleaning the spawning substrate should be routine. Clean substrate seems to stimulate the pair to lay eggs.

Broodstock diets are virtually the main keys to successful spawning. Eggs contain considerable lipids which are high and long lasting energy resources needed for the protracted development of the embryos within the eggs. These deposits are reserves to be incorporated within the eggs and provide energy to the female during her fasting period. Hence suitable diets and enough food must be fed to the broodstock fish. If broodstock fish are not properly fed, the results are directly reflected in the number of eggs laid, fertilization rate, hatch rate and the quality of hatched larvae. Poor quality eggs develop slowly, hatch late and often result in significant early larval mortalities. Conditioning food should be administered routinely to the brood stock clownfish three times a day – early in the morning, noon and around 5O'clock in the evening.

There is a misconception that *Artemia* is the food for conditioning and spawning tropical fish. Use of *Artemia* for conditioning should only be supplemental and not a steady diet. Live food imparts certain attributes not found in non living diets. But a steady diet of these foods usually leads to nutritional deficiencies and disorders. If brine shrimp or other live foods are included in the conditioning diet, they should be offered only as a supplement. Live foods are excellent carriers of nutritionally specific fats, oils and amino acids if they are programmed prior to feeding. A starving live animal is not a nutritionally balanced food. Essential fatty acids, micro algae, etc. can be administered to adult brine shrimp prior to feeding, to boost their food value. The

enriched food must be utilized within 3 hours or less to realize the potential of nutritional enhancement. Minimal disturbance of the pairs in the broodstock tank results in more consistent spawning and fecundity. Disruption during the spawning of clownfish often results in scattered, not fully fertilized eggs. Sight-seeing, just walking through broodstock areas, can have an effect on more sensitive species. Any movement can cause the pair to temporarily stop spawning and move away from the new nest. It is better that every effort should be made to keep the pair isolated from external distractions. Routine daily procedures of broodstock maintenance include feeding, checking for new spawns, checking the general health of the pairs and nest and adjusting air and water flows. Weekly routine maintenance includes siphoning of detritus buildup and uneaten food. Bi-weekly maintenance includes removal of dirty spawning substrates and replacing them immediately with clean ones and general bottom siphoning if needed. Every 12-18 months, the entire tank should be thoroughly cleaned, gravel removed and new or reclaimed gravel replaced. Disruption by moving of a mated pair usually results in no spawns for several months. Average recovery back to the spawning state was around 30 days.

Periodically adult clownfish consume their eggs hours after laying them. If this practice continues, it indicates a specific problem that must be corrected. There are several possibilities including being a newly formed pair, the pair was scared or nervous, too many tank inhabitants, diet deficiencies, parasitic destruction of eggs, poor water quality resulting in poor fertilization or the male is non functional. Diet is the most common problem. At times two females will reside together and spawn, obviously resulting in non fertilized nests which are usually consumed. Poor water quality can cause poor quality or unfertilized eggs. Low pH reduces sperm activity thus resulting in poor fertilization.

Pairs should be routinely checked externally for skin disorders, swelling and behavioural changes. Usually within spawning pairs of clownfish, the female is the most susceptible to diseases. The most susceptible adult fish are new ones, disrupted fish and non-spawning pairs. The most common disease problems in broodstock fish are due to old age. The female of a clownfish pair in most cases is the oldest individual and it is the female that spends most of the energy and is most susceptible to nutritional disorders which leads to weakness and eventual disease. Initial indicators of possible disease or stress include paleness or darkening of colour, excessive mucous or white surface film, dry looking skin especially around head region, exposed mucous pores around head region, rapid breathing, gills flared outwards, gulping air at the surface, hiding in the corners of the tank, resting on the bottom, swimming on the surface, erratic swimming or apparent loss of equilibrium, sunken abdomen, forehead or dorsal

crest, sores which do not heal quickly, bloated abdomen or raised scales, excessive scratching, white spots on surface of fins and body and disinterest in mate, usual habitat or feeding. The most common seasonal diseases of clownfish broodstock are white spot or saltwater itch and velvet disease. The white spot is caused by the ciliate *Cryptocaryon irritans* In the initial stages, large visible trophonts (active parasitic stage) and tomonts (reproductive stage) are confined to the caudal and pectoral fins and upper head region. In order to visually detect this disease, it must be observed in the early morning since most tomonts (white spot) drop off later in the day. These egg cases settle to the bottom, hatch out into free swimming tomites and reinfect the fish. This ciliate is very susceptible to hyper (>55 ppt) or hypo saline (<16 ppt) conditions. Use of high salinity dips for five to ten minutes will help to remove attached tomites. During treatment the tank should be drained, cleaned and refilled. Several dips may be required. Freshwater dips may also be used, provided that the pH is adjusted to 8.0 to 8.2. The velvet disease is caused by the dinoflagellate *Amyloodinium ocellatum*. In adult fish it manifests itself in high concentrations within the gill cavities and gill filaments. In later stages the fish characteristically have a dull velvet film on the skin with very small sparkling dots. Other symptoms include excessive scratching and laboured breathing. Quinine hydrochloride at 15 ppm is found to be very effective as a quick treatment followed by copper sulphate treatment for a long period. Popeye is another problem which is often induced by a mechanical injury from running into the tank walls or habitat. Initially opaque scar tissue or scrapes along the eye are evident. In later stages a slight swelling or bulge of the eye is evident which expose the underlying tissue. Treatment of popeye due to mechanical damage is simply rest, and care should be taken for not scaring the fish. Popeye can also be caused by a bacterial infection but, in this case both eyes usually swell evenly. This may be a secondary infection following a mechanical injury. There is no known cure and if the disease is aggravated the fish has to be removed. Popeye can result in the loss of sight or loss of an eye. Once cured, the loss of an eye is not a problem and the fish is able to spawn. Disease prevention is better than control. Disease prevention in broodstock is closely related to routine maintenance and water quality.

Recording and keeping data on broodstock is of prime importance. Initially data should be recorded for virtually every thing, since it is usually hard to determine what is important with regard to your specific conditions and fish. After 12-18 months, when you have formed a good picture of your specific conditions, you can start narrowing the amount of data you need to record. It is better to maintain monthly pair summary records. Pairs can be categorized into possible pair, new pair, spawning pair and inactive pair. The pair record is the birth certificate, diary and death certificate of a single pair. As long as the female of an established pair is alive, the pair data should remain intact.

Although record keeping on a pair is time consuming, it serves as a invaluable source of information for accessing the entire operation.

The period from larvae to sexual maturity and spawning varies from 9-15 months (normally 12) for clownfish. Some species like *A. ocellaris* reach maturity earlier. Sub adults generally take about 6 months to reach sexual maturity. Full size adults obtained from the wild usually take 2-3 months to begin spawning in captivity. If a consistent spawning pair is severely disrupted, such as tank change, illness, treatment, etc., they usually take 1-3 months to begin spawning again. Clownfish displays several common characteristics that indicate as spawning. Often the female takes the initial lead role in forming a new pair and in spawning. She will clean several hard surface areas and often pushes the male on his side or belly. Head shaking, standing on their heads, nipping and chasing are common prior to spawning, especially in new pairs. Excessive digging in the bottom substrate for cleaning of many areas usually occurs. As the pair matures, the male becomes more aggressive. Eventually the male assumes most of the nest cleaning and tending of the eggs. The clownfish normally spawn during forenoon. Once spawning commences, females press their body towards the substrate and slowly move in a rowing fashion using their pectoral fins. She moves in a circular path depositing a continuous spiral of eggs from the central outward. The male swims behind the female, releasing sperm over the newly deposited eggs. After spawning the male assumes a more dominant role. Although both tend the nest, the male becomes the real caretaker. He intermittently fans the nest with his caudal or pectoral fins. He also cleans the eggs by gently mouthing them without removing them. Dead and fungal infected eggs are routinely removed and eaten. Substrate around the nest is also often cleaned. While a nest is present, males do not feed aggressively. The male spends an average of 30-60% of its time during the day for tending the nest. Fanning the eggs is frequent on the day after spawning and diminishes considerably about mid way in the incubation period. On the day of hatch, fanning increases again. Nest can be located on any hard surface. Placing substrates usually helps to minimize spawning on the sides of the tank. Dirty spawning substrates should be avoided since it makes nest location harder to detect.

A key factor in regard to success and failure of the spawn is egg pigmentation. Egg pigmentation of benthic spawners is very common. Pigmentation has a direct relationship in the success of hatch and initial larval survival. Healthy, well fed clownfish pairs produce orange to bright red eggs depending on the species. The degree of intensity of colour is directly proportional to the amount of pigments within their diet. Highly coloured nests hatch better than pale whitish coloured nests. Addition of the pigment astaxanthin to the diet resulted in bright coloured nest within two weeks. In addition, nests became tighter, more compact, nests and eggs larger, hatches more regular, less

initial larval mortality, brighter larval colouration and faster larval metamorphosis. The egg colouration has a direct correlation with juvenile survival and growth. As fertilized clownfish eggs advances development, they change daily from initially yellow orange or red colour to a vivid black. Using these colour changes, it is possible to determine when the spawning occurred and when it will hatch. During early development, the larva's head is located at the attached edge of the eggs. As the development progress, they rotate inside the eggs so that their head is at the unattached tip. At hatching they push forward with their tails, breaking the unattached tip of the egg. Unfertilized or diseased eggs turn opaque within 24 hours. During incubation, eggs may die due to improper development, fungus, physical damage, severe water quality changes, lack of oxygen, lack of parental care and / or parasitic attack. Overall nest size depends more on the species, size and age of the female. In general, the clownfish eggs range in size from 2.0-2.4mm and 0.9mmwide. The newly hatched larvae measure around 4mm in total length, but may vary depending upon the size of brooder, previous spawning experience and broodstock nutrition.

The clownfishes live for longer periods. The age of captive *A. frenatus* is recorded as 17 years, *A. clarkii* as 14 years, *A. ocellaris* as 14 and *A. perideraion* as 21years. Clownfish are protracted spawners and produce one nest per month or less in the wild. Under controlled conditions and ideal consistent diets, they can be easily induced to spawn an average of 2.1 times per month. In captivity most pairs spawn a minimum of 11 months a year, regardless of the species. Individual pairs of clownfish seem to reach a typical reproductive pattern which remains fairly consistent for an extended period of time.

Eventhough adult clownfish pair for life, this does not mean that they will remain good commercial pairs throughout their lives. When a pair is no longer productive, it should be replaced or separated for repairing. The criteria that can be adopted for culling pairs include (1) A pair that remains as a possible pair over eight months (2) Pairs producing less than two spawns a month over the last four months. (3) Old pairs with greater than 50% of the nest gone over the last four months (4) Old pairs with consistently loose, scattered nest or small nests. (5) Pairs that continuously spawn on the side of the tank and (6) Unpopular species or overstock of a species.

Nests can tolerate more mechanical and chemical changes than newly hatched larvae. While within the egg, larvae can adjust more easily to water changes than after hatch. Determination of the time of hatch is dependant on visual appearances, temperature during incubation and the species. Hatching is also dependant on broodstock health, quality of the eggs, initial colour of the eggs, water quality and light. The longer the larva remains in the egg beyond the normal incubation, the weaker it will be at hatch. The duration of hatching of clownfish eggs from the day of egg laying for the common

species generally ranges from 6th day evening to 9th day evening, at a temperature range of 26-28°C. Hatching of clownfish eggs normally commences from 1-2 hours after dark. Hatching takes between 15-20 minutes. If larvae are allowed to remain in broodstock tanks overnight, numbers of larvae are significantly reduced due to predation or due to the filtration in the broodstock tanks. Scooping and siphoning hatched larvae is very impractical. The use of a net for removal of larvae is prohibitive due to the chance of severe mechanical injury to the delicate larvae. Whatever means are used, it is important to realize that larvae cannot tolerate being touched by a solid object like a net. Common practice is to allow the larvae to hatch within the broodstock tank, which is suitable for small scale operations. Since clownfish larvae are phototropic, they can be drawn to a specific spot for removal by using a flash light. The accumulated larvae can be collected by using buckets along with water. More sophisticated siphoning bucket includes using large diameter plastic tubes mounted above the nest area and equipped with a small light source at the top.

Hatching of nests can be done remotely outside the broodstock tanks also. Remote hatching is more advantageous especially to commercial operations. Larvae within the eggs are more tolerable than newly hatched larvae to physical, chemical and water quality change. Hatching nests within broodstock tanks may yield 100% hatch, but not 100% recovery of the larvae. Many larvae are consumed, drawn into the filters, become entrapped or die before being captured. Removing the nest and placing it into a flow-through hatching tank is better. To keep the eggs moving and well aerated, they are either aerated or incoming water flow is directed on to the eggs. Physically removing intact nests just prior to hatch and placing them directly in larval rearing tanks is also found to yield best successful hatching.

Larval rearing is the most critical, time consuming phase of marine fish culture. The major requirements are (1) to provide a simple, adequate stable environment that can be easily manipulated and maintained (2) to provide adequate, quality foods on a consistent basis and (3) to provide strict maintenance procedures on a daily basis. Water quality is the key environmental factor but can be easily controlled with simple water exchanges. Typically the bio-load in larval rearing tanks is insignificant when compared to broodstock or juvenile rearing tanks. Therefore deterioration of the water environment is easier to control. Success or failure in larval rearing is closely associated with availability of quantity and quality of live feeds and how they are administered. With out a ready, plentiful, nutritious live feed source your larval rearing attempts will be futile, erratic and very discouraging. To rear clownfish larvae, about 300-600 rotifers per larvae per day for a period of 5-10 days are required. In addition, the rotifers must be completely nutritious and balanced with essential fatty acids and micro algae. Larval

rearing is not simply putting fish larvae in the tank, feed them and watch them grow. It will be necessary to clean the tank daily of detritus and uneaten food. Water exchanges and air flows must be watched. Since benthic larvae like those of clownfish normally hatch with well developed functional eyes and fair swimming ability, there is no chance of larvae becoming trapped in the corners of the tank and hence rectangular or square tanks can be employed. Benthic larvae may feed from vertical surface areas of water column, but not normally from the bottom. Therefore deeper tanks are usually preferred. One of the extremely important criteria for selecting tank size is knowing the quantities of live feed we can provide on a daily basis and how many larvae you intend to rear in each tank. Larvae do not normally actively seek food but tend to be opportunistic feeders, patiently waiting for a food particle to come within striking distance. Placing 300 larvae in a 400 litre tank makes it difficult to provide proper densities of live feeds/ dry feeds with out polluting the water. Opportunistic contact between food and larva diminishes drastically when few are reared in a large tank. Forcing larva to swim considerable distances to seek food tends to drain their potential power supply quicker than it is replenished and results in eventual death or slow growth. Concentrating early stage larvae and food supplies minimizes production cost to provide sufficient live food organisms per unit area. By assuring more adequate food particles per unit area, energy expenditure of larvae to find food is minimized. This ensures faster growth rates and higher survivals.

Tank colouration can play an important part in the survival of larvae. Yellow, green and black coloured tanks are closer to natural environmental colours and showed better survival than darker red and brown coloured tanks. Usually light coloured tanks are considered detrimental in larval rearing since live foods are less visible against pale coloured backgrounds. In white coloured tanks, larvae died even without initiating feeding. An all-glass aquarium without background appears as a mirror to fish. Larvae tend to be drawn towards the mirror surface and just stick to the glass. This reduces feeding efficiency and is detrimental. Like wise they are often attracted to the bottom since it is transparent and reflects the colour of the surface on which the tank is placed. It is advantageous to reduce the mirror attraction and force the larvae to orient in the water column.

Clownfish larvae are highly sensitive to light. High light intensities or sudden lighting induces stress to the larvae. The light intensity just sufficient to see the live feeds is preferred. It is advantageous to have minimum of 14-16 hours light period to the larvae. Healthy clownfish larvae are clear yellowish in colour, with dark pigmentation around the stomach and eye region. Stressed larvae remain dark along the entire body. Body shape of well fed larvae is oval or round. Underfed or starving larvae are cone-shaped with a large head and a tapering body down to the tail. Clownfish mortalities are more common at hatch, day 2 after hatch when the yolk sac in almost gone, day 7-

9 at metamorphosis and around day 12. The most significant loses are on day 2 and 8. Clownfish larvae can quickly starve or loose strength when feeding is improper. For the first 9-15 days, they are basically pelagic. They scatter throughout the column and normally orient themselves head first toward the current created by the rising bubbles from the air stone. Early juvenile colouration is first detected by the development of the pale translucent white head bar. This occurs around day 7 to as late as day 15. At metamorphosis, larvae are about 9mm. Mortalities at metamorphosis are directly related to larval quality and water quality. Healthy larvae in healthy water conditions usually transform within in a 3 day period. Density of larvae in a rearing tank, how much food is fed and how it is presented have direct relationships to uniform growth.

The clownfish reach the juvenile stage, which can be transferred to grow out tanks around 13mm size when they are about 30 days old. Generally it takes a total of 4 months to rear to a marketable size of around 38mm. Juvenile growth and development are strongly influenced by water quality, food quality and the amount of food fed. A significant portion of juvenile culture facilities should be dedicated towards filtration. It is during juvenile growout that filtration capabilities become critical. Increasing bio-load, consisting of growing fish constantly demanding more food, increases biological oxygen demand required for respiration and oxidation of wastes. A conventional submerged undergravel filter is not advisable in growout tanks. Gravel, crushed coral, etc. is hard to clean and maintain and can easily clog, which can result in 'toxic tank syndrome'. It is advantageous to use a bare tank with a single large airlift sponge filter. The juveniles can be transferred very carefully with fine meshed net, to growout tanks. Clownfishes are territorial at very early age. The territorial problems can be prevented by (1) providing each fish with its own tank (2) providing a highly diverse habitat (3) increasing the tank volume so that each fish has several litres of water and (4) crowding them so that there is no territory to defend. The last method is preferred due to practical reasons of cost, space requirements and maintenance problems. Juveniles should be fed a minimum of 3 times a day to obtain maximum growth. Uneaten food and fecal matter should be removed each morning by siphoning. Harvesting large individuals from a single tank can go on for several weeks and then it advisable to cull remaining fish and condense them for a final growout. Sufficient pigmentation of juveniles is dependent on how they are maintained prior to hatch and during metamorphosis. Intense colouration is primarily developed through food. Fish grown in a very large tank (low density) have better colour than those in crowded conditions. Fish that grow slower than normal usually have more intense colouration. Fish grown in dark backgrounds develop dark colouration while those in light coloured have light pale colouration. Fish with excellent colouration that are moved to clear glass aquaria often become dull or light in colour in a matter of weeks. Stress plays a very important role in

colouration. Pigment cells expand and contract according to light intensity and background colouration. Wild caught fish often have brighter colouration but when maintained in the average aquarium the colour becomes dull. This has been attributed to colour changes but sometimes a combination of diet, tank background, lighting and/or water quality is responsible. It has been proved that astaxantin is the key pigment in clownfish. Products containing significant amounts of astaxanthin are most effective in enhancing pigmentation in clownfish. Frozen, freeze dried planktonic krill, lobster eggs, freshwater crayfish eggs and *Macrobrachium* eggs are also good sources of astaxanthin. Manipulation of diet, exterior environments, lights, water quality and maintaining healthy and unstressed fish can all contribute to colouration.

Juveniles are most prone to disease and health problems. Early detection of pending diseases or health problems is absolutely essential to success. Observations on mortality rate also help to indicate the nature of the problem. Quick significant mortalities are usually due to deteriorated water quality conditions. Parasitic diseases cause slow constant death patterns whereas bacteria infestations are quicker. Mortalities due to nutritional disorders are usually very slow and constant. Disease is usually a secondary result of prolonged stress. A low oxygen at present results in bacteria infections a week later. The main water quality stress situations and their possible management are as follows:

Observation	**Possible management**
Low dissolved oxygen(less than 5 ppm)	Increase aeration, stop feeding until corrected
High carbon di oxide	Increase aeration, stop feeding until corrected
Low pH (less than 8)	Add alkaline buffers (sodium bicarbonate etc., reduce feeding rate, check ammonia and nitrite concentrations
High ammonia (above 0.05 ppm as unionized)	Exchange water, reduce feeding rate, Check biofilter and pH
High nitrite (above 0.5ppm)	Exchange water, reduce feeding rate and add 5 to 6ppm chloride per ppm nitrite
Low alkalinity	Add alkaline buffers
Low hardness	Add calcium carbonate or calcium chloride

When environmental parameters are held constant and adequate diets are routinely provided, fish can resist most diseases and infections.

6. Hatchery production of Damselfishes

The damselfishes are very popular among aquarists due to their small size, bright colours, quick acclimation to captivity and interesting behaviour. The majority of species inhabit the Indo-Pacific region and about 100 species and 18 genera have been recorded from the Indian Ocean. More than 30 species belonging to the genera *Pomacentrus, Neopomacentrus, Chromis, Abudefduf* and *Chrysiptera* are commonly available from Indian coral seas.

Broodstock development and larval rearing were achieved for eight species of damselfishes *viz*. the three spot damsel (*Dascyllus trimaculatus*), striped damsel (*Dascyllus aruanus*), the blue damsel (*Pomacentrus caeruleus*), the peacock damsel *P. pavo,* the bluegreen damsel (*Chromis viridis),* the filamentous tail damsel *(Neopomacentrus cyanomos),* the yellowtail damsel (*Neopomacentrus nemurus)* and the Sapphire devil damsel (*Chrysiptera cyanea*).

(i) Striped Damsel, Three-spot Damsel and Blue Damsel

Broodstock development: Broodstock development was done in one tonne FRP tanks. These tanks were fitted with biological filters to maintain the water quality to the optimum level. The filtration rate was about 200 litres per hour. 6-8 fishes collected by traps were introduced in each tank for broodstock development. The ranges of water quality parameters of the broodstock were as follows:

Temperature	-	25ºC to 29.5ºC
pH	-	8.3 to 8.6
Salinity	-	28 to 31 ppt
Dissolved oxygen	-	4.5 to 5.1ml/litre

Water in the broodstock tanks was exchanged @30% once in a week. The broodstock tanks were kept under translucent roofing in order to reduce the light intensity. Feeding of the fishes was done once in a day @ 5-10% of the body weight. Various types of feeds like finely chopped fishes, shrimps and molluscan meat were given to the broodstock fishes. Substrata were provided in the broodstock tanks for the attachment of eggs during spawning.

Sub-adults of all the speceis spawned in captivity after 4-8 months of maintenance in the broodstock tanks. Previous day before spawning the parent fishes actively cleaned the site for attaching the eggs by rubbing it with their pelvic fins and picking off any

loose particles or algae with their mouths. During spawning, females attached their eggs on the cleaned site, which were immediately fertilised by the males. Spawning occurred during the morning hours. The development of egg took place in 3 days at 28 ^{0}C. During this period the parent fishes took care of the eggs by protecting them and by fanning them with the pectoral fins and tail.

Live feed culture: Live feeds like microalgae and copepods were cultured in order to develop green water in the larval rearing tanks and to feed the damsel fish larvae during initial larval phase. Pure cultures of micro algae *Nanochloropsis* sp. were maintained in indoor culture rooms by employing standard methods. These cultures were then scaled up in outdoor algal production facility to the required volume.

Hatching and larval rearing: The substratum with egg clutch was transferred to the larval rearing tanks containing sea water having the same physicochemical characteristics of the parent tank. A gentle air flow was created over the eggs by placing an air stone near to the egg clutch and the egg clutch was left in darkness. Generally hatching took place on the night of 4th day of incubation. Eggs can be hatched out in the broodstock tanks and immediately trasnferred to larval rearing tanks. Larval rearing was carried out in 5 ton FRP tanks. The inner side of the tank was light blue in colour in order have a better contrast between the live feed and the surroundings. The range of water quality parameters in the larviculture system were as follows:

Temperature	-	27ºC to 31.5ºC
pH	-	7.5 to 8.6
Salinity	-	28 to 34 ppt
Dissolved oxygen	-	4.5 to 5.1ml/litre

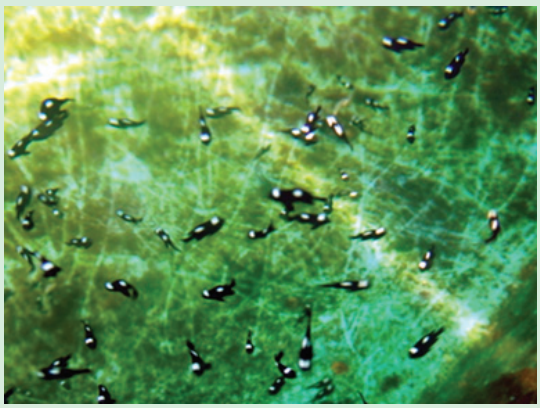
Green water technique using the microalga *Nanochloropsis* sp. was adopted for the larval rearing of damselfishes. The adults of two species of copepods *viz*. *Euterpina acutifrons* and *Pseudodiaptomus serricaudatus* were inoculated into the green water. When the copepods have started their growth phase, as was noted by counting the number of

Dascyllus trimaculatus

Newly spawned eggs

Fully developed eggs

Newly hatched larva



Hatchery produced juveniles of *D. trimaculatus*

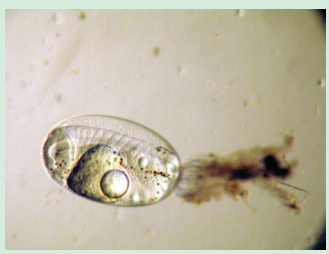
Adult of *D. trimaculatus*

egg bearing copepods and nauplii per 50 ml, the newly hatched larvae were introduced into these tanks. About 2000 larvae of each species were introduced into the respective tanks

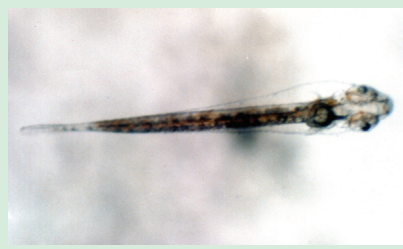
In the case of *D.trimaculatus* the larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larvae was 2.5mm. Mouth opening was formed on the second day and the gape measured around 150 µm. The larvae started feeding on copepod nauplii from the third day of hatching. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the green water was maintained at a range of 1 x 10^5 cells – 6 x 10^5 cells ml^{-1} . After twenty days when the average size of the larvae had reached around 4 mm with average mouth gape of around 450 µm, freshly hatched *Artemia* nauplii were fed *ad libitum*. Thereafter no mortality was noted. The larvae started metamorphosing from 35^{th} day of hatching and all the larvae metamorphosed by 40^{th} day. The just metamorphosed young one measured from 12 to 13 mm in length.

Dascyllus aruanus

Newly spawned egg



Fully developed egg



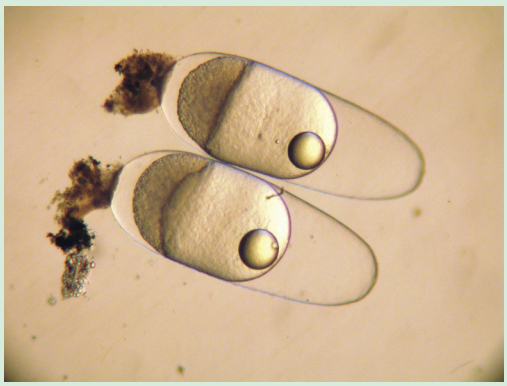
Newly hatched larva

Hatchery produced juveniles of *D. aruanus*

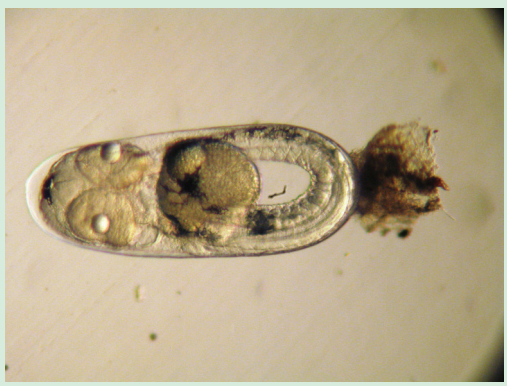
Adult of *D. aruanus*

In *D.aruanus*, the larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larvae was 2.4mm. The larvae were transferred to 5 tonne capacity rectangular FRP tanks in which mixed copepod cultures (*P.serricaudatus* and *E.acutifrons*) were maintained in green water. Mouth opening was formed on the second day and the gape measured around 160 µm. The larvae started feeding on copepod nauplii from the third day of hatching. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the green water was maintained at a range of 1×10^5 cells – 6×10^5 cells ml^{-1} . After twenty days when the average size of the larvae had reached 4 mm with average mouth gape of 450 µm, freshly hatched *Artemia* nauplii were fed *ad libitum*. Thereafter no mortality was noted. The larvae started metamorphosing from 25th day of hatching and all the larvae metamorphosed by the 31st day.

Pomacentrus caeruleus



Newly spawned eggs of blue damsel



Fully developed egg of blue damsel

Hatchery produced juveniles of blue damsel

Adults of blue damsel

In blue damsel, the newly hatched larvae measured about 1.2mm with an average mouth gape of 200µ. The larvae were transferred to 5 tonne capacity FRP tanks in which green water was developed and a mixed culture of copepods (*P.serricaudatus* and *E.acutifrons*) was maintained. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the green water was maintained at a range of 1 x 10^5 cells – 6 x 10^5 cells ml^{-1} . After fifteen days freshly hatched *Artemia* nauplii were also supplemented. Thereafter no mortality was noted. The larvae started metamorphosing from the 17th day and by 21st day all of them metamorphosed. The average length of just metamorphosed juvenile was 21mm.

(ii) Blue green damsel and Yellowtail damsel

Broodstock development and seed production methods were also developed for the blue green damsel *Chromis viridis* and the yellow tail damsel *Neopomacentrus nemurus*.

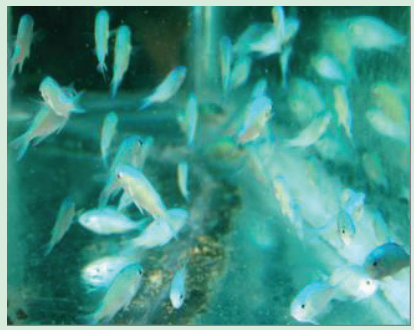
Chromis viridis

Freshly laid egg

Freshly hatched larva

2nd day larva

Tenth day larva - (Blue green damsel)



Hatchery produced juveniles of blue green damsel

The broodstock development of the green damsel *Chromis viridis* was carried out in 2 tonne FRP tanks fitted with biological filter and by feeding with special broodstock feeds. The fishes became broodstock at a total length range of 8 -9 cm. The average frequency of spawning was 5 per month with an interval of about 5 days. The egg was oval shaped and the average length was 502µ. The total number of eggs per spawning ranged from 1300 -1500 eggs. Hatching occurred on the evening of the fourth day of incubation. Larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larva was 2.25mm. The larvae were transferred to 5 tonne capacity round FRP tanks in which cultures of the harpacticoid copepod *Euterpina acutifrons* and the calanoid copepod *Pseudodiaptomus serricaudatus* were maintained in green water produced by adding *Nannochloropsis* culture. Mouth opening was formed

Neopomacentrus nemurus

Fully developed larva inside the egg of yellow tail damsel

Fully developed larva before metamorphosis (yellow tail damsel)

Hatchery produced juveniles of yellowtail damsel

on the second day of hatching and the gape measured around 190µ. The larvae started feeding on copepod nauplii from the 3rd day onwards. From the 32nd day of larval rearing freshly hatched Artemia nauplii was also supplemented. Metamorphosis started from 30th day and completed by 49th day.

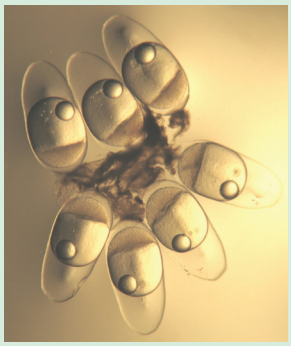
The broodstock of the yellowtail damsel *Neopomacentrus nemurus* was developed in 2 tonne capacity FRP tanks. The average interval of spawning ranged from 4 -5 days. The length of freshly laid egg was 870µ. The eggs hatched on the evening of the fourth day of incubation. The freshly hatched larva measured 1.8mm with a mouth gape of about 100µ. The larvae were transferred to 5 tonne capacity FRP tanks in which mixed culture of copepods were maintained in green water produced by adding cultures of *Nannochloropsis*. The larvae started feeding on nauplii of copepods from the third day of hatching. From the 12th day onwards the larvae were also fed *ad libitum* with freshly hatched artemia nauplii. From the 16th to 21st day of hatching the larvae metamorphosed into juveniles. The length of the just metamorphosed juvenile ranged from 10 -13 mm.

(iii) Sapphiredevil Damsel

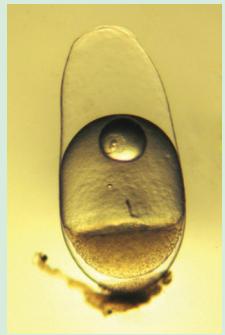
Broodstock development was done in two tonne capacity FRP tanks with biological filter and by feeding *ad libitum* with natural feeds. The size of broodstock fish ranged from 5 to 6.5cm. The number of eggs per spawning ranged from 2000 - 2500. The interval between successive spawnings ranged from 5 to 20 days. The eggs were either attached to the sides of the broodstock tank or on the substratum provided in the broodstock tank. The eggs were oval - shaped and measured around 1.3mm in length

and 0.6mm in width. Parental care by the male was noted. Hatching occurred on the night of the third day of incubation. The larvae were altritial type but with mouth opening at the time of hatching. The length of newly hatched larvae averaged to 2.5mm and the mouth gape around 150µ.

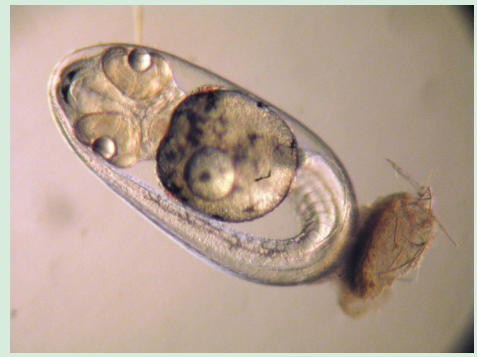
Chrysiptera cyanea



Bunch of freshly laid eggs



Freshly laid egg



Fully developed egg

Hatchery produced juveniles

Adult

Larviculture was done in five tonne capacity FRP tanks by employing greenwater produced by the microalgae *Nannochloropsis oculata*. Different larviculture systems were experimented by varying the cell counts of greenwater and the live feeds. The cell counts of green water employed for the experiments were 1×10^4 ml^{-1}, 1×10^5 ml^{-1} and 1×10^6 ml^{-1}. Four sets of experiments were conducted by feeding with different live feeds – one set with enriched rotifer (*Brachionus rotundiformis*) alone, the second set by employing mixed culture of two copepods species viz. *Euterpina acutifrons* and *Pseudodiaptomus*

serricaudatus, the third set by employing copepods and rotifers together as live feed and the fourth set with copepods as starter feed for the first six days followed by enriched rotifers from 7 -15 dph. The larval survival was recorded on 15th day of post-hatch. Feeding experiments with *B.rotundiformis* alone and those with *B.rotundiformis* and copepods together as live feeds were not successful. Co-culturing of the two selected species of copepods in the optimum range of cell count of greenwater gave the best survival. In this set, survival rate of larvae on 15 day post-hatch (dph) ranged from 5 to 8%. The maximum survival rate was 5-6% in the group fed with copepods as starter feed upto 6 dph followed by enriched rotifers from 7 to 15 dph. It was noted that a cell count range of 1 x 10^5 cells ml^{-1} to 9 x 10^5 cells ml^{-1} was the optimum which yielded the maximum larval survival in both these sets of experiments. After 15 dph the larvae were fed with freshly hatched *Artemia* nauplii and no further mortality was noted. Metamorphosis of larvae started from 24th day and all the larvae metamorphosed by 30th day.

The methodologies employed for the other damselfish species *viz. Neopomacentrus cyanomos* and *Pomacentrus pavo* are similar to the above. These methodologies developed can be scaled up for commercial level production.

7. Grow-out methods

Indoor grow-out systems

In the case of clownfishes, on 19-20th days of post hatching the larvae metamorphose into juvenile (size 1.0 to 1.2 cm) and shift from pelagic to epibenthic stages. The rate at which the juvenile fishes grow depends on the size of the tank and stocking density, the quality, quantity of food given and the water temperature. As the clown fishes exhibit social hierarchy, dominant clownfish will grow fast and suppress the growth of other fishes. This can be largely overcome by growing the fish together in a large tank with sufficient host anemones or dividing the juveniles into several groups in different rearing tanks of size 250 to 500 lit capacities fitted with biological filters. At this stage, the stocking density need to be reduced to 90-100 numbers of juveniles with single host sea anemone in 100 lit tank capacities for initial one to two months. On attaining a size of 24 to 35 mm in total length (TL), the stocking density needs to be reduced to 30 to 50 number with single sea anemone in 100 liter tank until marketing. In case of each 500 liter FRP tank, 130 to 150 juveniles can be reared with 3-4 sea anemones.

In the case of damselfishes, a total of about 500 nos. of size 0.8 to 1.2cm can be stocked in a 5 tonne capacity FRP tank for growing up to a marketable size of 2.5 to 3.5 cm in 3 months.

In the grow out phase, a survival of 70-100% can be obtained through proper feeding with different wet feeds like boiled sardine flesh, chopped clam meat, mussel meat and formulated dry feed, for 4 times a day *ad libitum.*

Grow-out in *hapas*

Grow out of ornamental fishes can be effectively practised in *hapas* installed in protected calm nearshore areas. The growth was found to be much faster. The major advantage is that the colour is much brighter in fishes grown in *hapas* due to natural light and good exchange of water.

Selection of site

The site should have at least 2 m depth of water, good dissolved oxygen content, free from industrial contaminants, low anthropogenic pollution and easy accessibility from land. A protected area is generally preferred.

Stitching of *Hapa*

Hapa of 2.5 m x 1.5 m x 1.5 m dimension

Installation of *hapa* in the sea

Construction of floating *hapa*

Rectangular shaped floating *hapa* (2.5 m x 1.5 m x 1.5 m) with PVC frames (dia 1.5 inch) for supporting the net bag structure and to retain the shape are used for the grow out phases of juvenile to marketable size within 2 months. Here the advantage is that it provides better water exchange and natural environment to the fishes.

Good quality HDPE net having 0.5 mm and 1 mm mesh size could be used to make the net bag. Double layered net bags are stitched in the diamention 2.5 x 1.5 x 1.5 m depending upon the design and requirement of the frame. The hapa can be moored properly at the suitable site.

Morning and late evening hours are better for stocking, as the temperature is comparatively low and chances of mortality will be less. A stocking density of 1000 fishes in optimal in the hapa of the diamention mentioned. Survival of 90-95% is obtained through proper feeding with different wet feeds like boiled sardine flesh, chopped clam meat, mussel meat and formulated dry feed, 2 times a day *ad libitum*. Fouling was a regular phenomenon and regular monitoring is advisable. Cleaning the net with coir brush has to be carried out on daily basis.

Floating *hapa* reared marine ornamental juveniles grow faster with increased survival rate and good colouration, thereby fetching better price in the market.

8. Issues and Challenges

The broodstock of the fish can be developed and spawning can be obtained by providing the environment and physical conditions of the fish to meet the species' minimum requirements for reproduction. The four basic criteria are :

(i) Adult fish in good health

(ii) Proper nutrition – quality and quantity

(iii) Suitable physical environment – light, temperature and ambient environment

(iv) Proper chemical environment –water quality

The most important points to bear in mind while trying to breed reef fishes are to provide an environment in which the fish feel comfortable and to feed them adequately with nutritious food. The breeding tanks should be of sufficient size for the concerned species. Breeding fish are territorial and extremely aggressive towards members of their own sex. Hence group spawners require enough space so that smaller individuals can form territories of their own. Pair spawners can usually be bred in relatively smaller tanks.

Information regarding size and age at first maturity, patterns of pair formation, spawning seasons and periodicity, feed preferences of adult fishes and larvae are the essential prerequisites for the captive breeding and rearing of fishes. Detailed information on the reproductive biological aspects are available only for a few species of pomacentrids. Pomacentrids characteristically attach their eggs to submerged objects such as coral pieces, rocks, algal mass etc. The spawning usually takes place in the morning and hatching after sunset. The nests are protected and cared by the parents , mainly by the male parent. Parental care includes defence of the nest from intruders, periodic fanning with fins and removal of dead eggs from the nest. Parental care of eggs are continued till hatching and hatchlings are dispersed by waves and currents.

The family Pomacentridae includes both hermaphroditic and gonochoristic members. Most anemonefishes are protandrous hermaphrodites and most damselfishes are protogynous hermaphrodites. The damselfish *Parva microlepis* is reported to be a gonochorist. The protandrous hermaphroditic anemonefishes exhibit monogamous mating system in which the fishes form permanent breeding pairs. Only one pair of mature fish will be present in a colony, normally the female being the largest. All other fishes remain as subadult males. Polygynous mating system is observed in protogynous hermaphrodites such as *Dascyllus reticulatus*. Here eggs from more than one female will be present in a males's nest. In this system more than one mature female will be present in a colony and the largest individual will usually be a male. Most of the pomacentrids have a very protracted breeding season. In captive conditions, they continue spawning for about 7 to 8 months which follows a pause of about three months. Majority of pomacentrids attain sexual maturity in the first year.

The size of the larvae and their behaviour are the important characters to be considered while selecting suitable rearing systems. Eventhough providing the required feed at adequate quantity without affecting the water quality is the key point in hatchery operations, other factors such as quality of water, water depth, water movements etc. also play crucial roles in larval rearing. Most damselfishes have initial pelagic larval phase, the duration of which varies with species. At the time of metamorphosis they become benthic and settle to the specific habitats of the species. Average duration of pelagic stage of 100 species of damselfishes studied from Indo-Pacific region showed that it varied from 13.1 to 35.2 days. Damselfishes are unable to postpone settlement by extending the pelagic phase unlike some other reef fishes

In nature, the larvae are directly released into the open sea and are exposed to water currents and waves. Therefore in captive conditions, larger water columns and area is

required for their survival and growth. The use of 2500 litres and 1000 litres tanks for rearing two damselfishes *viz*. *Dascyllus albisella* and *D.aruanus* has been repoted. Better results can be expected by providing a slight water turbulence rather than maintaining relatively stagnant water in the rearing system. But larger sized larvae such as the anemonefish larvae which measure about 4mm can be reared in small containers.

The most critical requirement for larval rearing tanks is to prevent headbutting syndrome. This is a phenomenon in which the larvae will swim towards any light reflected from off the sides or bottom of the tank and will continue to bash themselves against the sides of the tank until they die. 'Green water technique' is widely practised to avoid this phenomenon. In this system cultured green algae such as *Nannochloropsis, Chlorella, Tetraselmis* etc are added to the larval rearing tank. Though the larvae do not feed on microalgae, they act as water conditioner. It reduces the chances of headbutting syndrome by reducing light penetration. It also improves the water quality since the algae act as nutrient sink. The greenwater also improves the quality of food (rotifers and *Artemia*) and is reported to improve prey contrast and visibility. However aenemonefish larvae can be reared in clear water also if feed concentration is maintained at adequate levels.

Light intensity is another critical factor for larval rearing. The light intensity during the day has to be sufficient for the larvae to easily detect and capture food. The use of fluorescent tubes for 10 to 12 hours suspended above the larval rearing tanks is advisable. Providing low intensity diffuse lighting during night is also useful. This is especially important in earlier stages as it helps to keep the larvae swimming towards the surface at night rather than sinking to the bottom. While overnight lighting is preferable with the damselfishes and clownfishes it is essential for young cardinal fish larvae which otherwise show high overnight mortality. A common low intensity night light of around 10 watts works well if suspended above and away from the rearing tank. The light source must be indirect so that no light from it reflects directly off the sides or base of the tank.

The feed management of the newly hatched larvae when the yolk is just exhausted and the larvae have to resort to exogenous feeding is the the major bottleneck in rearing most marine fish larvae. At this stage the larval survival is entirely dependent on the availability and quality of food in required quantities. The gape of the mouth opening determines the size of the food that can be accepted by the larvae. The nutritional requirement of the fish larvae at this stage is expected to match with the composition of yolk that caters the needs of the pre-feeding fish. As the larva initiates exogenous feeding, the spurt in activity demands a great deal of energy and hence the larval nutrition is of

vital significance. An artificial feed catering to the nutritional requirements at this stage of the larvae is yet to be formulated and research over the past few decades revealed that live feeds can be successfully employed for the rearing of the larvae during the critical stage from endogenous to exogenous feeding. The live feeds that are used on a world wide scale are different species of microalgae, the rotifers *Brachionus plicatilis* and *B.rotundiformis* the brine shrimp *Artemia* and copepods. Considerable research input is needed to evolve suitable feeds and feeding schedules for the successful larval rearing of many species.

Pomacentrid larvae have very limited yolk reserves and they start feeding within few hours to few days after hatching. Anemonefishes are capable of capturing rotifers in the first week itself and feeding strikes started from day 2. Mouth gape of newly hatched anemonefish larvae is about 250 microns and they readily accept the rotifer *Brachionus rotundiformis*. The concentration of rotifers should be maintained at 4-5 numbers per ml because the larvae have very limited power of swimming and searching the prey. After about 10 days they attain the capability to chase the prey and can be fed with cultured *Moina micrura*. Eventhough *Moina* will not survive for long time in seawater, the late stage larvae are capable of locating and feeding them from the water column.

Most other damselfish larvae are too small to accept rotifers as the first feed since they have a mouth gape of less than 200 microns. However smaller strains such as Fiji strain rotifers and nauplii of suitable species of copepods are useful as the first feed for damselfish larvae. Two weeks old larvae can be fed with freshly hatched *Artemia* nauplii. *Dascyllus albisella* and *D.aruanus* were reared by feeding rotifers initially, gradually changing to larger wild caught zooplakton and finally *Artemia* nauplii till metamorphosis. *Abudefduf sexatilis* larvae were reared by using oyster trochophore, rotifers and *Artemia* nauplii. But larvae of many marine fishes including some species of damselfishes do not accept rotifers at any stage. In such cases maintaining a mixed culture of copepods with green water in the larval rearing tank is a positive alternative

Maintaining high water quality is another critical factor while rearing larval reef fish. Poor water quality management results in extremely high mortality. Three main steps should be taken to maintain high water quality. The addition of excess food can rapidly reduce the water quality. Reef fish larvae can survive at extremely low food densities. A bacterial build up on the sides and bottom of the tanks present as a slimy layer , can also affect water quality by producing compounds which may be toxic to the larvae. The sides and bottom of the tank should be wiped down on a regular basis to reduce bacterial build up. Regular water change must be carried out.

9. Packing and transportation

Live fish trade is emerging as a major business venture in most of the tropical countries. Broodstock and seed are widely transported by road, sea, rail or air to various destinations. Air lifting of live seafood, especially live lobsters, shrimp and mud crab for export market also increased during the last few years. Fin fishes like groupers are transported live, since they fetch premium price in the market. With changing life style of people, the demand from many of the affluent consumer markets gradually started shifting towards live fish and shellfish and this is showing an increasing trend. Marketing of live fish is regarded as value-addition procedure because live fish obtain substantially higher prices than fresh-chilled or frozen products

Concurrent with the increase in live seafood, the marine ornamental fish trade has been increasing at a frantic pace. Success of ornamental aquarium fish trade depends on effective packaging techniques based on sound scientific principles and careful handling practices to improve the survival before and after shipment which is critical to the industry. A thorough knowledge of the behaviour and physiology of the animal is necessary to minimize stress and to design most suitable, cost-effective, low stress method of transport to achieve maximum survival.

The freight cost of fish consignments is still a major cost in the ornamental fish trade. For consignments from Asia to the USA, shipping may cost more than the fish in the consignment. Since the air freight charges are very high, the exporters have no idea of the optimum number of a particular live fish to be packed in a container to reduce the transportation cost. Such information is also lacking, especially on Indian fishes, which are of great demand in the international market.

At present, the mortality rate during fish catching, collection and transportation is very high. The claim by the importers due to DOA (Death On Arrival) from India is on the higher side compared to the consignments from other developing countries. This is due to the lack of the use of appropriate technology for fish packing and transportation/ shipment. The research support and the technology provided has not improved in some of the major exporting and developed countries.

The basis of transporting live fish is to provide conditions, as similar as possible to their natural environment in order to keep them with minimum stress. A detailed understanding of the physiological behavior of the animal in stressful and low stress environment will provide an insight into the manipulations required to improve the survival and keep them in best conditions during live transport. Otherwise, transport of live fish is a stressful and traumatic procedure consisting of a succession of adverse stimuli including initial capture, loading, holding, packing, transporting, unloading

and restocking. Deterioration of water quality during transit may impose additional stress.

Several factors can become lethal agents during transportation. Deterioration of water quality during transport such as declining oxygen content, carbon dioxide build up, detrimental changes in pH, and accumulation of metabolic wastes resulting in increase in ammonia content are the major problems during transport. The loss of protective layer of mucus on fish can also be a problem. These can act individually or more frequently in combination to cause mortality.

A variety of methods are employed to manage the quality of water during transport. They include starving fish before packaging, lowering the temperature of transport water, addition of anesthetics, ion exchange resins, buffers or drugs in the transport water to minimize the metabolic activity of aquatic animals and build up of toxic chemicals.

Temperature reduction

Water temperature is an important factor as it determines the dissolved oxygen concentration. The lower the temperature, the higher is the oxygen level. Water temperature also decides the stocking density. Water can hold more oxygen in solution at low temperatures; however, fish requires more oxygen at higher temperatures. Therefore, a tank of a given volume can hold more fish at lower temperatures than it can hold at high temperatures. That is the reason why the temperature of water in transportation is always kept low according to the levels that the fish concerned can tolerate .

Lowering of metabolic rate

Discharge of metabolic waste may be controlled by lowering the metabolic rate of fish and also by using suitable substances to remove them. Reduction in metabolic rate can be achieved by lowering the temperature, addition of anaesthetic to water and through conditioning of the fish.

Use of Anaesthetics

With the use of anaesthetic, it is possible to increase loading density of fish. Also the tranquilising effects of anaesthetics reduce injury to large or excitable fishes when they are transported. Deep sedation which suppresses the reactivity of fish to external stimuli without upsetting equilibrium and which reduces oxygen consumption to basal rate seems best suited for transporting fish. The criteria for an ideal anaesthetic include rapid immobility, quick recovery, no toxicity to fish, low tissue residue and low cost.

Most of the anaesthetics that are used like quinaldine and MS-222 are expensive and can present a health hazard to the user. One promising anaesthetic is clove oil, a distillate of herbatious portion of the clove tree *Eugenia aromatica.* Clove oil has been used for a number of years to anaesthetize fish in seawater which is essential in some basic procedures in fish farming such as weighing, tagging experimental work and for transport. It considerably reduces the pathology risks from stress, injury and accident during handling. Additionally fish does not require a withdrawal period after exposure to the chemical. The method used consisted of introducing the active ingredient of clove oil into the fish's gills through water, i.e. 'anaesthesia by immersion'. The substance is absorbed through the gills and travels through the blood stream to central nervous system. The fish then goes through several anaesthesia stages ranging from balance loss to total motionlessness. Appropriate dose of clove oil can be added to the water holding container. The water was then agitated by vigorously stirring, after which the fishes were placed in the container. Use of ethanol at 1:5 ratio facilitate easy dissolution. Successful induction to anaesthesia was determined as the stage where total loss of equilibrium first became evident (i.e. the fish could no longer swim or maintain a vertical position in the water). Concentrations of clove oil was considered suitable if induction to anaesthesia was<3min.

Conditioning

Conditioning for a period of time before packing reduces stress to fish and the metabolic rate and fouling during transit. A few days before transport, the fish are kept in clear running water in separate tanks. This helps in removing of flavour in the fish. Weak or diseased fish are removed. Feeding is stopped at least 24 hours before transport; this empties the gut of the fish. The temperature of the holding tank is lowered gradually.

Conditioning lowers stress, the metabolic rate and oxygen consumption. This keeps the mortality rate low, makes it possible to ship consignments over longer distances and to increase the packing density. The packing density of live fish can be greatly increased by proper conditioning as the oxygen demand will be lowered .

Removal of ammonia can be accomplished by biological means during transportation of marine fishes by introducing nitrifying bacteria cultured on solid substrate in to the seawater.

Transport of reef fishes with 'live rocks' (2-3 pieces placed in containers) had significantly prevented possible damages during transit. The live rock probably had provided shelter besides acting as water conditioner and maintaining water quality.

Use of Buffers

Rapid changes in pH stress the fish; hence buffers can be used to stabilize the pH of water during fish transportation. The organic buffer tris- hydroxyl- ethyl- amino-methane is quite effective in freshwater and seawater . Actual amount of buffer which will be consumed during any transport operation is dependent up on the pH, the natural buffering of the transport water, the temperature and the duration of transport.

Packing

Most fish are packed in double polyethylene bags filled with one-third water and two thirds oxygen, sealed and placed in a cardboard box (often reinforced with polystyrene foam for added insulation). Aggressive species are placed in opaque bags. To avoid putting the health of fish at risk, a recommended maximum travel time of 40 hours has been recommended for shipments (with 24 hours being considered as reasonable).

Acclimation on arrival

Bags ready for transport

Packed boxes for transport

The main reason for mortalities after arrival is hasty transfer from transport water to the new water. By the time of arrival, the fish would have become acclimatized to the conditions in the transport bag, *viz*. high concentration of carbon dioxide and ammonia and low pH (5-6). These high concentrations may be reduced by a simple method. First, the bags are opened and left in box or baskets. Then new water is poured from the tanks into the bags, until the water volume is 3 – 4 times the initial volume. This process should last for at least half an hour. The transport water must not be aerated as this would drive out carbon dioxide, increase the pH and turn harmless

ionized ammonia into poisonous unionized ammonia. The water in the bag is gradually replaced with new water. The fish are then transferred to the tanks. The tanks should be kept dark and covered carefully, to avoid stressing the fish and preventing them from jumping out of the tank. The fish should not be fed immediately on the day after arrival .

10. Maintaining marine ornamental fishes in aquarium

Marine aquarium keeping has been rapidly expanding in recent years, mainly due to its added attractiveness when compared to freshwater aquarium. Successful marine aquarium keeping is now possible mainly due to the recent scientific knowledge on various aspects of biological filtration and also to the advent of an array of aquarium gadgets. Now it is well known that the maintenance of marine aquarium require a very different type of management and equipments than those of freshwater aquarium. The major modern developments which enable us to maintain marine aquarium effectively are adequate aeration techniques, formulation of synthetic sea water salts, the use of all-glass tanks pasted with silicon rubber cement, adequate filtration methods especially biological filtration, the methods of efficient and rapid transport of exotic specimens, availability of suitable feeds and the successful treatment of some of the more common diseases of marine fishes.

The Aquarium

Sea water aquaria should be fairly large, as the marine fishes are used to a larger habitat and they cannot be crowded with fishes. A large tank is also more stable in the constitution and temperature of water than a smaller one. A tank capacity of about 200 litres is the minimum size (90cm length x 40cm width x 50cm height), which can be used as a home marine aquarium. A shallow tank is more advisable because the water surface is the place where oxygen enters and the carbon-di-oxide leaves. The shape of the marine tank can also affect the filtering capacity of the tank. The more the surface area in the filter, which covers the bottom of the tank, the greater the number of fish that the tank can safely support. Therefore a low flat tank with an undergravel filter has a slightly greater filtering capacity than a high sided tank of the same volume. The tank can be of any shape provided that the filter is large enough to carry the biological load of the tank.

An all-glass tank is widely employed for marine aquarium in recent years. They are non toxic, relatively inexpensive and can now be made to any desirable size or shape. A tank of the size 90cm x 40cm x 50cm can be fabricated with 6mm glass and larger tanks of 500 litres and 1000 litres can be fabricated with glass plates of thickness 8mm and 12mm respectively. The glass plates can be glued together with silicon rubber

cement. It is advised to use only a product that specifies itself as an aquarium sealer on the label. The edges of glass plates are ground with grinding stone, to remove the sharp edges of cut glass plates. Building the aquarium should be done all at one time so as to get a continuous seal from sealant. 5 – 10 minutes can be taken to put the glass plates together and thereafter leave it undisturbed until the silicon cures. Larger tanks should have belts around the top and one or two cross bars connecting the belt. The tank must be evenly supported on a resilient sheet of thermocol, to take up any slight irregularities. Make sure that this support is at dead level, as any slight departure will result in an ugly looking slant to the water line.

Lighting

The aquarium should not be installed in a place where there is much day light, in particular direct sunlight, because it may overheat the water of the tank. Lighting is usually provided by a fluorescent tube or tubes with a reflecting hood. If only fishes are kept, a single tube of any type may be used. If you want to grow sea-anemones, sea-weeds and live corals, more light is needed and it must be provided in sufficient intensity by special lamps which can emit lights of red and blue wave lengths of visible spectrum. The light should be on for at least 12 hours per day and should not be switched on and off suddenly in a darkened room. A dimmer switch can be used to avoid this and to give the fishes a chance to wake up or settle down.

Heating

Temperature fluctuations may be detrimental to many species of fishes and hence it is important to have a heater / thermostat combination in the tank. It is preferable to have the heater / thermostat that is totally submersible and guaranteed suitable for salt water. A heater lying in the bottom of the tank is more efficient and less obtrusive than an upright one. But it should not be covered by gravel. Heaters of appropriate power should be selected according to the tank capacity. For example, for a 100 litre tank, we can use a 100 watt heater, for a 200 litre tank we can employ a 150 watt heater and for a 300 litre tank, a 200 watt heater can be used. It is better to use an alcohol thermometer instead of a mercury thermometer, which may poison the water if it breaks. A tropical marine tank generally needs a temperature range of 25 – 28^0C.

Aeration

Aeration is a must in a marine tank and its purpose is to keep the water moving and exchanging gases with the air. This occurs in the surface, not between the bubbles and the water unless these are very dense. Aeration is often combined with filtration but it is better to provide air stones to add the effects of filter / filters. Air stones come in all shapes and sizes, but what is most important is that they should give medium sized

bubbles between ½ and 1 mm in diameter and these should move the water most efficiently. Very fine bubbles are good but form a mist in open water. A good brand of diaphragm pump with a volume control can be used for both air stones and filters.

Water Quality

Sea water is an extremely complex and dynamic fluid which is constituted by a number of inorganic and organic components. The components of natural sea water can be put into four broad classifications. The first of these is **pure water** which represents about 96% of sea water. The second component of sea water can be broadly termed as **inorganic solids and gases**. All the dissolved salts, trace elements, inorganic pollutants and dissolved gases belong to this category. Only seven salts *viz.* sodium chloride, magnesium chloride, magnesium sulphate, calcium sulphate, potassium sulphate, calcium carbonate and potassium or sodium bromide make up over 99.5% of all the conservative salts in sea water. The conservative salts are those that do not change in proportion to each other regardless of the total amount of dissolved matter. The remaining 0.5% of the inorganic solids is made up of at least 60 elements found in such tiny amounts that they are called trace elements, and a variable amount of pollutants such as mercury, pesticides and petroleum. Eventhough the trace elements are present in extremely small amounts, some of them especially zinc, copper, iodine, strontium, vanadium, cobalt, molybdenum and arsenic are essential to many living organisms. The third basic component of sea water is **dissolved organic substances**. These are compounds such as amino acids, proteins, enzymes, vitamins and pigments. Inshore water carries a greater load of dissolved organics than clear offshore waters. Natural toxins are sometimes found in sea water, especially during blooms of micro algae. **Life** is the fourth component of sea water. However clear the sea water may be, numerous varieties of living things will be present in the same. Bacteria and microscopic plants and animals occur in each drop of inshore water.

Water from a marine aquarium has the same four basic categories of constituents – water, dissolved inorganics, dissolved organics and life. Water and basic salts are the same in natural sea water and aquarium sea water, but the other constituents differ greatly in both the waters. Dissolved organics are not as diffused or reused in a marine aquarium as they are in the sea, thus they accumulate from the wastes of animals to a great degree. These wastes are converted to basic nutrients by proper biological filtration, but the concentrations of these can be much greater in aquarium water than in raw sea water. Bacteria are the primary life forms in aquarium water. The number of bacteria in one cubic centimeter of sea water varies from less than 10 in offshore waters to several 100s in clear inshore waters. In contrast marine aquarium water may contain several hundred thousand or more bacteria in each cubic centimeter.

Collection of natural sea water

The dissolved organics and planktonic life forms present in seawater cause problems in the marine aquarium. When the seawater is used in the aquarium, most of the planktonic plants and animals die and bacteria proliferate. Eventually all the remains of planktonic creatures are decayed by bacteria, which also utilize some of the dissolved organics.

Most bacteria need some sort of a substrate to form a colony and grow and the sides of the aquarium tanks, detritus and dead plankton provide much more surface area for their multiplication. These factors result in a tremendous proliferation of bacteria in aquarium seawater, some times reaching to levels of several millions per cubic centimeter. Hence it is advisable to keep the newly collected seawater in the dark for two weeks or more before use. After that period most of the organic matter are utilized, oxidized and precipitated, and all dead plankton consumed by bacteria. A brownish flocculent material accumulates at the bottom of the container and only the clear sea water can be removed without stirring of the sediments from the bottom.

The best seawater for aquarium is the clear offshore water that requires a boat for collection. But seawater collected from inshore water also can be employed provided that the water is carefully collected to avoid contamination. Take the water from an area that have good tidal flushing and no nearby sources of pollution. Avoid sewage outlets, industrial plants and freshwater inlets. Don't collect water from areas that show an oil film on the surface. The natural sea water may contain more plankton and particulate materials which are not suitable. Hence it is advisable to filter the water through a fine mesh at the time of collection. The best way to treat the water after collection is to store it in the dark for about 2-3 weeks prior to use in the aquarium. Another treatment of collected seawater is chlorination and de- chlorination. Chlorine kills all lives in the collected water including bacteria and oxidizes the organic matter dissolved in natural seawater, including toxins. For chlorination and de-chlorination we require bleaching powder, sodium thiosulphate and a test kit for chlorine. Add a small quantity of bleaching powder to newly collected seawater until there is at least 5 parts per million chlorine as measured by the test kit. Keep the water with light aeration for 12 – 24 hours and test once again for chlorine. If no chlorine is indicated, this means that the water has a high organic load and it should be treated once again with chlorine. After chlorination is completed, add sodium thiosulphate in small quantities until your test kit indicates that no chlorine remains. Now the water is sterile and may have a slightly cloudy appearance, which will clear by itself if the water is left to settle for a day or two, or it can be filtered through activated charcoal for a few hours to clear faster.

Artificial Sea water

There are a number of sea salts readily available in the market. Synthetic sea water may not be the same as natural seawater, the major brands of sea salts available today will support the life of marine aquarium almost as well as natural seawater, even better in some circumstances. Synthetic seawater differs from natural sea water because concentrations of the major inorganic salts are not exactly the same, inorganic trace elements are not the same in number or concentration and there are no dissolved organics. If any impurities are present in the 'make up water' it will affect the quality of artificial seawater. Follow the manufacture's instructions when you mix the salts and add the trace elements. If you are just setting up a new tank, you can mix the water to be used right into the tank, but if you are changing water in an established tank, it is best to mix the salt in a plastic container. Never use a metal container to mix the salt water. Wait until the solution clears and all the elements are dissolved before adding the newly mixed water to your tank. Some of the elements may not dissolve even after 24 hours of aeration and will form a white precipitate in the bottom of the container. We can ignore about this residue unless it is remarkably excessive. It is also good to let the newly mixed salt water to age for a day or so, to let the pH stabilize before adding into the tank.

Filtration

Efficient filtration is mandatory in a marine aquarium. There are two basic types of contaminates in aquarium water – suspended, physical particles and dissolved chemical compounds. The dissolved contaminants are mainly produced by the inhabitants. They are created from the metabolic waste materials of fish, invertebrates and plants, and also develop from the activity of bacteria on waste organic matter produced in the tank. These dissolved chemical compounds include ammonia, nitrite, nitrate, urea, proteins, fatty acids, phenols, dyes and many other less abundant compounds. Filtration can be classified into three types.

(i) Mechanical filtration

Mechanical filtration removes suspended particulate matter from the aquarium water and keep it clear. The efficiency of the filter depends on how fast water moves through the straining surfaces, the surface area of the filter and the size of the trap for the particles. A mechanical filter can use sand, gravel, sponge or glass-wool to strain particles from the water. A good mechanical filter that removes very small particles with a rapid water flow maintains high water clarity, removes free-swimming parasites and accumulated dirt and detritus from the aquarium. Power filters with motor can even remove algae and bacteria. If the filtering material is not cleaned or exchanged frequently

the filter clogs and the efficiency of mechanical filtration is lost. Hence the filter must be cleaned every few days which should not exceed a week.

(ii) Chemical filtration

Chemical filtration removes dissolved compounds, toxins and colour and regulate pH. This is a chemical action and is exerted by substances like activated carbon. High grade activated carbon removes over 50% of its own weight of toxins, gases, colouring matter and many other organic compounds from the water. Finely divided activated carbon comes in a pin-head size and consists of dull looking granules that have incredibly large surface area. The adsorptive properties of activated carbon change as the carbon is used. New carbon has a greater ability to adsorb gases than old carbon and in general picks up more molecules at a faster rate. Activated carbon does not effectively remove ammonia, nitrite and nitrate and cannot be a substitute for biological filter. The greatest danger in using activated carbon is that it is so efficient in clearing and cleaning the water that it hides the need for occasional partial water changes. Use it sparingly on invertebrate tanks because invertebrates seem to be more dependent on trace elements in water than fish. Never use carbon filtration on medical treatment tanks. A carbon filter generally uses a sandwich of activated carbon between two filter mats. The mats filter mechanically but they are mainly used to retain the granular carbon. The top mat may need replacing every week as it clogs up. 100 – 200 grams of activated carbon is needed for a medium sized aquarium and should be renewed every three months.

The carbon filter or box filter or canister filter may be located either inside or outside the aquarium. A central stem carries a rising stream of bubbles that causes water to flow through a perforated lid, down through the filter bed and up through the central stem back into the tank. If it is placed inside the tank it can be placed in one corner at the bottom rear of the aquarium. Hidden by rock or coral, this filter is quite unobtrusive. The carbon filter can be fitted outside of the tank also. The main advantage is that the filter can be turned off, removed and cleaned without disturbing the tank.

(iii) Biological Filtration (Undergravel filtration)

The nitrogen cycle in the aquarium is concerned with the breakdown of nitrogen containing substances like proteins and their end products, the principal of which is ammonia. Ammonia is very poisonous and also raises the pH making the water more alkaline. In seawater, a given amount of ammonia is over ten times as toxic as the neutral water, as sea water has a pH of approximately 8.3. Ammonia is then toxic in less than 1ppm (1ml/litre). The biological (under gravel) filter uses the gravel at the bottom of the aquarium as the filter bed and allows the growth of vast number of

beneficial bacteria that primarily convert the end product of the decay of excreta and uneaten food or dead animals, to first nitrites, less toxic but still dangerous and then to nitrates which are harmless to fishes and many invertebrates unless present in large amounts (over 40ppm). This concentration is taken care of by periodic water changes and sometimes by the growth of algae that use it as food.

Biological filtration is the transformation of toxic waste substances, primarily ammonia into relatively non- toxic substances through the activity of living organisms, primarily nitrifying bacteria. These bacteria in the genera *Nitrosomonas* oxidise ammonia to nitrite and *Nitrobacter* oxidize nitrite to nitrate. All we need to provide is a surface for bacteria to colonize and a source of ammonia. Ammonia occurs in two states depending on pH, the un-ionised state (NH_3) and the ionized state (NH_4^+). The unionized state is more toxic than the ionized state because it can invade body tissues more readily. But almost all free ammonia is in the ionized state at the normal pH of sea water. As pH increases, the non-toxic form of ammonia rapidly decreases and the toxic form rapidly increases. Thus a lethal level of toxic ammonia may be present at a p^H of 8.4, while the same total amount of ammonia may be tolerable at 7.8. Fish that are susceptible to ammonia poisoning may suddenly suffer symptoms if the pH increases rapidly when significant levels of ammonia are present. The levels of ammonia and nitrite are always very near to zero in the aged and balanced marine aquarium.

The *Nitrosomonas* bacteria are the first to populate the filter and rapidly begin oxidizing ammonia to nitrite. *Nitrobacter* is inhibited by the presence of ammonia and doesn't begin rapid population growth until the ammonia levels begin to fall. These bacteria cannot begin their growth unless ammonia and nitrite are present in the tank. After the populations of *Nitrosomonas* and *Nitrobacter* are well established, oxidation of ammonia and nitrite occurs almost, as these compounds are formed, thus they never accumulate in the system and only the end product nitrate can build to high levels. Accumulated nitrate can be removed by dilution through partial water changes or since it is a basic plant nutrient, algal growth can utilize a lot of nitrate that is produced.

A biological filter is a living thing. It consumes oxygen, feeds on the wastes of the animals in the system and excretes wastes of its own. The nitrifying bacteria in the filter are dependent on the oxygen contained in the water flowing through the filter. If this flow of oxygenated water stops, the good bacteria die, the water fouls, and the entire tank eventually dies. Because of this great demand for oxygen, there must be a rapid flow through the filter at all times. The amount of water flowing through a filter designed for a fresh water tank is not adequate for a marine tank, and the proper populations of nitrifying bacteria will not be established. The biological filter in the marine aquarium should get maximum flow rate that your equipment can deliver. The

object behind establishing a biological filter is to bring the nitrifying capacity of the filter into an equilibrium with the waste production of the tank's inhabitants. The more efficient the filter, the more fish the tank can support. Each individual undergravel filter will have a maximum potential carrying capacity, which depends on many things. First of all, the extend of the surface area of the filter is more important than the depth of the filter bed. This is because the bacteria need oxygen to function, and as the water flows through the filter bed, oxygen is depleted and nitrification decreases, thus the top half inch of the filter bed does almost the entire work. Other factors of importance are the size and shape of the filter gravel, the rate of water flow, whether the filter is new and clean or old and dirty and how the filter was established. The gravel size should be small enough to provide a large amount of surface area for a high bacteria population and to provide some mechanical filtration, yet large enough to allow a good water flow with some freedom from particulate clogging. Irregular gravel of about 1-4mm in diameter is a good size for a marine undergravel filter. It allows a lot of water to pass through and keeps the filter bed well oxygenated. Large air lift tubes of about ¾ inch internal diameter and a strong air flow broken up into small bubbles are essential to provide the necessary water flow through the filter bed.

There are two main types of under gravel filters. One consists of a network of perforated tubes buried in the gravel that suck the water through them by means of one or two air lifts and return it to the surface(tube filter). The other often preferable in the marine aquarium is a perforated plate or plates covering the entire base of the aquarium and raised about 1cm above the glass bottom(plate filter). Air lifts also perform the same function as in the first type. Power heads may be used to run under gravel filters. Instead of the simple air lifts to draw water through the gravel, the power head draws water from under the filter at a much greater speed, thus providing a great deal more filtration and oxygenation. Coral sand or any other suitable alkaline material is placed to a depth of about 2 inch on average above the plate. If it is coarse enough (2-4mm diameter), it will not fall through the plate, which should have slots of about 1mm. Besides providing a bed for bacterial growth the coral sand helps to maintain the mildly alkaline p^H of sea water. The filter works in aerobic conditions so that as the water passes through it, it will lose oxygen and gain carbon-di-oxide. The water should pass through the filter at about 3 times the tank volume per hour to give the best results.

The biological filter is a living community, consuming oxygen and containing vast numbers of organisms that can die and foul the water instead of purifying it. They will die if under-oxygenated, poisoned or subjected to prolonged antibiotic or disinfectant treatment. Lack of oxygen in an undergravel filter will be followed by serious

consequences within a few hours. A biological filter should remain undisturbed as long as possible. Removing, washing and replacing the same gravel from the tank, clears off many beneficial bacteria and the function of the filter is seriously impaired. As the biological filter gets aged, the top layers of the filter get filled by debris and it may slow the passage of water excessively. When this occurs, siphon off much of the loose debris and some of the top gravel, but leave most of the gravel untouched. Wash gently and replace the gravel removed. By such methods a tank may remain without complete overhaul for many years. If a complete overhaul becomes imperative replace the old gravel without more washing than is necessary, so as to start off with as good a bacterial population as is possible.

New tank syndrome

A new tank which has been carefully set up and fishes are introduced without maturation of the tank will become unhealthy after a few days and fish mortality will result. Old tanks will have a nicely balanced bacterial population with the nitrogen cycle proceeding satisfactorily and hence no build up of ammonia or nitrites. As ammonia is produced, it is converted rapidly into nitrites which in turn are converted into nitrates which are comparatively nontoxic. In a new tank there will not be an inadequate population of any type of bacteria and the first thing is the growth of bacteria which decompose organic matters such as fish feases, uneaten food or any kind of decaying matter. The end product of such decomposition is ammonium hydroxide.

$$NH_4OH \underset{\rightarrow}{\leftarrow} NH_4^+ + OH^- \underset{\rightarrow}{\leftarrow} NH_3 + H_2O$$

The ammonia is the main toxin that is not tolerated by fishes even in fractions of a ppm. The higher the pH the more ammonia can be formed. Sea water with a higher pH, is more able to accumulate ammonia gas than freshwater. When sufficient ammonium hydroxide accumulates to form a substrate for the growth of the bacteria of the group *Nitrosomonas*, these will multiply and convert much of it to nitrous acid and nitrites. So the amounts of NH_4OH, NH_4^+ and NH_3 will fall. But nitrites are also highly toxic. When they in turn accumulate sufficiently, bacteria of the group *Nitrobacter* multiply and convert them to nitric acid and nitrates. These are not toxic until relatively very high levels are reached (40-60 ppm). So in the new tank we get a wave of ammonia production lasting for several days according to conditions followed by a way of nitrite production lasting for several days followed by subsidence of nitrites and lasting high level of nitrates, which is comparatively harmless. Hence in a tank with newly set biological filter, the filter acts only as a mechanical filter and leads to fish mortality due to ammonia toxicity. To avoid this, the tank has to be matured.

Maturation of the tank

Several days/weeks are required to mature the tank in order to get the nitrogen cycle in the tank going safely.

(i) Old method: Put in a few tough fishes like clownfishes or *Dascyllus* and let them start things up, gradually introducing others over the next few weeks. Even just some rotting fish or meat or any thing which produces ammonia could be used instead.

(ii) New method (Chemical Maturation): Here, ammonia producing chemicals are employed for maturation of the tank. The main advantage of this method is that, by using as high an amount of ammonia as feasible from the start, more of the bacteria in the gravel will be of the desired type, which increases the efficiency of the filter. Make up a 10% solution of ammonium chloride or a 15% solution of ammonium sulphate. 250 ml of either solution is required per 100 litres of sea water to complete the treatment. Starting the treatment add 2ml per 100 litres on days 1 and 2; 4ml on days 3 and 4; 6ml on days 5 and 6; 8ml on days 7 and 8; from day 9 onwards add 10 ml per day and continue at that level. Using a nitrite test kit start measuring the nitrite level from day 18 onwards. The peak of nitrite will rise from almost 0 to 10 or 20 ppm and then fall again. When the level of nitrite has fallen nearly to nil (less than 1 ppm), stop ammonia treatment and put in the fishes with in a day or two.

Aquarium Accessories

There are a number of equipments available now, especially for marine aquarium. Most are designed to remove more efficiently the waste products produced or to sterilize the water as far as possible.

1. Powered Canister Filters: They force the water through the fine filter blocks that remove even bacteria and really clean the water. Activated carbon provides large surface areas for adsorption of unwanted materials. Special activated carbon is now being especially packed for the marine aquarium. Power filters are sometimes used one behind the other with generally two or three containing different filter media. This helps to detach each unit separately for cleaning while the others are kept running. Many aquarists use combinations of filter methods. For example an under gravel filter can be combined with a canister filter.

2. Protein Skimmer: In a protein skimmer fine bubbles of air are passed through a column of water. Many organic molecules stick to the bubbles since they are attracted to surfaces. The pre-filter skims off those that collect on the water surface. In a skimmer the resulting foam is collected in a cup at the top of the column of water. It can then be discarded with all the particles adhering to it such as proteins, amino acids, phenolic

compounds and pigments. The importance of protein skimming is that it removes matter that would break down into nitrogen cycle compounds if left. It therefore greatly lowers the load on biological filtration.

Protein skimmer

1. **Denitrator:** Denigrators remove nitrates. A slow flow of water from the aquarium passes through these filters where anaerobic bacteria converts nitrates back to nitrogen. The nitrogen then passes into the air.
2. **Ozonizer:** Ozone is a super active form of oxygen produced by special ozonizing equipments. It kills bacteria and parasites and oxidizes toxins. It should be used with a protein skimmer followed by a carbon filter. This prevents a damaging amount of ozone from entering the tank. As it oxidizes vitamins as well as pollutants, ozone should be used with caution. It raises the redox potential also, sometimes to dangerous levels.
3. **UV Sterilizers:** Ultra Violet light is another sterilizer which is applied out side the aquarium as it is harmful to life if it is exposed directly to water. This is a safer method than using ozone. UV is used in sterilizing water in quarantine setups and as a cure for diseases.
4. **Other auxiliary equipments:** Test kits for measuring salinity, pH, ammonia, nitrite and nitrate concentration are needed. Hand nets, siphons for clearing tanks, dip tubes for removing uneaten food and long plastic tongs are also required.

Setting up of a tank

1. Thoroughly wash everything in cold fresh water. Never use a house-hold disinfectant except hydrogen peroxide or a chlorine bleach only if necessary, followed by several rinses in fresh water. Wash the coral sand very thoroughly; it may need a dozen or more washings until the water runs off clear. Failure to do this can result in cloudy water that is difficult to get cleaned.

2. Fit the under gravel filter close against the back and sides of the aquarium, but leave a small gap at the front so that it will not show. Connect up air lifts.

3. Cover the filter with wet gravel, slopping it gently from back and sides to middle front so as to form a shallow half basin not more than 2-3 inches deep anywhere. This helps an even flow of water, looks neat and encourages collection of wastes at the front. Seed the gravel with desirable bacteria that will convert ammonia to nitrates. This can be done with a few pinches of garden soil, a commercial bacterial preparation or a handful of gravel from an established disease-free tank.

4. Place all equipments, coral rocks, etc. in position. Make sure that they are thoroughly cured, coral in particular. The coral should have been soaked in a bucket of freshwater to test whether it is cured. You will see or smell if it is not clean.

5. If artificial salt is used for making seawater, calculate the actual volume of the tank, subtract 20% as a rough estimate as the volume occupied by the gravel, etc. and then dump 28 gram/litre of high grade salt mix. There is no need to dissolve salt at this stage as the water can be added to the aquarium.

6. Place a bowl in the centre front and lead the salt water hose into it, so that water runs slowly over the top of the bowl with least possible disturbance to the gravel.

7. Turn all equipments on and check that all is well.

Fish capacity and Crowding

The numbers of fishes of different sizes that can be safely housed in a marine aquarium depend mainly on the species and on feeding rates. The feeding rate has a big effect – an unfed fish produces less than half the pollutants than a fish receiving 2.5% of its own body weight per day produces, and less than a third of those produced if 5% is fed per day. It is advisable to start a conditioned tank with a load of not more than 1 inch to 2 inch fish per 20 litres of water. As the tank matures, 2 inch to 4 inch fish in 12 liters of water is safe. At the start don't put more than 5 fishes in a 100 litre tank and better to avoid more delicate species. Don't over feed but follow the rule of giving a bit less than they would eat straight away, twice a day utmost. After a few weeks, if

fishes are really growing fast, we can cut the food down slightly. Fishes are very accommodative and do not suffer if kept on a low diet as long as it is nourishing.

If the tank is overcrowded, it means that the capacity of the filter bed and the tank water to maintain the existing animal load in good health is nearly exhausted. If the animal load exceeds the capacity of the system to process their wastes, then the tank balance is destroyed, bacteria populations change drastically, oxygen decreases, toxins appear and fish die. This can happen suddenly due to the introduction of a number of new fish into an already overcrowded tank or as a slow break down when a well stocked tank is overfed and not maintained. Hence the total animal load occupying the tank should be well within the maximum carrying capacity of the tank. There are so many variables determining the optimum carrying capacity and these changes with the age, maintenance schedule and fish and algal growth. The following are the major factors that can limit or reduce the carrying capacity of the aquarium.

1. Filter bed too thin or too thick.
2. Filter bed gravel too small or too large.
3. Filter bed heavily clogged with detritus.
4. No algal growth.
5. Consistent over feeding.
6. Infrequent or no water change.
7. Poor quality lighting.
8. Poor water flow due to small air lift tube, constricted air line or weak air or water pump.
9. Filter bed bacterial action inhibited because of medication.

The following are some indicators of over crowding :

1. Reduction of green algal growth and more rapid growth of red and blue green algae.
2. Development of yellow water coloration before the next scheduled water change.
3. Persistent, rapid drop in pH below 7.8.
4. Rapid accumulation of nitrates and persistent traces of ammonia or nitrite.
5. Distress behaviour in fish, fading colors, loss of appetite, hyper activity, rapid respiration.

Maintenance

An aquarium needs regular attention. Depending on exactly how it is set, the attention required will vary but the following can be a reasonable schedule.

Daily

Check the temperature and general appearance. Look for each fish and large invertebrates to see that all is well. Feed sparingly morning and evening and see that each fish eats. Never feed more than that is consumed almost immediately and remove any uneaten food.

Weekly

Clean the front glass. Check the pH for the first few weeks and correct if necessary. Whatever the pH add 5 ml of Sodium bi carbonate per 100 litres of water to keep the buffer capacity of the sea water. Add more than 5 ml if the pH needs to be increased.

Monthly

Siphon off 20-25% of water and replace it with new water that has been aerated and is at correct temperature. When siphoning lift rocks and coral where feasible and disturb the top of the gravel to remove debris. Check the pH, nitrate level and specific gravity after replacing the water. It may be necessary to add a little freshwater to make up for evaporation loss. See, if the top pad of the carbon filter needs replacing.

Quarterly

Renew the carbon filter completely, wash the new carbon before use. If the rocks and coral are getting covered by too much algae, remove some of it but not all.

Yearly

Siphon off about 1/3rd of the gravel. If a lot of sediment swirls up when you disturb it, wash it and replace it. Never remove most of the gravel at one time or you will lose many of the beneficial bacteria. After such a clean up, feed very lightly for the next week, as you have weakened the capacity of the undergravel filter to deal with ammonia. Check overall equipments carefully and renew any damaged diaphragms, valves, tubes, air stones, etc. Air stones may need more frequent cleaning or replacements.

Toxic tank syndrome

Even in well-managed aquaria, sometimes, a sudden unexplainable loss of almost all fish occurs with in 12-24 hours and this is known as toxic tank syndrome. The syndrome most often occurs in tanks heavily populated with young fish of a single species, although it can occur in any type of marine closed systems. Water is often very clear and uncolored, with no trace of ammonia or nitrites and it usually has an acceptable pH when the syndrome occurs. This may be due to a virulent toxin released on the biological filter and is often species specific. It is probable that a substance released from the fish stimulates the filter bacteria to produce a toxic substance or a new type of

toxin producing bacteria is stimulated into a population bloom. Fish showing early symptoms of toxic tank syndrome that are removed and placed in a totally different system almost invariably recover and those left in the affected tank will die. It is probable that the bacteria in the genus *Vibrio* possibly *V. anguillarum* may be the causative organism of this syndrome. It proliferates rapidly, displaces other species of bacteria, attacks fish externally or internally and produces a toxin that is quickly lethal to fish. Reducing the amount of toxin present by moving the fish to a new system, changing water, cleaning the bio-filter and treating the fish with an antibiotic reduces the toxic effect on the fish and release the symptoms. The best treatment is the transfer of fish to a new unaffected system. Modern well-maintained systems fitted with protein skimmers, ozone reactors, trickle filters and activated carbon, limit accumulation of detritus and dissolved organics and control bacterial blooms much better.

11. Reef Aquarium tank

A **reef aquarium** or **reef tank** is an aquarium containing live corals and other animals associated with coral reefs. These aquarium setups attempt to recreate marine life like

Reef Aquarium tank





that of the natural coral reef, often spectacularly colored mixed reef that blend hard and soft corals. Unlike the marine aquarium, which are built to house various types of fish, the main attraction in many reef tanks are the varieties of coral and other invertebrates.

Some points to consider before setting a reef tank are the following:

(i) Power access

A reef tank requires a lot of power unless you use natural lighting, and even then it can be substantial. A 1000 lit reef tank uses close to 30 amps, which means that you will need at least two dedicated breaker circuits of at least 15-20 amps each. One can also expect fairly hefty power bills for the tank.

(ii) Structural support

Make sure that the location where you plan on placing the tank will support its weight. As long as the tank is not too deep (greater than 30"), or you don't plan to place the tank in the middle of the room, you should be okay for loading. If either or both of the above mentioned conditions are true, and then you need to make sure the actual loading (total tank weight/unit area) is within your floor's capability.

(iii) Evaporation rate

A large tank evaporates a significant amount of water on a daily basis. One should try to have some sort of automated top-off system planned and plumbed unless you are ready to add this much make-up water to it every day or two.

(iv) Maintenance

Make sure that all pumps, outlets, filters, and especially the sides of the tank that need to be cleaned are readily accessible. Make sure that the tank layout and positioning allows you to reach most points in the tank for maintenance as well as specimen positioning. One of the keys to making the tank accessible for cleaning, as well as getting to specimens, is to have a canopy or lighting system that is easily removed, or constructed so as to not hinder access by allowing it to be opened or hinged in some fashion.

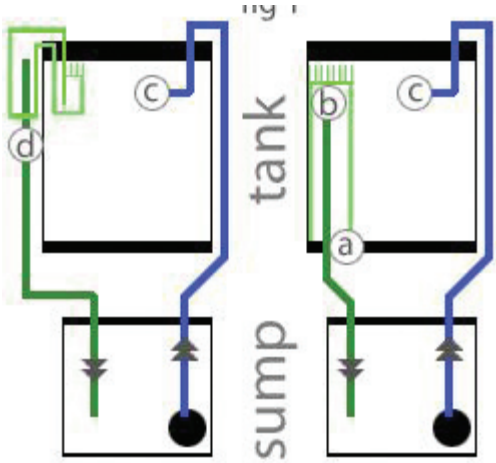
(v) Redundancy/safety precautions

Try to have back-up systems wherever possible. The cost and effort put into stocking a large tank are such that you do not want a single failure in any one piece of equipment to cause your system to crash. Use multiple pumps from the sump as well as within the tank itself for circulation. Have multiple heater units. Place the various pumps and heaters, as well as lighting fixtures, on multiple electrical circuits (you have to for a large tank anyway), so that if any one circuit trips due to short or other failure mode, not all the critical equipments will be shut down.

Components : (i) Aquarium (ii)Filtration (iii) Water movement (iv) Lighting (v) Cooling

Aquarium

Glass or acrylic tanks are used for reef aquariums; these usually include an internal overflow made of plastic or glass which encloses holes that have been drilled into the bottom glass to accommodate a drain or standpipe and a return line. Water pours over the overflow into and down the standpipe through PVC piping, into a sump, which generally houses various filtration equipment, through a return water pump and chiller and finally back via more piping through the second hole into the aquarium. Alternatively, aquariums sometimes employ an external "hang-on" overflow with a U-tube that feeds water via continuous siphon to the sump which returns it via a water pump. Regarding size, for reef tanks usually bigger is better, the greater water volume of larger aquariums provides a more stable and flexible environment.



Water flow is a reef tank

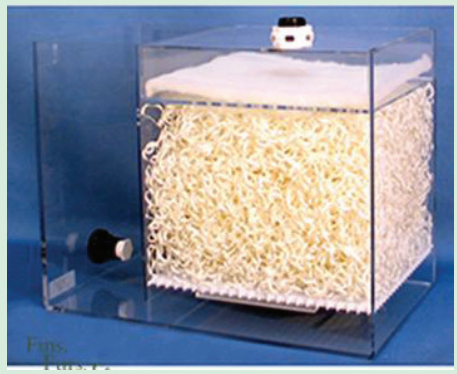
Large main pump

Filtration

The primary filtration for reef aquariums usually comes from the use of large amounts of live rock which come from various rubble zones around existing reefs or more recently aqua cultured rock which is supplemented by protein skimmers. This method first came from Germany and is termed the Berlin Method. In addition, a refugium which houses many species of macroalgae is sometimes used to remove from the water excess nutrients such as nitrate, phosphate, and iron. Some aquarists also advocate the use of deep sand beds.

Usually combined mechanical/biological filtration is avoided because these filters trap detritus and produce nitrate which may stunt the growth or even kill many delicate corals. Chemical filtration is used sparingly to avoid discoloration of the water, to remove dissolved matter (organic or otherwise) and to help stabilize the reef system.

Modern reef filter



Easily removable drip plate and pre-filter

The following is an overview of the components for a modern reef filter.

1. Live rock, 0.5-1kg/10 lit.
2. Large protein skimmer capable of turning over water in the tank 6 times per hour.
3. Easily removable drip plate and prefilter material to clean or change once a week.
4. Large main pump capable of turning over water in the tank 6 times an hour.
5. Large sump box providing considerable turbulence, and capable of holding all the overflow of water from the tank, including the "working water."
6. De-nitrification areas.

Power head

Canister filter

Water movement

Water movement is important in the reef aquarium with different types of coral requiring different flow rates. At present, many hobbyists advocate a water turnover rate of 6x per hour, where x is the aquarium capacity in litres. This is a general rule with many exceptions. For instance, Mushroom Coral requires little flow and is commonly found in crevices near the base of the reef. Species such as *Acropora* and *Montipora* thrive under much more turbulent conditions in the range of 30 to 40 times more flow, which imitates breaking waves in shallow water near the tip of the reef. The directions to which water pumps are pointed within an aquarium will have a large effect on flow speeds.

To create turnover many reef aquarists use an overflow (internal or external) which drains water into a sump where it is then pumped back into the tank. Tanks that come equipped with an internal overflow and pre-drilled holes are known in the hobby as "Reef Ready" or simply "Drilled" tanks. Of the many methods of creating the required flow, one of the most popular is by using multiple powerheads which are simply small submersible water pumps. The pumps may be randomly switched on and off using a wave timer, with each aimed at the flow of another power-head or at the aquarium glass to create flow in the tank. Another method gaining popularity is the closed loop in which water is pulled from the main tank into a pump which returns the water back into the aquarium via one or more returns to create water turbulence. Only recently submersible propeller pumps are gaining popularity due to its ability to generate large volume of water flow (turbulent flow) without the intense directed force (laminar flow) of a power head. Propeller pumps are more energy-efficient than powerheads, but require a higher initial investment.

Another recent method is the gyre tank. A gyre tank encourages a maximum amount of water momentum through a divider in the center of the aquarium. The divider leaves an open, unobstructed space which provides a region with little friction against water movement. Building water momentum using a gyre is an efficient method to increase flow, thus benefiting coral respiration and photosynthesis.

Water flow is important to bring food to corals, since no coral fully relies on photosynthesis for food. Gas exchange occurs as water flows over a coral, bringing oxygen and removing gases. Water flow assists in reducing the risk of thermal shock and damage by reducing the coral's surface temperature. The surface temperature of a coral living near the water's surface can be significantly higher than the surrounding water due to infrared radiation.

Light source

Most aquarium corals contain within their tissue the symbiotic algae called zooxanthellae. These zooxanthellae require light to perform photosynthesis and to produce simple sugars that the corals utilize for food. The challenge for the hobbyist is to provide enough light to allow photosynthesis to maintain a thriving population of zooxanthellae in a coral tissue. Though this may seem simple enough, in reality this can prove to be a very complex task.

Zooxanthellae are golden-brown intracellular endosymbionts of various marine animals and protozoa, especially anthozoans such as the scleractinian corals and the tropical sea anemone, *Aiptasia*.

Hermatypic (reef-building) corals have zooxanthellae and are largely dependent on them, limiting their growth to the photic zone. The symbiotic relationship is probably responsible for the success of corals as reef-building organisms in tropical waters. However, when corals are subjected to high environmental stress, they can lose their zooxanthellae by either expulsion or digestion and die. The process known as coral bleaching occurs when the zooxanthellae densities within the coral tissue become low or the concentration of photosynthetic pigments within each zooxanthella decline. Color loss is also attributed to the loss or lowering of concentrations of Green Fluorescent Proteins (GFP) from the cellular pigments of the cnidarian itself. The result is a ghostly white calcareous skeleton, absent of zooxanthellae, with the inevitable death of the coral unless conditions improve, allowing for the zooxanthellae to return.

Temperature changes have provided the most stress to the zooxanthellae-coral relationship. A rise in temperature of 1-2 degrees Celsius for 5-10 weeks or a decline in temperature of 3-5 degrees Celsius for 5-10 days has resulted in a coral bleaching event. Strong temperature changes shock the zooxanthellae and cause them to suffer cell adhesion dysfunction which sees the detachment of the cnidarian endodermal cells from the zooxanthellae.

Some corals such as the Mushroom Coral and Coral Polyps require very little light to thrive – conversely, corals such as Brain coral, Bubble Coral, Elegance Coral, Cup

Coral, Torch Coral, and Trumpet Coral require moderate amounts of light, and Small Polyp Stony Corals (SPS) such as *Acropora*, *Montipora*, *Porites, Stylopora* and *Pocillopora* require high intensity lighting.

Of the various types, most popular aquarium lighting comes from metal halide, very high output or VHO, compact fluorescent lighting systems. Recent advances in lighting technology have also made available a completely new technology for aquarium lighting: light emitting diodes (LEDs). Although LEDs themselves are not new, the technology has only recently been adapted to produce systems with qualities that allow them to be considered viable alternatives to gas- and filament-based aquarium lighting systems. The newness of the technology does cause them to be relatively expensive, but these systems bring several advantages over traditional lighting. Although their initial cost is much higher, they tend to be economical in the long run because they consume less power and have far longer lifespans than other systems. Also, because LED systems are comprised of hundreds of very small bulbs, their output can be controlled by a microcomputer to simulate daybreak and sunset. Some systems also have the ability to simulate moonlight and the phases of the moon, as well as vary the color temperature of the light produced.

The choices for aquarium lighting are made complicated by variables such as color temperature, (measured in kelvins), color rendering index (CRI), photosynthetically active radiation (PAR) and lumens. Power output available to the hobbyist can range from a meager 9 W fluorescent lamp to a blinding 1000 W metal halide.

Luckily, the choice of lighting systems for a hobbyist can usually be narrowed by first determining which types of corals the hobbyist plans on keeping, since this is the primary factor in determining the lighting needs.

When choosing any type of lighting, you should use a bulb whose Kelvin rating is no less than 6500 K. Lower Kelvin ratings will provide you with a light that is yellow to very yellow. Light source of 6500 K to 10,000 K are adequate for most situations. There are 20,000 K bulbs available, but they tend to be quite blue and, as in the case of a <6500 K bulb, the color rendering may not be proper. When choosing a compact fluorescent system, you should attempt to find bulbs of a color temperature of 6700K and 7100K. A 1:1 combination of these bulbs is ideal for reef tanks.

Heating & cooling

Most hobbyists agree that a reef tank should be kept at a temperature between 25 and 27 ^{0}C (75-80 ^{o}F). Radical temperature shifts should be avoided as these can be particularly harmful to reef invertebrates and fish. Depending on the location of the tank and the conditions therein (i.e. heat/air conditioning), you may need to install a

Chiller

heater and/or a chiller for the tank. Heaters are relatively inexpensive and readily available at any local aquarium store. Aquarists frequently use the sump to hide equipments such as heaters. Chillers, on the other hand are expensive and are more difficult to locate. For many aquarists, installing surface fans and air conditioners suffice in the place of a chiller.

Reef aquarium tanks

Because of the delicate inhabitants extra attention to maintain water quality is essential in reef tanks compared to usual marine fish only aquariums. Many experienced reef aquarists recommend testing the water fortnightly, with partial water changes at least every month. In particular, ammonia, nitrite, nitrate, pH, salinity, alkalinity, calcium and phosphate levels should be monitored closely. When it comes to reefs, even minute changes in water conditions such as mild temperature fluctuations can be problematic.

Reefs also require extra care in the selection of occupants. There are two major factors to be considered: biological load, i.e. the ability of the tank to process the wastes produced by the occupants, and species compatibility. These issues, though present in normal tanks, are magnified in the reef tank. Species considered reef safe and able to coexist in larger tanks may not do well in smaller reef tank due to their close physical proximity. For this reason, smaller species of fish such as gobies and clownfish are popular choices due to their relatively small size and ability to coexist peacefully with other tank inhabitants.

12. Feeds and Feed Management

Feed management of aquarium fish is as vital as the water quality management in aquarium. In nature, fish are adapted physiologically and ecologically to certain types of food organisms. These natural diets provide the amount and balance of proteins, fats, carbohydrates, vitamins and minerals that each species needs to maintain good health and reproductive capability. Most marine aquarists, however do not employ feeds according to the exact composition but feed their fish with commercially prepared diets or a fish food mix.

Fish with broad, unspecialized dietary requirements usually adjust easily to the foods and conditions of captivity , while fish with highly specialized diets may have difficulty. A fish may be limited by its adaptive behaviour patterns to certain food organisms, but still be capable of digesting and utilizing other foods. There are two problems that must be overcome in feeding captive marine fish- the first is to get the fish to accept a substitute for the natural diet and the second is to provide all the nutritional requirements in the substitute food.

Feeding Habbits

(i) Algae and seagrass feeders: The macroalgae are larger which range in size from thumbnail sized growth to kelp plants 40ft or more in height. The sea grasses – turtle grass, eel grass etc. are not algae but are true aquatic flowering plants. They often cover vast areas of shallow bottom around coral reefs. Fish such as parrotfish and surgeonfish that primarily feed on algae are true herbivores, the browsers and grazers of the sea bottom. These fish can get along for a while on standard aquarium diet, but nutritional deficiencies (sunken stomach, loss of colour, inactivity) eventually develop. Marine algae are the best vegetable matter to add to the diet, but leafy vegetables or even freshwater aquarium plants can be substituted.

(ii) Algal feeders: There are many species of macroalgae that make up part of the diet of omnivorous fishes. Some of these are ingested incidentally as the fish feeds on small crabs, shrimps and mollusks and others are deliberately eaten. These algae may

make up 10 to 50% of the diet of many species and hence vegetable matter should be a basic part of their diet in captivity. Clownfish, batfish and angelfish are good examples of fish that normally include a high percentage of algae in their diet.

(iii) Algae and Detritus feeders: Detritus is composed of a great variety of organic matter. Bits of algae, organic flocculants, solid wastes from fish and inveretebrates, coral slime, bacterial debris and small worms and crustaceans accumulate in sheltered nooks about the reef. This detritus and the tiny algae and invertebrates associated with it are a food source for many small fish. Some gobies, blennies and damselfish are among those that utilize this resource. Detritus accumulates in all aquariums, especially in well lighted tanks and serves to supplement the diet of species that normally feed on it. These species do well on normal aquarium foods, but may require old , well established aquariums.

(iv) Sponge feeders: Sponges are incidentally ingested in small volume by most herbivorous fishes as they graze algae, but only adult angelfish consume sponges as the major dietary component. The diet of sponge feeders cannot be easily provided in captivity.

(v) Plankton feeders: Many small reef fishes are zooplakton feeders (eg. Sea horse, pipefish etc). Some plakton feeders in aquarium do well on bits of shaved shrimp and fish, processed flake food and other foods that drift about in the water before settling. Many plankton feeders will not take up food items once they have landed on the bottom.

(vi) Generalised bottom feeders: This category comprises of large non-specific carnivores such as sharks and groupers and includes many groups whose juveniles are important to marine aquarist. These fish are opportunistic feeders although they may have a general preference for specific group of food organisms. In nature, they feed on whatever foods are most abundant and available – small fishes, shrimps, juvenile lobsters, polychaetes etc. The flexibility of their food requirements make these fish easy to feed in captivity. Frozen shrimps, clams, fish and other seafood will keep these fish healthy in the aquarium.

(vii) Fish Feeders: Very few marine aquarium fish are exclusively piscivorous. The lionfishes, anglerfishes, frogfish and sargassumfish are close to being piscivorous. Small freshwater fishes like guppies and bait minnows can be fed which live quite long enough in sea water tank to get eaten by the piscivores

(viii) Coral feeders: To feed on coral polyps a fish must be able to either crush the hard coral or reach a tiny mouth into small protected areas to remove the animals therein. Unless care and attention is taken in developing a diet for these fish, they will not be able to obtain proper nourishment. The tiny mouthed butterflyfish are the most difficult in this category to feed. Bits of shrimp, mussel or live *Tubifex* pressed into coral or irregular rock surfaces often stimulate these fish to begin feeding.

(ix) Crustacean feeders : Small shrimps and crabs represent the major part of the diet of this group and hence some crustacean flesh should be a staple part of their diet in captivity. Frozen shrimp or krill is the easiest way to crustacean material into the diet of this group.

(**x) Generalised Inverebrate feeders :** Most tropical marine fish feed on a variety of small invertebrates with a little bit of algae. The organism of choice often changes as the fish gains in size and changes its habitat. Since the specific natural diets of most fish at different stages of their life are relatively unknown, a variety of foods that include some item of each basic group of invertebrates is most likely to provide all the basic nutritional needs.

(xi) Parasite pickers : A few popular marine aquarium fishes make part of their living, either as adults or during the juvenile stage by removing external parasites from large fish. This cleaning behaviour is quite fascinating and can readily be observed in aquarium if neon gobies or cleaner wrasses are kept with larger fish. However parasite pickers do very well on a varied basic diet when parasites are not available.

Natural Foods

Natural foods are obtained fresh, frozen or freeze dried and fed fresh, thawed or cooked. Typical natural foods are leafy green vegetables and fed fresh or thawed after freezing, cooked or fresh fish and invertebrate flesh and, freeze dried brine shrimp and other zooplankton.

Green Leafy vegetables: Raw leafy green vegetables are composed mostly of water and are low in energy, protein and lipid, but contain relatively high concentration of carbohydrate, ash, fibre and certain vitamins.

Fish and Invertebrate flesh: Captive fishes can be fed a variety of seafoods either fresh, thawed or cooked. Cooking does not alter the proximate composition much and the energy content per gram actually increases because the percentage of tissue water is lowered. Raw seafoods have been implicated in the transmission of certain infectious diseases to captive fishes and their use is not recommended.

Freeze-dried foods: Freeze dried adult brine shrimp is available. Freeze dried and vacuum dried brine shrimp nauplii retain the same fatty acid composition and approximately the same total lipid concentration as freshly killed brine shrimp nauplii.

Moist Feeds: Moist feeds have a texture closer to natural foods and sometimes are readily accepted by fishes. Factors to consider are (i) digestibility of the ingredients (ii) acceptability to fishes (iii) physical stability and natural retension in water (iv) nutrient balance and (v) ease of mixing and storage.

Basic aspects of Marine ornamental fish feeds

Proteins: The purpose of adding proteins to feeds is to supply EAAs. Protein quality is a measure of the relationship between the amino acid composition of food and the amino acid content of the animal to which it is fed. The highest quality protein contains an amino acid composition that most closely matches that of the recipient. The proximate composition of whole fish is similar among species and hence fish meal provides the highest quality protein available for fish feeds.

Lipids: Dietary lipids in excess of physiological requirements are deposited in tissues, resulting in reduced activity and abnormal fatty acid ratios. Fatty acids of the ω-3 series are required by all fishes. Fish oils are high in PUFAs of the ω-3 series. Direct addition of C_{20} and C_{22} ω-3 PUFAs to feeds is the best procedure and this is accomplished easily by using fish oil as the sole source of lipid. Lipids should make upto 10-20% of the diet.

Carbohydrates: Seawater fishes apparently do not require carbohydrates and hence their addition to feeds is not mandatory. Energy can be provided easily and more effectively in the form of lipids.

Vitamins: Vitamins are added to moist feeds as premixes. Vitamin deficiencies are less likely if live feeds are fed regularly as dietary supplements.

Carotenoids: The normal skin colouration of some fishes can be intensified by adding carotenoids to feeds. Several carotenoids are available as powder for addition to feeds.

Binders: No feed is suitable if the ingredients do not hold together long enough to be ingested by a fish. In dry and moist feeds this function is performed by binders, which are proteins or carbohydrates derived from animal process wastes, sea weeds or exudates of terrestrial plants. The most useful of these are alginates.

Leaching: Leaching is the diffusion of dissolved nutrients from foods and feeds into water. Water soluble vitamins and EAAs are the principal substances lost. The problem of leaching has been largely overcome in aquaculture feeds by encapsulation. During encapsulation feed particles are encased individually in digestible walls composed of synthetic polymers such as ethyl cellulose, polyvinyl alcohol or natural polymers. When manufactured correctly, the products retain nutrients exceptionally well in water and have a spongy texture that seems attractive to fishes.

Storage and handling: Moist feeds should be cut into pieces of convenient size, sealed tightly in heavy plastic and frozen. Dry feeds should be kept cool and dry. Flakes containing PUFAs should be packed at the factory in air tight containers charged with dinitrogen to prevent in vitro oxidation and subsequent rancidity.

Prepared Diets

There are three main categories of aquarium feeds – prepared diets, plant feeds and animal feeds. The fish in a traditional community tank should receive two small

feedings of a prepared diet each day and one feeding from each of the other two categories once a day or at least every other day to keep them in top condition. Eventhough three or four relatively small feedings of varied foods per day is best, most marine fish can be in good condition on two feedings per day. A good dry flake food feeding in the morning and a feeding of natural animal and plant foods in the evening will keep most fish in good condition.

The prepared diet specially compounded for marine fish can be very convenient to the marine aquarist. But total dependence on a prepared diet can cause nutritional problems.

(i) Dry flake and pallet feeds: This is the most widely used prepared diet. These are relatively inexpensive and contain a wide variety of nutrients. The flake chosen should be of high quality, 35 to 45% protein and contain some plant material including marine algae and carotene. There are now a number of dry flake and pellet feeds that are compounded just for marine fish. Care should be taken not to overfeed with these feeds. Coral reef fish do not normally feed on the surface and when they do take floating food, they may also ingest air bubbles which can cause excessive buoyancy and disturb the digestive process. If this is a problem, soaking the feed in a small volume of water before feeding eliminates floating feeds and surface feeding. Small fish such as young clownfish , may gorge on dry feed and develop bloats as the feed expands in their stomachs. When this happens the abdomen of the fish is greatly distended and the fish continually fights to keep from floating at the surface. The cure of this condition is to stop feeding for a day and then feed mostly a wet feed mix and only an occasional light feeding of dry feed.

(ii) The seafood paste mix: The basic ingredients to this mix are fresh frozen shrimp, clams, fish, mussel, lobsters and other crustaceans. Animals that break down to a paste-like consistency when blended such as shrimp are preferred. Clean the meat of all shell, scales or skin and blend it with a little water to a paste like consistency. It is better to use two or three types of sea food to provide a variety of nutrients. To this mixture 20% by volume of vegetable material (preferably marine algae) can be added. Blend the mixture and add multi vitamin mineral mix. The mixture is then frozen and individual cubes can be stored in a plastic bag. At feeding time one of the cubes is removed and small pieces of the right size for the fish are snipped off and dropped into the aquarium.

(iii) The gelatin mix: Here the ingredients are held together with gelatin. Dissolve the gelatin in warm water and add the melted gelatin to the other ingredients. Then add vitamin and mineral supplement and if desired a little green or red food colouring. The colouring aids the fish in seeing the food and helps the aquarist to find uneaten

food for removing before it decomposes. Pour the completed mix into a tray and chill until set. The mix can then be cut up into cubes and stored in freezer.

(iv) The plaster mix: Most herbivorous fishes and some coral and sponge eaters spend a great deal of time biting and scraping at the reef to consume the proper amount of coral polyps and algae. In the aquarium the fish miss this extensive feeding activity. The plaster mix allows these fish to feed under simulated natural conditions and gives them the exercise they need. Prepare a small quantity of plaster of paris(calcium sulphate). Add the food ingredients , trace elements and food colouring materials while the plaster of paris is still fluid. Make sure that all excess water is removed from the ingredients before feeding them into the hardening plaster. The food ingredients should only make up 10 to 15% of the total volume. Store the mix in freezer. This diet is most used by public aquariums that keep large fish , but may be useful to the advanced aquarist who keeps surgeonfishes and parrotfishes.

Live Animal Foods

Most coral reef fish consume animal food as a major portion of their diet, thus animal foods are most important to the marine aquarist. Fish consume entire live prey organisms when feeding. This gives them access to minerals in hard parts and shells, plus vitamins from internal organs and proteins and food energy in the flesh and fat. Obviously a variety of live feed organisms is the best possible diet for most fish.

Brine Shrimp: Brine shrimp (*Artemia*) is the most common live food marine aquarists offer their fish. Although live, clean and well fed adult brine shrimp are generally considered as an attractive and nutritious food for most marine tropicals, some aquarists fear introduction of bacteria and parasites from unclean cultures of brine shrimp. Adult brine shrimp can also be obtained in frozen and freeze dried forms. Frozen brine shrimp can lose much of its nutritive value if they are not handled properly. Thawing and refreezing or slow initial freezing causes ice crystals to form in the tissues. Ice crystals rupture cells and internal organs and cause most of the fluids to leave the shrimp as it is thawed before feeding. Thus the nutritional value of thawed and refrozen brine shrimp is limited to the protein in the exoskeleton.

Newly hatched brine shrimp can be very valuable to the marine aquarist. They can be used to feed small plankton feeding fish such as damselfish, many filter feeding invertebrates and young fish of many species. The dried *Artemia* cysts are introduced into seawater (salinity 30-35 %) at the rate of 1 g/litre and provided with aeration and light which stimulates embryonic development. The free swimming nauplii are hatched out within 12 to 36 hrs after hydration in sea The nauplii which congregate near light are siphoned out and used for feeding the fish.

Live fish: A number of popular marine aquarium fish *viz.* lionfish, groupers, snappers, sargassum fish feed on small live fish. It is best to feed with small freshwater fish because they are less expensive than marine fish and most important , do not carry marine diseases and parasites.

Small shrimp and other crustaceans: Small shrimps, amphipods etc. are excellent livefood organisms for many marine aquarium fishes. These organisms may be given a quick dip in freshwater for incapacitating the organisms so that the fish can snap them before they find shelter in the bottom of the tank.

***Tubifex* worms:** One very useful method of feeding *Tubifex* worms is to press a small ball of worms deeply into a coral skeleton and then place it in the tank. Hard to feed fish such as butterflyfish are often attracted by the movement of the worms and begin to feed quickly. *Tubifex* worms can quickly foul a tank if overfed to marine fish

Ornamental fish feed prepared by CMFRI

Ornamental fish feed preparation in CMFRI

Feed Management and Water Quality

The feed management in the aquarium plays a key role in maintaining the water quality of the aquarium. As a thumb rule, feeding can be done @ 2-3% of the fish body weight once in a day. The excess feeds in the aquarium decay and foul producing poisonous gases like ammonia which is toxic to fishes. While feeding aquarium fishes care should be taken to feed them only with the required food. It is more dangerous to overfeed the fish than to underfeed them. If some excess feed is present in the tank it should be siphoned out from the tank daily.

For feeding marine ornamental fish CMFRI has scientifically evaluated feeds containing not less than 30 % protein, 9 % fat, 39 % carbohydrates, 7 % ash (minerals) and less than 2 % fiber. These feeds are made up of marine protein, soy protein, wheat flour, oil, vitamins, minerals, color imparting nutrients, immune promoters, an anti-oxidant, antifungal and probionts. They are sold in packets of 50g capacity.

Technology commercialization package is available for production and marketing of this product with CMFRI as knowledge partner.

13. Diseases and health management

The commonly recognized diseases of aquarium fishes are those that cause visible symptoms externally, whether physical or behavioral. Some of these can be cured or alleviated, while others warn the aquarist to get rid off the fish suffering from them. If the aquarist has no quarantine facilities, it may be necessary to treat the whole tank, but it is preferable to medicate the individual fish. When it is necessary to treat the tank as a whole it is better not to use a medicine that will not stain the fittings, colour the water deeply or kill the bacteria in the nitrifying filter. The biggest danger of in-tank treatment is damage to the biological filter. Carbon filters must be turned off during treatment, as they remove medicines.

The disease problems fall under 3 major categories:-

1. Problems caused by poor environmental conditions.
2. Problems caused by poor nutrition.
3. Problems caused by an organism that causes disease.

Usually poor environmental conditions and/poor nutrition create stress and reduce the natural resistance of the fish to diseases. The very best disease control in an aquarium consists of prevention, and the very best disease prevention techniques consist of providing good nutrition and good environment, and using a quarantine procedure.

There are 3 steps to solve a disease problem:-

1. Determining that a problem exists.
2. Identifying the cause of the disease or source of the distress
3. Successfully curing the fish and eliminating the disease or cause of distress.

Behaviour is often the best first indicator of the disease and hence watch for lack of feeding, rubbing against rocks, colour changes and other unusual appearance and behavioral pattern. The quicker a disease is identified, the better the chances for treatment and recovery. Treatment should be started only after making sure of the cause of the distress.

When a fish is sick, we cannot attribute that the sickness is caused by disease or parasites. The fish may be suffering from poisoning due to bad maintenance, malnutrition or an incurable genetic disability such as tumour or curved spine, etc.

Disease Symptoms and Treatments

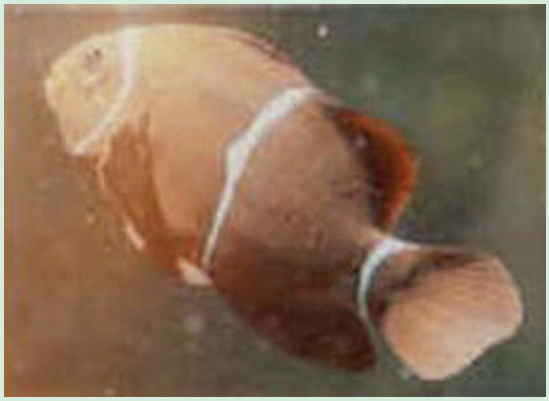
Coral fish disease

It is also known as velvet disease, coral disease or salt water itch, and is caused by *Amyloodinium ocellatum*. *A. ocellatum* is a marine dinoflagellate. It is parasitic on fish during one stage of its life cycle and can complete its life cycle in the aquarium. A few numbers of this parasite would have a little effect on a fish in the open ocean; but in a closed system aquarium this parasite can reach population that totally infects any fish that may be present.

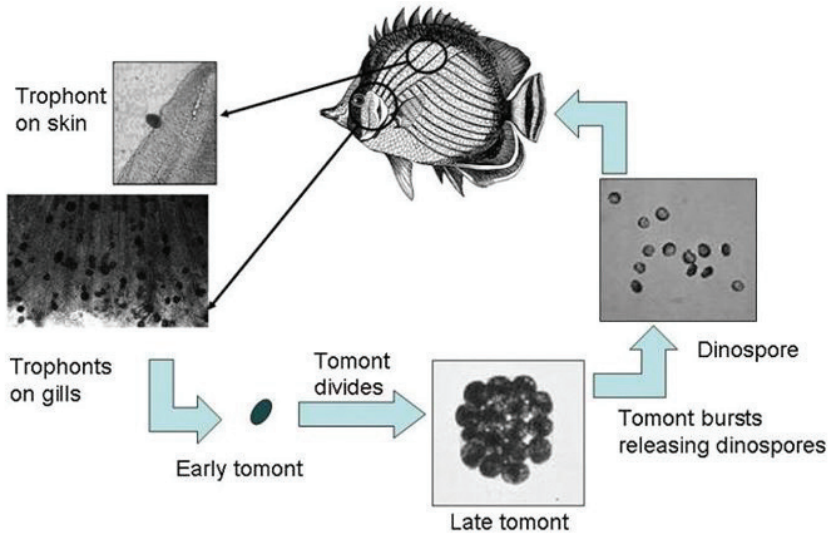
Amyloodinium infestations typically begin in the gills. Damage to the delicate gill tissue stimulates fish to produce excessive mucus in the gills, and this condition restricts the exchange of respiratory gases and increases the respiratory pace. As the infestation progresses the cysts become visible on the fin membranes and on the body surfaces. Infected fish often scratch their sides on the bottom or on rocks, and sometimes shake while swimming. As the infestation progresses, colours fade, a powdery or dusky appearance becomes very noticeable and secondary bacterial infections often develop. Respiration is now very rapid and the fish begins to lie on its side on the bottom of the tank. It is too late to save that particular fish but some of its less infected tank-mates can be saved if treatment is quickly provided. The period between the first observation of rapid respiration and terminal infestation may be as short as 3-4 days.

A successful treatment for *Amyloodinium* is treating both the infected fish and the infected tank. If the parasite is not eradicated from the tank, reinfection occurs no matter how effectively the fish have been treated. *Amyloodinium* can be treated

successfully with formalin, copper, hydrogen peroxide, malachite green and a number of other compounds. The most common treatment used in large and small marine systems is copper in the form of cupric sulphate complexed with citric acid or chelated with EDTA. A most effective treatment is freshwater bath which can be done as follows:-



Clown fish affected by velvet disease



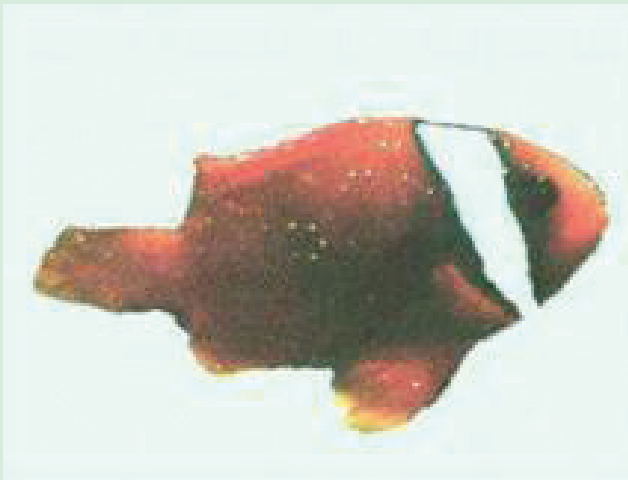
Life cycle of *Amyloodinium ocellatum*

Take freshwater and dechlorinate if necessary. Remove all fish from the infected tank and give them a 1-2 minute bath in the fresh water. The fish can easily withstand the abrupt change in the external osmotic pressure, but the parasites have no protection. They quickly swell and burst. After the freshwater bath the fishes are placed in the

treatment tank. The treatment consists of a 3 week exposure to a copper level of 0.2-0.3ppm to destroy all dinospores. Then the fish may be exposed to an antibiotic (Neomycin, Erythromycin, Tetracycline, etc.) treatment to control secondary bacterial infections.

White spot disease

White spot disease or *Cryptocaryosis* is caused by the ciliate *Ichthyopthirius multifiliis*. It can also complete its life cycle in a closed system aquarium. The tomite is the motile infective stage of the life cycle. They are small ciliated protozoans about 50 microns long, and their function is to find a host within a day or two. Once they attach to the gill or body of a host, they develop into the second stage trophont. This stage burrows into the host and feeds on host tissues. The well fed trophont stops feeding and encysts and becomes the tomont stage. Within 6-10 days, about 200 new tomites may emerge from the tomont and seek another host fish to begin the life cycle again.



Clown fish with white spot disease

The first sign of the disease is usually several to a dozen white spots on the body and fins of the host fish. These become more numerous as the disease progresses. The gills become clogged with tomonts, mucus and tissue debris. Bacterial infections invade the lesions caused by the trophonts and the fish decline rapidly. Scratching on the bottom or on rock is a common symptom. Loss of colour also occurs.

The traditional treatment for this disease consists of a one hour formalin bath every other day for a total of 3 baths and a copper treatment for the aquarium. Prepare the formalin bath by adding 1ml of formalin for 4 litres of sea water. Aerate this preparation

and carefully give the treatment. Another effective treatment for *Cryptocaryon* and other ciliates is a 5 day exposure to malachite green. 1 or 2 drops per 4 litres of a 1% solution can be used for treatment.

Clownfish disease

Brooklynella hostilis is another ciliate which occurs on clownfish and causes 'clownfish disease'. The symptoms appear as small whitish spots with indistinct borders on the sides and sometimes on the fins. These whitish areas begin to enlarge and soon mucus and skin erode off and the affected areas become red. The disease advances rapidly and the fish usually dies within a few days. The formalin treatment recommended for *Cryptocaryon* is suitable for this disease also.

Viral diseases

Lymphocystis virus causes cell to swell enormously and groups of them become tumours composed of connective tissue, at first looking like a fluffy fungus. White or grey fluffy patches are the main symptoms. It is best to destroy the infected fish to prevent infection to others. Lateral line disease is thought to be a virus disease, which is sometimes cured by change of environment. The disease usually starts at the head and progresses down the lateral line. There is no specific cure for this disease.

Bacterial disease

A common bacterial disease is the tail rot or fin rot in which red streaks appear on the body or fins which later become ulcers and leads to loss of fins. This is caused by *Pasteurella, Vibrio, Pseudomonas*. Treatment is likely to succeed if trouble is noted very early. Treatment is mainly by disinfection with acriflavin or monacrin. Make up either drug as a 0.2% solution in distilled or tap water and give upto 5ml/4litres, to be repeated for about 3 days. If there is no cure, switch on to other treatments. It is best not to use antibiotics in the aquarium as general treatments because of their relative ineffectiveness in salt water, the danger of culturing resistant strains and of effects on filters. Instead, it is better to give them through food in a concentration of about 1%. Chloromycetin, Neomycin or Gentamycin can be used. The best food to mix is a flake, feed twice a day.

Fish tuberculosis is a wide spread marine fish disease caused by the bacterium *Microbacteria marinum*. The disease causes hollow belly, ragged fins and skin blotches. If the disease is caught in the early stages, treatment with streptomycin and isoniazid may be an effective cure. Add 40 mg of isoniazid and 40 mg of streptomycin/ 4 litres of seawater to the treatment tank and the fishes are transferred. Change water every 3 days. Return the fish to the display tank when they are recovered.

Dropsy is a swelling of the abdomen usually caused by the kidney disease that in turn leads to accumulation of body fluids. *Corynebacteria* are responsible for this disease. Erythromycin treatment should be given by mouth.

Fungal diseases

The main fungal infection in marine fishes is caused by *Ichthyosporidium hoferi*. The pathogen enters through food, invades the blood stream and settles down in the liver. From there it spreads everywhere forming cysts. Early symptoms are sluggishness, hollow belly or loss of balance. An infected fish should not be left in the aquarium or it will infect others. The addition of chloromycetin at 1% in the food is recommended.

Exophthalmos (Pop eye)

This condition is a symptom and may accompany various diseases. One is that both the eyes protrude from the sockets, sometimes proceeding to blindness or loss of the eye. When not caused by disease, it may be due to toxic conditions in the aquarium, to gas bubbles or to copper poisoning. The treatment depends on the cause of the disease.

Clownfish with pop eye

Black itch

This is a disease caused by parasitic flat worms of the genus *Paravortex*. These parasites can complete their life cycles in closed aquarium systems and if left untreated

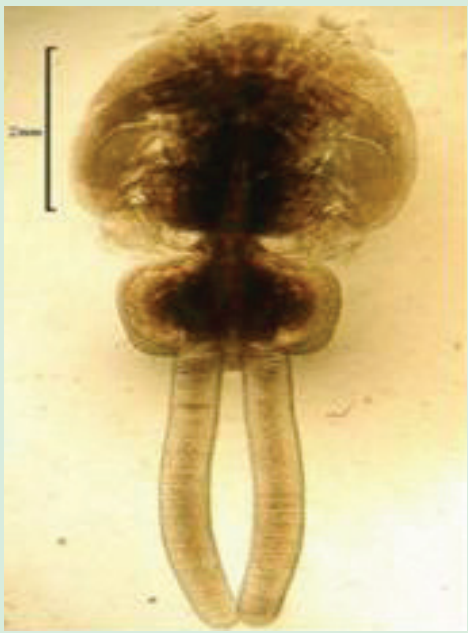
can destroy fish in the aquarium. A tiny free-swimming worm first finds and attaches to the skin or gills of a fish host. It feeds on the tissues of the host. An infested fish displays numerous tiny dark spots usually on the side of the body, just behind the gill openings or on fins. Infected fish display scratching behaviour and will not feed properly. Formalin treatment is effective for the disease.

Fish flukes

Benedenia melleni are flattened transparent parasites which attaches to the gills and external surfaces of the fish. Fish that are infested actively scrape and swim against the bottom attempting to dislodge the parasites. Severe infestations may lead to bacterial infection by stressing and weakening the fish as well as by breaking through the skin and mucous layers. The best immediate treatment is freshwater bath which can be repeated every day or whenever the flukes are observed on the fish. The traditional formalin bath is also an effective treatment.

Copepods

Various species of *Argulus*, the fish louse, are fairly large and should be removed by forceps. Sea horses are their favorite hosts. Remove them as soon as they are detected, as eggs are soon laid in the tank and can result in an infestation that is very hard to cure



Argulus sp. - parasitic copepod

Clownfish with isopod infection

chemically. Visible signs of copepods may be egg sacks hanging from the fish, the rest of the animal being beneath the skin. In other cases the whole animal may be seen hanging from the gills or body. Affected fish shows discomfort, sometimes dashing around the aquarium. Freshwater and formalin bath as done in the case of fluke infestation is effective for copepods.

External poisoning

Various house-hold materials can poison the fishes, especially ammonia containing substances – cleaners, insecticides, etc. Detergents on improperly rinsed hands, tobacco, etc. can all be introduced by accident. Copper poisoning can also happen from the water obtained through copper pipes. Symptoms of severe poisoning include violent swimming with heavy respiration. Fish will frequently jump from the tank, shake and finally die. The first step of treatment is to find and remove the source of poisoning. A good filter cleaning and water change will be sufficient to solve the problem.

Internal poisoning

Clogged filters, dying of algae, overfeeding, a dead fish or invertebrate, over crowding can all cause the release of toxins. Ammonia, hydrogen sulphide and phenols are usual causes of trouble. These toxins should be detected when they are at a very low level. Otherwise, things degrade quickly and toxins reach a level where they cause severe distress and even death. Generally two problems are caused by the internally generated toxins. These are ammonia and/nitrite poisoning (New tank syndrome) and a sudden unexplainable loss of almost all fish in a healthy looking tank, within 12 – 24 hours (Toxic tank syndrome). Ammonia/nitrite poisoning leads rapidly to bacterial disease because of impaired functioning of the kidneys and the liver. Excessive mucus is also produced in the gills and rapid respiration is one of the first signs of ammonia / nitrite toxicity. The fish may also keep their mouths open and move restlessly about the tank. In extreme cases, movement is rapid and fish may try to jump from the tank, eventually colours fade, eyes get dull and the fish goes into shock and dies. The toxic tank syndrome progresses very rapidly. The early symptoms are very rapid respiration and disturbed swimming movements. Lowering the pH will bring some relief to the fish suffering from ammonia /nitrite poisoning. The best treatment is removal of the fish to a balanced, ammonia/nitrite free environment. The only way to save the fish suffering from toxic tank syndrome is immediate transfer to totally separate system. Water changes slow the progress of the syndrome but do not prevent its re-occurrence. There are two methods for treating the tank. The first is a good filter cleaning and water change. The second is the sterilization of the entire system and re-establishment of the biological filter.

Nutritional disorders

A diet that provides all the essential nutrients is necessary to keep a marine fish in good health for a long period. Fish suffering form malnutrition becomes susceptible to many other maladies, and although death may be caused by a specific disease, the under-lying problem is a fish weakened by malnutrition. Except for total starvation, nutritional deficiencies do not occur quickly. They are the result of habitual poor feeding practices like under-feeding. Most fish need to be fed at least once a day, twice is better. Fish don't have to eat a lot to be healthy, but they do need right foods on a constant schedule. Overfeeding is bad for the fish and for the tank. Small fish that consume too much flake food have a tendency to bloat after feeding and become so buoyant that they have difficulty in staying near the bottom. Feeding the same food week after week without change promotes under-feeding and results in nutritional deficiencies. Many species require vegetable matter, preferably algae in the diet to provide roughage and proper balance of nutrients. Fatty degeneration of liver is one of the common nutritional disorders of marine fish. Feeding marine fish with a diet high in animal fats gradually causes fat to infiltrate the liver and eventually the liver stops functioning. Then the fish is very susceptible to stress and often falls in deep shock. The common symptoms of dietary deficiencies are a tendency to bloat after feeding, a sunken stomach, overall thinness, fading colours, loss of colours in blotchy areas, erosion of the skin behind the head and general restlessness. Providing a good, varied diet is the solution for nutritional disorders.

Tank sterilization

Tank sterilization may be required in the case of toxic tank syndrome or due to an extremely persistent bacterial or parasite problem. When a tank is sterilized, everything in the tank will be killed, and hence we have to make sure that all the animals of the tank are removed. The agent of the sterilization is chlorine. There must be high concentration of chlorine for sterilization. The first thing to do is to remove all the coloured plastic ornaments if any in the tank, as they may be discolored by chlorine. It is better to remove as much as the accumulated organic matter as possible before sterilization. The easiest way to remove the organics is to scrape the sides and stir the bottom well, then siphon out all the accumulated debris and dirty water. Refill the tank with freshwater, put everything else that need sterilization into the tank. Add one table spoon of dry, granular chlorine for 100 litres of water and keep it for 12 to 24 hours. The next thing is neutralizing the chlorine. Add sodium thiosulphate or any other commercial dechlorinator until chlorine is gone. When the chlorine has been neutralized, the tank should be siphoned again and everything including the gravel filter should be rinsed with one or two changes of fresh water. Then the tank can be refilled with salt water

and the process of conditioning the filter with nitrifying bacteria can be started.

Precautions

1. Immediately remove dead and dying fish from aquaria.
2. Isolate fish for treatment.
3. Identify the disease problem before treatment.
4. Change water in the treatment tank every 2 or 3 days.
5. Keep the bottom of the treatment tank clean.
6. Provide shelter for the fish in treatment.
7. Keep light intensity low in the treatment tank.
8. Monitor ammonia and nitrite levels in the treatment tank.
9. Keep the fish isolated until the cure is complete.
10. Monitor copper levels in treatment water every 1 or 2 days.
11. Rinse any external filters with fresh water and change the media to prevent reinfection of a tank after the treatment is complete.
12. Do not medicate unless necessary.
13. Do not continue to add copper without testing the current copper level. The copper level should not exceed 0.3ppm.
14. Do not use antibiotic in a tank with a biological filter.
15. Do not use an activated carbon filter with any type of medication.
16. Do not use a UV- filter with any type of medication.

14.Setting up of a small-scale hatchery

Small-scale hatcheries for marine ornamental fish are those where the capital costs and technologies are accessible for relatively low cost which focuses on broodstock development, larviculture, nursery rearing and grow-out to marketable size. The small-scale hatcheries can be easily adapted to culture a range of different species.

A typical small-scale hatchery for marine ornamental fish consists of the following units.

1. Broodstock tanks
2. Larviculture tanks

3. Nursery rearing and grow-out tanks
4. One sand filter
5. Outdoor live feed (Phyto and zooplankton) production tanks
6. Seawater and freshwater supply system.

Advantages of small-scale hatcheries

1. Low capital inputs
2. Simple construction
3. Ease of operation and management
4. Flexibility
5. Quick economic returns.

Site Selection

A site suitable for a small-scale marine ornamental fish hatchery should have the following characteristics:

(i) Good water source – both seawater and access to freshwater

(ii) Good infrastructure such as road, electricity, etc.

(iii) Free from industrial and other pollution

Hatchery lay out

The hatchery should be laid out in such a way that it provides for ease of operation and it should also be free from work hazards. The essential types of tanks required for a small-scale hatchery are the following:

i) Sand filter tank

Small-scale hatcheries may use a gravity sand filter to initially remove coarse particles and organisms from the source water. Such filter tanks are usually made of concrete and the filter medium comprises a layer of coarse material such as stones at the bottom and gravel and sand layers respectively. The water inlet to this filter is at the top of the tank to allow water to filter from top down before going to the larval rearing tank.

ii) Larval rearing tanks

Larval rearing tanks are generally cement tanks, rectangular or square in shape. They range in size from 6 to 10 m^3 capacity. Usually, larval rearing tanks are 1 m in depth, but nursery tank can range between 0.5-1 m deep. All cement tanks used in hatcheries need to be finished internally with food grade epoxy paint to prevent the

water from coming in direct contact with the cement. It is better to paint the inside of the tanks with blue or green colour.

iii) Live feed production tanks

Microalgal production tanks normally make up about 30% of the total production volume of a small-scale hatchery. These tanks are usually located outside the hatchery and are not roofed. Capacity varies from 10m^3 to 20m^3. Generally the rotifer culture area will take up about 10% of the total hatchery area. Rotifer tanks can be 5-6 m^3. Artemia are hatched in fiberglass or plastic tanks. These tanks range from 20 to 500 litres.

Hatchery equipment and accessories

(i) Water Pump: Two types of pumps are required for the small-scale hatchery operation. A pump of 5HP is required to pump seawater to the hatchery's sand filter tank. A separate submersible pump is required to distribute water within the hatchery system.

(ii) Generator: A generator of 1 KVA is essential as backup electricity supply for the hatchery.

(iii) Aeration system: Small 100 watt air pump with at least one backup is needed.

(iv) Other hatchery equipments

a. An ordinary microscope.

b. Thermometer

c. Salinometer

d. pH meter

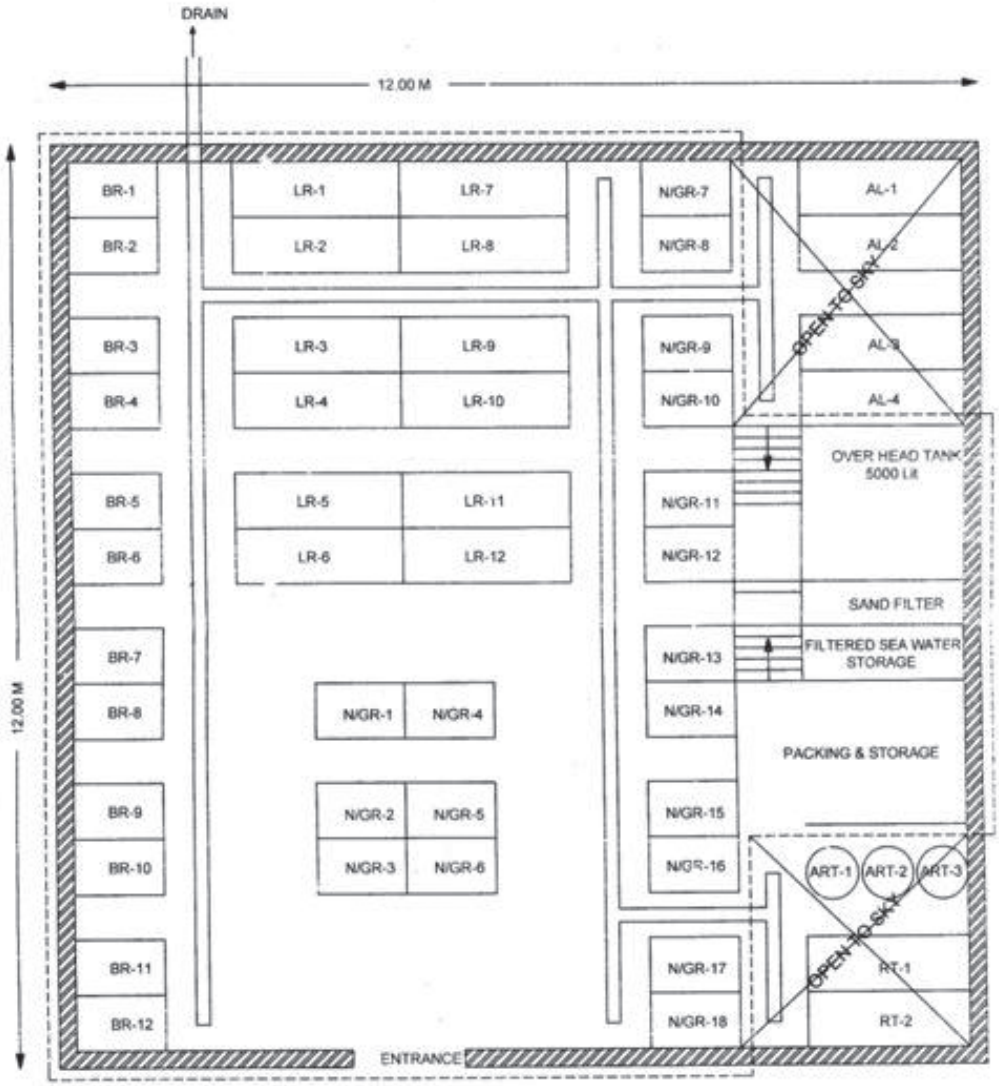
e. Water analysis kit

f. Hand nets

g.Plastic wares like buckets, bins, hoses etc.

(v) Manpower: The small scale hatchery can be managed by two full time staff – One technician and two workers. Basic training on technical aspects is needed for day-to-day hatchery operation. Daily routine works include cleaning broodstock and larval tanks, feeding broodstock and larval tanks, harvesting microalgae, rotifers, *Artemia* etc.

LAY-OUT OF A SMALL-SCALE MARINE ORNAMENTAL FISH HATCHERY



BR-1 to BR-12 - BROOD STOCK TANKS 1.00X0.50X0.60 M
LR-1 to LR-12 - LARVAL REARING TANKS 2.00X0.50X0.60 M
N/GR-1 to N/GR-18 - NURSERY/ GROW-OUT TANKS 1.00X0.50X0.60 M
AL-1 to AL-4 - ALGAL CULTURE TANKS 2.00X0.50X1.00 M
ART-1 to ART-3 - ARTEMIA CULTURE TANKS 0.75 M dia
RT-1 & RT-2 - ROTIFER CULTURE TANKS 2.00X0.50X1.00 M

15. Economic Assessment

The candidate speceis selected for economic analysis is the true clown *Amphiprion percula*.

Capital Investment

This component involves all the expenditure on the infrastructure and establishment of the hatchery. The items included in this component generally have a life span larger than one year and they are used to generate the future income from the hatchery. The items include

Capital Investment items	Quantum	Cost in Rupees
Temporary Shed	144m^2 (12 X 12m)	1,10,000
Cement tanks for		3,40,000
i. Broodstock	12	
ii. Larval rearing	12	
iii. Nursery and grow out	18	
iv. Microalgae (outdoor)	4	
v. Rotifer (outdoor)	3	
vi. Sand filter /Over head tank	1	
Artemia hatching tanks (Transparent Perspex)	3	10,000
Power installation		10,000
4 HP diesel pump	1	19,000
1/2 HP submersible pump	1	6,000
Generator 2 KVA	1	30,000
Air pumps	2	40,000
PVC piping, plastic wares (water supply/aeration/drainage)		45,000
Netting, miscellaneous etc.		40,000
TOTAL COST		**6,50,000**

Operating expenses

This component is for the expenses that are spent during each production cycle and are essential for the routine operation of the hatchery. The items included are:

	Items	1st year	2nd year	3rd year
1.	Broodstock fishes/Anemone	25,000	5,000	5,000
2.	Feeds	12,000	12,000	12,000
3.	Artemia	4,000	12,000	12,000
4.	Chemicals for microalgal culture	6,000	6,000	6,000
5.	Electricity	36,000	36,000	36,000
6.	Diesel	24,000	24,000	24,000
7.	Maintenance	12,000	18,000	18,000
8.	Workers salaries(1xRs. 5000; 2xRs.3000)	1,32,000	1,32,000	1,32,000
10.	Miscellaneous expenditures	12,000	12,000	12,000
	TOTAL	**2,63,000**	**2,57,000**	**2,57,000**

Non-operational expenses

These are related to the capital cost and investment write off. There are two items under this component for small-scale hatcheries.

i) Depreciation

ii) Interest on capital investment

Technical assumptions for production

It is assumed to be an indoor system located in a coastal area with access to both salt and freshwater and easy transportation access to market.

There are 12 broodstock pairs. At any time there are 10 active spawning pairs. Each pair will spawn 2 times per month. An average of 400 larvae are produced during each spawn. The survival rate of the larvae to the grow out phase is 50%. The period from larvae to juvenile is 30 days.

There is a 60% survival rate for juveniles to market size, which are saleable. The period from nursery to market size is 120 days. In a month, 240 saleable sized fishes can be produced from one pair of clown fish. Each fish can be sold at a rate of Rs.100.

The sale of the fishes will start from second year onwards. The first year of operation will be construction and set up of the building, procurement of equipment and collection and maintenance of brooders. The first spawning is expected in eighth month of first year. The first harvest and sale will occur at the first month of second year.

	Amount in Rs.		
	Year 1	**Year 2**	**Year 3**
Revenue			
Sale of clownfish fingerlings @ Rs.100/fingerlings(240 juveniles x 10 pair x12 month =28,800 numbers 28800 x Rs 100 = Rs. 2880000)		28,80,000	28,80,000
Non operating expenses			
a. Depreciation (20%)	1,30,000	1,30,000	1,30,000
b. Interest rate on capital investment @12%	78,000	78,000	78,000
Operating costTOTAL EXPENSES	2,63,000	2,57,000	2,57,000
	4,71,000	4,65,000	4,65,000
Profit	———	**24,15,000**	**24,15,000**
Pay back period	**5.28 months**		

Profit and Loss

This consists of the revenue generated from sales of clownfish young ones minus all the operating and non-operating expenses. The payback period can be used to measure how rapidly a small-scale hatchery can provide a return to the farmers or investors.

Payback period (PP) = (Capital Investment / Profit) x 12 months= (6.5/14.53)*12 = 5.28

Return on investment or pay back period for the small-scale hatchery based on the above calculations is about six months. It is evident that the capital invested for the small-scale hatchery can be recovered fully within six months from the start of earning.. The only assumptions made are that the hatchery operations are running smoothly and the price of *A.percula* juveniles remain stable during the period.

Environmental Impacts

The ornamental fish culture programmes will not make any significant environmental hazards. On the contrary the practice of exploitation of ornamental fishes from the coral reef habitats can cause destruction of the reef habitats and endangering of many reef species.

16. Acknowledgement

The authors wish to express their sincere thanks to Dr. G. Syda Rao, for his constant encouragement for bringing out this compilation. They also thank Dr. P. Vijayagopal, Sr. scientist, Dr. R. Narayan Kumar, Sr. Scientist for their contributions. They are also indebted to Shri. V. Edwin Joseph, Officer in-charge, Library and Documentation, Shri. J. Narayanaswami, Technical Officer, for their technical efforts to bring out this publication.

17. Suggested Reading

Colette, W., M. Taylor, E. Green and T. Razak, 2003 *From Ocean to Aquarium: a global trade in marine ornamental species*. UNEP World conservation and monitoring centre (WCMC), 65 pp

CMFRI. Annual Reports 2004-05, 2005-06, 2006-07, 2007-08, 2008-09

Emmens, C.W. 1994. *The Modern Reef Aquarium*, TFH Publications, Inc.,One TFH Plaza, Neptune City, NJ 07753.

Emmens, C.W. 1995. *A step by step book about setting up a marine aquarium*,TFH Publications, Inc., One TFH Plaza, Neptune City, NJ 07753.

Emmens, C.W., 1995. *Marine aquaria and miniature reefs*, TFH Publications, Inc.,One TFH Plaza, Neptune City, NJ 07753.

Frank.H.Hoff. Jr. 1996. *Conditioning, spawning and rearing of fish with emphasis on Marine clownfish*. Aquaculture Consultants Inc., 33418 old Saint Joe Rd.,Dade City, FL 33525.

Martin A.Moe, Jr. 1995. *Marine Aquarium handbook – Beginner to Breeder*, GreenTurtle Publications, P.O.Box 17925, Plantation, Florida 33318.

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
Post Box 1603, Ernakulam North P.O., Kochi – 682 018, Kerala , India