SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I - AQUACULTURE – SBT1608

UNIT 1 INTRODUCTION TO AQUACULTURE

Aquaculture: Definition-Site selection, design and construction of aquaculture pond - Criteria for selecting the candidate species for aquaculture - Types and methods: Extensive, semi-intensive and intensive culture - Composite fish culture and integrated fish farming - Types of culture systems: pen culture, Cage culture, raft culture and Pond culture

---

AQUACULTURE

Aquaculture may simply be referred as 'Underwater Agriculture'. Over the years, the enormous increase in the growth rate of aquaculture has been in response to declines in commercial harvests of wild stocks of fish and shellfish. Top aquaculture producers In 2008 were China with 62 percent of world aquaculture production of fish, crustaceans and molluscs (32.7 million tonnes). Other countries producing over one million tonnes in the same year are India (3.5 million tonnes), Vietnam (2.5 million tonnes), Indonesia (1.7 million tonnes), Thailand (1.4 million tonnes) and Bangladesh (1 million tonnes). Carps are the most cultured species in the world with 39 percent of production by volume. Other major groups cultured include shellfishes (oysters, clams, mussels and scallops), other freshwater fish includes tilapias, followed by shrimps, prawns and salmons. At single species value level, white leg shrimp generated the highest value (USD 9 billion) in 2008, followed by Atlantic salmon (USD 7.2 billion), grass carp (USD 4.8 billion), silver carp (USD 4.8 billion).

India is a major maritime state and an important aquaculture country in the world. It is also home for more than 10% of global fish biodiversity. India has achieved considerable production increases in aquaculture, especially in the production of freshwater fishes and shrimps. While progress in research and development of new technologies have already made in mollusk culture, seaweed culture, and in culture of certain marine fishes like seabass these have not yet taken off on commercial scale. The production gap in aquaculture between China and India or other important Asian countries are very wide, so effective utilization of the diversity of our marine living resources for aquaculture, in the long coastline will increase Indian aquaculture production.

Advantages of aquaculture

- Aquaculture is the important source of excellent quality protein and healthy oils
- Future for fish production is dependent on aquaculture

- Due to production of fish at low cost, it can be supplied at an affordable price even to poorer peoples
- Cultured fishes are safe from captured fish because cultured fishes are free from pollutants
- Aquaculture provides good quality food for the growing population
- Increases employment opportunity

Disadvantages of aquaculture

- The infrastructure development for aquaculture will affect the local flora and fauna like wetlands and mangroves
- The untreated effluent discharged with heavy organic load will adversely affect the local ecosystem
- Farming of exotic species would bring with new pathogen to the new environment
- Disease and parasite transfer from captive stock to wild

In the present era of food insecurity, aquaculture shows enormous potential to feed not only the ever increasing human population but also the aquaculture products can be utilized as a feed ingredient in the diets of different domesticated animals of high commercial value. The global developments and the strategic importance of aquaculture in terms of food security contribute to give aquaculture a promising future. The aquaculture sector has become a modern, dynamic industry that produces safe, high valuable and high quality products, and has developed the means to be environmentally sustainable. Aquaculture over recent years has not only led to substantial socioeconomic benefits such as increased nutritional levels, income, employment and foreign exchange but has also brought vast un-utilized and under-utilized land and water resources under culture.

In near future world will have to face the challenge of “food gap” which is the difference between production and demand for food. This could be more than double in the developing world during in the next 25 years and increasing dependence on imports from developed countries to the under developing countries will be more. To fill the “food gap” the productions from Agriculture and Aquaculture have to improved in a sustainable way.

Different systems of Aquaculture

- **Introduction**

3.1.1. Different systems of aquaculture

Aquaculture practices are classified in several ways, depending upon the different aspects and situations involved in the culture practice. Some major and important classifications are given below based on the different factors involved in aquaculture.

On the basis of salinity

- Freshwater farming
- Brackishwater farming
- Marinewater farming

On the basis of intensity

- Extensive fish farming system
- Semi-intensive fish farming system
- Intensive fish farming system

On the basis of fish species

- Monoculture
- Polyculture

On the basis of enclosure

- Pond culture
- Cage culture
- Pen culture
- Race-way culture

On the basis of integration

- Agriculture cum fish farming
- Animal husbandry cum fish farming

Introduction

On the basis of salinity

Freshwater Farming

Farming of aquatic animals and plants in zero saline water, mostly fresh water farming is inland based. Catla, Rohu, Mrigal, Silver carp, Grass carp, Common carp and Fresh water prawn are mainly farmed in fresh water.

3.1.2.1. Brakishwater Farming

Brakishwater is a mixture of seawater and freshwater with a salinity less than 30ppt. All estuaries, backwaters, creeks and mangrove waterways are brakish in nature. Over 25 species of commercially important fishes, shrimps, crabs and mollusks offer a wide scope for farming in brakishwater.

3.1.2.2. Marinewater farming

Farming of aquatic animals and plants in sea water is commonly known as marinewater farming or mariculture. In mariculture rearing of commercially important fishes and shell fishes are done in open sea by installing cages.

3.1.3. On the basis of intensity of inputs and stocking density

Extensive fish farming system

Extensive fish farming system is the least managed form of fish farming, in which little care is taken. This system involves large ponds measuring 1 to 5 ha in area with stocking density limited to only less than 5000 fishes/ha. No supplemental feeding or fertilization is provided. Fish depends only on natural foods. Yield is poor (500 to 2 ton/ha) and survival is low. The labour and investment costs are low and this system results in minimum income.

3.1.3.1. Semi-intensive fish farming system

Semi-intensive fish culture system is more prevalent and involves rather small ponds (0.5 to 1 hectare in area) with higher stocking density (10000 to 15000 fish/ha). In this system care is taken to develop natural foods by fertilization with/without supplemental feeding. However, major food source is natural food. Yield is moderate (3 to 10 ton/ha) and survival is high.

3.1.3.2. Intensive fish farming system

Intensive fish farming system is the well-managed form of fish farming, in which all attempts are made to achieve maximum production of fish from a minimum quantity of water. This system involves small ponds/tanks/raceways with very high stocking density (10-50 fish/m^3 of water). Fish are fed completely formulated feed. Good management is undertaken to control water quality by use of aerators and nutrition by use of highly nutritious feed. The yield obtained ranges from 15 to 100 ton/ha or more. Although the cost of investment is high, the return from the yield of fish exceeds to ensure profit.

3.1.4. On the basis of number of species stocked for farming

Monoculture

Monoculture is a fish production system in which only one fish species is reared in a culture system. The major fish varieties reared in monoculture system are trout, tilapia, catfishes, carps, shrimp etc. Monoculture of high-value, market-oriented fish species in intensive system is a common practice throughout the world. Supplementary feeding is compulsory to ensure production.

3.1.4.1. Polyculture

Polyculture is a fish production system in which two or more different fish species are farmed or culture of fish along with some other aquatic animals like shrimp or prawn. In this system of culture species with different habitats and different food preferences are stocked together in such densities that there will be almost no competition for food or space. Polyculture practices give higher yield than monoculture under the same conditions for freshwater carp farming.

3.1.4.2. Biological basis of polyculture

Common fish species in Indian polyculture are catla, rohu, mrigal, silver carp, grass carp and common carp, and this system is sometimes called as composite fish culture. The biological basis of polyculture is different fish species grow together in a pond with difference in feeding and living behavour.

The principal requirements of the different species in combination for polyculture are

- They must be different in feeding habits
- They should occupy different columns in a pond system
- They should attain marketable size at the same time
- They should be non predatory in behaviour

3.1.5. On the basis of enclosure used for culture

Pond culture

It is the most common method of fish culture. In this case water is maintained in an enclosed area by artificial construction of dike/bund, where aquatic animals are stocked and grown. Ponds are usually filled by rain, canal water and by man made bores. They differ widely in shape, size, topography, water and soil qualities.

3.1.5.1. Cage culture

Cage culture is rearing of fish from juvenile stage to commercial size in a volume of water enclosed on all sides including bottom, while permitting the free circulation of water. Cage culture is readily adapted to water areas which cannot be drained. Fish culture in cage is an innovative concept to exploit the potential of lakes, reservoirs and riverine pools. Cage culture of fish and other aquatic organisms is popular in many countries. Japan, South Korea, China, Philippines, Thailand, Malaysia, Germany, Norway, USA are some of the countries where cage culture is well developed. In principle, almost every cultivable species of fish can be cultured in cages, such as carps, tilapia, trout, catfishes, etc. depending on socioeconomic, ecological and technical suitability.

Advantages of Cage Culture

- Use existing waterbodies
- Technical simplicity with which farms can be established or expanded
- Lower capital cost compared with land-based farms
- Easier stock management and monitoring compared with pond culture

Disadvantages of Cage Culture

- Stock is vulnerable to external water quality problems eg. Algal blooms, low oxygen
- Stock is more vulnerable to fish eating predators such as water rats and birds
- Growth rates are significantly influenced by ambient water temperatures

3.1.5.2. Pen culture

Pen culture is defined as raising of fish in a volume of water enclosed on all sides except bottom, permitting the free circulation of water at least from one side. This system can be considered a hybrid between pond culture and cage culture. Mostly shallow regions along shores and banks of the lakes and reservoirs are used in making pen/enclosure using net/wooden materials where fish can be raised. In a fish pen, the bottom of the lake forms the bottom of the pen. Pen has the advantage of containing a benthic fauna which serves as food for the fish and polycultue can be practiced in pens as it is in ponds. The environment in fish pen is characterized by a free exchange of water with the enclosing water body and high dissolved oxygen concentrations.

Advantages:

a. Intensive utilization of available space : Stocking density can be increased compared to that of a pond culture system

b. Safety from predators: Within the enclosure the predators can be excluded. In the larger pens this would be more difficult, but in smaller pens this can be done as efficiently.

c. Suitability for culturing many varied species : Due availability of more space and the natural water system

d. Ease of harvest : In the large pens the harvest may not be as easy as in cage rearing but it more controllable and easier than in the natural waters.

e. The flexibility of size and economy : When compared with the cage, pens can be made much larger and construction costs will be cheaper than that of the cages.

f. Availability of natural food and exchange of materials with the bottom : Since, the bottom of the pen is the natural bottom, the pen cultured organisms are at an advantage that they can procure food/exchange materials from the natural bottom.

Disadvantages:

a. High demand for oxygen and water flow

b. Dependence on artificial feed

c. Food losses : Part of the feed is likely to be lost uneaten, and drifted away in the current, but the loss here would be less than in floating cages.

d. Pollution : Since a large biomass of fish are cultured intensively a large quantity of excrements accumulate in the area and cause a high BOD - also substances such as ammonia and other excreted materials, if not immediately removed/ recycled. They pollute the water and cause damages.

e. Rapid spread of diseases : For the same reason of high stocking density in an enclosed area, any disease beginning will spread very quickly and can cause immense mortality of stock and production decline.

f. Risk of theft : Since the fish are kept in an enclosed area, 'poaching' and thefts can take place more frequently than in natural waters, but perhaps less than those from cages.

g. Conflict with multiple use of natural waters : In locations where a pen is constructed, if the water is used for multipurpose like irrigation and recreational activities, such as swimming, boating etc. may lead to conflicts.

3.1.6. Raceway

Raceway culture is defined as raising of fish in running water. It is a high production system in which fishes are grown in higher stocking density. Raceways are designed to provide a flow-through system to enable rearing of much denser population of fishes.

Raceway ponds are basically of two types:

Linear type : Ponds arranged in sequence. In a linear type, the volume of water entering each pond is larger and as the same water is used repeatedly from pond to pond, occurrence of disease in initial ponds may directly affect the other connected ponds

Lateral type : Ponds laid out in parallel.n a lateral or parallel type the volume of water entering each pond is smaller but a fresh supply of water is always ensured, and no transfer of disease from one pond to another.

3.1.7. Recirculating Aquaculture system (RAS)

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

3.1.8. On the basis of different farm integration

Fish farming with agriculture

In the fish integrated agriculture system, fish culture is integrated with agricultural crops such as rice, banana and coconut, thereby producing fish and agricultural crops. Agriculture based integrated systems include rice-fish integration, horticulture-fish system, mushroom-fish system, seri-fish system.

3.1.8.1. Rice-Fish integrated farming

In this system of farming fish is farmed in paddy fields, not all paddy varieties are suitable for integrated fish farming. Varieties with strong root system like Tulsi, Panidhan, CR260 77, ADT 6, ADT7, Rajarajan and Pattambi 15 and 16 are suitable for farming in combination with fish because it has strong roots to withstand flood conditions. The fish species such as Common carp, Tilapia and Murrells are most suitable for culture in rice fields.

3.1.8.2. Horticulture-Fish integrated farming

The dykes and the adjoining areas of the ponds can be best utilized for horticulture crops. The top, inner and the outer dykes can be planted with dwarf variety coconut, mango and banana. And the side by land can be used for planting pineapple, ginger, and turmeric and chilly. The exchanging water can be used to water the plants which is rich in organic load. The residues from the vegetables cultivated could be recycled into fishponds, particularly when stocked with fishes like grass carp.

3.1.8.3. Mushroom-Fish integrated farming

Cultivation of mushroom requires high degree of humidity and therefore its cultivation along with aquaculture tremendous scope. *Agaricus bisporus* , *Voloriella* spp. and *Pleurotus* spp., are commercially cultured mushrooms in India.

3.1.8.4. Seri-Fish integrated farming

In this faming system silk worm is cultured along with fish. Here the mulberry leaves produced is primarily consumed by the silk worm and the faeces of the silk worm is directly applied to the fish pond to increase of natural food organism-detritus and bacteria in fishpond.

3.1.9. Livestock integrated fish farming

Livestock integrated fish farming system includes cattle-fish system, pig-fish system, poultry-fish system, duck-fish system, goat-fish system, rabbit-fish system. In this integrated farming the excreta of ducks, chicks, pigs and cattle are used directly in ponds to increase plankton production which is consumed by fish or serve as direct food for fish. Hence, the expenditure towards chemical fertilisers and supplementary feeds for fish ponds are totally avoided reducing the production cost.

3.1.9.1. Cattle-Fish integrated farming

Cow dungh is the most widely used manure, in fish ponds all over the world. A healthy cow excretes over 4,000-5,000 kg dung, 3,500-4,000 litre urine annually. For 1 ha pond 5-6 cows can provide adequate manure. An additional income is generated from milk (9,000 litres/year) and fish production ranges from 3,000-4,000 kg fish/ha/year.

3.1.9.2. Pig-Fish integrated farming

In this farming system 60-100 no of pigs are enough to fertilize one hectare area fish pond. A floor space of 3-4m^2 is required for a single pig. Five tones of pig manure is required for manuring 1 ha fish pond for 1 year. Pigs are fed with kitchen waste, aquatic plants and crop wastes. The waste produced by 30-35 pigs is equivalent to 1 tonne of ammonium sulphate. Exotic breeds like White Yorkshire, Landrace and Hampshire are reared in this farming system. Grass carp, silver carp and common carp (1:2:1 ratio) are suitable for integrated farming with pigs.

3.1.9.3. Poultry-Fish integrated farming

Chicken droppings are rich in phosphorus and nitrogen, so chicken manure is an effective fertilizer. For 1ha fish pond 25,000 chicks can be reared. Poultry shed is constructed above the pond with bamboo flooring to facilitate the direct fertilization of the pond. One chicken produces 25 kg poultry manure per year. From poultry 90,000 to 1,00,000 eggs and 2500 kg meat can be produced and 3000 – 4500 kg of fish can be produced without any chemical fertilizer and supplementary feeding.

3.1.9.4. Duck-Fish integrated farming

In Duck-fish integrated farming, ducks provide a safe environment to fish by consuming juvenile frogs, tadpoles and dragonfly in the pond. As the duck spends most of its time swimming in the pond the dropping goes directly in pond, which in turn provides essential nutrients to stimulate growth of natural food in the fish pond. The duck dropping contain 25 per cent organic and 20 per cent inorganic substances with a number of elements such as carbon, phosphorus, potassium, nitrogen, calcium, etc. Hence, it forms a very good source of fertilizer. To fertilize 1 ha fish pond number of ducks required is between 100 and 3,000, depending on the duration of fish culture and the manure requirements.

Small ruminants such as goats and sheep are integrated with fish culture is practised, but on a very small scale. Integrated rabbit-fish farming is also practiced only on a very small scale. This system has up to now not received much attention.

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – II - AQUACULTURE – SBT1608

UNIT 2 HATCHERY AND GROW- OUT PRODUCTION OF AQUATIC ORGANISMS

Design and construction of a fish hatchery - Types of hatcheries and management practices - Live feed culture: culture of microalgae, rotifers and crustaceans (Artemia) - Selection of brooder, nutrition, gonadal changes, hormonal regulation - Culture of economically important marine species: *Litopenaeus vannamei* (shrimp), *Lates calcarifer* (seabass), Lobster, seaweeds - Culture practices of freshwater species: Prawns, carps, catfish, murrels and ornamental fishes.

LIVE FEED CULTURE

Introduction

The early life stages of seabass and gilthead seabream are zooplankton-feeders, i.e. they prey on small free living planktonic animals. As no artificial larval diet can at present totally fulfil their nutritional requirements, their successful rearing still depends on an adequate supply of high quality live feeds, usually in the form of rotifers (fed on unicellular algae) and brine shrimp *(Artemia spp).*

This chapter describes the equipment and operation to mass produce these organisms, whose biology has been presented in Part 2. The design of the hatchery sections for production of live feed is described in the Design and Engineering part of this manual.

The technology for phytoplankton and zooplankton mass production has become very reliable and the production of live feeds is part of the standard working procedures in Mediterranean hatcheries. The efficiency of this part of the hatchery mainly depends on the implementation of standard procedures by well trained staff.

As live preys for first postlarval stages of seabass and gilthead seabream, two small animals are extensively used:

- all-female (amictic) populations of the rotifer *Brachionus plicatilis* (60 - 350 mm in length);
- larval stages (nauplii and metanauplii) of a small crustacean, the brine shrimp *Artemia spp.* (length: 400 - 800mm).

In farms dealing with seabass only, live feed production is often limited to the hatching of *Artemia* nauplii, which are obtained by incubating their dry resting eggs (cysts). The mouth of the seabass at first feeding is large enough to gulp brine shrimp nauplii and these larvae do not require the smaller rotifers as first feed, as it is in the case of gilthead seabream. The hatcheries working on both species, or on gilthead seabream alone, have to produce rotifers as well as microalgae.

As said in previous sections of the manual microalgae are now used not only for rotifer production (see below), but also to improve water quality in the larval tanks, creating the so called “green water”, which is used during the initial rearing phases.

In case of rotifer mass production, clear advantages of this organism are given by its fast reproductive rate and by the high densities that can be reached in the rearing facilities, up to 1,000 rotifers per ml and over. The daily increase of their populations ranges between 50 and 150%, depending on the production technique chosen and the nutritional value of their diet. Their main drawback is that to culture them microalgae are needed as food, at least in the initial steps. However, for their final production process in large volumes (see below) there are now good artificial feeds which can replace algae, whose mass production remains unavoidable at least in gilthead seabream production for the “greenwater.

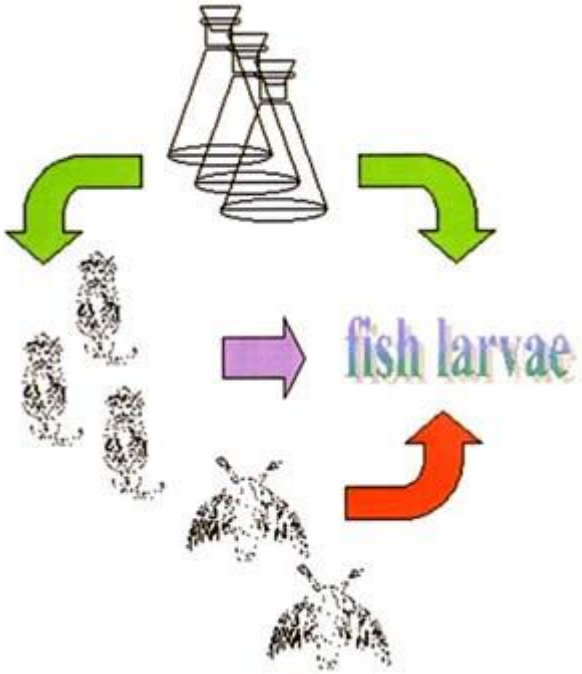


Fig. 23.00 A sort of simplified trophic chain is established in the hatchery.

On the other hand, the production of *Artemia* is greatly facilitated by the availability of dry resting eggs, which can be purchased from specialised suppliers. If properly canned and stored, brine shrimp cysts can remain viable for years.

As rotifers and nauplii are produced to fulfil the needs of the larval rearing unit, they have to be available at given times, in pre-set quantities and with their nutritional quality intact. To achieve this, the design and operation of the culture systems should pay special attention to the following points:

- adequate dimensioning of the production facilities, including additional space for back-up cultures as a precaution against culture collapses;
- proper daily renewal and up-scaling of the cultures (standard operating procedures);
- maintenance of strict hygienic conditions in the culture environment (cleaning procedures);
- close control of culture conditions (monitoring procedures).

Mass culture of microalgae

Mass production of phytoplankton for rotifers and "green water" in most Mediterranean hatcheries is limited to a few species such as: *Chlorella sp*, *Isochrysis galbana*, *Pavlova lutheri*, *Nannochloropsis oculata* and *N. gaditana*, *Dunaliella tertiolecta* and *Tetraselmis suecica*. These species have been selected on the basis of their size, nutritional value, culture easiness and absence of negative side effects, such as toxicity. Their nutritional value shows a great variability not only among different species, but also in genetically different populations of the same species (strains). For hatchery purposes, the species to be cultured should both fit well the local rearing conditions and have a high nutritional value for rotifers. The increasing availability of nutritional boosters as enrichment diets for both rotifers and brine shrimps, has made this choice easier.

Fig. 23.01 Mass culture of microalge (photo STM Aquatrade)

Population dynamics

Microalgae population dynamics can be described by different phases:

- the lag-phase, where, just after the inoculum, the cells increase in size, but not in number, and begin to absorb the nutriens supplied with the culture medium;
- the log-phase (or esponential phase), where cells reproduce very fast and population growth is exponential;
- the transitional phase (or declining growth phase), where growth rate slows down;
- the stationary phase, where cells remains constant in number and reproduction is balanced by death;
- the decline phase, where cell number decreases since death rate exceeds growth.

It is advisable to harvest phytoplanktonic organisms during their log phase, since in the new culture they will grow more rapidly and will yield a more viable population.

Mass production systems

For aquaculture purposes, microalgae are mass produced in three main ways: (i) batch (or discontinuous or multistep back-up system) culture, (ii) semi-continuous culture, and (iii) continuous culture.

In the batch culture a small axenic stock culture produces a series of cultures of increasing volume where the algal population of each culture vessel is entirely harvested at or near its peak density, i.e.

while still conserving a good growth potential, to be used either as inoculum for other culture vessels, or to feed rotifers or be used in fish larval tanks. It typically makes use of small (few liters) to medium size (500 liters) containers, and it is kept indoor and under strictly controlled, if not properly axenic, conditions. It is considered by many authors the easiest and most reliable method of algal production, provided that the working protocol is strictly enforced. Algal quality is less erratic than in the semi-continuous method, even if the latter is more productive for any given volume.

In the semi-continuous system the algal population, when mature, is partially harvested at intervals. The harvested culture volume is replaced by fresh medium to keep growth going on. This culture is adopted to produce large amounts of algae and frequently uses large outdoor tanks. Their main drawbacks are: (i) the unpredictable duration, (ii) the risk of contamination by other organisms as competitors (other microalgal species), contaminants (bacteria) and predators (ciliate protozoa feeding on the algae), as well as (iii) the building up of metabolites, which can affect quality.

The continuous system is a steady-state continuous flow culture in which the rate of growth is governed by the rate of supply of the limiting factor. It is a balanced axenic system where the algal population is harvested and fertilised continuously. This method, though the most efficient over extended periods, produces limited amounts of high quality cells and requires complex equipment as well as advanced management. A relatively recent development of this system is represented by the photo-bioreactor, a continuous culture device that increases the density of cultured microalgae to very high levels under predictable environmental and microbiological conditions.

The microalgae produced can be concentrated to a dense liquid suspension by centrifugation, and can then be stored for more than one month in the refrigerator, still giving excellent viability when used. A new industry is now appearing, whose concentrated algal products can also fulfil the hatchery needs, saving the time-consuming and expensive production of microalgae in the hatchery.

The system described below is the batch culture, by far the most widely adopted method by Mediterranean hatcheries. Before its description, additional instructions are given concerning facilities, the preparation of the culture medium, and the equipment required.

Fig. 23.02 Old fashioned unit using artificial light for algae mass culture (photo M. Caggiano)

Mass culture facilities for microalgae

Algae are cultured in a dedicated sector of the live feeds production section, which is made of three working areas inside the hatchery building: a lab for duplicating small cultures, a conditioned room to maintain small culture vessels and pure strains and finally a large area for the mass cultures in PE bags or, less frequently, tanks. In the warmest Mediterranean areas, a light greenhouse can replace the latter.

Small volume cultures are kept in vessels ranging from 20-ml test tubes up to 18 l carboys. They can be made of borosilicate glass, polycarbonate, PET or any other material able to stand a sterilization process. These vessels are placed on glass shelves lightened by fluorescent tubes and equipped with a CO_2 enriched air distribution system.

Hot-extruded tubular PE film is utilised for larger volumes bags. The film is usually 0.25 mm thick and its stretched width ranges from 45 to 95 cm. Two bag designs are widely adopted in Mediterranean hatcheries: the smaller suspended bag and the larger one placed within a steel wire cylindrical frame. The first type has a capacity of 60 I (single) to 150 I (double or U-shaped), whereas the latter, that stands on a saddle-like GRP base to improve circulation, can contain up to 450l. Their top is closed by a plastic cover to prevent contamination.

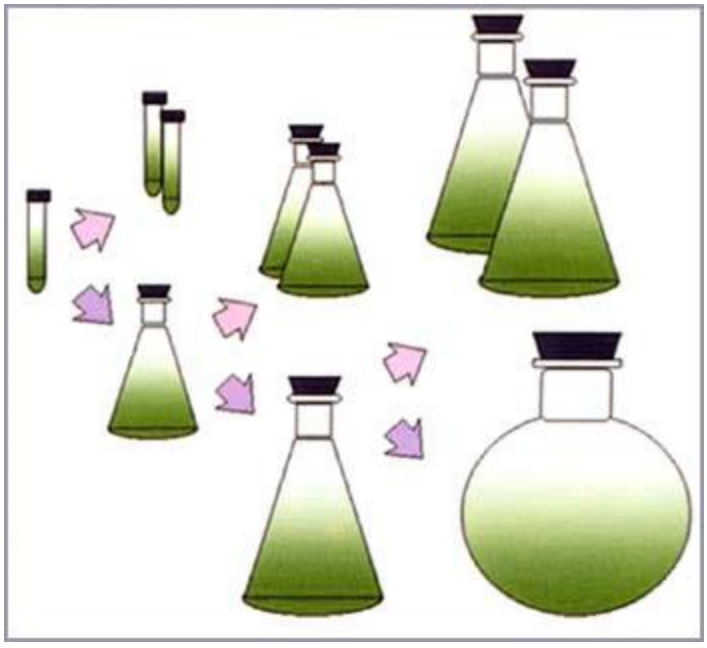


Fig 24.00 A typical scheme of a batch type production

All units are equipped with artificial lights, usually fluorescent tubes, an aeration system, often with an additional source of carbon dioxide, and stands for the culture vessels, i.e. light shelves for small volumes and metal racks or wired frames for PE bags.

The unit also stores the special equipment to process pre-treated seawater, such as fine filters and sterilizers, as well as a laboratory where nutrients and glassware are prepared and stored, and where the necessary monitoring operations are performed. Standard cleaning procedures have to be strictly followed to maintain proper hygienic conditions

---

4.1.4.1. Production unit setup

Standards: The production unit should manage the surrounding environment from any impacts like escapement of cultured organism, spreading of disease, avoiding the use of synthetic and chemical fertilizers and paints. Consideration for the surrounding environment is crucial for positioning and management of the organic unit.

4.1.4.2. Environment / Water Quality

Standards: The water system must be loaded to the minimum possible extent with feed wastage and faeces that can cause over-fertilization or other disturbances to natural environment. Aqua farmers should not deplete nor excessively exploit the available water resources, and must preserve the natural water quality.

4.1.4.3. Breeds and Breeding

Standards: Breeds that are adjusted to local conditions should be used. Breeding must be done on a large number of breeding pairs to prevent inbreeding, enetic damage and loss of genetic variation. Triploid, genetically modified and sex reversed organisms should not be used.

4.1.4.4. Feed and feeding

Standards: The feed should consist of organic raw materials originating from wild aquatic stocks. For management of fishery resource, aquatic raw materials from stocks that are not used for human consumption and from by-products must be used for feed preparation. Feeding must be performed in a way that allows natural feed available in pond system also gets consumed with minimal wastage of the supplemented feed.

4.1.4.5. Additives

Standards: Additives such as growth promoters, hormones and appetizers should never be used and the usable additives like vitamins, minerals, antioxidants and colouring agents should be of natural in origin or it should be close to natural form as possible. Synthetic and unnatural additives must not be used in organic farming system.

4.1.4.6. Treatments and animal health welfare

Standards: Considering the health management in aquatic organisms “Prevention is better than cure” concept should be followed so that there will not be any need for medication. If there is still sign of disease, suitable measures shall be adopted immediately. Drugs with the minimum environmentally harmful effect and with the minimum risk to human and animal health should be used for treatment.

4.1.4.7. Record keeping

Standards: It is a very important protocol to be followed in feed management and in disease management of the aquatic organism. For disease management record must have all the details related to disease and its treatment procedures followed. The farm manager should maintain a monthly record of the feed type, feed producer, and quantity fed used till the end of the crop.

A universally accepted standard for organic aquaculture practices does not currently exist . To risk investment in this sector, producers require formally recognized standards in order to communicate the advant ages of the organic aquaculture products to consumers. The key to the continued growth and development of organic aquaculture lies in resolving a number of issues that currently stand in the way of instituting internationally accepted certification standards .

Unit 5: Site selection and construction of fish farm

5.1.1. Site selection

Proper selection of suitable site for farming is the important factor in determining the success of a fish farm. The selection of suitable site for construction of pond depends on two main factors such as the water retention capacity of the soil and its inherent fertility and also its capacity to respond readily to organic and inorganic fertilization. Besides these, there should be dependable perennial source of adequate water supply to fill in ponds at any time of the year. The site should preferably be gently sloping, so that self draining ponds can be possible on higher elevation. In swampy and marshy areas, bunds have to be piled up by depositing earth to construct ponds of required size. The site should be easily accessible by road or rail with market nearby the farm for easy disposal of fish. The necessary inputs like feeds, fertilizers and building materials for construction and other accessories should be available near the site. There should not be any industrial, domestic or insecticide and pesticide pollution at the site. The farm should have additional scope for integration of aquaculture with agriculture and animal husbandry. Site selection also depends on the kind of fish planned to be farmed. For site selection and pond construction following factors are to be considered,

1. Ecological factors

Soil

Water

Land

Climate

2. Biological and operational factors
3. Economic and social factors

5.1.1.1. Ecological factors

Soil

The quality of soil influences both productivity and water quality in a pond. However, it must also be suitable for dike construction. To determine soil suitability the most important properties to be examined are soil texture (particle size composition) and porosity or permeability (ability to let water pass through). The pond bottom must be able to hold water and the soil should also contribute to the fertility of the water by providing nutrients (Clay soil is more fertile) so the best soil for pond construction should contain more of clay. Mostly s ilt clay, clay-loam, loamy soils are generally suitable for a fish pond. A good quality soil should consist of 90% soil fraction and gravels not exceeding 10%. Rocky, sandy, gravel and limestone areas are to be avoided. In case of porous soil, pond bottom may be treated with bentonite, clay or other soil sealants. Plastic film liners can be used to reduce the rate of seepage. But such treatment apart from being expensive, prevent exchange of 'soil-water minerals and nutrients making the pond less productive. The three ways one should follow to predict whether the soil will be suitable for pond construction are:

a. Squeeze method

b. Ground water test

c. Water permeability test

a. Squeeze method

Wet hand full of soil is taken and squeeze the soil by closing your hand firmly, if it holds its shape after opening the palm of your hand, the soil will be good for pond construction

b. Ground water test

For reliable results this test should be performed during the dry period. Dig a hole with a depth of one meter and cover it with leaves for one night. If the hole is filled with ground water the next morning a pond could be built. But for draining probably the pond may need more time due to the high ground water levels. If the hole is still empty the next morning, then the site will be suitable for farming. Now as a next step soil should be tested for its water permeability.

c. Water Permeability test

To perform the permeability test fill the hole with water to the top and cover the hole with leaves. Next day if water level reduces then the soil has more water seepage. To reconfirm refill the hole with water to the top Cover it once more with leaves check the water level the next day. If the water level is still high, the soil is impermeable enough and is suitable for pond construction. If the water has disappeared again, the site is not suitable for fish farming, unless the bottom is first covered with plastic or heavy clays.

5.1.1.1.1. Water

Availability of an adequate and dependable source of water is another prerequisite for setting up a fish farm. The pond should be filled with water at regular intervals so as to adjust water depth. The usual source of water supply to fish farm are reservoirs, streams, springs, canals, surface run off (rain), wells, tube wells etc. However, the best thing to do is to have as far as possible natural, preferably rain Water and other large water bodies, because in natural 'water bodies, dissolved oxygen content, pH, water quality and water temperature are more stable and suitable for fish to grow. On the contrary, the water from underground source is often deficient in dissolved oxygen. The waste water discharged from factories and mines usually contain harmful chemicals which are not suitable for fish farming. The ponds should be at a safe distance away from rivers and reservoirs so as to avoid water flooding during rainy season. Water should not be either highly acidic or alkaline. It should be corrected if acidic by the application of lime and if alkaline by adding organic manure. Water parameters should also be taken into consideration in selection of an area for faming

(a) Water temperature

The water temperature is an important condition in assessing whether the fish species selected can be raised. A water temperature between 20°C and 30°C is generally good for fish farming.

(b) Water salinity

Variation in water salinity (amount of dissolved salts in the water) is also an important factor which must be considered. Some fish species can withstand a wider salinity range than others: e.g. tilapia and catfish can withstand a wide range from fresh- to seawater while carp can only withstand freshwater.

Fresh water sources for fish farm and their main disadvantages

Sl.No	Water source	Disadvantage
1	**Rainfall -** "sky" ponds rely on rainfall only to supply water	**Dependency -** The supply depends heavily on amount of rain and seasonal fluctuations
2	**Run-off -** Ponds can be filled when water from the surrounding land area runs into them	**High turbidity -** Turbidity is the amount of mud in the water. In case of run-off the water may be muddy. Danger of flooding and pesticides (or other pollutants) in the water
3	**Natural waters -** Water can be diverted and brought in from streams, rivers or lakes	**Contamination -** Danger of pesticides (or other pollutants) animals, plants and rotting organisms can cause diseases. in the water
4	**Springs -** Spring water is water under the ground that has found a way to get out. Spring water is good for fish ponds because it is usually clean.	**Low oxygen level and low temperature**
5	**Wells -** Wells are places where ground water is pumped up.	**Low oxygen level, low temperature and toxic gases presence**

5.1.1.1.2. Land (Topography)

Topography of the land determines the type of pond to be constructed. The land selected for farming should be with slope not steeper than two percent. The land should be free from high flooding; the high flooding recorded for the past ten years should not be higher than the top of the dyke planned for the pond construction. The land with regular shape is more suitable for farm construction. A with scope for industrialization which may cause pollution in future should be avoided. Likewise area already located with industries must be avoided. Areas having underground oil pipe lines, high

electricity power poles and radio masts are not considered suitable for farm. Lands with heavily rooted vegetation are also not suitable for construction of farm.

5.1.1.1.3. Climate

The following important climatological factors are to be collected from the metereological station or from the past incidences from the selected area for farm construction

a. Temperature
b. Rainfall
c. Evaporation
d. Humidity
e. Sunshine
f. Wind speed and direction
g. Incidence of high rainfall, wind/heavy storms and earthquake

5.1.1.2. Biological and operational factors

Before initiation or finalization of a site for farm construction the following factors are to be ascertained

a. Species to be cultured
b. Availability of seed for farming
c. Type of project based on investment
d. System of culture to be adopted

Based on these biological factors only an area has to be selected for farming

5.1.1.3. Economic and social factors General standards for Organic aquaculture production

Land without any legal problems should be selected; the selected area for farming should not have any local problems or conflicts with the local population residing. The selected site should have road facility which is accessible in all weather conditions, availability of electricity, communication

facility (Telephone or mobile), availability of construction material, equipments, labour (skilled and semi skilled), staff, hospital, school, bank, transport etc and all other facilities required to run a farm.

5.1.2. CONSTRUCTION

Before construction of a pond a proper design and lay out should be prepared. The size and shape of the pond should be designed in such a way that the excavated earth should be used for construction of dyke. The land is to be excavated with a gradual slope towards the outlet for proper draining facility. Construction of pond must be planned in such a way that, construction must get completed in summer so the pond can be used for stocking.

5.1.3. Different kinds of ponds and its requirement ratio in a farm unit

Fish culture commences with the construction of ponds. Specific types of ponds are required based on species of fish and various life history stages. Various ponds like nursery, rearing, stocking and treatment ponds are required for a fish farm. The ratio of these ponds varies in relation to farming practice, stocking density and survival.

The ideal shape of the pond should be rectangular. Length and breadth in the proportion of 3:1 is ideal. In any case, the breadth should not be more than 30-50 meter as it is difficult to operate efficiently a net longer than 50 meter. The corners of the pond should be rounded, so that while operating net, fish may not escape. In Indian conditions the total fish farm should be constructed consisting of nursery pond (5% of the total area), rearing pond (20% of the total area), stocking ponds (70% of the total area) and bio pond or treatment pond (5% of the total area).

5.1.4.Size and depth of the different kinds of pond

Other factor involved for site selection, is the purpose of farm. The purpose of the farm depends how large is the size of the area. A Scientifically constructed fish farm has 3 types of ponds, which comprise of nursery, rearing, stocking, brooder pond and breeding ponds etc., a large area is required.

Nursery pond

From hatchery spawn which is a 3 day old larvae are produced. The larvae are reared till fry stage (2-3 cm length) for 11 to 30 days. During this period, they are reared in nursery ponds. The ideal size of each fry pond is 0.01 to 0.05 ha and their number varies in accordance to target production with a

water depth of 1-1.5 m. The shape of a nursery pond is regular as a rectangle and flat even bottom for easy netting operation.

Rearing tank

Similarly the fry are reared to fingerling stage in the rearing tank (10-15 cm length) and the time period taken varies from 2-3 months. The size of each fingerling pond varies from 0.05 ha to 0.1 ha with water depth of 1.5 - 2 m and their number varies in accordance to target production of fingerlings. The shape of a fingerling pond is rectangular with flat even bottom for easy netting-operation.

Stocking pond

The stocking pond, each having an area of 1 ha to 2 ha is considered to be an optimal size for intensive culture of food fish with water depth of 2.5 - 3 meter. Fingerlings of 10-15 cm length are stocked in this pond until it reaches table size or marketable sized fish.

The number of stocking ponds varies according to the target fish production. The time period of rearing varies from 8-10 months. The stocking ponds are used as breeding pond or brooder raising pond as per the requirements. However, there is no hard and fast rule regarding the size of the ponds.

Bio pond or treatment ponds

Nowadays apart from the above mentioned fish pond in a fish farm a special type of pond- Bio pond is also seen in some farms. It acts as a large settling tank, where the water used for fish ponds of a farm is purified biologically. On need basis it may be used as stocking pond also. The area covered by this type of pond is 7- 10% of the total productive area of a fish farm.

5.1.5. Positioning of different kinds of ponds in a fish farm

The location of different kinds of ponds in a farm is of considerable importance for easy operation and minimization of operational cost. The detailed contour survey of the area is required before fish farm construction. This will minimize filling or digging and movement of earth, which all cause expenditure on labour or mechanical earth moving. .

The deeper and lower area of the farm site should be developed into stocking ponds. The appropriate higher areas can be developed in to rearing ponds and areas higher still into nursery ponds. The highest of the areas are to be developed for office building, staff quarters, laboratory and hatchery.

5.1.6. The above mentioned pond of a fish farm is constructed in 2 ways and they are

Dug out pond: Dug out ponds are constructed in a plain area by digging soil. This type of fish pond is more suitable for fish farming as they can be constructed by the fish farmer based on their requirements scientifically by maintaining the shape, size, depth, etc.

Embankment pond: This type of pond is constructed in hilly areas. This is constructed by erecting dyke on 2 sides or in 1 side of the selected place on need basis. This is economic to dig out pond from the construction side, but it is not good from the fish culture point of view. This is because the size, shape, depth, etc. cannot be fixed as per the scientific fish culture specification, which are depends upon the site's configuration. Normally this type of pond is constructed in hilly places by erecting embankments to a suitable height for fish culture with provisions of inlet and outlet. Here in the inlets and out lets small mess size bamboo made or nylon made screen is tied. This prevents the entry of unwanted fish, aquatic insects, etc. into the culture system and also stops the escaping of cultured fishes from the culture system.

5.1.7. Steps to be followed in construction of a fish pond are

1. Prepare the site by removing trees, bushes, rock and mark the area for pond construction
2. Construction of clay core to build leak free and strong dyke
3. Digging pond and construction of dyke over the clay core
4. Construction of Inlet and outlet
5. Protect the pond dyke by covering with soil and plant grass such as Rhodes grass (*Chloris gavana*) or Star grass (*Cynodon dactylon*). Do not use long rooted trees or plants as this will weaken the dyke.
6. Fencing of pond will protect from theft and predatory animals

5.1.8. Dyke

Dyke should be compacted, solid, leak proof so as to conserve fertile water. Dyke should be stable in all weather conditions. Height should be more than 0.3 - 0.7 m above the maximum flood water level. For dyke construction, a soil containing 15-30 per cent silt, 45-55 per cent sand and 30-35 per cent clay is most suitable.

A berm of sufficient width should be provided for stabilizing the slope. Wider berm also helps in operating the net in the pond. The berm should be sufficiently wide and in no case should be less than 1 m. The slope of the embankment in horizontal to vertical axis should be 2:1 in good quality clay soil. The slope (base: height) in loamy silt or sandy soils should be 3:1. While raising the dyke, the clay puddle (mixture of 1:2 of sand and clay) is deposited in 10-15 cm thick layers with a precaution that it should be adequately moistened before next layer of clay puddle is laid. The puddle may be at the centre or in the water side of the pond. The crest or crown of the dyke should be sufficiently wide, so that allied farm activities can be taken up gradually and the minimum· top width of the embankment should be about 1m. Excess water outlet pipes can be provided on the embankment, as safety measure so as to safeguard against damage due to excessive rise in water level.

Short creeping grass is recommended to be grown in the top and sides of dyke to check soil erosion. On wider embankment, commonly-banana and coconut trees are planted so as to avoid shade in the morning hour when photosynthesis starts. Even in wider crest and slopes, terrestrial grasses like hybrid Napier, gunny grasses and elephant grasses are cultivated to supply feed wholly or partially to herbivorous fishes like grass carp reared in cultured tanks.

5.1.9. Inlet canals

Feeder canals are essential, except for those ponds which are filled directly by spring or by rainwater. Feeder canal should be constructed in such a way to provide the required quantity and quality of water to the pond. The inlet pipe should be placed above the maximum water holding level of the pond. The inlet pipe should be provided with screen to filter the pumping water.

Standard inlet pipe size used based on the size of the pond

POND SIZE (m2)	INSIDE DIAMETER OF PIPE (cm)
<200	not less than 10

200-400	10-15
400-1 000	15-20
1 000-2 000	20-25
2 000-5 000	25-30
>5 000	40 or more

5.1.10. Outlet canals

Outlet system or the drainage canal is used to empty the pond during harvest or for partial draining of the pond water for exchange of water during culture period to maintain the water quality of the pond. A outlet is constructed before construction of the pond dyke.

5.1.11. Different outlet canals or draining systems

a. Rivaldi valve

Rivaldi valve is flexible pipe system named after the farmer from Paraguay, who first used this drainage system. The flexible is placed on the ground before construction of dyke and the pipe is turned up and tied to a stake or a pole above the pond's maximum filling level. At the time of draining the stake is removed and the pipe is lied down. This also allows the excess water to flow out during heavy rainy season.

b. Sluice

Sluice can function as both inlet and outlet canal based on placement in the pond. A sluice is a cement structure mostly used as outlet system of a pond; it consists of different slot or grooves structures which are closed by wooden gates. The wooden gates are normally made of different pieces which are placed in the groves and the space between the two wooden gates is filled with earth. Apart from this a wooden framed meshed gate is also placed in one of the groove to avoid escapement of fish during exchange.

c. Monk

Monk is similar to that of the sluice but built inside the pond dyke and on the dyke of the pond. Monk can never be used as inlet as sluice can be. The monk structure consists of a horizontal drainage pipe

and the vertical structure, or monk. The drainage pipe runs from back side of the monk. Monk structure is constructed in such a way it is closed on three sides and open on one side. The two parallel sides and the bottom of the cement structure have grooves, which are placed with wooden and meshed gates.

Major Cultivable Freshwater Fishes

8.2.1. Carps

8.2.1.1. Indian Major carps

8.2.1.1.1. Catla

Common name : catla
Kingdom :Animation
Phylum :chordate
Sub Phylum :Vertebrata
Class : Actinopterygii
Order :cypriniformes
Family :cyprinidae
Genus : catla
Species :catla
Scientific Name :catla catla
Specific characters:

Distribution:

Tropical freshwater in India, Pakistan, Burma.

Physical appearace and special features

Body is deep. Head is large and very curspicuous. Mouth is large and upturned. Lips are nonfringed and no barbles Body is greenish dorsally and silvery on sides and ventrally. Structure with a big head, strong fins with more body depth and big scales.

Habitation in pond

Pelagic habitat

Feeding behavior

surface feeder. It filters the plankton available esp. zooplankton. Gill rakers are specially adapted for filtering specific food organism from the water.

stage of maturity

catla attains maturity by the end of its 2nd year.

Fecumdity

fecundity is very high; 2 lakh to 4.2 lakh.

g.Catla grows to a length upto 45 cm, weighing more than a kilogram in one year and attains 2.2 kg and 6.5 kg at the end of 2nd and 3rd year respectively.

Breeding:

catla breeds naturally in the open waters like the rivers, in particularly during monsoon season. The technique of induced breeding called hypophysation is successful in this species.

8.2.1.1.2. Rohu

Common Name	:Rohu
Kingdom	:Animalia
Phylum	:chordata
Sub Phylum	:Vertebrata
Class	: Actinopterygii
Order	:cypriniformes
Family	:cyprinidae
Genus	: labeo
Species	:rohita
Scientific Name	:labeo rohita

Specific characters:

Distribution

Tropical freshwaters of India, Pakistan and Burma. It is also cultured in India and in countries like Srilanka, Malaysia, Japan and Thailand.

Physical appearance and special features

Head is small and pointed. Mouth is terminal. The intra orbital space is flat. Lower lip of the mouth is fringed. Scales are light red in colour.

Habitation in Pond

This forms one of the component species of composite culture where it occupies middle column layer.

Feeding behavior

It is column feeder. It feeds on both phytoplankton and zooplankton. It is considered as the tastiest fish of all the carps.

Stage of Maturity

It attains the maturity by the end of 2nd year.

Fecundity

Fecundity ranged from 2 to 5 lakhs.

Growth

It is one of the fastest growing carp, it attains a length of 35 to 45 cm with a weight of 0.7kg to 1kg in the 1st year depending upon the conditions. At the end of 2nd year it reaches around 2 kg.

Breeding

It is capable of breeding in ponds after inducement by pituitary gland extract. In natural conditions it spawns once in a year. But by induced breeding, it can breed twice in a year.

8.2.1.1.3. Mrigal

Common Name	:Mrigal
Kingdom	:Animalia
Phylum	:chordata
Sub Phylum	:Vertebrata
Class	: Actinopterygii
Order	:cypriniformes
Family	:cyprinidae
Genus	:**Cyprinidae**
Species	:**mrigala**
Scientific Name	:**cirrhinus mrigal**

Specific characters:

Distribution

Fresh water; Mrigal is distributed in India, Pakistan, Malaysia, Japan etc.

Physical appearance and special features

The body is narrow and linear. Head is small and the snout blunt. The mouth is terminal. Lips are thin and nonfringed. Dorsal fin is with 12-13 branched rays. Body is bright silvery in colour. The tip of the head is flattened and upper jaw is fringed.

Habitation in Pond

A component of composite fish culture where if occupy bottom zone.

Feeding behavior

It feeds on plant and animal materials in the pond bottom. Relatively it consumes more quantities of decaying organic and vegetable matter.

Stage of maturity

It attains maturity at the end of the 2nd year.

Fecundity

4-12 lakhs

Growth

It grows to a length of 40cm recording 1 kg in the 1st year.

Breeding

It breeds in the natural waters by the end of the 2nd year following maturity. It also responds to induced breeding technique in confined water such as ponds.

8.2.1.2. Exotic carps

8.2.1.2.1. Silver Carp

Common Name	:Silver Carp
Kingdom	:Animalia
Phylum	:chordata
Sub Phylum	:Vertebrata
Class	: Actinopterygii
Order	:cypriniformes
Family	:cyprinidae
Genus	:**Hypophthalmichthys**
Species	:**Molitxix**
Scientific Name	:**Hypophthalmichthys molitxix**

Specific characters:

Distribution

It is cultured in fresh water. It is a native carp of China, introduced and cultured in countries like India, Thailand, Malaysia, Japan, Srilanka, Pakistan, Nepal, Phillippines, U.S.S.R.(Russia), Burma, Hongkong, Singapore, Egypt etc. It can also tolerate lower salinity of brackish waters.

Physical appearance and special features

Small Silvery Scales, hence the name Silver Carp. Head is small, cylindrical. Mouth is small and upturned. Scales are very small and silvery in colour.

Habitation in pond

A component of composite fish culture. It occupies the surface layer of the pond.

Feeding behavior

It feeds mainly on phytoplankton present in water surface. Its rate of filteration of water is around 32 L per day. Silver Carp is able to feed more and grow fast.

Growth

In first year it grows to 1.5 to 2 kg.

Stage of Maturity

It attains maturity by the end of the 2nd year.

Fecundity and Breeding

It breeds by the end of the 2nd year. It also responds to induced breeding. Due to its high fecundity more seeds can be produced in the hatchery.

8.2.1.2.2. Grass Carp

Common Name :Grass Carp
Kingdom :Animalia
Phylum :chordata
Sub Phylum :Vertebrata
Class : Actinopterygii
Order :cypriniformes
Family :cyprinidae
Genus :**Ctenopharyngodon**
Species :**idella**
Scientific Name :**Ctenopharyngodon idella**

Specific characters:

Distribution

It is distributed in India, Japan, Russia, Vietnam, Thailand, Malaysia, Srilanka, Burma, Hongkong, Phillippines, Singapore, Yugoslavia, Hungary, Rumania etc.

Physical appearance and special features

The body is cylindrical and elongate. Snout is rounded. Upper Taw is slightly longer than the lower jaw. Scales are medium sized and they are light greenish in colour.

Habitation in pond

It is a freshwater species, also a component of composite fish culture. It is a bottom dewellers.

Feeding behavior

It feeds on both soft and hard aquatic weeds. It also accepts terrestrial grass growing on the funds. It is omnivore during their early stages. Fry feeds on organisms like Cyclops, Daphnia etc. However in the later period its food habit changes towards aquatic weeds. The feeding rate is completely different and extremely higher than other carps. It needs a minimum feed of 25% of its total body weight daily because of this nature it is beneficial in biologically controlling the aquatic weeds and it also serves as a 'living green manuring machine'

Stage of maturity

It attains maturity at the end of 2^{nd} year.

Fecundity

The fecundity rate is upto 6,18,000

Maximum growth in a year(culture condition)

1.5 kg

Maximum growth in a year (maturally)

4 and 7 kg

The growth is faster than silver carp

Breeding season

It responds to induced breeding.

8.2.1.2.3. Common Carp

Common Name	:Common Carp
Kingdom	:Animalia
Phylum	:chordata
Sub Phylum	:Vertebrata
Class	: Actinopterygii
Order	:cypriniformes
Family	:cyprinidae
Genus	:**Cyprinus**
Species	:**carpio**
Scientific Name	:**Cyprinus carpio**

Specific characters:

Distribution

It is a native of China and Russia. It is also distributed in Europe, Germany.

Physical appearance

The body is deep and the head is short. The scales are large. Mouth is terminal. Two pairs of barbles are present on the lips. The scales are prominent all over the body. Dorsal fin is long. Lateral line is complete. Tooth is absent. Its head and belly are big.

Habitation in pond

Benthic region of pond

Feeding behavior

It is omnivorous in food habit, eating zooplankton, insect larvae, warms, mollusks and also the submerged plants. It never completes with mrigal but utilizes mainly the natural foods which mrigal is avoiding.

Stage of maturity

Unlike major carps, it matures early when it is about 6 months old and is capable of reproducing in confined water.

Fecundity

It's fecundity is high (upto 20 lakhs)

Growth

It attains 1 to 1.5 kg of weight and in the 2nd year it grows more than 2 kg. Unlike other carp, common carp can be cultured twice in a year.

Seeds can be produced throughout year. Eggs are adhesive.

Breeding season

After 6 months. One of the six components of composite fish culture.

8.2.1.3. Minor carps

Calbasu (Labeo Calbasu)

Fringe lipped carp (Labeo finbriatus)

Whit carp (Cirrhinus cirrhosa)

8.2.2. Tilapia

Tilapia (orcochromis mossambicus)

8.2.3. Air breathing fishes/live fishes

8.2.3.1. Cat fishes

Magur (Clarias batrachus)

Singhi (Heteropneustes fossilis)

8.2.3.2. Murrels or Snakeheads

Giant Murrel (Channa marulius)

Striped murrel (Channa Striatus)

Sported murrel (Channa punctatus)

Climbing perch (Anabas testudineus)

8.2.4. Sport fishes/Cold water fishes

8.2.4.1. Trouts

Brown trout (Salmo trutta fario)

Rainbow trout (Salmo gairdueri)

8.2.4.2. Mahseers

Red fined mahseer (Tor tor)

Yellow finned mahseer (Tor putitora)

Mahseer (Tor Khudree)

8.2.5. Crustaceans

Giant freshwater prawn (Macrobrachium rosenbergi)

Indian freshwater prawn (Macrobrachium malcolmsoni)

Ganga river prawn (Macrobrachium choprai)

8.2.5.1. Giant fresh water prawn

Common Name	:Freshwater prawn
Kingdom	:Animalia
Phylum	:Arthropoda
Sub Phylum	:crustacca
Class	: Malacostraca
Order	:Dacapoda
Family	:palaemonidae
Genus	:**Macrobrachium**
Species	:**rosenbergii**
Scientific Name	:**Macrobrachium rosenbergii**

Specific characters

Distribution

It is distributed in the Indo-west pacific countries, Pakistan, India and Bangladesh. In India, the most common in the river Godavari, Cauvery and their estuaries and lakhs of Chennai and Andhra Pradesh and also in Chilka Lake.

Physical appearance

Rostrum is long, slender, slightly upturned. Extend antennal scale. The dorsal margin is armed with 11-14 teeth.

Habitation in pond

Bottom of the pond

Feeding behaviour

Accepts artificial feeds. Omnivorous, Cannibalism observed during moulting.

Stage of Maturity

The minimum size of maturity for & and % prawns is 153 mm and 175 mm respectively.

Fecundity

Fecundity 150,000 – 5,00,000.

Growth

Growth is in inverse exponential pattern. Made attains a length of 108 mm, 146 mm and 233 mm at the ends of 1^{st}, 2^{nd} and 3^{rd} year respectively.

Females attain a length of 82,5 mm, 130 mm, 168.5 mm in the corresponding year.

Breeding season

Mating prawn migrates to the estuarine region for spawning; and the breeding season coincides with the monsoon. Breeding period December to July.

It can be cultured in ponds as poly monoculture. Cultured prawns can be harvested after 6 months of growth. Culture period is between 180-240 days. Survival rate about 75% upto 97%.

8.2.6. Molluscs

Freshwater mussel (Lamellidens marginalis)

8.3.1. Finfishes

Eel (Anguilla bicolour)

Madavai (Liza tade)

Estuary grouper (Epinephelus tauvina)

Indian Salmon (Eleutheronema tetradactylum)

8.3.1.1. Milk fish (Chanos chanos)

Common name	-	Milk fish
Kingdom	-	Animalia
Phylum	-	Chordata
Subphylum	-	Craniata
Class	-	Actinopterygii
Order	-	Gonarynchiformes
Family	-	Chanidae
Genus	-	Chanos
Species	-	Chanos
Scientific name	-	Chanos chanos

Specific characters:

Distribution: advance fry and finger lings occur in good quantities in estuaries in southindia. In india it is collected from natural soaurces on the east and west coasts during april to June.

Physical appearance: It is elongated and compressed. Mouth is relatively small, anterior and transuerse in position. Upper jaw situated over hanging lower jaw. Pectoral fin pointed with elongated scaly appendages at base.

Special features: Tolerate wide ranges of salinity. It can also be acclimatized to fresh water and grown. One of the additional species in composite fish culture and its performance has been similar to common carp and mrigal.

Feeding behaviour: Generally they feed on lab-lab. The algal mat, consists of a complex animal-plant combination material. The young larvae feed on algae belonging to bacillariophyceal,

myxophyceal and chlorphyceae. Fry and fingerlings feed upon diatoms, algae, lamellilranchs, fisheggs etc. It is primarily a phytoplankton feeder.

Stage of maturity: The smallest matured female so far recorded was 95 cm length.

Fecundity: Fecundity has been observed to vary from 1,93,550 to 57,00,000 eggs from fishes ranging in size from 110 to 157 cm. Eggs are pelagic about 1.2mm in diameter.

Growth: Growth is very rapid on an average, it grows to 350mm in length and 250 gm weight in the first year.

Breeding: The fish doesnot breed in confined waters. It spawns in the inshore waters of the sea. The seeds of chanos chanos occur generally along shallow coasts, tidal creeks and estuaries during march to august and October to December.

8.3.1.2. Sea bass (latus calcarifer)

Common name	-	Sea bass, barramundi
Kindgom	-	Animalia
Phylum	-	Chordata
Subphylum	-	Craniata
Class	-	Actinopterygii
Order	-	Perciformes
Family	-	Centro pomrdae
Genus	-	Latus
Species	-	Calcarifer
Scientific Name	-	Latus calcarifer

Specific charachers:

Distribution: Thakurn, Matlah, Westbengal, Chilka

Physical appearance: Gray with a dash of green color along the back and silvery color on the abdomen region during the monsoon time it has a tingle as purple color. The immature are usually darker than the adults. Scales are large. Teeth are uiliform.

Special features: Iuryhaline nature

Feeding behaviour: Predatory nature, carnivorous and highly predaceous feeding in the column. Feed on small fishes and decapods crustaceans, worms and snails.

Stage of maturity: Attains maturity when it reaches a size of 20-24 inches in third year of age.

Fecundity: 0.75 to 1.5 million eggs in single spawning.

Growth: Fast growth rate. It grows to 400 cm during the first year and 500 cm during the second year in the natural environment.

Breeding: It does not greed in confined waters. In nature it spawns in the sea and post. Larvae enter the estuary. Spawn in june/july with supplementary spawning in Jan/March.

8.3.1.3. Mullet (Mugil parsia)

Common Name	:	Mullet
Kingdom	:	Animalia
Phylum	:	Chordata
Subphylum	-	Craniata
Class	-	Actinopterygii
Order	-	Perciformes
Family	-	Mugilidae
Genus	-	Mugil
Species	-	Parsia
Scientific name	-	Mugil Parsia

Specific Characters

Distribution

Albania, Erris, Ireland, Dam or sea cortez colorodo, lagura dam, Main-steam and lateral canals in the gila river region.

Physical appearance:

Males are generally smaller and slender whereas, in females belly shall be distended and swollen during spawning season.

Feeding behaviour

Fillter feeders, feeding on organisms low in food chain.

Stage of Maturity

Attain maturity at 25-50 cm length weighing 12-2.0 million eggs.

Fecundity

2 million eggs.

Growth

Grow to 570mm in first year, and 512 mm in the second year in culture ponds.

Breeding

Does not breed in ponds or rivers in West Bengal, it breeds from April to May.

8.3.1.4. Pearl spot (Etroplus suratensis)

Common name	: Karimeen, Chromidae(striped)
Kingdom	:Animalia
Phylum	:Chordata
Sub phylum	:Craniata
Class	:Actinopterygii
Order	:Perciformes
Family	: Ciclolidae
Genus	:Etroplus
Species	:Suratensis
Scientific name	: Etroplus suratensis

Specific characters

Distribution

Large quantities of fry of pearl spot are collected from the western shore of the chilka lake(Orissa) in its northern sector during summer and atutumn months. At Chennai the fry are collected during November to feb and in Adayar throughout the year. Fingerlings has been reported to be fairly abundant in high tide during March in the Pulicat Lake (Toulore).

Physical appearance: Body very deep, short, oval, and strong. Eyes large. Mouth small, Teeth villiform, caudal fin slightly emarginated. Scales are ctenoid.

Special features: A cup like excavation is made in the soil for the attachment of eggs and look yellowish patch with size of 15-17 cm and parents for about a month guard the eggs. The female lies flat in the nest and attaches to the eggs one by one to the object. The eggs are brownish in colour, 1-2 mm in dia and hatchout within 3-5 days.

Feeding behaviour: Advanced fry take in plenty of aquatic insect larvae but when they are about 2 cm long, there is a shift in feeding habit and they start feeding on filamentous algae and vegetable matter. Adults are Lerbivorous feeding on Myxophyceal, chlorphyceae and decaying organic matter.

Stage of maturity: Sexual maturity is first attained at a length of about 15 cm.

Fecundity: Fecundity is between 2000 and 6000 eggs.

Growth: Males are generally larger than females.

Breeding: During breeding, the brooders swim about in pairs to select suitable site in shallow water for breeding.

Maximum growth (culture) 40 cm

Maximum growth (nature)

10-12 cm, 113 gm in one culture.

8.3.1.5. Butter fish (Scatophagus argus)

Common name : Butter fish
Kingdom :Animalia
Phylum :Chordata
Sub phylum :Craniata
Class :Actinopterygii
Order :Perciformes
Family : Scatophasidae
Genus :Scatophagus
Species :argus
Scientific name: Scatophagus argus

Specific characters

Distribution

Indo pacific region. As juveniles they live in fresh water and as they mature they move to salt water environment.

Physical appearance: Small mouth with rectangular body profile. Black spots all over the body. Pelvic fins and anal fin with 1 strong spine followed by rays.

Feeding behaviour: Omnivores

Stage of maturity: 2nd year

Fecundity: Ranging from 4 lack to 5 million eggs/female with an avg. of 1.0-2.0 million eggs/kg female

Growth: 6 to 9 inches(in cultured condition)

1.75-20 inches(in natural water)

Breeding: Summer (july)

8.3.2. Crustaceans

8.3.2.1. Shrimps

Green tiger shrimp (Penaeus semisulcatus)
Kurume shrimp (Penaeus japonicas)
Banana shrimp (Penaens merguiensis)
Jinga shrimp (Metapenaeus affinis)
Kadal shrimp (Metapenaeus dobsoni)
Yellow shrimp (M. Bolevicoruis)
Speckled shrimp (M. Monoceros)

8.3.2.1.1. Black Tiger Prawn (Penaeus monodon)

Common name: Black Tiger Prawn

Kingdom :Animalia
Phylum :Arthropoda
Sub phylum :Crustaceans
Class :Malacostraca
Orde r:Decapoda
Family : Penaeidae
Genus :Penaeus
Species :monodon
Scientific name: Penaeus monodon

Specific characters

Distribution

In India, it is distributed in the east and west coasts. It forms a large scale fishery in the theries of West Bengal, Chilka Lake in Orissa and the coastal A.P.

Physical appearance: The rostrum of this species is armed with 7-8 teeth on the dorsal side and 3 or 4 teeth on the ventral margin Rostral ridge lacks a distinct groove behind it. Telson has a groove but is without lateral spines. Carapace and abdomen have black bands. Periopods may be red.

Special features:

It is known as giant tiger shrimp because of the greatest size it grows among shrimps and the transverse markings on its abdominal segments.

Feeding behaviour: Omnivorous with preference for animal matters like polychaetes, crustaceans, insects and small mollusks than plant matter.

Stage of maturity: 5-22 days

Attain maturity at 60 gms.

Fecundity

Its fecundity ranges from 68,000 to 7,31,000.

Growth

Its maximum growth recorded is 336 mm in length and 130 g in weight. The rate of growth varies from 18 to 55 mm/month. Under short-term culture conditions, 40-50 mm juveniles attain a size of 130-140 mm in 2 months period in well-prepared ponds. It grows to a length of about 180-250 mm.

Breeding

It breeds in the sea close to the river mouth. After breeding Post larvae of 10-20 mm size migrate into the estuaries, lakes, backwaters and mangroves and among these the mangroves serve as the best natural nursery.

8.3.2.1.2. Indian white shrimp (Penaeus indicus)

Common name: Indian white shrimp

Kingdom :Animalia
Phylum :Arthropoda
Sub phylum :Crustaceans
Class :Malacostraca
Order :Decapoda
Family : Penaeidae
Genus :Penaeus
Species :indicus
Scientific name: Penaeus indicus

Specific characters

Distribution

In India, it is available both in the east and west coasts. It is abundant in the coastal zones of Karnataka, Kerala, TamilNadu, Andhra Pradesh, Orissa and West Bengal. It forms a considerable fishery in the estuaries and backwaters.

Physical appearance: The rostrum of this species is slender and long with 7-8 teeth on the dorsal side and 4-6 teeth on the ventral margin. Overall creamy white. Legs may be red and the rostrum region brown. Gastro orbital ridge is well defined and hepatic ridge is absent.

Special features:

Comparatively, this is the best species for culture due to its abundant seed availability. Again this is the most suited and first ranking species due to the tolerance of salinity, which will normally be high in the tropical region. Its disease resistance character is again a more desirable quality.

Feeding behaviour: Omnivorous, feeding mainly on detritus, small crustaceans, polychaets etc.

Stage of maturity: Does not attain maturity in ponds. Attains maturity when it reaches a size of about 130 mm.

Fecundity: Its fecundity ranges from 68,000 to 7,31,000.

Growth: Maximum recorded size 230 mm. Attains marketable size of 80-120 mm in 90-100 days under culture condition. However, the normal culture period of a 'crog' is about 4 months.

Breeding: Migrates into the inshore waters for breeding. Unlike other prawn species, the juveniles of P.indicus can be collected throughout the year. Controlled breeding has also been successfully carried out.

8.3.2.1.3. American white shrimp (Litopenaeus Vannames)

Common name: white leg shrimp, camaron patibianco

Kingdom :Animalia
Phylum :Arthropoda
Sub phylum :Crustaceans
Class :Malacostraca
Order :Decapoda
Family : Penaeidae
Genus :Litopenaeus
Species :vannamei
Scientific name: Litopenaeus vannamei

Specific characters

Distribution

Ecuador, Mexico, Panama, Columbia.

Physical appearance

Rostrum curves down slightly and has 8-9 dorsal teeth and 1-3 ventral teeth. Overall white, with white legs.

Special features

Instead of crawling, it swims in the water.

Feeding behavior

Natural feeds available in the pond.

Stage of maturity

Six nauplii, three protozoeal and three mysis stage.

Fecundity

1 lacks – 2.5 lacks

Growth

Maximum size 23 cm, with maximum cl of 9cm.

Breeding

Breed throughout year Breed in deep sea. Breeding is effected by temperature and salinity. Open thelycum is present.

8.3.3. Lobsters

Green lobster/scalloped spiny lobster (Panulirus homarus)

Ornate spiny lobster/Bamboo lobster (Panulirus ornatus)

Mud spiny lobster (Panulirus polyphagus)

Pronghorn spiny lobster (Panulirus penicillatus)

8.3.4. Crabs

Mud crab (Scylla serrata)

8.3.5. Molluscs

Indian pearl oyster (Pinctada fucata)

Green mussel (Perna viridis)

Brown mussel (Perua indica)

Edible oyster (Crassostrea madrasenis)

Big clam/Hard clam (Meretrix meretrix)

Backwater clam (Meretrix Casta)

Mud clam (Katelysia opima)

Malabar clam (Paphia malabirica)

Blood clam (Anadara granosa)

Giant clam (Iridacna gigas

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – III - AQUACULTURE – SBT1608

UNIT 3 POND MANAGEMENT AND POST HARVEST TECHNOLOGY

Pond management: Nursery and grow-out pond maintenance, pond fertilization. Water quality management: Dissolved Oxygen, CO2, Ammonia, pH, salinity, temperature and turbidity. Harvest and post-harvest technology: Types of harvest, sorting, cleaning, packing, transportation of live organisms and preservation. Fish processing: Types of processing and canning, Quality assurance: Standards of sanitation and hygiene. Implementation of HACCP (Hazard Analysis and Critical Control Point) concept and food safety in fish industry.

5.1.12. Maintenance

In course of fish culture practice, the pond bottom accumulates considerable amount of silt and organic matter. Which need to be removed periodically in every 3 to 4 years. If excessive pond silt is there, it should be desilted. It is better to expose the dry pond bottom to sunlight by drying. The pond dyke should be repaired annually after monsoon. The dyke crest has to be leveled, thoroughly rolled and grass planted to bind it. The holes created in pond sides or embankments should be filled up by clay or loam .

Unit 6: Pre-stocking management

- **Chapter 1. Introduction**

6.1.1. Introduction

Pre-stocking management means management before stocking. Broadly it can be said that all the management practices involved in fish culture before stocking of fry in order to prepare the water body and its surrounding environment for living and growth of the fry.

Pre-stocking management is actually the pond preparation phase which includes preparation of pond, eradication of undesirable fishes and aquatic weeds, liming of pond, water filling and basal manuring and fertilization.

Chapter 2. Pond preparation

6.1.2. Pond preparation

Pond preparation is the main function of Pre-stocking management.

Pond drying / Dewatering

Pond drying is one of the most important physical development works of pond preparation. The objectives of pond drying are

Ø To kill undesirable species and predatory fishes from the pond.

Ø To help the works of dike and predatory fishes from the pond.

Ø To help for liming and fertilizing to the soil

The pond bottom should be allowed to dry in sunlight at least for 15 days.

Desilting /Bottom mud excavation

Ø After dewatering the pond the excess mud of the bottom should be removed.

Ø After drying generally 10-12 cm mud is removed

Ø It can be done by physical labor.

Desilting process will remove the unwanted silt/bottom mud which is rich in nutrients without interference to the next crop. The removed materials have to be taken away from the ponds and not spread over the dike /bunds so that they are not washed back into the pond during rain. The pond bottom should be made even to allow effective rutting and harvesting of fish.

Dike and canal reconstruction

The broken pond dyke must be repaired and well raised to prevent the outward migration of fish under normal flooding situation. Grass or other vegetables can be planted on the dykes, which would help prevent erosion of dyke in monsoon months and alleviate turbidity problem as well.

Dike reconstruction is the primary work of pond preparation. It is very essential for the following reasons

Ø To prevent the pond from overflowing during the rainy season.

Ø To prevent the breaking of the dike.

Ø To give a certain shape to the pond.

Ø To maintain a certain slope of the pond.

Process

Ø Dike reconstruction should be done during the end of the day season.

Ø February-April is the better time for drying and reconstruction of the pond.

Ø The inner side of the dike should cut properly or make smooth by hitting mud or soil.

Ø Now the dike must to be as high as it can prevent the flood water.

Ø The slope of the dike must be 1:2 of the pond depth.

Ø The top of the pond dike should 3 feet wide

Ø It is necessary to repair the existing dike by filling new soil

Ø Well rooted grass should cultivate in the dike of the pond.

Ploughing / Tilling of the bottom

When the bottom soil takes the crack after removal of the bottom silt, tilling / ploughing the bottom soil at the top 5-8 cm is done diagonally two times. Then it is dried for 3-10 days.

Chapter 3. Control measures

Control of aquatic weed

It is one of the important works for pond preparation. Clean and excess aquatic weed free pond is very essential for getting a better production from it. All the aquatic vegetations (floating, submerged or emergent) should be removed from the pond. They hamper primary productivity by absorbing available nutrients from water and soil and hinder normal penetration of sunlight and mind action.

It is necessary:

Ø To ensure the entrance of sufficient sunlight on the pond surface.

Ø To produce more natural food.

Ø To increase the fish production.

Although some aquatic weeds are the source of food for some fishes such as grass crap all weeds are not necessary and beneficial for a pond. All aquatic weeds are nourished from the water and soil of a water body. It is very harmful for the reproduction and growth of plankton. More over sufficient sunlight cannot enter in to the pond because of excess aquatic weed which is also harmful for the growth of plankton. They also decrease the dissolve oxygen of water and make disturbance for the movement of fishes. There are three types of aquatic weeds found in a pond they are

1. Floating

2. Submerged

3. Emergent

Control of predators and undesirable species

Predators

Predators are those species which take pray as these foods by hunting. As for example shoal, boal, chital etc.

Undesirable species:

Those species which are not expected during the culture of a specific or desirable species and those which grow naturally in a pond with cultured species are known as undesirable species or weed fishes.

Reasons for control

- Predatory fishes eat the fry of cultured species as for e.g. Predators eat 10-12 kg of other fishes for their 1 kg growth.

- Undesirable species share the food of cultured species.

- Undesirable species breakout disease for other cultured species.

Eradication of undesirable fishes

All the predatory and unwanted fishes must be eradicated from the pond prior to stocking the pond with the fingerlings of desirable species. This can be done either by complete dewatering the pond or by poisoning. Some commonly used efficient fish toxicants are

Rotenone

Rotenone kills all the fish species except shrimps when applied @ 2-3ppm. The killed fish is also suitable for human consumption. However, higher cost and unavailability are the negative points. Toxicity lasts for about 10-12 days.

Tea seed cake

In tea seed cake the active ingredient responsible for killing the fish is the saponin. Tea seed cake is effective at 75-100ppm (5-10ppm saponin content). Before application, the tea seed cake should be soaked overnight and then broadcast over the pond surface. The toxicity lasts for about 10-12 days and the killed fish is fit for human consumption.

Mahua oil cake

Mahua (Basia latipolia) oil cake contains 4-6% saponin and kills fishes when applied @ 250 ppm. The toxicity lasts for about 10-15 days under normal conditions. Killed fish is fit for human consumption. It also serves as a base manure in the pond.

Liming

Liming is an important step in preparation of ponds. The basic objectives of liming in the pond are

- To maintain the p++ of soil and water above 6
- To increase the function of fertilizer
- To remove the turbidity of water
- To control decrease and toxin gases.
- To make the pond environment clean
- To increase the productivity

Liming of a fish pond is highly recommended because of its following advantages.

Lime neutralizes soil acidity and creates a buffer system to prevent marked diurnal fluctuations of the water from acidic to alkaline conditions.

- Destroys fish pathogens and their intermediate life stages.
- Converts unsuitable acidic condition of water to suitable alkaline condition.
- Neutralize iron compounds which are undesirable in fish ponds.
- Promotes mineralization of soil which is desirable in fish ponds.
- Settle excess dissolved organic matters and thereby reduces incidences of oxygen depletion
- Acts as determinants and improves hygienic

Apart from other advantages, the buffering action of calcium is the most important. Lime serves both the prophylactic and therapeutic purposes. Lime treatment for ponds should be done before initial manuring.

Water filling

When a pond is fully prepared for stocking of fish then water filling is done. Initially before manuring/fertilizing the water depth should be maintained as low as possible so that the effect of nutrients for natural food production is fully realized. After 1 to 2 weeks of manuring / fertilizing the water depth has to be raised to the required level before stocking the seeds for fish culture. The average water depth in a pond is an important factor in fish culture. This generally depends on various factors like rainfall, evaporation losses, seepage, use of water for irrigation, etc. If necessary, water may be let in from nearby available sources during summer or drained out during monsoon to maintain desirable water depth in the pond.

Manuring / Fertilizing

Manuring / fertilizing is another important step in pond preparation. Zooplankton and phytoplankton are the main natural food of fish.

Objective of fertilizing

· To produce natural food.

· To increase the amount of nitrogen, phosphorous, potassium etc.

Types of fertilizing

Mainly two types of fertilizing are done.

Organic manures

Organic manures are directly used for the development of natural food. For the pond culture cow dung is better to use. During pond preparation poultry manure can also be used.

Inorganic fertilizer

Generally urea can be used at 20-30kg/ha. It should be dissolved in water and spread over the surface of the pond. Similarly 10-15kg/ha of super phosphate is used during preparation of pond.

In undrainable ponds where the frequent change of water is a Impossible. The physico-chemical properties of pond water governing the biological production cycle are more or less a reflection of the bottom soil. Therefore, the fertilizer requirement varies depending on soil productivity levels.

Organic manuring, is being important as a means of adding the nutrients in water is also equally important for improving the soil texture. If there is shortage of organic manures, the application of inorganic fertilizers is recommended. A generalized schedule can be adopted in the abundance of detailed soil and water analysis data. The proper mode and timing of application of fertilizers are very important in order to get good results as well as avoid water quality problems. Manures / fertilizers should be applied only when the other environmental conditions of water are suitable such as sunshine, good oxygen content and adequate water level etc. The best way of applying is to dissolve the fertilizers in water and spray throughout the pond surface. The best time for manuring is morning with 9-10 o'clock. Application of manure / fertilizer in late afternoon or evening may cause oxygen depletion in the early hours of the following day because of faster decomposition at night. During fully cloudy and rainy days, manuring has to be suspended. In case of algal blooming the manuring fertilization has also to be lessened.

Manure and fertilizers are best utilized when the desired total dose is given in small portions. Daily manuring with small quantity has been found best for keeping optimum level of fish production period. For example, the best utilized manure is from the animals released together with fish under the integrated livestock – cum – fish culture system.

The average water depth in a pond is an important factor in fish culture. This generally depends on various factors like rainfall, evaporation losses, seepage, use of water for irrigation etc. If necessary, water may be let in from nearby available sources during summer or drained out during monsoon to maintain desirable water depth in the pond heavy accumulation of metabolites at the bottom of ponds may deplete oxygen in the pond water during low water depths, adversely affecting fish growth. However, such problems generally not occur in seasonal ponds.

Unit 7: Post stocking management

7.1.1. Post stocking management

This phase includes the activities to be undertaken from stocking of fingerlings up to the final harvesting of fish from the pond. The activities are manuring, feeding, growth and health monitoring, water quality monitoring and harvesting.

7.1.2. Manuring / Fertilizing

Besides, application of high dose of basal manuring / fertilizing before stocking, regular addition of manure / fertilizer in small quantities is required in order to ensure in tempted supply of natural fish food. Organisms in the pond manuring / fertilizing should be done monthly or fortnight at regular intervals and the quantities should be in split doses.

7.1.3. Supplementary feeding

The need for supplementary feeding in aquaculture depends on the intensity of fish culture. After certain level of fish biomass increase the available natural food organisms in a pond are not sufficient to support further growth of fish. Oil cakes, rice / wheat beans, grain fodders and other agricultural by-products and available slaughter house by-products (blood, rumen content, Viscera etc) may be utilized as fish feed ingredients. The required can be either farm made or can be produced from feed manufactures. Feeding is the most expensive operation in aquaculture. So cave has to be taken to supply the required quantity and quality of feed to the species culture. Under feeding will result in poor growth of fishes whereas, overfeeding will increase the cost of feeding. Hence, feeding assumes prime importance in improving the yield and the profitability of aquaculture. The required quantity of feed has to be estimated based on the biomass available and feed has to be given in internals based on the species cultured.

7.1.4. Storage of feed

The price of feed stuff show seasonal variation. Therefore it is better to by a larger quantity when the prevalent price is low. However, without proper storage, the nutrient values can deteriorate rapidly. A decomposed fungal infected feed must not be given to the fish. Feed ingredients should be stored in places which are dry and well ventilated. Feed should be stored always 10-15 cm above the floor level.

7.1.5. Regular sampling of fish

In a proper fish production management system, periodic sampling at regular internal is very important with a view to

- Checking the health condition of the fish

- Monitoring the growth rate of fish

- Calculating the quantity of supplementary feed to be applied in accordance with the increasing biomass of fish

- Estimating survival and mortality of fish in the pond

Periodic sampling of fish should be done at least once in a month. In each sampling 10-20 fish of every species should be taken for growth measurement. For sampling, complete netting of pond by seine net is better. However, partial netting of pond also serves the purpose of sampling. During each sampling data relating to fish health and growth rate has to be properly recorded. Any undesirable fish, if some how get into the pond, must be removed if found in the sample netting. In case of some fish exhibit the symptoms of any disease, suitable curative measures should be taken immediately. However, prophylactic treatment measure such as giving the fish dip in potassium permanganate at 250-500ppm / minutes should be strictly followed before releasing the fish back in the pond.

7.1.6. Harvesting of fish

Harvesting of fish means the complete removal of fish from the pond at the end of production. A single stocking and a single harvesting are the common practice in existence. However, the technique of partial harvesting and restocking is now being practiced and has been found to yield better results in terms of fish production per unit area. Bigger size fishes should be harvested and sold in batches and the pond should immediately be restocked with the same number of fishes of such species.

7.1.7. Benefits of partial harvesting & stocking rate

- Allow smaller fish to grow faster.

- Increase carrying capacity of a pond and thus the total production become higher per unit area.

- Farmers some cash return from the pond within a short period of 4-5 months. This encourages them to reinvest the money in Improving his production capacity

- All the tropic and special niches of the pond are fully utilized throughout the culture period maximizing production.

Harvesting of fish is related to biological productivity and carrying capacity of the pond, when the pond is overcrowded and the productivity of pond cannot support further growth of fish biomass. In rearing pond, relatively bigger sized fishes must be harvested in order to leave available space and food for smaller fish to growth further. Thus, partial or total harvesting of fish can be done at any time when the carrying capacity of a pond is saturated.

Harvesting should be done by seine net preferably in the morning, when pond environmental conditions remain good. During harvesting, marketable fish should be sorted out first and then small size fish should be returned to the pond. The total operation should be done as possible so that the fishes returned back to pond are not stressed.

Unit 8: Selection of candidate species for Aquaculture

8.1.1. Criteria for selection of species

Profitable fish culture aims at the production of maximum quantity of edible fish flesh form a given quantity of organic matter in the shortest possible time. Therefore, the species selected for culture should have certain essential qualities like,

8.1.2. Rate of growth

Fishes which grow to big size in a short period of time are the most suitable for cultivation. Eg. Indian major Carps.

8.1.3. Short food chain

Fishes with short feeding chain are ideal. This will help to reduce the loss of energy from the passage of one link of production to the next. Because at every tropic level, there is loss of 90% of the energy. Fishes feeding on detritus, plankton or vegetation have additional advantage (of being tolerant of other species in a pond)

Raising carnivores in expansive – trout, salmons, snake heads.

8.1.4. Adaptation to climate

This is an essential condition which limits the use of both cold and warm water species. Salmonids which are cold water species cannot tolerate warm water. Similarly warm water species like Tilapia spp, Indian major carps etc., can't tolerate the cold climates of temperate countries.

8.1.5. Consumer liking

It is absolutely essential to bear in mind the consumers liking, when a species is selected for culture. Eg. Silver carp (not liked because of spines, low keeping quality etc.,

8.1.6. Aptitude for artificial food

In order to obtain a high production rate, it is necessary to rear the fishes, which accept artificial feed.

8.1.7. Tolerance to fluctuations in physic – chemical conditions of water

This is highly desirable quality of a species selected for culture. Such fishes with stand handling and transportation stress.

8.1.8. Resistance to common fish diseases and parasites

Catla is more susceptible for Lernea infection, rainbow trout (RBT) is better resistant than Brown trout (BT) to IPN virus.

8.1.9. Easy reproduction under controlled conditions

In order to ensure an easy and constant supply of fish seed for rearing purposes, it is ideal of the fishes cultured reproduce in captivity. However if the reproduction is too prolific it will create population density problem.

8.1.10. Amiability to live together

The quality to live together without troubling other species is especially required in fishes used in poly culture. In this respect, carnivores fishes should be cultured separately.

8.1.11. More edible flesh per unit weight

Species which gives more flesh per unit weight is more economical.

Soil and water quality parameters in aquaculture

- **Chapter 1. Introduction**

Introduction

Maintenance of a healthy aquatic environment and production of sufficient fish food organisms (plankton) in ponds are two factors of primary importance for successful aquaculture culture operation. The nutrient status of water and soil play the most important role in governing the production of plankton organisms or primary production in fish ponds. The bottom soil governs the storage and release of nutrients to the overlying water through various chemical and biochemical processes for biological production in the environment.

Chapter 2. water quality parameters

Physical parameters

Water

The Physical condition of water is greatly influenced with depth, temperature, turbidity and light. These constitute the more important physical parameters on which the productivity of a pond depends.

Depth

Depth of a pond has an important bearing on the physical and chemical qualities of water. Depth determines the temperature, the circulation pattern of water and the extent of photosynthetic activity. In shallow ponds, sunlight penetrates up to the bottom, warms up the water and facilitates increase in productivity. Ponds shallower than 1 m get overheated in tropical summers inhibiting the survival of fish and other organisms. Generally a depth of about 2 meter is considered ideal from the point of view of biological productivity of a pond.

Temperature

Water temperature generally depends upon climate, sunlight and depth. That too, the intensity and seasonal variations in temperature of a water body have a great bearing upon it productivity. The temperature in fish ponds is generally less during the early hours of morning and reaches the maximum value in the afternoon showing diurnal fluctuations. Compared to the yields of fish in

ponds in temperate zones, the natural water in tropical areas generally show a higher production due to more heat budget in the ponds system. A part from these, temperature plays very important role in physiological processes for breeding in fish both under natural and artificial conditions. The chemical changes in both soil and water are greatly influenced by temperature. Decrease in DO_2 is directly related to increase in temperature. Fish display great variability in their tolerance to temperature. Indian major carps usually tolerate wide range of temperature and are called eurythermal.

Turbidity

The turbidity of water bodies may be either due to suspended inorganic substances like silt, clay and planktonic organisms. Turbidity of water varies greatly with the nature of basin and inflowing sediments. Ponds with clay bottom are likely to have high turbidity that restricts the penetration of light, therefore reduces the photosynthetic activity hence acts as a limiting factor for productivity.

Light

Light is another physical factor of importance. Availability of light energy to a fish pond greatly influences its productivity. Penetration of light is determined by turbidity which is measured optically and represents the resultant effect of several factors such as suspended clay and silt and dispersion of planktonic masses.

Chemical Parameters

Among the chemical factors influencing aquatic productivity, pH, Alkalinity, dissolved gases like Oxygen, Carbon dioxide and dissolved inorganic nutrients like P, N are considered to be important.

pH (Hydrogen ion concentration)

The pH of water is defined as the logarithm of the reciprocal of hydrogen ion concentration. It may be expressed mathematically as $pH=Log\ 1(H)^{+}$. The pH of neutral water is 7, below 7 is acidic and above 7 is alkaline. The pH of pond water undergoes a diurnal change; it is being alkaline in mid afternoon and acidic just before day break. High yield of fish crops are usually produced in water which is just on the alkaline side of between 7.0 and 8.0. The limit above or below which pH has a harmful effect is given as 4.8 and 10.8.

Alkalinity, Carbonate, Bicarbonate and Free Carbon dioxide

Alkalinity or acid combining capacity of natural freshwater ponds is generally caused by carbonate (CO_3) and Bicarbonate (HCO_3) or hydroxides of calcium, Magnesium, Na, K, NH_4 and Fe, calcium being from the major constituent. Bicarbonate and carbonate are the major constituent of pond water and their concentrations are expressed as total alkalinity. In general, calcareous water with alkalinities more than 50ppm are most productive. Waters with an alkalinity less than 10ppm rarely produce large crops, water intermediate between these 10-50ppm may produce useful results.

Dissolved oxygen

Among the chemical substances in natural water, O_2 is of primary importance both as a regulator of metabolic processes of plant and animal community and as an indicator of water condition. The pond water receives oxygen mainly through (1) interaction of atmospheric air on the surface water (2) by photosynthesis. Photosynthesis, respiration and slow rate of diffusion cause a fluctuation of dissolved oxygen in water and accordingly remain optimum during morning and gradually increase to attain maximum in the afternoon and declines thereafter during night to reach minimum before dawn. It is possible that below 3.0 ppm of DO_2, asphyxia from low O_2 can be expected and to maintain a favorable condition for a varied warm water fish fauna, 5.5 ppm of DO_2 is required. Sometimes fishes congregate near the surface for respiration in such low DO_2 ponds. For average or good production ponds should have DO_2 concentration above 5.5 ppm.

Total hardness

In principle hardness is defined as the total of soluble Calcium and Magnesium salts present in the water medium. In most natural water, usually HCO_3 anions are associated with Ca, Mg, Na and K cations. Usually bicarbonates of Ca and Mg cause temporary hardness. Permanent hardness of water is due to soluble Ca and Mg carbonates and salts of inorganic acids ($CaSo_4$). The pond water having a hardness of 15 ppm or above are satisfactory for growth of fish and do not require addition of lime, but water having hardness, less than 1.1ppm require liming for higher production of fish. Water having, hardness less than 5 ppm, cause slow growth, distress and eventual death of fish.

Dissolved Nitrogen and its compounds

The importance of dissolved nutrients especially nitrogen is well recognized. It is an important element influencing the growth of phytoplankton in aquatic environment. As constituent of protein, Nitrogen occupies a highly important place in aquatic ecosystem. Pond having dissolved nitrogen below 0.1ppm does not indicate productive condition, while the range of 0.1-0.2 ppm an average production is expected but above 0.2ppm is considered favorable. However optimal limit of nitrogen can be in the range of 0.3-1.3ppm

Phosphorous

The phosphorus fertility less then 0.02ppm is low productive, 0.02-0.05 ppm is fairly productive, 0.05-0.10 ppm is good productive and above 0.20 ppm excessive. Besides the absolute concentration, the ratio of nitrogen and phosphorus concentration is likely to influence aquatic productivity. Nitrogen and phosphorus are utilized for plankton growth at a ratio of 3:1 to 6:1.

Chapter 3. Soil quality parameters

Soil quality parameters

Soil

Soil plays an important role in regard to the fertility of fish ponds. Types, characteristics and chemical conditions of soil influences the pond productivity. The physico-chemical properties of pond water are more or less a reflection of the properties of the bottom soil. In this respect the major chemical factors of importance are pH, total nitrogen, total phosphorus, organic carbon, available N_2 and available P.

Hydrogen ion concentration (pH)

The pH of soil depends on various factors. The release of essential nutrients at soil water interface is greatly hampered due to low pH. pH range of 5.5 is (highly acidic) 5.5-6.5 (moderately acidic), 6.5-7.5 (nearly neutral) and 7.5-8.5 (moderately alkaline) has been considered favorable for fish ponds, whereas above 8.5 is considerable highly alkaline.

Phosphorus

The importance of available phosphorus in soil for increasing productivity is well recognized. The phosphorus in soil is in both inorganic and organic forms. The organic form constitutes about 35-40% of the total phosphorus content of the soil. The available soil phosphorus ($P2O_5$) below 3 mg/100gm (30ppm) as poor productivity, 3-6mg/100gm (30-60ppm) as average, above 6-12mg/100gm (60-120ppm) as high productivity and above 12mg/100gm (120ppm) as excess.

Nitrogen

Nitrogen in soil is present mostly in organic forms as amino acids, peptides and easily decomposable proteins. The conversion of complex organic forms of nitrogen to simple inorganic forms is carried out by anaerobic microbes. Hence, it is important to know available nitrogen than the total nitrogen in soil. The range of available nitrogen is 50-75mg/100gm of soil relatively more favorable for pond productivity.

Organic carbon

Compared to the mineral constituents of the soil, organic compounds are more varied and complex. Very high organic content is also not desirable for a pond soil. However, organic carbon less than 0.5% may be considered poor, 0.5-1.5% as average while 1.5-2.5% appeared to be optimal for good production.

Unit 10: Physical, chemical and biological factors affecting productivity of ponds

- **Chapter 1. Factors affecting productivity of ponds**

10.1.1. Introduction

Water is the primary requisite for the existence and growth of aquatic animals. The elements present in water considerably influence the biological production in aquatic environment. The soil provides all these elements for biological production in aquatic environments. The quality of soil is important because of its influence on productivity and quality of overlying water. It has also has a great influence on construction and maintenance of pond bundles.

10.1.2. Quality

A satisfactory pond soil is one which part from being impervious to water, permits rapid mineralisation of organic water, adsorbs nutrients, loosely bound and releases them slowly over a

long period of time. The ability of the pond to retain the required water level is also greatly affected by the physical characteristics of soil such as texture and porosity. Soil texture depends on relative proportion of particles of sand, silt and clay. Fine textured soils such as silly clay, clay loamy, silty-clay-loamy and sandy clay are more suitable for fish pond construction because of high water retention capacity. Gravelly and sandy soils having poor water retaining capacity and higher rates of seepage are not suitable for fish culture.

Chapter 2. Physical factor

10.2.1. Soil

10.2.1.1. Texture of pond soil

The proportionate composition of the mineral fraction of the soil particles (sand, silt and clay) is denoted by the texture of the soil and is an indicator of the water holding capacity of the soil. Pond soil for aquaculture should certain 35% clay.

10.2.2. Water

The important physical properties of water are depth, temp and turbidity.

10.2.2.1. Depth

Depth of water in a pond is most important since penetration of light to the bottom contributes in large to the pond productivity. Water layer below 3 to 4 meter in temperature countries and below 1.5 to 2 meter in tropical region have little significance in biological productivity. Shallow water gets warm up rapidly and provide optimum conditions for aquatic life. The primary production takes place from surface to a depth of 1 meter. However, fish ponds should not be too shallow in tropics, as extremely high temperature adversely affect production and may lead the fish mortality. A water depth of 1.5 to 2 meter is considered congenial (agreeable) from the point of biological productivity of a pond.

10.2.2.2. Temperature

Temperature greatly influence the biological activities of fish notably their respiration, growth and reproduction.

Fish can perceive water temperature changes which are smaller than $0.1^{O}C$. Every species has its own characteristic optimum temperature range which might change seasonally. Fish is a cold blooded animal and unlike mammals and birds their body temperature is not internally regulated. Hence its body temperature varies with the temperature of water. This results in changes in metabolism. The temperature of water therefore has profound effect on the life.

The oxygen consumption of fishes increased with rise in temperature. At the same time, the amount of oxygen that water can dissolve and hold decreases with increase in temperature.

Ex : $10^{O}C$ – 10.92 mg/l

$20^{O}C$ – 884 mg/l

$30^{O}C$ – 7.53 mg/l

Processes of fish such as growth and development. Several species have a wide range of temperature tolerance. However growth is usually optional within a limited range. The Indian major carps usually tolerate wide range of temperature, but thrive well in the temperature range of 18 to $38^{O}C$. lethal temperature limits for cold water species like trout and salmon is around $25^{O}C$. For carps and Tilapia, the lethal limits are around $40^{O}C$.

(Lethal – causing death, fatal – resulting in death)

10.2.2.3 Turbidity

Turbidity is a condition of water resulting from the presence of suspended matter. It may be due to suspended clay, silt and finely divided organic matter and plankton. It may be temporary due to rains, floods and drainage inflow or permanent an account of nature of soil and constant wind and wave action. Turbidity is measured by secchi disc visibility optimum secchi disc visibility in fish ponds is considered to be 40 to 60 cms.

Turbidity is an important binding factor in the productivity of a pond. Light can penetrate deeper into cleaner water and induce the growth of plants. Photosynthesis will be very much reduced in turbid waters. Turbid water gets heated up quickly and trap nutrients and cause siltation leading to ageing of pond. Turbidity suppresses or destroys planktonic organisms by suffocation. Waters containing > 400 mg/l of suspended solids (matter) are not productive.

Chapter 3: Chemical factor

10.3.1. Soil

10.3.1.1. pH

The soil pH influences transformation of soluble phosphates, response of different nitrogenous fertilizers, adsorption and release of nutrients at the soil water interface including bacterial activity in soil and is maximum at neutral pH. The ideal pH is 6.5 to 7.5 for fish ponds.

10.3.1.2. Nitrogen

Nitrogen is required to stimulate primary production in aquatic environment. Soil is the main source of this element. However, a major fraction of this element remain in complex organic substances and bacterial degradation of organic matter present in soil cause the release of mineral nitrogen for utilization. The available nitrogen contents in the range of 25 to 50 mg/100 g is considered favourable for average production.

10.3.1.3. Phosphorous

Phosphorus is another element required for all aspects of cellular metabolism, respiration, cell division and growth, synthesis of protein and incorporation in all living tissues. The low status make this element as a limiting factor in biological production. The nature of phosphorous status of soils is generally low compared to other major elements and also due to reactive nature of phosphate ions, it becomes unavailable as insoluble phosphates. Due to these factors available soil phosphorus is considered for biological production rather than total phosphorus content of soil. The

available soil phosphorus in the range of 3 to 6mg/100g is considered desirable for average productive soil.

10.3.1.4. Organic carbon

Bacterial activity depends on the carbon content of the soil utilising it as a source of energy. In fish ponds, the process of sedimentation and decomposition of organic matter takes place and as a result various nutrients are released from complex organic forms to simple inorganic compounds. The unproductive nature of both newly constructed ponds and order ponds are mainly due to the low and high organic carbon content of the soil respectively. Organic carbon content in the range of 0.5 to 1.5% is considered is ideal for average fish production.

10.3.2. Water

Dissolved oxygen, pH, Carbon dioxide (CO_2), Ammonia (NH_3), alkalinity, dissolved organic matter etc., are the important chemical properties of water.

10.3.2.1. Dissolved Oxygen: (Do)

The most common kind of deficiency in water is lack of dissolved oxygen usually caused by decay of organic matter.

Even though oxygen is a major component (20.95% of air) but is sparingly soluble in water. The solubility has inverse ratio with temperature. There is a well known diurnal (24 hrs) variation in DO during day due to active photosynthesises, DO can increases reaching peak in the afternoon. (from dusk to dawn). T here is gradual decrease in DO content.

10.3.2.1.1. DO depletion

Main cause of depletion is organic matter load and its decomposition. Usage of high dose of organic manure should be done carefully. Second cause is algal bloom die off.

Prolonged exposure to sub lethal concentration of oxygen is harmful to fish which more often go unnoticed. Feed consumption, growth, feed conversion and disease resistance are reduced in sub lethal concentration under culture conditions.

10.3.2.1.2. Do needs of different sps.

The rate of respiration (O_2 consumption) varies with species, size, activity, temperature, nutritional status etc., Younger fishes being more active consume more O_2 than starved ones. If oxygen concentration in water is high fish tend to consumer more oxygen.

A minimum concentration of 5 mg/l is sufficient for warm water fishes, while 9 mg/l is required for cold water sps. (For short periods even if DO falls by 2-3 mg/l below the minimum levels fishes won't die). Experiments have shown that flucations of DO both below and above the optimum range has adverse effects on growth, appetite, feed conversion.

Fishes with accessory respiratory organs like in species clarias, Heteropneustus, channa, Anabas etc., can survive in poorly oxygenated waters. Tilapia species can survive well at DO concentration as low as 1 mg/l .

10.3.2.1.3. Methods of increasing DO

a) Aerators – Increase air-water interface water bubbles into small- more surface area.

b) Pumping fresh water

c) Splashing water surface

10.3.2.2. pH – puissance dihydrogen (Concentration of Hydrogen)

The pH or hydrogen ion concentration is often used as an index of water conditions in fish pond. The pH is defined as the negative logarithm of hydrogen ion concentration. The substances dissolved in water gives it an acid, neutral or alkaline reaction.

The pH value varies between 0 & 14. While a pH of 7 indicates a neutral reaction. pH above 7 shows an alkaline reaction and below 7 an acid reaction. Alkaline or neutral water is more productive than acid water. Water with pH ranging from 6.5 to 9.0 is most suitable for fish culture. In waters more acidic than pH 6.5 or more alkaline than pH 9.5 for long period, reproduction and growth will be diminish. Water more alkaline than pH 9.5, CO_2 becomes unavailable. Acid waters also affect fish indirectly by its adverse affects on fish food organisms.

10.3.2.3. Carbon dioxide (Co_2)

CO_2 is highly soluble in water, but it is only a minor constituent of atmosphere (0.03% of air). The major sources of CO2 in water are from the decomposition of organic matter and respiration of aquatic animals and plants. Plants use CO_2 for photosynthesis and release oxygen. Accumulation of CO_2 generally takes place in the night.

CO_2 occurs in water in 3 closely related forms, namely (a) free CO_2 (b) bicarbonate ion (HCO_3) (c) Carbonate ion CO_3 (band C are band form)

The concentration of free CO_2 usually does not exceed 20mg/l. However, it may be as high as 50mg/l in organically polluted water. High concentration of free CO_2 interfaces with respiration in fishes leading to mortality. Fish can tolerate high concentration of CO_2 if the DO concentration is high.

CO_2 is not highly toxic to fish. Concentration between 50-100 mg/l can cause respiratory stress.

Between 100-200mg/l CO_2 is fatal. Most spp. will survive for several days when concentration is upto 60 mg/l, provided dissolved oxygen is plenty.

10.3.2.4. Organic matter

Organic matter is present as living plankton, suspended particles of decaying organic matter (detritus) and dissolved organic matter, BOD (Biochemical oxygen demand) is frequently mentioned in connection with organic matter in water.

10.3.2.5. BOD

The Biochemical oxygen demand is used up oxygen by unstable organic matter for its stabilization in water brought about by aerobic bacteria. For Aquaculture waters. BODs should be 20 ppm, incubation for 5 days difference between initial DO and final DO give BODs is 20 ppm.

10.3.2.5.1. Hydrogen Sulphide (H_2S)

Fresh water fish ponds should be free from hydrogen sulphide. H_2S is produced by chemical reduction of organic matter that accumulates and forms a thick layer of organic deposit at the bottom.

Unionized hydrogen sulphide is toxic to fish, but the ions resulting from its dissociation are not very toxic 0.01 to 0.5mg/l – lethal to fish and any detectable concentration of hydrogen sulphide in water creates stress to fish.

10.3.2.5.2. Rectification of H_2S

1) Frequent H_2O exchange to prevent building up of hydrogen sulphide in the water.

2) When pH of water is increased by liming, the toxicity of hydrogen sulphide decreases.

3) Potassium permanganate (6.2 mg/l) can be used to remove H_2S (1 mg/l) from water.

10.3.2.6.1. Ammonia (NH_3)

The major source of NH_3 to water is decomposition of organic manure, feed and excretion by fishes.

Ammonia occurs in 2 forms unionized Ammonia (NH_3) and Ionized Ammonia (NH_4). Ammonia refers to combed concentration of unionized and ionized Ammonia ($NH_3 + NH_4$). Only the unionized Ammonia is toxic to fish.

0.02 to 0.05 mg/l – safe concentration for many tropical fish species

0.05 to 0.4 mg/l – sub-lethal effects depending on the species

0.4 to 2.5 mg/l – lethal to many fish species.

10.3.2.6.2. Measures to maintain safe NH_3 level

Normally high DO_2 and high CO_2, the toxicity of Ammonia to fish is reduced

1) Aeration

2) Healthy phytoplankton population removes NH_3 from water.

3) Biological filters (convert NH_3 to NO_2)

4) No excess feeding (go for high protein feed)

5) Excessive liming should be avoided.

6) Water exchange

10.3.2.7. Total Alkalinity

The term total alkalinity refers to the total concentration of bases in H_2O expressed in mg/l of equivalent calcium carbonate. Even though small amounts of carbonates of magnesium, sodium and potassium may slightly influence the alkaline reserve for all practical purposes. It can be expressed as the calcium content of the water in most waters.

Total Alkalinity is a measure of productivity of a pond. Productive waters have alkalinity values upto 100 ppm or 100 mg/l.

< 20 mg/l stress to fish

20-300 mg/l ideal for fish

> 300 mg/l stress to fish.

Chapter 4. Biological factor

10.4.1. Biological factor

· Biological factors of water which influence fish production are tied up with the capacity of the surrounding environment to supply essential food to cultured species. They are therefore concerned only with rearing operations where no supplementary food is given and the energy requirements are met through the phytoplanktonic primary production. Photosynthesis which transforms mineral salts into carbohydrates under the influence of light given the energy necessary for the development of plants. The biomass of phytoplankton various seasonally in general reaching its highest levels in spring and summer. The density is highest in superficial layers of water (0-10m) and deceases with depth. The appearance of different populations is linked in part to the characteristics of the surrounding water temperature, turbidity and depletion of nutrient.

— The diseases in fishes and prawns are caused by bacteria, virus, fungi, protozoa and crustacean parasites. These parasites enter into the pond along with water, fish or prawn seed and nets from other infected ponds.

— Excess growth of aquatic weeds in fish pond is not a good sign in aquaculture systems. Weeds utilize the nutrients and compete with desirable organisms.

10.4.2.1. Estimation of productivity

It is estimated by estimating the primary production to refer to the rate of synthesis of organic matter from the inorganic constituents of watery by the plants (phytoplankton) in the presence of sun light. Organic production by plants is the first step in trapping energy by living beings from non-living natural resources and hence called primary productivity. The methods followed are Dark and light bottle method and C techniques.

10.4.2.2. Light and Dark bottle method (Garden and Gran, 1930)

The amount of oxygen liberated by phytoplankton during photosynthesis is considered as a .....of primary production. Water samples are collected in three BOD bottles namely light, dark and control at depths at which productivity as to be measured. Zooplanktons are removed by filtering through plankton net (300 ....). Water samples is the control bottle is immediately fixed by using Winkler's fricatives. The dark bottle is wrapped with aluminium foil and kept in a black bag to protect from light. The light and dark bottles are then suspended on to a raft and anchored. The bottles are incubated for a period of 4 to 6 hrs between dawn to midday or between midday to sunset in the respective depths from where productive measurement need to be carried out. After incubation period, the bottles are taken out and fixed with Winkler's fricatives. The oxygen content in the sample is determined by using Winkler's method (other method of Do estimation can also be used)

Primary production is carried out as follows.

Let the initial oxygen level be IB

Let the final oxygen level be DB

Let the oxygen level in light bottle be LB

Net oxygen production = LB-IB

Gross production of oxygen = LB-DB

Let 't' be the time kept for incubation

Gross primary productivity

$$= \frac{(LB-DB)x1000x0.375}{1.25xt} = mgc/m^3/hr$$

Net primary productivity

$$= \frac{(LB-IB)x1000x0.375}{1.25xt} = mgc/m^3/hr$$

Unit 11: Nutrition

- **Chapter 1. Fish Nutrition**

11.1.1. Nutrition requirements

The nutritional requirement of fish are all to those of terrestrial animals for growth producers and other normal physiological functions they need to consume protein, carbohydrates, fat minerals, vitamins and growth factors and energy sources. Deficiencies of one or more of the essential nutrients result in disease or even death. These nutrients may come from artificial or prepared diets or from natural aquatic organism source of energy.

All animals use chemical compounds to supply energy and for tissue building. They must obtain their energy directly by eating plant material or by eating other organisms' naturals. These organic maters belongs to these major groups (i.e.) proteins, carbohydrates, and fats in which energy is stored.

11.1.2. Other essential minerals

Dietary requirement for most of the other minerals have not been established for fish difference in growth response have been obtained by changing the dietary levels of Mg 1K, Cu, and Ve for several sp. of fish.

· Forage materials Gef. Grasses & Macrophyty which may be introduced into culture system or made to grow in the culture system (e.g. in F. H2O way fish culture)

· Prepared feed including a wide array of feeds, lagging from simply on from based mixtures of a few ingredients to micro en-capsulated diets.

Chapter 2. Feed

11.2.1 Feed

The great bulk of fed used in aquaculture to the last category and are compounded using a number of ingredients.

11.2.2. Feed ingredients

A wide variety of ingredients are available for use in fish and crustacean feeds

1. Grasses

2. Pulses and Legumes

3. Miscellaneous fodder plants crop

4. Fruits and vegetables

5. Root crops

6. Cereals

7. Oil-bearing seeds and oil cakes

8. Animal products

9. Miscellaneous feed stuff

10. Additives

11.2.3. Feed Materials

Feed formation is followed by manufacture; their technology will differ, at least in details, depending on the type of feed to be manufacture.

Chapter:3 Organic compound

11.3.1. Vitamins

Vitamins are essential growth factors that are required in the diet in only very small quantities. The essential vitamins required in fish feed formation are Vitamins-A,D,E,K (fat soluble), Thiamine (Vitamin B1), riboflavin (vitamin B2), Phydoxine (vitamin B6) choline, Niacin, Biotin, Pantothenic Acid, inositol, Cynacopalamine (Vitamin B12), folic acid, Ascorbic acid (Vitamin c) one of the 1st systems of deficiency of practically any of the 13 to 15 essential vitamins for warm water fish is depressant appetite and reduced growth rate and their common symptoms are abnormal colour, back of co-coordinator nervousness, harmostrage fatty acid and increased susceptibility to baternoil arfectors.

11.3.2. Minerals

Fish probably requires the same minor as warm-blooded animals for tissue formalin various metabolic process. In addition, fish use inorganic element to maintain osmotic balance between fluids in their body and the H_2O , Mineral require is fish may he classifies as hulk elements as Ca, P, K, Na, Mg and Cl and race element like Cu, Co, Fe, I, Mn, Se, Zn, Ai, Cr, Vandium.

11.3.2.1. Ca & P

Fish like mammals require large amount of Ca and P for the growth and devil. Most fish appear to be able to absorb enomy calcium from the $_{HO}$ through the gells for normal growth, except when H_2O is unusually low in Ca.

Level of dissolved phosphorous are very low in natural H_2O in relation to cacium consequently, the H2O in fish culture envin is not a significant source of phosphorous dietary deficiency in phosphorous have caused reductions in growth late, body content of ca. d. Phosphorous and appetite in fish.

11.3.3. Fats

Fats are the principal for of energy storage in planted of animals' fat contains more energy/unit at than any other biological product the exclusion of fat usually increases the palatability of a feed. Tineralty fats are well digested and utilized by fish.

11.3.4. Carbohydrates

Carbohydrates are the cheapest and the most abundant source of energy for animals. Most of plant material is carbohydrate is feeding range from easily digested sugars to most complex cellulose molecules which cannot be digested by animals. It is only through their symbolic relation bacteria that nutrient animal can utilize large amounts of cellular. There is controversy as the value of carbohydrate in fish food.

11.3.5. Protein

Protein is the gain consistent of the fish body, thus a generous dietary supply needed for required growth. Protein is expensive than carbohydrate or fat, that the amount of protein in the diet limited to that extent which is needed growth and tissue repair and the entire come from the cheaper sources.

11.3.5.1. Protein level in fish diets

Fish require a higher of protein in their diet than do not blooded animals for eg. The optimum protein in practical diets for Harvest fish is 30 to 36%

11.3.5.2. Protein quality

Protein quality is includable primarily by amino acid compositors. They are made up of 20-25 amino acids. It can synthesize 10 of them (by interconversions) from each other or from other molecules of intermediary but the other 10 (essential amino acids) cannot be synthesis in the fish must be provided in the diet. They are methionine, Arginine, Threonine, tryptophan, isolucine, Leucine, valine, phenylalanie, histidine and lysine (TAMIL TV HPL).

Chapter 4. Types of feeds

11.4.1. Types of feeds

Feeds can be classified based on the stage on the life cycle, at which they are largelted. Accordingly they are

1. Starter

2. Fly

3. Figerling

4. Growthout

5. Brood stock

This does not necessarily comply that for the production of a culture sp all 5 types feeds are fly feed may be same while grow-out and broad stock feed may also the same.

In addition, product quality feeds are used in many cultures to increase the quality of the final sale able product. Starter feeds should be complete, easily digestible and be of the appropriate particle size.

Natural feed staffs are usually adult in K, Mg, Na and Cl of normal growth of animals, unless there is high rate of mineral loss. These element are probably available in sufficient quantity in practice fish feed count mineral supplementation. However, in fish feeds low in animal products (fish meal, meat and bone break) May be deficient in tall minimum when losses than 15% of the ratio in composed of animals products a brace mineral supplement is recommended.

Chapter 5. Feed formulation and Processing

11.5.1. Feed formulation and Processing

Following points need to be considered in fish feed formation

1. Cost of feed ingredients

2. Nutrient content of feed ingredient

3. Nutrients requirement of the animal (protein energy, vitamins, minerals, amino acid)

Available of nutrients to the animal from various feed materials. Cost nutrient content are readily available for most commercial feed stuff information is avail on nutrient requirements for several fish sp, enough to formulate reasonably satisfactory production ratios.

The Basic information requirement for feed formulation is,

· Nutrients requirement of the sp. Cultural

· The feeding habit of the sp.

· Local avail cost & nutrient composition of ingredients

· Ability of the cultured organism to utilized nutrients from various ingredient as well as prepared diet.

· Expected feed consumption

· Feed additives need and

· Type of feed processing desired

11.5.2. Aims of Feed Preparation

Proper nutrition is one of the most important function influenced the ability of cultured organism to attain the potential for growth, repro, and survival. The nutrients requirement varies between sp. and different stage of it life cycle.

11.5.3. Forms of diets

Diets supplied to aquatic organisms could to vary in form possible diet include.

· Live food generally required for the culture of most aquatic organism at their larval phase.

Starter feed are generally in the forms of fine consumable or flakes

11.5.4. Forms of feeds

Feed types can take numerous forms

However, they basically fall in one of 2 general forms

a. Dry

b. Moist

Basic steps

(a) Grinding

Grinding reduces particle size and increase the surface area of ingredient, thereby facilitating, mixing, pulleting & digestibility.

(b) Mixing

Ground ingredients are mixed in desired proportion to form hormony blend.

(c) Pelleting

Defined as the compacting of feeds formed by extruding in ingredients or mixture of ingredients feed storage.

Chapter 6. Feed storage

11.6.1. Feed storage

Manufactured diet require storage at least at the place of Manufacture and one of the food feeds are compared of punishable biological material which deteriorates storage. It always desirable to minimize storage lime

11.6.2. DETERIORATIVE EFFECTS DURING STORAGE ARE CAVSFED BY

· Oxidative damage

· Microbial damage

· Insect and or rodent damage/ infestation and

· Other chemical changes during storage

11.6.3. Proper storage

Good feed storage should provide protection against high temper humidity, moisture and insect and rodent enfestation. Feed stuffs as far as possible be stored for a minimum length of time. Materials such as trash fresh should be used immediately as kept frozen until used.

Chapter 7. Feeding and Food conversion Ratio (FCR)

11.7.1. Feeding

Feed assumes more than 50-60% of cost production in most aquaculture practices. Hence great car has to be taken in providing feed to the cultured organism under feeding may result in poor growth sutitmal deficiency, disease and even mortality whereas over feeding may incase the cost of production reduce the H2O quality breading to stress and poor growth.

Quantity of feed given to depend on total biomass available on the pond expected growth and conversion rates. Based on periodic sampling the total biomass of fish available in the ponds is estimated and then quantity of feed to gn is estimated. The daily ratio is given in split doses depending on the feeding habit of culture organism for crustaceans having nocturnal feeding habit, feeding during high is also sea meals whereas for all other special day feeding is followed,

Feed is either provided by broad casting or placed in bays or in feeding bays so that the feed in completely fixed by the factor fishes cultured.

In intestine fish crustaceans use of demand feeders and automatic feeders is also followed to increase the efficiency of feeding.

11.7.2. Food conversion Ratio (FCR)

Food conversion ratio in which the feed given is converted to flush-tt is an indicator of quality of feed gn and efficiency of cultured fish to convert into biomass

Total Quantity of feed gn

Food conversion Ratio (FCR) =----------

Total biomass increase

Feed in which the FCR is usually low in the best quality feed for sp. Cultured.

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV - AQUACULTURE – SBT1608

UNIT 4 CHROMOSOME MANIPULATION AND FISH BIOTECHNOLOGY

Genetic improvement: Inbreeding and cross breeding; Hybridization, Genetic manipulation: Sex-reversal and sex control; role of steroids in sex reversal, chromosomal manipulation, polyploidy, androgenesis and gynogenesis; cryopreservation of gametes. Fish Biotechnology: Production of transgenic fishes, micro injection technique, Cloning and expression of GnRH.

FISH GENETICS:

Gynogenesis

Gynogenesis is the process of embryonic development with solely the maternal genome and without paternal genetic input, a phenomenon similar to parthenogenesis. Gynogenesis occurs in nature and can also be induced.

Mitotic gynogenesis

Mitotic gynogenesis can be used to create mitotic gynogens (all genes come from the mother), fish that are 100% inbred. The technique that is used to accomplish this with species that have the XY sex-determining system (females are XX and males are XY; virtually all aquacultured species have this system of sex determination) is outlined in Figure.

The first step in this breeding programme is the production of first-generation mitotic gynogens. Ultraviolet radiation is used to destroy the DNA (the genes) in sperm. The irradiated sperm are then used to activate eggs. An irradiated sperm cannot fertilize an egg because its genes have been destroyed. The activation causes the egg to undergo the equational division (second meiotic division) and to extrude the second polar body. The egg now contains only a haploid egg nucleus; this produces a haploid zygote (the zygote contains only a single chromosome (homologue) from each chromosome pair, and each chromosome comes from the mother, which is why they are called “gynogens”).

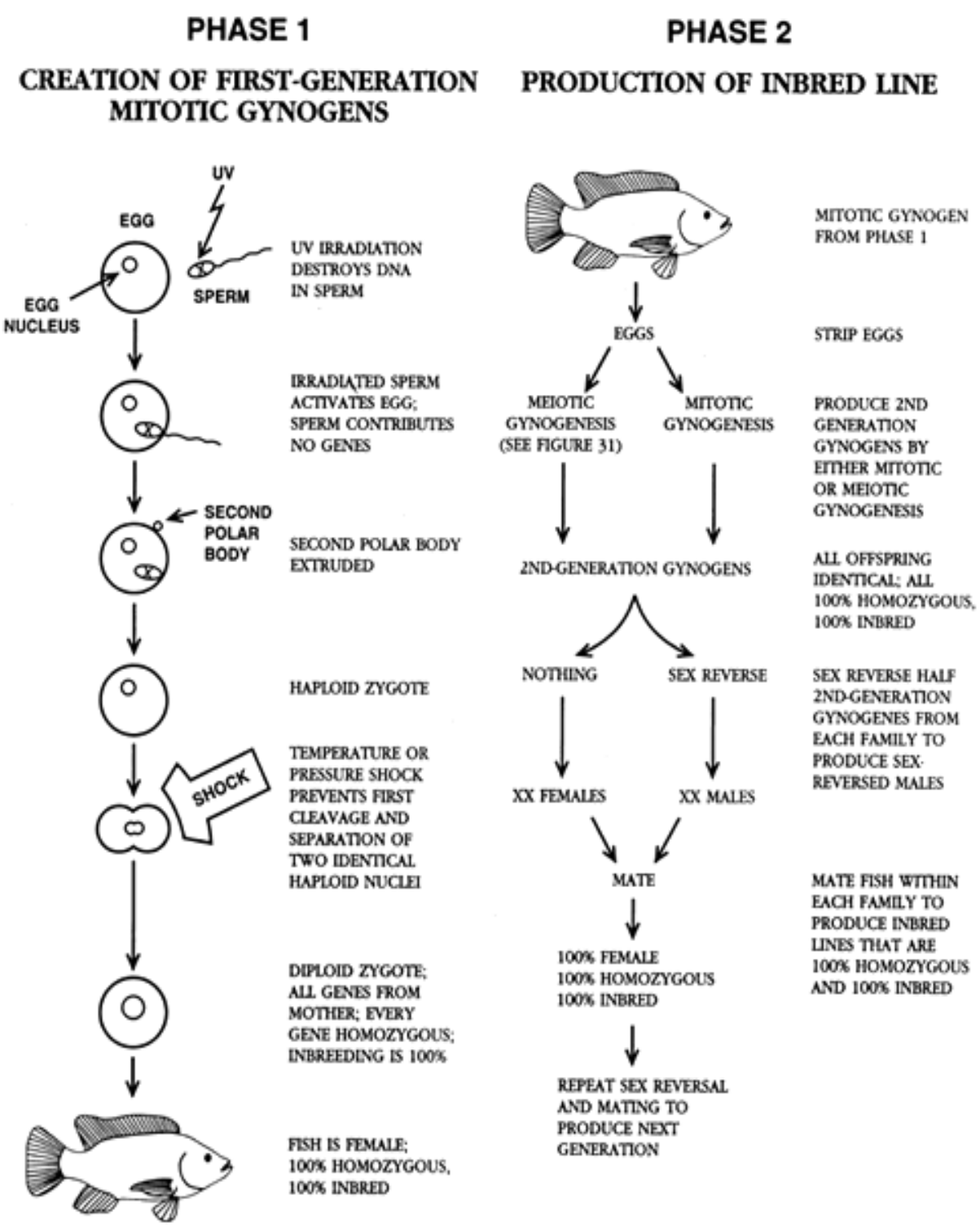
When the haploid zygote undergoes first cleavage, a pressure or temperature shock is used to prevent the haploid zygote nucleus from dividing into two daughter nuclei.If the shock is timed perfectly, the haploid zygote nucleus has replicated its chromosomes so that each daughter nucleus will have a full and identical set of chromosomes, but the haploid zygote nucleus has not divided.By preventing first

cleavage, the zygote remains a zygote, but the chromosome number of the zygote has doubled from the haploid state to the normal diploid state, which means that each chromosome occurs as a pair.

Since mitosis (first cleavage is a mitotic cell division) produces two identical sets of chromosomes, each chromosome pair is composed of two identical chromosomes. Consequently, every gene comes from the mother, and every gene is homozygous; the mitotic diploid gynogen is 100% homozygous and 100% inbred. If first-generation mitotic gynogens are to be used in a breeding programme to create inbred lines, a second phase of gynogenesis followed by sex reversal is needed in order to produce the lines of 100% inbred fish, in which all fish within each inbred line are genetically identical.

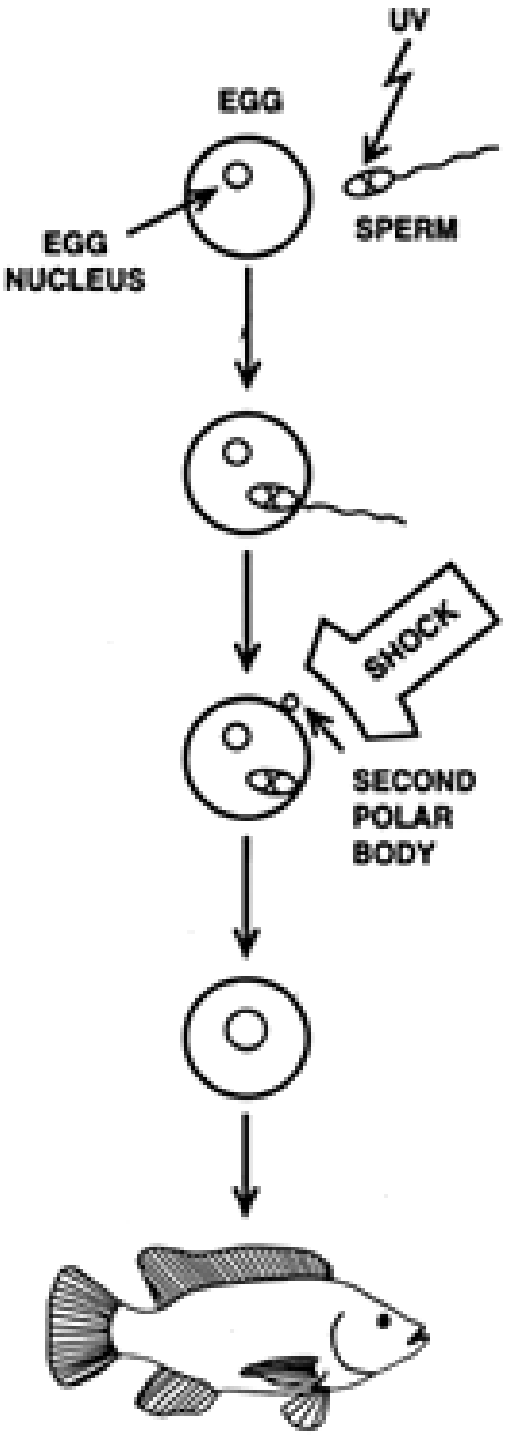
Each first-generation mitotic gynogen will be used to create a unique line of 100% homozygous and 100% inbred fish by utilizing either of two possible types of gynogenesis. When the first-generation mitotic gynogens mature, their eggs are stripped, and either mitotic gynogenesis is repeated to produce second-generation gynogens or meiotic gynogenesis is used to create second generation gynogens.

Half of the second-generation gynogens from each family are sex-reversed with anabolic androgens (steroid hormones) to produce XX sex-reversed males. The sex-reversed males are genetic females but phenotypic males. The fish that are not treated with hormones are raised normally. Within each family, the sex-reversed males and their sisters are genetically identical (genetically, they are all identical sisters); when they mate, they produce an inbred line of genetically identical fish that is 100% female, 100% homozygous, and 100% inbred. Sex-reversed males must be created every generation, because it is the only way males can be produced, and it is the only way each inbred line can be perpetuated without additional chromosomal manipulation.



Meiotic gynogenesis

Gynogenesis can be used to create another type of inbred fish-meiotic gynogens. This type of chromosomal manipulation is easier than mitotic gynogenesis, and meiotic gynogens have a higher survival rate than mitotic gynogens because they have less inbreeding.Meiotic gynogenesis is less useful in producing inbred lines because it is difficult to accurately predict the exact amount of inbreeding produced, and the inbreeding produced each generation is quite variable.Since regular systems of inbreeding are most useful when they produce reliable and predictable amounts of inbreeding, meiotic gynogenesis is less useful than mitotic gynogenesis for producing inbred lines. However, one to three generations of meiotic gynogenesis can be used to produce highly inbred fish.

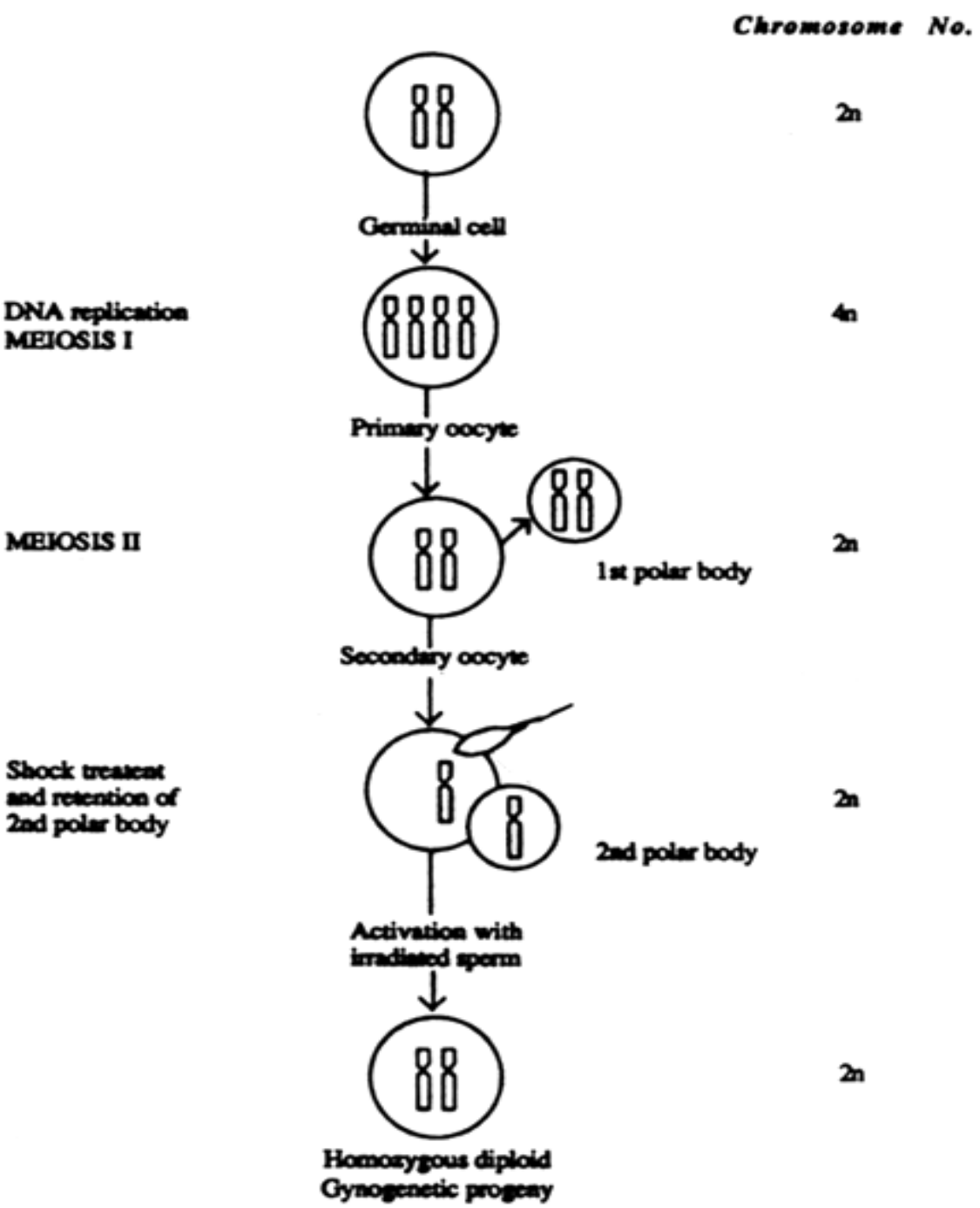


UV IRRADIATION DESTROYS DNA IN SPERM

IRRADIATED SPERM ACTIVATES EGG; SPERM CONTRIBUTES NO GENES

TEMPERATURE OR PRESSURE SHOCK PREVENTS EXTRUSION OF SECOND POLAR BODY

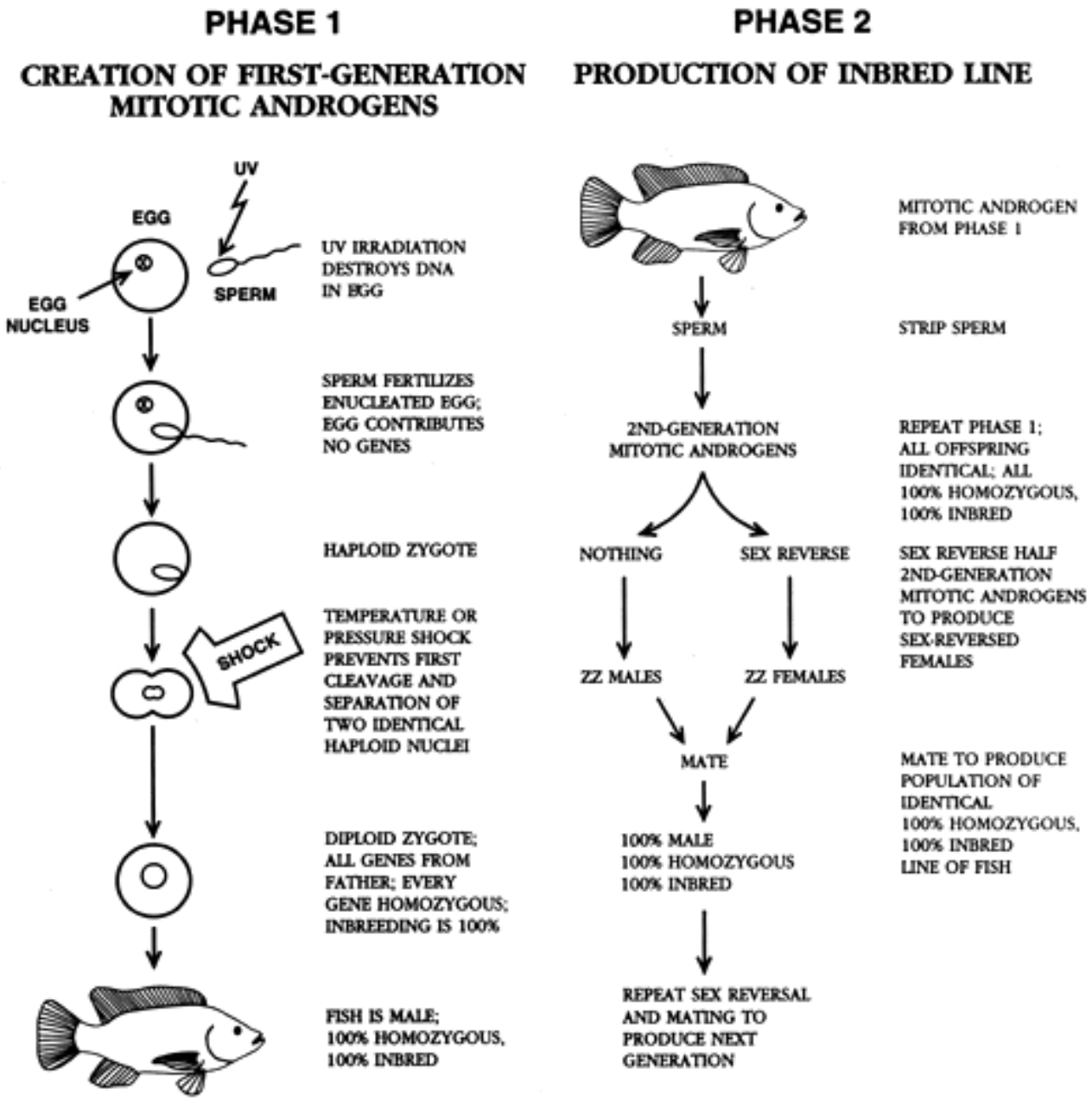
SECOND POLAR BODY NUCLEUS FUSES WITH EGG NUCLEUS TO FORM DIPLOID ZYGOTE; ALL GENES FROM MOTHER


Chromosome No.
2n
Germinal cell
DNA replication
MEIOSIS I
4n
Primary oocyte
MEIOSIS II
2n
1st polar body
Secondary oocyte
Shock treatent
and retention of
2nd polar body
2n
2nd polar body
Activation with
irradiated sperm
2n
Homozygous diploid
Gynogenetic progeny

****

Mitotic androgenesis

Mitotic androgenesis can be used to produce mitotic androgens (all genes come from the father), fish that are 100% inbred. The technique that is used to produce mitotic androgens for species with the WZ sex-determining system is outlined in Figure



Phase 2 of this breeding programme uses a second round of mitotic androgenesis, and half the offspring from each family are sex-reversed with anabolic estrogens to produce sex-reversed ZZ females. These females are genetic males but phenotypic females. Within each family, the sex-reversed females are mated to their genetically identical brothers to produce each inbred line; fish in each inbred line are genetically identical, 100% homozygous, and 100% inbred and all are males. As was the case with mitotic gynogens, each line is genetically unique.

****

Polyploidy

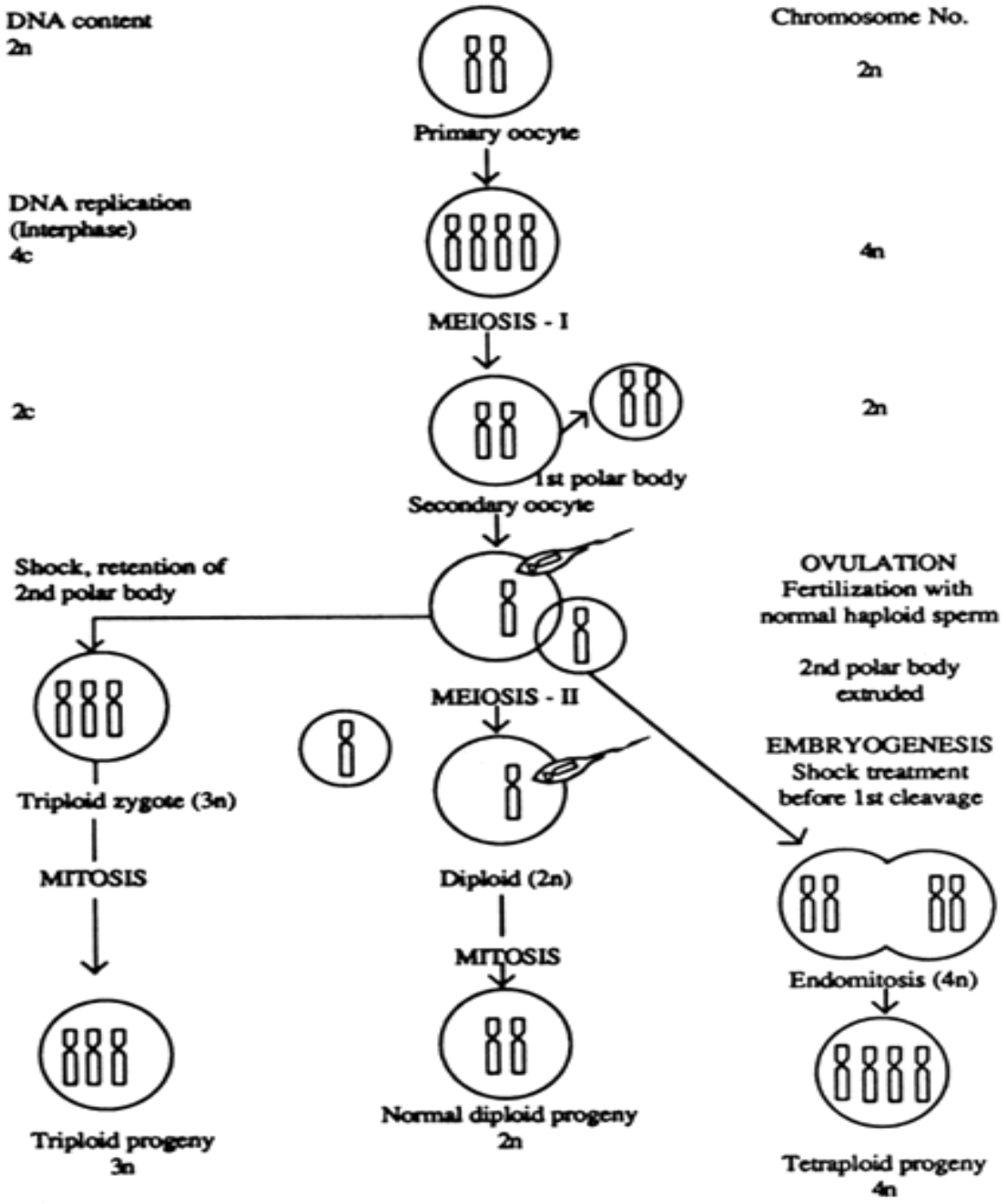
An increase in the level of ploidy of an individual by the addition of one or more set(s) of chromosomes refers to polyploidy resulting usually either in triploidy or tetraploidy.Sometimes it may also result in penta or hexaploidy.

Natural polyploidy (triploidy/tetraploidy)

Polyploidy has been observed to occur in nature in some species of fish like the common carp (*Cyprinus carpio*) and trout mainly due to chromosomal translocation and when two distantly related fish species are crossed.The crosses between grass carp and bighead carp had produced triploid hybrids (Marian and Krasznai, 1978).However, none of the interspecific nor intergeneric hybrid crosses among Indian carps have been reported to produce such allotriploids.

Artificial induction of polyploidy in Indian major carps

Some preliminary attempts have been made to induce artificial induction of triploidy and tetraploidy in Indian major carps with varied degrees of success


DNA content
2n
Chromosome No.
2n
Primary oocyte
DNA replication
(Interphase)
4c
4n
MEIOSIS - I
2c
2n
1st polar body
Secondary oocyte
Shock, retention of
2nd polar body
OVULATION
Fertilization with
normal haploid sperm
2nd polar body
extruded
MEIOSIS - II
EMBRYOGENESIS
Shock treatment
before 1st cleavage
Triploid zygote (3n)
MITOSIS
Diploid (2n)
MITOSIS
Endomitosis (4n)
Triploid progeny
3n
Normal diploid progeny
2n
Tetraploid progeny
4n

Conclusion:

Chromosomal manipulation can be used to quickly produce highly inbred lines of fish.One generation of mitotic gynogenesis or mitotic androgenesis will produce fish that are 100% homozygous and 100% inbred. A second generation of chromosome set manipulation is needed to produce 100% inbred fish that are capable of reproducing.

One generation of meiotic gynogenesis will produce fish that have large, but unknown, levels of inbreeding; the amount of inbreeding produced by meiotic gynogenesis is variable and depends on crossing over frequencies.The use of chromosomal manipulation to produce inbred lines should be done only by scientists at large agribusinesses or at research stations.

****

Artificial insemination

Artificial insemination (the collection of spermatozoa and ova and their mixing together in various media that keep spermatozoa motile) is carried out in only a few species (mostly freshwater), such as

salmonids, cyprinids and acipenserids. Traditionally, fresh water (or sea water for marine species) is used as the medium in which the male and female gametes are mixed. However, fresh water is not a very favourable medium because hypotonic shock causes the sperm structure to deteriorate in several minutes and the egg is activated quickly. These problems can be avoided by using as media various saline solutions of different composition, depending on the species (125 mM NaCl pH 9 for salmonids; 50 raM Nacl pH 8 for cyprinids). These media prevent sperm deterioration, prolong slightly the duration of motility, and prevent or defer the cortical reaction. These solutions also prevent the yolk of crushed eggs from precipitating when it comes into contact with the water, limit motility and block the micropyle. Fish farmers are beginning to use these media, so significantly increasing the fertilization rate while reducing the number of spermatozoa used for insemination. The length of gamete survival is an important factor to consider in carrying out artificial reproduction. Gamete survival *in vivo* (after the release of sperm and oocytes from cysts and follicles) varies with the species. Sperm fertilizing ability decreases during the spawning period in sea bass and trout but not in carp. Ovum survival in the general or ovarian cavity is from one to several weeks in salmonids, several hours in carp at 20°C and only 30 min in Chinese carp. *In vitro* survival is from one to several weeks for sperm (under oxygen and with antibiotics added) and several hours for ova (2–4 h in carp and 12–24 h in trout). The spermatozoa of several species of teleosts have been stored deep frozen, but the quality of the sperm is not as good and more spermatozoa per ovum have to be used to obtain the same percentage of fertilization as with non-frozen sperm.

INDUCED BREEDING AND LARVAL REARING
of
Clarias macrocephalus

by

Vijai Srisuvantach
Manob Thangtrongpiros
Regional Lead Centre in Thailand
National Inland Fisheries Institute
Bangkhen, Bangkok 10900
Thailand

1. INTRODUCTION

Walking catfish in English, or “pla duk” in Thai, is a generic name for a number of species belonging to the family Clarridae. Five are encountered in Thailand, two of which are popular sources of animal protein, Clarias batrachus and Clarias macrocephalus, locally known as “pla duk dan” and “pla duk oui”, respectively. C. batrachus fry is easily obtained from the spawning pond. Unfortunately, C. macrocephalus do not readily reproduce themselves in captivity. However, it can be induced to breed if injected with extracts of fish pituitary glands containing gonadotropin sex hormones.

Thai consumers have a preference for C. macrocephalus but, because of bottlenecks in fry availability and slow growth, its culture is still limited in comparison to C. batrachus.

Our biologists have worked for 20 years to develop artificial breeding methods. This attempt was first successful for Pangasius sutchi and Chinese Carps in 1965. Further studies were continued. Up to now, induced spawning is a rutine work for our aquaculturists.

The purpose of this review is to summarize the induced breeding and larval rearing of C. macrocephalus practices in Thailand.

2. Characteristics and Biology

The species is closely related to C. batrachus from which it can be distinguished by the wide occipital process (Fig. 1). Body is elongate with head broadly depressed, four pairs of well developed barbells, and small eyes. Dorsal and anal fins are long without spine. Pectoral fin has a pungent spine with serrated on its inner edge. Caudal fin is not confluent with dorsal or anal fin (Fig. 2). Body color is dark brown with purplish tint and about tent transverse rows of small white spots on the side.

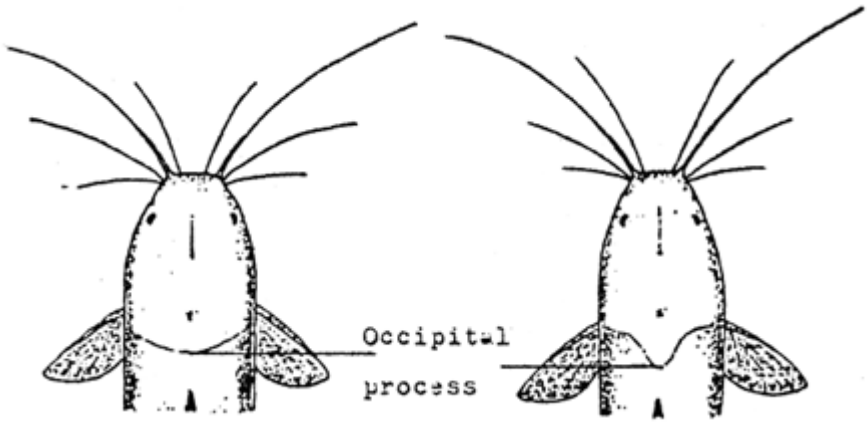


Fig. 1. Occipital process of Clarias macrocephalus (left) and Clarias batrachus (right).

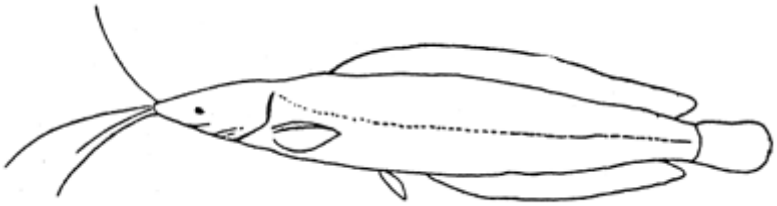


Fig. 2. Clarias macrocephalus Günther

The species is distinguished by their ability to survive in a wide range of water conditions. It requires a relatively small area for culture and can be stocked more densely than many other species. They can live out of water for several hours or in waters of low oxygen content as they have accessory organs that enable them to breathe atmospheric air. Its range of distribution includes the areas from Indochina penninsular. Thailand and the Philippines.

The spawning season is between May and October. The female makes a small round hollow nest with grassy bottom about 30 cm in diameter and 5 – 8 cm deep in shallow water. The eggs are deposited in the nest and attached to the roots of aquatic vegetation in the nest. The male will take charge of these eggs until they are hatched out. The egg can be hatched out within 20 hours at temperature of 25 – 30°C. A female weighing 300 – 800 gm can produce between 5,000 – 10,000 eggs. The natural diet is wide-ranging, it includes worms, insects, shrimps and decayed matter.

3. Selection of Spawners

The essentials in fish induced spawning are fully ripe mature brooders both female and male. Brood fish should be carefully tended for two to three months before induced spawning operations are

carried out. Males and females should be segregrated and stocked in separate ponds. Selection of spawners is one of the most important stages in induced spawning operations. It is necessary to know how to select healthy males and females in order to obtain maximum production of fry.

Determination of ripeness is an art and requires experience. To be good brooders the firsh must be more than one year old or 150 gm. Sex can be distinguished by the shape of the genital papilla (Fig. 3). The male genital papilla is pointed. The female papilla is oval shape. The following characteristics can be used as guidelines to ascertain that the female is ready for induced spawning operations. It has a bulging abodomen. It is clastic and soft to the touch. The cloaca is reddish and prominent, and the contour of this ovary can be seem on both sides of the abdomen.

4. Obtaining the Pituitary Gland

Pituitary gland contains hormone namely gonadotropin which stimulate the production of sex steroids in the gonad which responsible for the maturation of gametes. Gonadotropin is composed of follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are responsible for egg development and egg ovulation respectively.

Pituitary gland of common carp, Chinese carp, Indian carp, and Panqasius sutchi can be used for induced spawning. The fish from which the hyopphysis is to be collected is weighed and placed on a shopping board. The skull is cut open with a knife (Fig. 4). After removing a piece of the skull, fatty tissue and blood are wiped off with a cotton pad. The pituitary gland can be seen after the mid-brain has been folded back by using forceps.

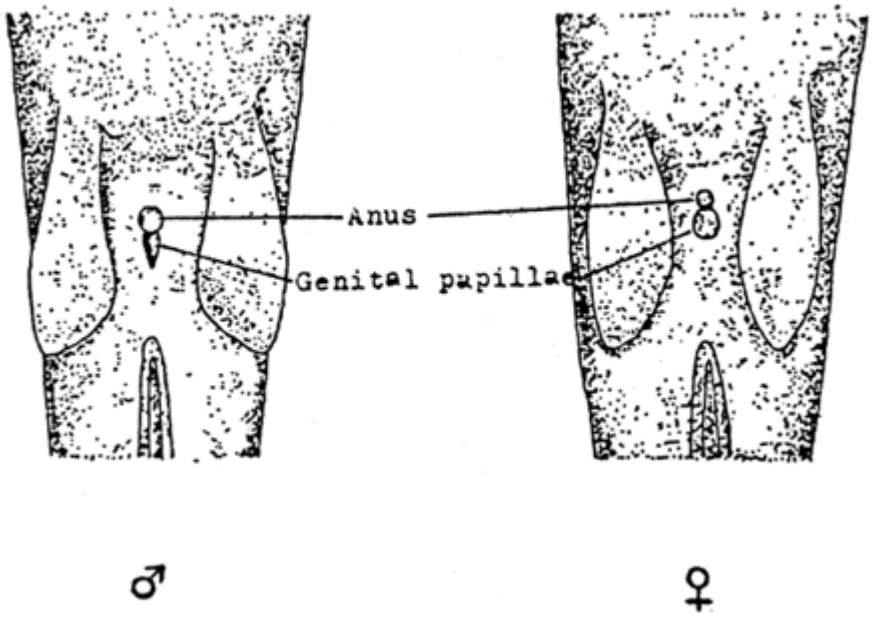


Fig. 3. Genital papillae of the male and female Clarias macrocephalus

5. Amount of Pituitary Gland Solution Injected

One concentration or one dose commonly used can be expressed as follow:

$$\text{one dose} = \frac{\text{weight of donor fish}}{\text{equivalent weight of recipient fish}}$$

The gland is ground in the homoginizer: distilled water is added and the gland is again ground. A syringe is used to take up the solution for injection (Fig. 5). The female can be injected with aypophysis of Chinese carp in a dosage of 1.0 for the first injection and 2.0 for the second injection with the time interval of 6 hours. Aliquots of isotomic saline solution or distilled water is added depending on the weight of recipient, about 0.5 ml for fish weigh less than 1.0 kg of body weight and 1.0 kg body weight and 1.0 ml for fish weigh 1 – 3 kg.

The intramuscular injection is given in the area between the base of the dorsal fin and lateral line (Fig. 6).

6. Artificial Insemination

Ovulation occurs about ten hours after the second injections. The water on the female's body should be wiped off with a towel. As the abdomen is being pressed, the stripped eggs should be collected in

a dry plastic container (Fig. 7). At the same time, the milt is made to drip on the eggs by grinding the testis with fingers and pouring the water through the fine mesh cloth. Eggs and sperm are mixed and stirred gently with a feather. Next, a little clean water is added and gently mixed again. After one to two minutes, water is added two or three times to cleanse the fertilized eggs. The fertilized eggs are transferred to the hatching hapa (Fig. 8). Most of the fertilized eggs hatch out within 24 hours. Figure 9 shows the location of testis.

7. Fry Mursing

After yolk resorption, usually within 2 days, the larvae are transferred from hapa to the nursery fiber glass tank. The fry develop feeding behaviour at about the same time their yolk was absorbed. The food to be given for the first 3 weeks is live moina. Usually 3 weeks old fry with the size of 2 – 3 cm are distributed to the fish farmers.

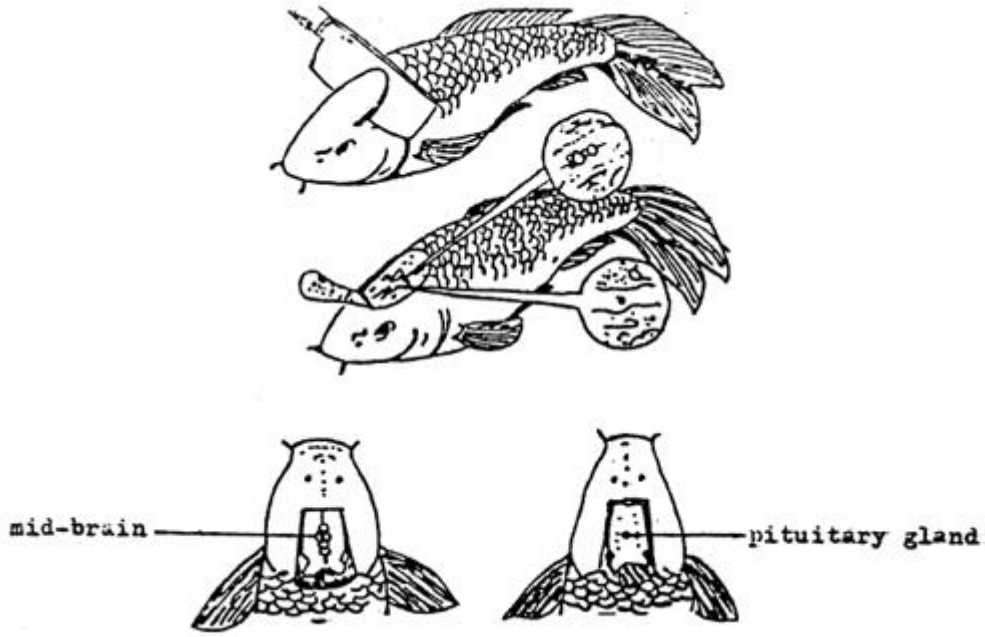


Fig. 4. Extraction method for removing the pituitary gland, showing transverse cut, mid-brain and pituitary gland location.

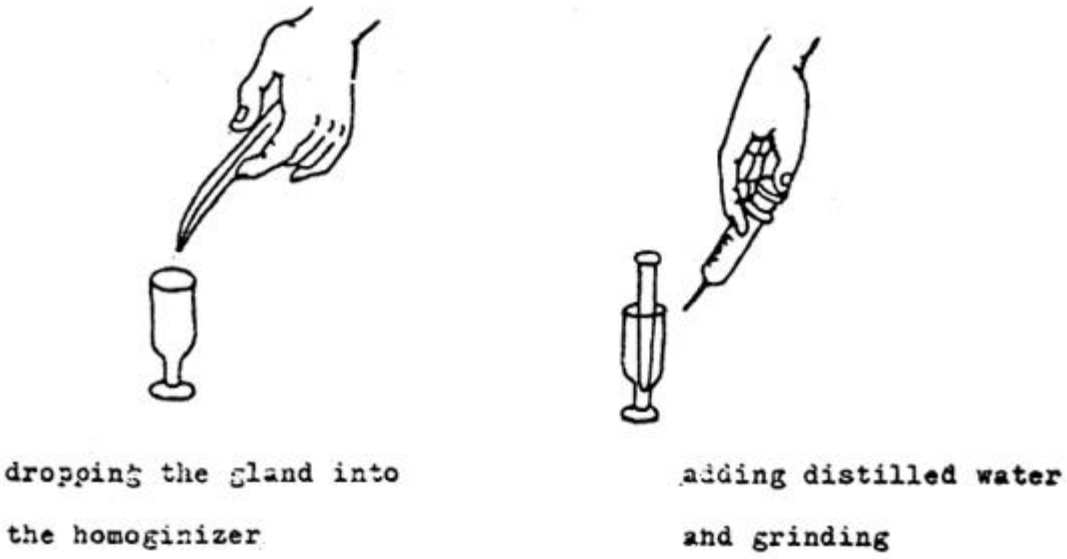


Fig. 5. Preparation of the pituitary gland solution

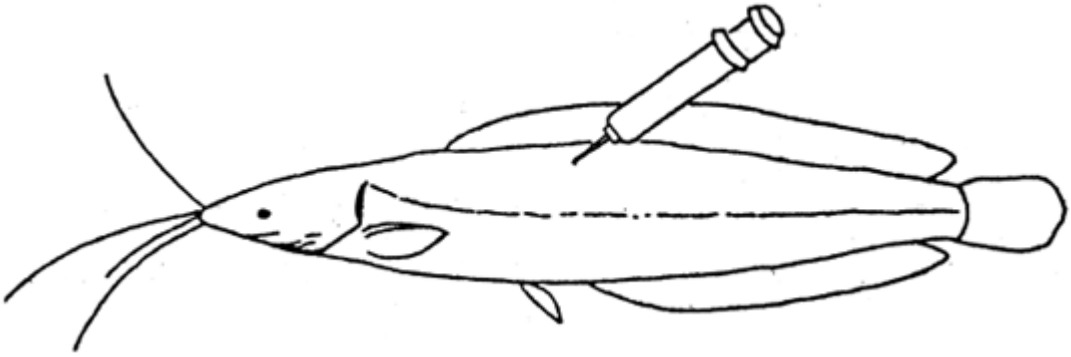


Fig. 6. Location for intramuscular injection.

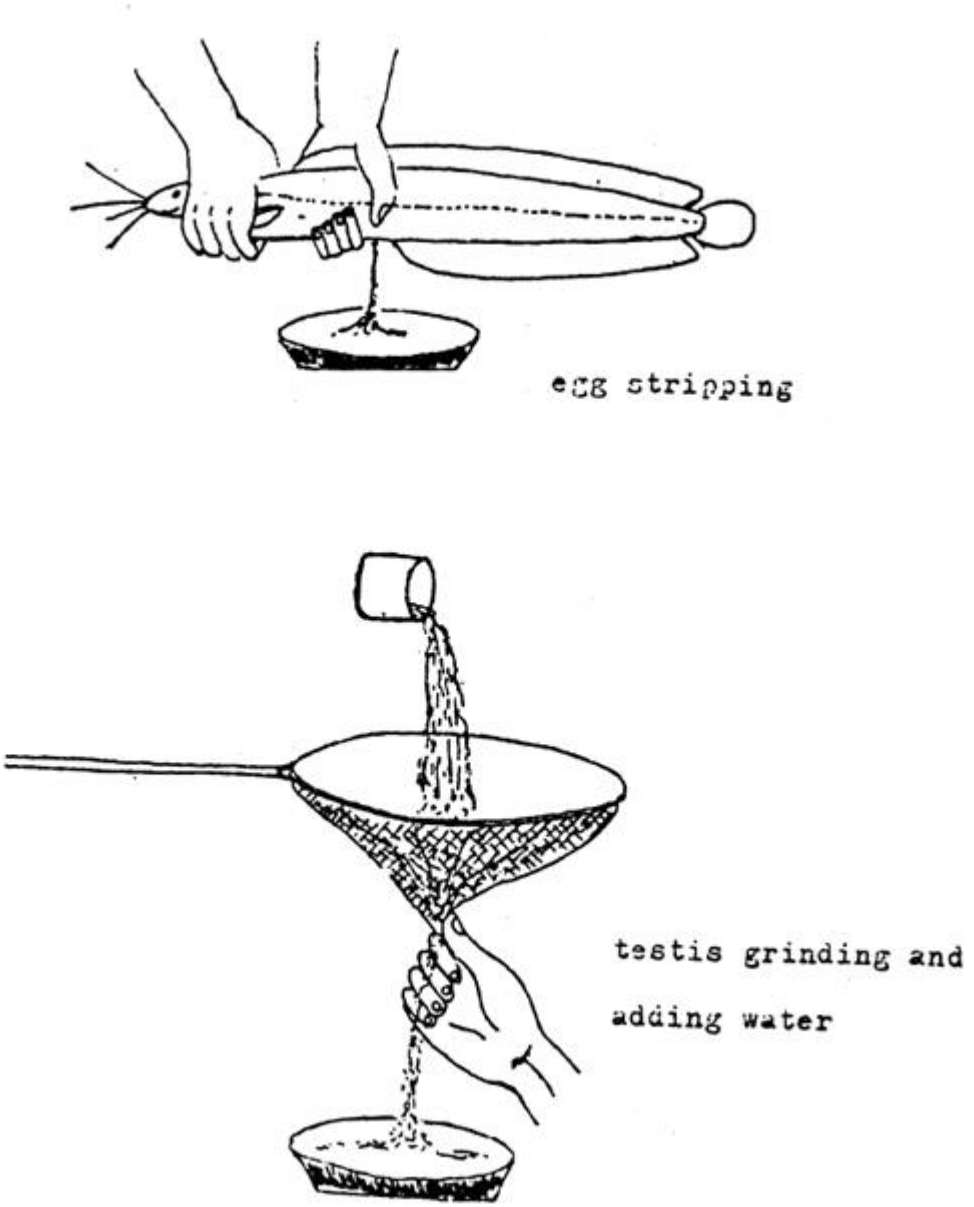


Fig. 7. Artificial insemination.

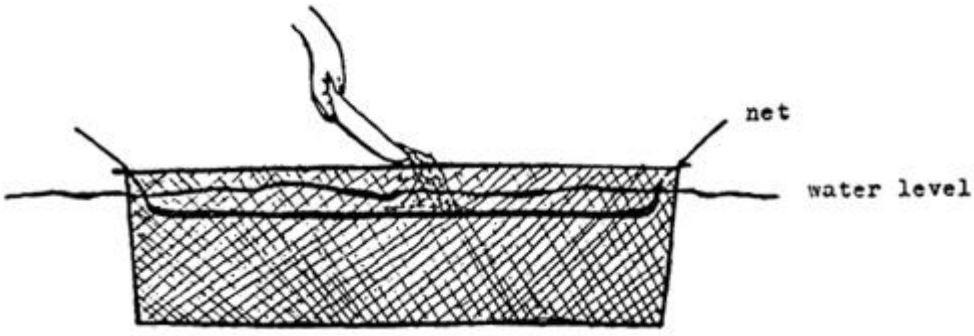


Fig. 9. Transferring fertilized eggs to incubator hapa.

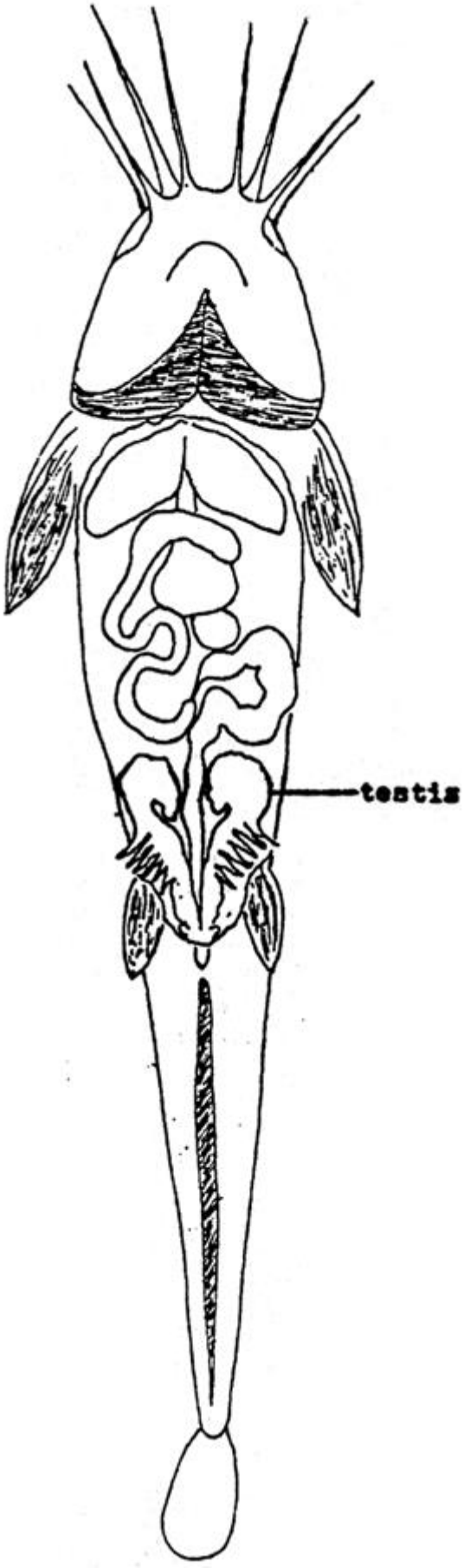

testis

Fig. 8. Location of testis with the digestive track folded up.

8. Egg Development

The stages of egg development are given in Fig. 10.

Fig. 10. Egg development of Clarias macroccphalus, 70 X

Eyestalk ablation

The process of unilateral eyestalk ablation is used in almost every marine shrimp maturation/reproduction facility in the world, both research and commercial, to stimulate female shrimp to develop mature ovaries and spawn. This method of inducing females to develop mature ovaries is used for two reasons:

1. most captive conditions cause inhibitions in females which keep them from developing mature ovaries in captivity
2. even in conditions where a given species will develop ovaries and spawn in captivity, use of eyestalk ablation increases total egg production and increases the percentage of females in a given population which will participate in reproduction

Hormonal effects of eyestalk ablation

The most commonly accepted theory is that a gonad inhibitory hormone (GIH) is produced in the neurosecretory complexes in the eyestalk. This hormone apparently occurs in nature in the non-breeding season and is absent or present only in low levels during the breeding season. By inference, then, the reluctance of most penaeids to routinely develop mature ovaries in captivity is a function of elevated levels of GIH, and eyestalk ablation lowers the high hemolymph titer of GIH. The effect of eyestalk removal is not on a single hormone such as GIH, but rather effects numerous physiological processes (Bray & Lawrence, 1992).

Indirect effects of eyestalk ablation

Considering that eyestalk ablation affects the hormone balance for numerous physiological processes in addition to stimulation of gonadal hypertrophy, what are the practical effects of

this operation, and at what cost do we achieve induced ovarian development using eyestalk ablation? The following observations have been made concerning use of eyestalk ablation in captive reproduction, and may be related to either captive conditions, eyestalk ablation, or both:

- Captive spawn size (number of eggs per spawn) is smaller than in wild-matured females, regardless of whether eyestalk ablation is used.
- Eyestalk ablation increases total egg production in captivity by producing more frequent spawnings, but not larger spawns.
- There is not a strong trend toward diminishing spawn size over time.
- Molt cycle duration is shorter in eyestalk-ablated females than intact females.
- Higher mortality of eyestalk ablated females is often, but not always, reported.
- Eyestalk ablation has been suggested to deteriorate female condition.
- Eyestalk ablation in some instances has been observed to produce lower hatch rate of eggs than unablated females.
- Hatch rate has been observed to decline over time under captive conditions.
- Ovarian color in captive females, especially in eyestalk ablated females, is often rather different than wild-matured (Bray & Lawrence, 1992).

There is strong circumstantial evidence that part of the problems seen with captive reproduction are related to a simple inability of current diets to supply required nutrients as rapidly as required for the gonadal hypertrophy stimulated by eyestalk ablation. In nature, an organism would not be anticipated to develop eggs, constituting some 10% of female body weight, unless nutrients are available for first, metabolism, second, growth, and third, reproduction. Eyestalk ablation accelerates the production of ova, regardless of whether the proper types and balance of nutrients are available, and regardless of whether those ova are even capable of fertilization. Dietary factors clearly have been shown to influence percentage hatch and percentage of females spawning (Bray & Lawrence, 1992).

Eyestalk ablation techniques Shrimp should be ablated only when hard-shelled, never when in post-molt (newly molted or seft-shelled) or premolt stages. Because molting and reproduction, especially in females, are both energy demanding processes, they appear to be antagonistic in terms of biological programing.

Unilateral eyestalk ablation is accomplished in the following ways:

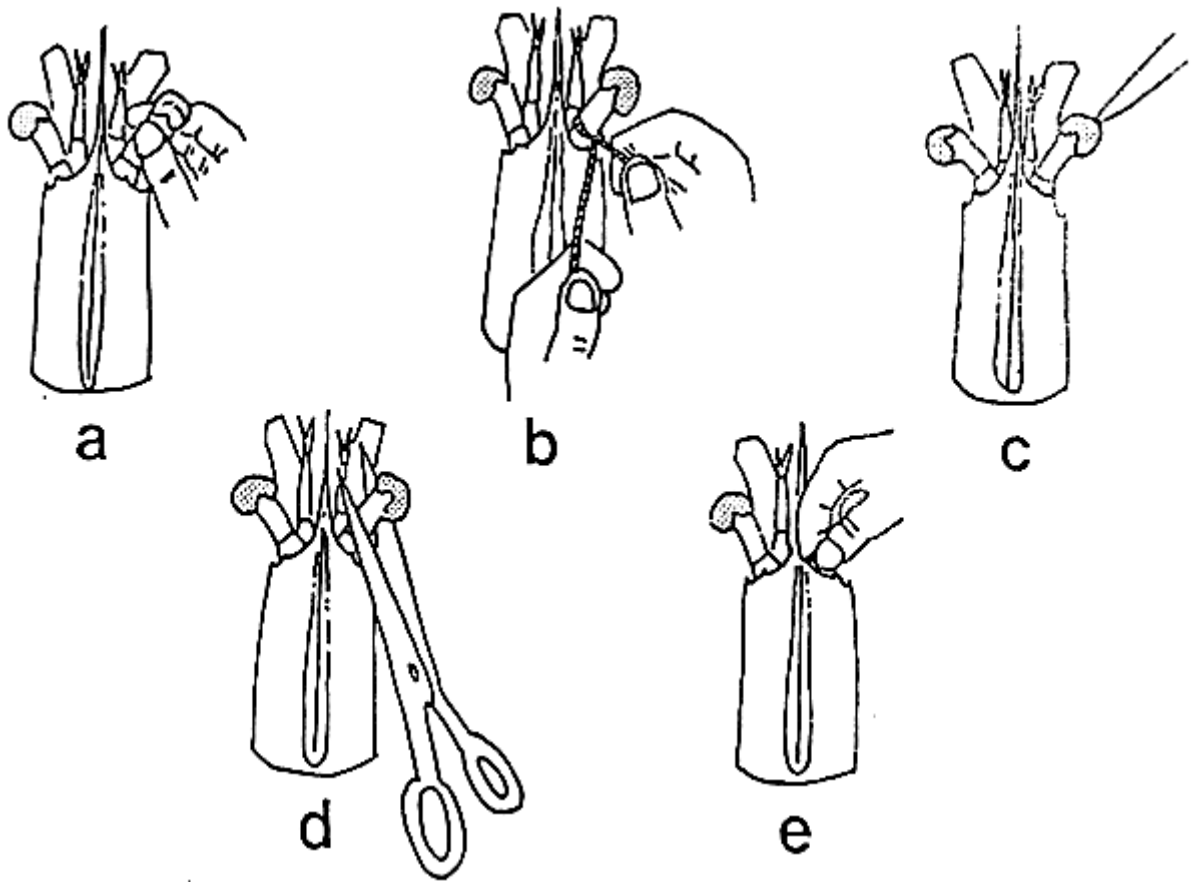
1) Simple pinching of the eyestalk, usually performed half to two-thirds down the eyestalk. This method may leave an open wound.

2) Slitting one eye with a razor blade, then crushing eyestalk, with thumb and index fingernail, beginning one-half to two-thirds down the eyestalk and moving distally until the contents of eyes have been removed. This method, sometimes called enucleation, leaves behind the transparent exoskeleton so that clotting of hemolymph, and closure of the wound, may occur more rapidly.

3) Cauterizing through the eyestalk with either an electrocautery device or an instrument such as a red-hot wire or forceps. If correctly performed, this method closes the wound completely and allows scar tissue to form more readily. A variation of this technique is to use scissors or sharp blade to sever the eyestalk, and then to cauterize the wound.

4) Ligation by tying off the eyestalk tightly with surgical or other thread. This method also has the advantage of immediate wound closure (Bray & Lawrence, 1992).

Latency period: eyestalk ablation to ovarian development
Once females have been subjected to eyestalk ablation, complete ovarian development often ensues within as little as 3 to 10 days, assuming the animals were removed from a breeding or ready-to-breed population, of adequate size for reproduction, and not subjected to too much transfer stress. If the animals have been removed from non-conducive environmental conditions (e.g, cold, non-breeding season temperatures, or hypersaline conditions), a longer than normal latency period between eyestalk ablation and ovarian development can be anticipated, probably due to seasonal hormonal cycling. Duration of the latency period between eyestalk ablation and maturation of ovaries is determined by the readiness of the population at the time of eyestalk ablation (Bray & Lawrence, 1992).



Methods of eyestalk removal in Penaeids:

a) eyeball incision and squeezing;

b) ligation or tying

c) electrocautery or using a silver nitrate bar;

d) cutting;

e) pinching-crushing.

Transgenic Fish and Aquaculture

Transgenic fish species can be routinely produced by transferring foreign DNA into developing embryos via microinjection or electroporation. This technology offers an excellent opportunity for modifying or improving the genetic traits of commercially important Fishes, mollusks, and crustaceans for aquaculture. Studies have shown that administration of recombinant fish or mammalian growth hormone (GH) to juvenile fish or oysters resulted in significant growth enhancement. Thus, it is possible to improve the growth rates of marine animals by manipulating GH or its gene. This paper reviews the results of studies to determine the efficacy of recombinant fish GH in improving the growth rates of fishes, mollusks, and crustaceans, and of gene transfer technology in producing fast-growing transgenic animals.

Introduction

The worldwide harvest of fishery products traditionally depends upon the natural populations of fishes, mollusks, and crustaceans from fresh and marine waters. Due to the rapid increase in consumption of fishery products by the general public, as well as uncontrolled fishing and poor management, the total annual harvest has already approached the maximum potential of about 150 million tons as forecast by the US Department of Commerce. Accumulation of chemical pollutants in aquatic environments has further affected the fisheries production. A number of regions have experienced significant declines in the catches of important species such as salmon, striped bass, sturgeons, eels, jacks, mullets, mackerel, abalones, oysters and crabs (FAO 1986). Fishing fleets now travel great distances to exploit more productive areas. They have switched to alternative species and begun to employ a variety of sophisticated technologies. These developments have caused significant increases in fish prices. In the past decades, many countries have turned to aquaculture to increase fish production. In 1992, the world production from aquaculture exceeded 17 million tons, about 32.2% of the total fisheries production (Csavas 1995). Thus, aquaculture has the potential to substantially meet the world demand for fishery products.

The success of aquaculture depends on: (1) complete control of reproduction and the life cycle, (2) excellent genetic background of the broodstock, (3) efficient detection and effective prevention of diseases, (4) thorough understanding of the optimal physiological, environmental, and nutritional conditions for growth and development, (5) sufficient supply of good quality water; and (6) innovative management techniques. By improving some of these factors, the aquaculture industry has already made impressive progress over the last several years. The application of molecular biology and biotechnology will further speed up the expansion of the industry. These applications

include enhancing growth rates, controlling reproductive cycles, improving feed composition, producing new vaccines, and developing disease-resistant and hardier genetic stocks. Over the last several years, our laboratory and others have been searching for innovative strategies to increase fish production by applying the methods of contemporary molecular biology and biotechnology. In this paper, I will summarize results of our studies and those of many others to demonstrate the efficacy of modern biological techniques, including transgenic fish technology, in increasing the production from aquaculture.

Effect of Recombinant Fish Growth Hormone on Somatic Growth

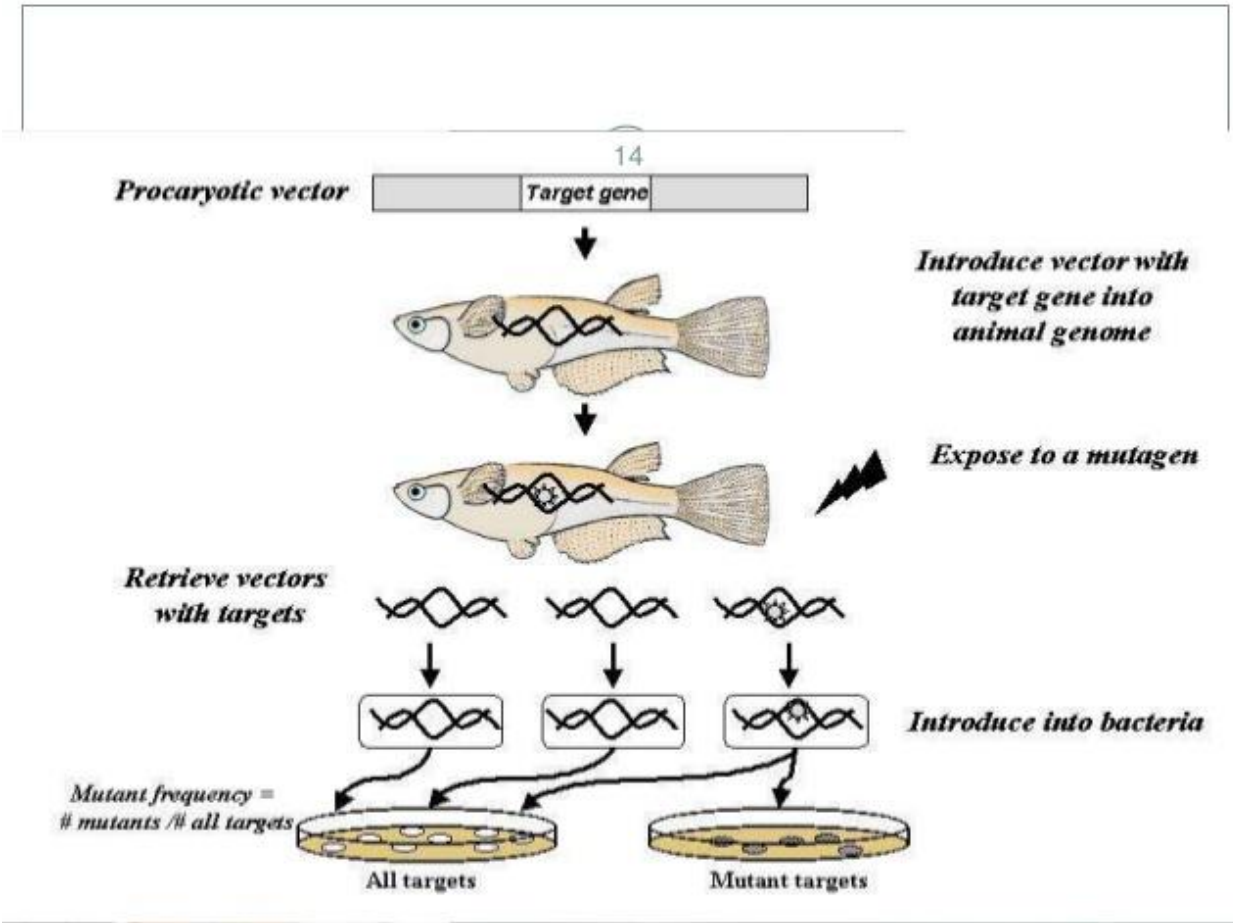
Thanks to the rapid advances in recombinant DNA technology, complementary DNA (cDNA) and the genomic sequence of growth hormone (GH) have been isolated and characterized for several fish species in recent years (Gill et al. 1985, Agellon and Chen 1986, Gonzales-Villaseñor et al. 1986, Momota et al. 1988, Watahiki et al. 1989). Our laboratory has prepared biologically active recombinant GH by expressing rainbow trout (rt) GH1 cDNA in E. coli cells (Agellon et al. 1988). Since rainbow trout GH molecule is highly hydrophobic, the resulting polypeptide synthesized in E. coli cells forms insoluble inclusion bodies, which are inactive but can be easily recovered by differential centrifugation. The protein is dissolved in a 100 mM Tris buffer pH 9.0 with 8 M urea. Renaturation of the recombinant hormone is carried out by slowly diluting the solution to 4.5 M urea and the protein concentration below 0.5 mg/ml while stirring gently at 40°C for 24 hours. The biological activity of the resulting hormone preparation is assessed by its ability to stimulate the uptake of radioactive sulfate into gill cartilage in vitro and the accumulation of insulin-like growth factor (IGF)-I in the liver in vivo.

Agellon et al. (1988) showed in a series of studies that application of this recombinant hormone to yearling rainbow trout resulted in a significant growth enhancement. After treatment with the recombinant rtGH for four weeks at a dose of 1 mg/g body weight each week, the weight gain among the hormone-treated rainbow trout was two times greater than among the controls. Significant length gain was also evident in hormone-treated animals. When the same recombinant hormone was administered to small juvenile rainbow trout by immersing them in GH-containing solutions, the same growth-promoting effect was also observed (Table 1; also Leong and Chen, unpublished results). These results are in agreement with those reported by Sekine et al. (1985), Gill et al. (1985), and others (Schulte et al. 1986, Sato et al. 1988a, 1988b, Moriyama et al. 1990). However, it is important to mention that the growth enhancement effect of the biosynthetic hormone was markedly reduced when more than 2 mg/g was applied to the test animals (Agellon et al. 1988). These results

suggest that when the total amount of GH exceeds the maximal threshold level, homeostasis is disturbed and growth is affected.

Transgenic Fish Harboring Growth Hormone Gene

Although exogenous application of recombinant GH results in significant growth enhancement in fish, it may not be cost-effective. If new strains of fish producing elevated but optimal levels of GH can be produced, it would bypass many of the problems with exogenous GH treatment. Moreover, once these fish strains have been generated, they would be far more costeffective than their ordinary counterparts because the fish would produce and deliver the hormone and transmit the enhanced growth characteristics to their offspring.



Gene transfer methodology

Animals into which a segment of foreign DNA has been introduced and stably integrated into the host genome are called 'transgenic' Since Constantini and Lacy's (1981) transgenic mice, many other transgenic animals including livestock and fishes have been constructed successfully (Palmiter et al. 1982, Gordon and Ruddle 1985, Hammer et al. 1985, Ozato et al. 1986, Dunham et al. 1987, Pursel

et al. 1989, Chen and Powers 1990, Chen et al. 1993). These animals play important roles both in basic research as well as in biotechnology application. Various methods have been used to deliver foreign DNA into somatic cells and germlines of mammals and other higher vertebrates. The methods include direct microinjection, retrovirus infection, electroporation, calcium phosphate precipitation, and particle-gun bombardment. Direct microinjection of DNA into the male pronuclei of the fertilized eggs has been the prevalent method. The microinjection method has been used to deliver foreign genes into several fish species in recent years. These include goldfish (Zhu et al. 1985), medaka (Ozato et al. 1986, Lu et al. 1992), rainbow trout (Chourrout et al. 1986), salmon (Fletcher et al. 1988), tilapia (Brem et al. 1988), zebrafish (Stuart et al. 1988), common carp (Zhang et al. 1990, Chen et al. 1993), and catfish (Dunham et al. 1992, Powers et al. 1991). In general, gene transfer in fish by microinjection is carried out as follows. Eggs and sperm are collected into separate dry containers. Fertilization is initiated by adding water and sperm to eggs and stirring gently. Eggs are waterhardened for various periods of time and then rinsed. Microinjection is done within the first two hours after fertilization. The equipment consists of a dissecting stereomicroscope and two micromanipulators, one with a microneedle for injecting DNA into the embryos and the other with a micropipette for holding the embryos in position during the injection. Since the male pronuclei of the fish embryos studied to date are not visible, the foreign genes are usually injected into the egg cytoplasm and the amount of the DNA injected into each embryo is in the range of one million copies or higher. In zebrafish and medaka, natural spawning can be induced by adjusting photoperiod and water temperature and newly fertilized embryos can be readily collected for microinjection. Within the first two hours after fertilization, the micropyle of the embryo is still visible under the microscope. The DNA solution can be easily delivered into the embryos with a microneedle through the micropyle (Stuart et al. 1988, Lu et al. 1992). Although the microinjection method is successful in transferring foreign DNA into fish embryos, it is very laborious and time-consuming. There is interest in developing convenient mass gene transfer technologies for use in fish transgenesis. Among the mass gene transfer methods are particle-gun bombardment, electroporation, and those mediated by retroviruses, liposomes, or sperm. Electroporation is the most effective means of transferring foreign genes into fish embryos. This method uses a series of short electrical pulses that change the membrane permeability and thereby permit the entry of DNA molecules into embryos. Lu et al. (1992) showed that the rate of foreign gene integration in transgenic medaka produced by electroporation was 20% or higher. Powers et al. (1992) recently reported a much higher rate of gene transfer in common carp and channel catfish with the same electroporator. The rate of transgene integration in transgenic medaka produced by electroporation was only slightly higher than that of microinjection, but it takes much less time to produce large numbers of transgenic fish by electroporation.

TRANSGENIC - GloFish

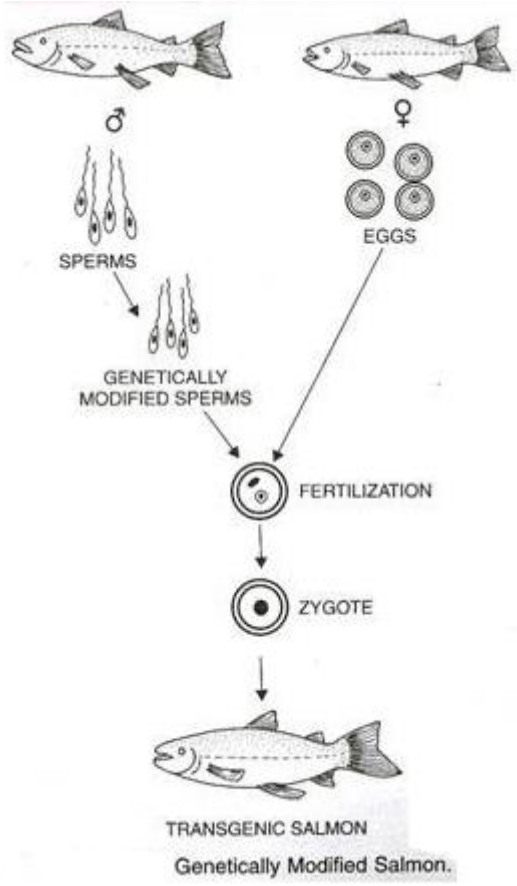
- GloFish, originally developed in Singapore as a way to monitor water pollution
- Produce by integrating a fluorescent protein gene from jelly fish into embryo of fish.
- The normally black+-and-silver zebrafish was turned green or red by inserting various versions of the GFP gene
- Glofish are on sale throughout the US except in California
- Glofish retail for about $5 per fish. Normal zebrafish cost around one tenth of the price

http://www.nus.edu.sg/corporate/research/gallery/research12.htm

Growth Performance of Fish Transgenic for Growth Hormone

Since the site of transgene integration differs among the individuals in any population of P1 transgenic fish, they should be considered as totally different transgenic individuals and cannot be directly compared for growth performance among themselves. Instead, the growth studies should be conducted in F1 transgenic and non-transgenic siblings derived from the same family. Chen et al. (1993) evaluated the growth of F1 transgenic carp in seven families. In these experiments, transgenic and non-transgenic full siblings were spawned, hatched, and reared communally under the same environment. Results showed that growth of F1 transgenic individuals in response to rtGH1 cDNA varied widely. The seven trials showed that transgenics grew 20, 40, 59, and 22% faster, or 27, 15 and 2% slower than non-transgenic full siblings. In three of the four families where F1 transgenics grew faster than their non-transgenic full siblings, the highest and lowest body weights of the transgenics were larger than those of the non-transgenics. In the fourth family, the minimum but not the maximum body weight of the transgenics was larger than that of the non-transgenics. In two of the three transgenic families in which the transgenic siblings grew slower than the non-transgenics, the lowest and highest body weights of the transgenics were less than those of the non-transgenics.

In the third family, however, one of the F1 transgenics was the largest fish in the family. Since the response of the transgenic fish to the insertion of the RSVLTR-rtGH1 cDNA appears to variable, as a result of random integration of the transgene, the fastest growing genotype will likely be developed by utilizing a combination of family selection and mass selection of transgenic individuals after the insertion of the foreign gene. Among transgenic medaka carrying chicken b-actin gene promoter human GH gene construct, the F1 transgenics grew significantly faster than the non-transgenic siblings (Lu et al. 1992). In an effort to study the biological effect of elevated levels of IGF-I on somatic growth, transgenic medaka harboring trout IGF cDNA driven by carp b-actin gene promoter have been produced in our laboratory. Both P1 and F1 IGF-I transgenic medaka hatched two days earlier than their non-transgenic siblings. The P1 transgenics also grew faster than the non-transgenics.



Genetically Modified Salmon.

General Conclusion and Prospective

Transgenic fish technology has great potential in the aquaculture industry. By introducing desirable genetic traits into fishes, mollusks, and crustaceans, superior transgenic strains can be produced for aquaculture. These traits include faster growth rates, improved food conversion efficiency, resistance to some known diseases, tolerance to low oxygen concentrations, and tolerance to extreme temperatures. Our laboratory and those of others have shown that transfer, expression and inheritance of fish growth hormone transgenes can be achieved in several fish species and that the resulting transgenics grow substantially faster than their non-transgenic siblings. This is a vivid example of the potential application of the gene transfer technology to aquaculture. However, to realize the full potential of the transgenic fish technology in aquaculture or other biotechnological applications, several important scientific breakthroughs are required. These include: (1) more efficient technologies for mass gene transfer, (2) targeted gene transfer technologies such as embryonic stem cell gene transfer or ribozyme gene inactivation, (3) suitable promoters to direct the expression of transgenes at optimal levels during the desired developmental stages, (4) identified genes of desirable traits for aquaculture and other applications, (5) information on the physiological, nutritional, immunological and environmental factors that maximize the performance of the transgenics, and (6) safety and environmental impacts of transgenic fish. Once these problems are resolved, the commercial application of the transgenic fish technology will be readily attained.

****

DNA vaccines for aquacultured fish

Deoxyribonucleic acid (DNA) vaccination is based on the administration of the gene encoding the vaccine antigen, rather than the antigen itself. Subsequent expression of the antigen by cells in the vaccinated hosts triggers the host immune system. Among the many experimental DNA vaccines tested in various animal species as well as in humans, the vaccines against rhabdovirus diseases in fish have given some of the most promising results. A single intramuscular (IM)
injection of microgram amounts of DNA induces rapid and long-lasting protection in farmed salmonids against economically important viruses such as infectious haematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV). DNA vaccines against other types of fish pathogens, however, have so far had limited success. The most efficient delivery route at present is IM injection, and suitable delivery strategies for mass vaccination of small fish have yet to be developed. In terms of safety, no adverse effects in the vaccinated fish have been observed to date. As DNA vaccination is a relatively new technology, various theoretical and long-term safety issues

related to the environment and the consumer remain to be fully addressed, although inherently the risks should not be any greater than with the commercial fish vaccines that are currently used. Present classification systems lack clarity in distinguishing

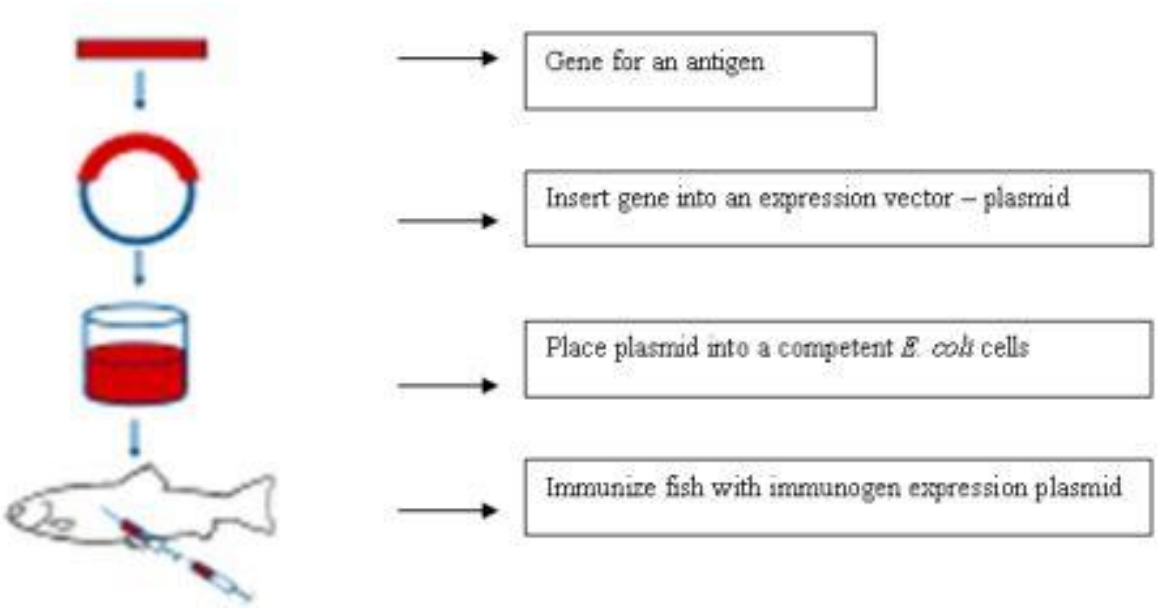
DNA-vaccinated animals from genetically modified organisms (GMOs), which could raise issues in terms of licensing and public acceptance of the technology. The potential benefits of DNA vaccines for farmed fish include improved animal welfare, reduced environmental impacts of aquaculture activities, increased food quality and quantity, and more sustainable production. Testing under commercial production conditions has recently been initiated in Canada and Denmark.

Table I

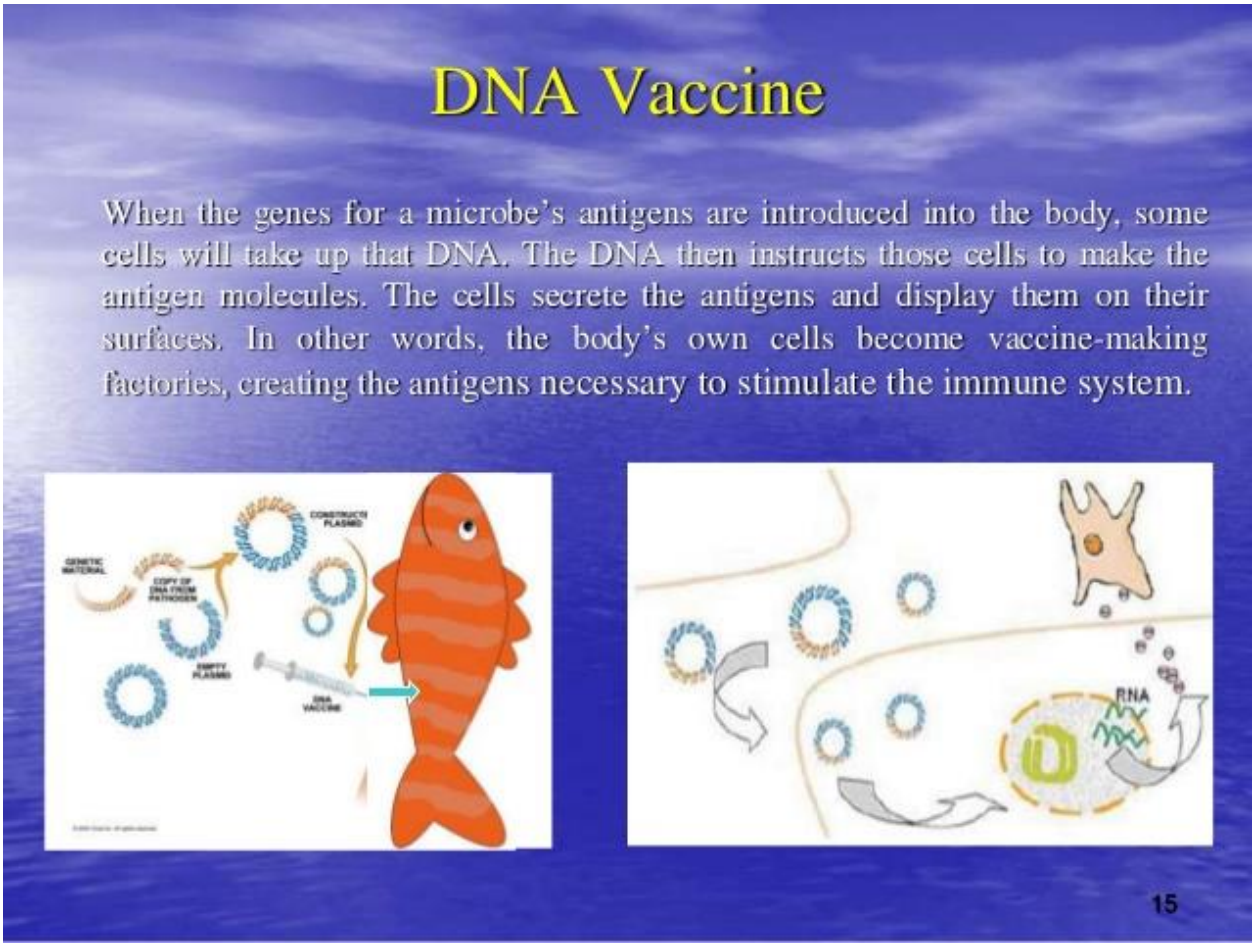
Advantages and disadvantages of deoxyribonucleic acid (DNA) vaccines

Advantages	Disadvantages/current problems
Generic and simple principle	Difficulty/cost of delivery; need for new strategies for mass vaccination of small fish
High level of safety – no risk of infectious disease	Not efficient for all pathogens
Combination of advantages of traditional killed and attenuated vaccines	New concept – long-term safety issues remain to be analysed
Can be successful when traditional vaccine strategies fail	Official distinction between DNA-vaccinated animals and genetically modified organism (GMO)´s not always clear
Possibility of incorporating molecular adjuvants such as CpG motifs	Public aversion to ingredients from GMOs in food products, which might influence consumers' acceptance of veterinary DNA vaccines
Activation of both humoral and cellular mechanisms *	No regulatory precedents yet available for DNA vaccines for husbandry animals
Multivalent vaccination possible by simple mixing of DNA vaccines *	Possible complications of intellectual property rights affecting commercialisation of veterinary DNA vaccines
Good effect when given at an early life stage *	
Protection induced shortly after vaccination and is also long lasting *	
Protection induced at both low and high temperatures *	
Protection efficient across serotype variations *	
Ability to prepare vaccines for new pathogen variants quickly at low cost	
High stability of purified product	
Relatively low cost; easy production/quality assurance	

*Specifically demonstrated in the case of DNA vaccines for fish



In contrast to many DNA vaccines tested in other animal species, the DNA vaccines against rhabdoviruses in aquacultured fish have proved to be very effective in the target species. A single 1 μg dose of plasmid DNA promptly stimulates immunity, which appears to persist throughout the normal lifespan of a cultured food fish. As traditionalvaccines against fish rhabdoviruses have not been successful,the DNA vaccine technology could provide a valuable toolfor more sustainable production of farmed fish. Althoughthere has been preliminary testing using IM injection under field conditions, more suitable delivery methods need to be developed in order to make vaccination of small fish (below 5 g) economically feasible. Other requirements that will present an important challenge for authorities and scientists working in fish vaccinology are to achieve transparency of regulatory and safety issues, and to ensure public dissemination of information about the positive effects of DNA vaccines in aquaculture.



****

CRYOPRESERVATION

ABSTRACT

Cryopreservation is a long-term storage technique to preserve the biological material without deterioration for extended period of time at least several thousands of years. The ability to preserve and store both maternal and paternal gametes provides a reliable source of fish genetic material for scientific and aquaculture purposes as well as for conservation of biodiversity. Successful cryopreservation of fish sperm have been achieved for more than 200 fish species and many fish species have been adequated for the purpose of cryobanking. Cryopreservation of fish embryo is not viable, mainly because of the same limitations as in fish oocytes, i.e., high chilling sensitivity and low membrane permeability. However, cryopreservation of isolated embryonic cells is another option for preserving both maternal and paternal genome. In this paper, an overview of the current state of aquatic species is followed by a discussion on the sperm, embryos, oocytes and embryonic cells - blastomeres.

Key words: cryopreservation, sperm, embryo, oocyte, blastomere

INTRODUCTION

Cryopreservation is a long-term storage technique with very low temperatures to preserve the structurally intact living cells and tissues for extended period of time at a relatively low cost. Cryopreservation is to preserve and store the viable biological samples in a frozen state over extended periods of time. A very important part research in cryopreservation is to reveal the underlying physical and biological responses of the cell and cause of cryoinjury, especially those associated with the phase change of water in extracellular and intracellular environments (Mazur 1984). From the original slow-cooling study, another cryopreservation approach has moved to easier and more efficient technique-vitrification, Cryoprotective agents has to gain access to all the parts of the system. Cryopreservation considers the effects of freezing and thawing. Therefore, the diffusion and osmosis processes have important effects during the introduction of cryoprotective agents, the addition or removal of cryoprotectants, the cooling process, and during thawing. These phenomena are amenable to the experimental design and analysis. Thus, reliable methods can be developed for preserving a very wide range of cells and some tissues. These methods have found widespread applications in biology, biomedical technology and conservation.

Germplasm cryopreservation includes storage of the sperm, eggs and embryos and contributes directly to animal breeding programmes. Germplasm cryopreservation also assist the *ex situ* conservation for preserving the genomes of threatened and endangered species. The establishment of germplasm banks using cryopreservation can contribute to conservation and extant populations in the future. Since the first successful cryopreservation of bull semen (Polge et al. 1949), cryopreserved bull semen has been used to propagate the rare and endangered species using assisted reproduction techniques. Every year, more than 25 million cows are artificially inseminated with frozen-thawed bull semen (Foote 1975) and many bovine calves have been produced using the transfer of cryopreserved embryos into cow (Mapletoft and Hasler 2005). Tissues, cultured cell lines, DNA and serum samples could be frozen and store in cryogene bank. For example, mice and sheep have been generated from frozen-thawed pieces of ovary that have been replaced in a female and stimulated to ovulation. (Gosden et al. 1994; Candy et al. 2000; Sapundzhiev 2008). The principle of testicular cell freezing and transplantation has been demonstrated and is currently used for human male infertility (Clouthier et al. 1996). Significant efforts are being made on non-mammalian species using cryobiology techniques. In fish aquaculture, the successful cryopreservation of gametes and embryos could offer new commercial possibilities, allowing the unlimited production of fry and

potentially healthier and better conditioned fish as required. Cryopreservation of reproductive products of many aquatic species has been successfully achieved. Cryopreservation of aquatic sperm is relatively common in the breeding and management of fish species, including salmonid, cyprinids, silurids, and Acipenseridae (família) is well documented (Magyary et al. 1996; Tsvetkova et al. 1996). However, cryopreservation of embryos and oocytes of aquatic species have not been successful, except for eastern oyster eggs (*Crassostrea virginica*) (Tervit et al. 2005), larvae of eastern oyster (Paniagua-Chavez and Tiersch 2001) and larvae of the sea urchin (Adams et al. 2006).

Cryopreservation technology applied to the preservation of fish gametes in aquaculture plays an important role in seed production, genetic management of broodstock and conservation of aquatic resources. Fish germplasm also plays a significant role in human genomic studies because its relatively small size of the genome makes it easier for sequencing and ideal models for studying the human disease. This would help in identifying the roles for human genes from fish mutations and also in fish models for genes identified by human disease (Brownlie et al. 1998; Barbazuk et al. 2000). Aquatic species preservation would assist the development, protection and distribution of research lines and would offer benefits for restoration of endangered species.

Sperm

In 1949, Polge et al. (1949) successfully cryopreserved the avian spermatozoa using glycerol as a cryoprotectant. Thereafter, cryopreservation of male gamete became possible. Blaxter (1953) applied a similar approach for fish gametes and reported success with Atlantic herring spermatozoa, achieving approximately 80% cellular motility after thawing. Since then, cryopreservation of fish sperm has been studied and has been successful in more than 200 species (Kopeika et al. 2007; Tiersch et al. 2007; Tsai et al., 2010) and techniques of sperm management have been established for freshwater and marine fish species, including carp, salmonids, catfish, cichlids, medakas, white-fish, pike, milkfish, grouper, cod, and zebrafish (Scott and Baynes 1980; Harvey and Ashwood-Smith 1982; Stoss and Donaldson 1983; Babiak et al. 1995; Suquet et al. 2000; Van der Straten et al. 2006; Bokor et al. 2007; Tsai et al. 2010). Many studies on cryopreservation of fish sperm have been carried out on economically important freshwater species and attempts to cryopreserve sperm from the marine fish species tended to be more successful when compared with those obtained from the freshwater fish (Tsvetkova et al. 1996). Although freshwater fish sperm are generally more difficult to cryopreserve, the fertilization rates obtained from the cryopreserved marine fish sperm are similar to those obtained with mammalian species (Tsvetkova et al. 1996). Controlled-rate slow cooling in

cryopreservation has been mainly used for fish sperm. Common carp has been studied using frozen-thawed sperm with 95% fertilization and hatching rate.

Salmonid species spermatozoa have been successfully cryopreserved (Lahnsteiner 2000). Another well studied cryopreserved group is cyprinids and some of these cyprinid fishes are widely farmed throughout Asia and Europe. A fertilization and hatching rate of 95% using the frozen-thawed sperm has been reported for the common carp and these results are not significantly different from fresh sperm (Magyary et al. 1996). Tilapias are among the exotic freshwater fishes that have been successfully established for fish farming in Taiwan; they have been cryopreserved successfully and produced 40-80% motility with cryoprotectant DMSO (Chao et al. 1987). The sperm of more than 30 marine fish species have been cryopreserved successfully (Suquet et al. 2000; Gwo 2000; Van der Straten et al. 2006). Generally, high survival and fertilization capacity has been obtained in frozen-thawed spermatozoa when compared to freshwater species (Drokin 1993; Gwo 2000).

Successful cryopreservation of the sperm of aquatic invertebrate has been carried out for sea urchin, oyster, starfish, abalone and coral (Adams et al. 2004a; Adams et al. 2004b; Gwo et al. 2002; Hagedorn et al. 2006; Kang et al. 2009). Dimethyl sulfoxide has also been reported as a successful cryoprotectant for sperm cryopreservation; the concentration range used was 5 to 30% for these species. Various levels of motility, ranging from <5% to 95%, have been reported for the cryopreserved aquatic invertebrate sperm (Dunn and McLachlan 1973).

Embryos

Cryopreservation of embryos has become an integral part of assisted reproduction. Successful cryopreservation of embryos is important because the biodiversity of both the paternal and maternal genomes will be preserved. While cryopreservation techniques have been largely established for the mammalian embryos, successful cryopreservation of intact fish embryos has not yet been achieved. Factors limiting fish embryo cryopreservation include their multicompartmental biological systems, high chilling sensitivity, low membrane permeability and their large size, which gives a low surface area to volume ratio (Zhang and Rawson 1995). The effect of such low ratio is a reduction in the rate at which water and cryoprotectants can move into and out of the embryo during cryopreservation (Mazur 1984). Fish embryos are osmoregulators; they are released into the external medium and activated. Then the vitelline envelope separates from the plasma membrane and forms chorion. Studies on the chorion permeability of zebra fish embryos clearly showed that it was permeable to electrolytes and a range of cryoprotectant, including propane-1,2-diol, methanol, DMSO, ethylene

(Zhang and Rawson 1996). The chorion structure plays a crucial role as flexible filter for the transport of some materials (Toshimori and Tsuzumi 1976) and protects against the microorganisms (Schoots et al. 1982) Studies on zebra fish embryos have shown that the water permeability of the plasma membrane at different developmental stages remained relatively stable. The permeability to methanol (cryoprotectant) appeared to decrease during embryo development (Zhang and Rawson 1998). This also indicated that there was a gradual reduction in the permeability following the fertilization in zebra fish embryos, as opposed to the generally held belief that the membrane permeability of fish embryos reduced rapidly to minimum shortly after the fertilization (Alderdice 1988).

The studies on the kinetics of subzero chilling injury in *Drosophila* embryos (Mazur et al. 1992) and chilling sensitivity of zebra fish embryos have demonstrated that chilling injury plays an important role in reduction of embryo survival during the exposure to subzero temperatures (Zhang and Rawson 1995; Hagedorn et al. 1997). Chilling sensitivity has been shown for many species and has been analyzed in fish embryos, including brown trout (*Salmo trutta f. fario*) (Maddock 1974), rainbow trout (*Oncorhynchus mykiss*) (Haga 1982), carp (*Cyprinus carpio*) (Dinnyes et al. 1998), fathead minnows (*Pimephales promelas*) (Cloud et al. 1988), goldfish (*Carassius auratus*) (Liu et al. 1993) and zebrafish (*Danio rerio*) (Zhang and Rawson 1995; Zhang et al. 2003). These studies demonstrated that the later stages (after 50% epiboly) were less sensitive to chilling, but chilling sensitivity increased significantly as the temperature fell below zero. The high chilling sensitivity of fish embryos, especially at early stages, their complex membrane structure and large yolk are the main obstacles to achieve successful cryopreservation of these embryos (Zhang and Rawson 1996). Chilling injury in embryos has been linked to the inhibition of metabolic and enzymatic processes from low temperatures injuries which could be detrimental in the embryonic development such as fish embryos (Dinnyes et al. 1998). Cryoprotectant toxicity follows a similar pattern to chilling sensitivity with later stages being less sensitive to cryoprotectant (Zhang et al. 2005; Zhang et al. 1993; Liu et al. 1993; Suzuki et al. 1995). Several studies have determined membrane permeability for zebra fish embryos (Zhang and Rawson 1998; Hagedorn et al. 1997) and membrane permeability to water and most cryoprotectants has been shown to be low (Zhang and Rawson 1996; Zhang and Rawson 1998). Studies on the cryopreservation of zebra fish embryos demonstrated 8% embryo survival in 2M methanol at -25 °C; however, no embryo survival was observed when frozen to -30 °C or below (Zhang et al. 1993).

Cryopreservation studies on the embryos and larvae have been conducted on marine invertebrate such as oysters, sea urchins, polycheate worms, coral and penaeid shrimp species (Liu et al. 2001; Gakhova

et al. 1988; Lin et al. 1999; Olive and Wang 1997; Paniagua-Chavez and Tiersch 2001; Hagedorn et al. 2006; Tsai and Lin 2009). However, survivals of most of these species has been inadequate in maintaining the structure and activity of embryos and larvae after freezing to cryogenic temperatures. Embryonic and larval development of marine invertebrates after cryopreservation often showed abnormalities in structure and colour (Odintsova et al. 2001). The problems with invertebrate embryo cryopreservation associated with those identified with the fish embryos are their low membrane permeability and high chilling sensitivity. Although cryopreservation of the embryos has not been fully achieved, considerable progress has been made in understanding the conditions required for fish embryo cryopreservation and this would undoubtedly assist the successful protocol design in the future.

Oocytes

Oocyte cryopreservation is potentially the best way to preserve the female fertility. Cryopreservation of fish oocyte has been studied (Isayeva et al. 2004; Plachinta et al. 2004; Zhang et al. 2005; Guan et al. 2008; Tsai et al. 2009) which offers several advantages such as the smaller sizes range, much lower water content in oocytes and absence of a fully developed chorion that the permeability to water and solutes in oocyte is higher than embryo. Fish embryos are too large to apply traditional cryopreservation protocol. Immature oocytes can be an alternative for the mature eggs because of their smaller size (Hagedorn et al. 1996). However, there is no practical technique available to induce the small oocyte to mature *in vitro*. A technique to obtain the mature eggs from the late stage oocytes is available. Thus, the combination of this technique and their cryopreservation could be a breakthrough. However, at present, late stage oocytes cannot be successfully cryopreserved because their size is still not small enough to result in much lower surface area to volume ratio. These reduce the rate at which water and cryoprotectant move into and out of oocytes during the cryopreservation. Developing the methods for cryopreservation of oocytes requires the screening of potential cryoprotectant treatments, evaluation of tolerance to chilling, determination of the appropriate rate of freezing to cryogenic temperatures and rate of thawing. Viability assessment methods of oocytes with trypan blue (TB), fluorescein diacetate (FDA) + propidium iodide (PI) and adenosine triphosphate (ATP) content assay have been developed for quick assessment of viability (Plachinta et al. 2004; Zampolla et al. 2006; Guan et al. 2008; Tsai et al. 2008; Tsai et al. 2009; Tsai et al. 2011; Tsai and Lin 2012). A functional test based on *in vitro* maturation, followed by germinal vesicle breakdown (GVBD) has also been shown effective for late stage III oocyte (Plachinta et al. 2004).

The permeability of the zebra fish oocyte membrane to water and cryoprotectants has been studied (Zhang et al. 2005) and membrane permeability was shown to decrease with the temperature and permeability was generally lower than those obtained from sea urchin eggs (Adams et al. 2003) but higher than the immature medaka oocyte (Valdez et al. 2005). Studies on zebra fish oocyte chilling sensitivity showed that those oocytes were very sensitive to chilling and their survival decreased with decreasing temperature (Isayeva et al. 2004). Chilling sensitivity in zebra fish oocytes was thought to be due to lipid phase transition of the oocyte membrane (Pearl and Arav 2000). The phase transition in zebra fish oocytes showed that chilling injury could occur when oocytes were exposed to temperatures between 12 to 22°C above the water freezing temperatures (Drobnis et al. 1993; Pearl and Arav 2000). Cryopreservation of late stage zebra fish oocytes has been studied using the controlled slow cooling and an optimum cryoprotective medium and cooling rate identified. Guan et al. (2008) showed that although the oocyte viability obtained immediately after freeze-thawing was relatively high with 88% using TB staining; oocyte viability decreased to 29.5% after 2 h incubation at 22 °C. The study also showed that the ATP level in the oocytes decreased significantly after thawing and all oocytes became translucent. Cryopreservation of early stage zebra fish oocytes using the controlled slow freezing has been reported by Tsai et al. (2009). The results suggested that 4M methanol in KCl buffer was identified as the optimum cryoprotective medium. Although results obtained after the cryopreservation using trypan blue and FDA+PI staining were promising with 69% and 54%, especially with stage II ovarian follicles, the ADP/ATP ratio assay showed that the energy system of these follicles had been compromised. Apparently the ADP/ATP ratio could be a valuable measure of cellular injury after post-thaw incubation period as it reflected the metabolic and energy status of population as well as indicating some measure of the potential for repair. Furthermore, *in vitro* culture method is effective for assessing early stage zebra fish oocytes growth competence *in vitro.* The early stage zebra fish oocytes can be cultured *in vitro* for 24 h, stage I and II oocytes can grow to the sizes of early stage II and stage III oocytes after hCG treatment. and also can be used for other teleost species (Tsai et al. 2010).

Studies on the cryopreservation of invertebrate oocytes and eggs over the past several decades have been extraordinarily difficult to achieve (Koseoglu et al. 2001; Tsai et al. 2010; Lin et al. 2011; Lin and Tsai 2012). However, it was found that intracellular crystallization occurred in the starfish oocytes at relatively high temperature that was very close to the temperature of extracellular ice formation (Koseoglu et al. 2001). In order to avoid this problem, Hamaratoglu et al. (2005) successfully cryopreserved starfish oocytes using ultra-rapid freezing technique, called vitrification. High chilling sensitivity (Tsai et al. 2009) and low membrane permeability (Guan et al. 2008) of

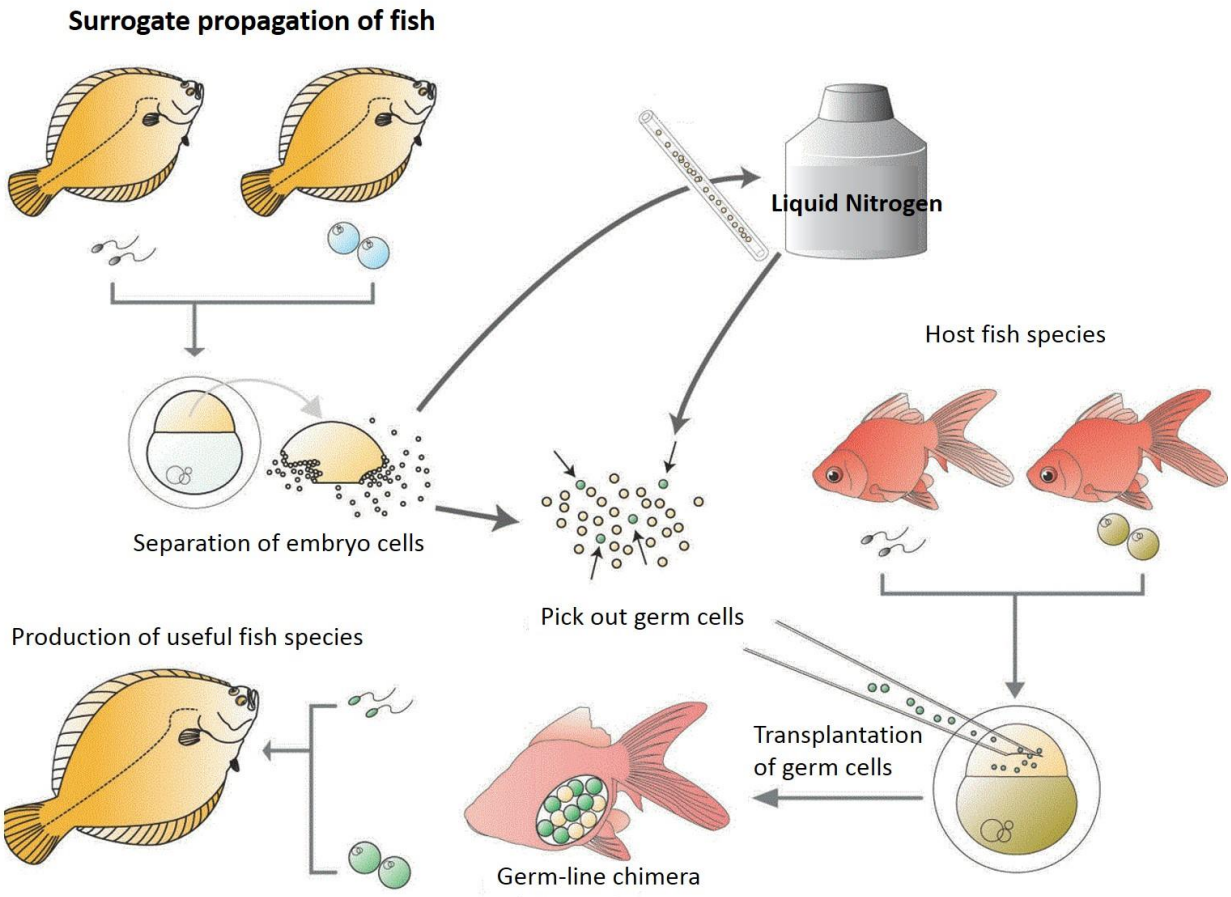
zebra fish oocytes are major obstacles to the development of a successful protocol for their cryopreservation as chilling sensitivity or cold shock can hinder slow cooling processes. Vitrification may be another option to achieve successful cryopreservation for the oocytes.

Blastomeres

Blastomeres are the cells produced as the result of cell division and cleavage in the fertilized egg. They are totipotent and pluripotent (depending on the stage of embryonic development) having the ability to differentiate into any of the three germ layers or entire organism. They are different from the muscle cells, blood cells or nerves cells. Although cultured somatic-cells from fish have been cryopreserved successfully, their value is limited because of loss of development potential. Cryopreservation of blastomeres can maintain the genetic diversity of both, nuclear genome and mitochondrial DNA (Nilsson and Cloud, 1992). Blastomeres from the early embryos of fish still retain pluripotency (Ho and Kimmel 1993) and their cryopreservation may be a promising approach to preserve the genotypes of zygotes and reconstitution of the organism. Indeed, there are several reports of germ-line chimeras created using the transplantation of blastomeres into goldfish (Yamaha et al. 1997; Kusuda et al. 2004), zebra fish (Lin et al. 1992), medaka (Hong et al. 1998; Wakamatsu et al. 2001) and rainbow trout (Takeuchi et al. 2001) embryos. Kusuda et al. (2004) transplanted the frozen-thaw blastomeres into goldfish embryos and the blastomeres differentiated into primordial germ cells. This report demonstrated that germ-line cells from the cryopreserved blastomeres could develop into mature gametes of chimeric fish because the blastomeres were not damaged by cryopreservation. Therefore, the cryopreservation techniques are very important.

Cryopreservation of blastomeres has been successful in several fish species. In the first reported studies, Harvey (1983) used a two-step freezing procedure, with ice-seeding at -6°C, and cooling to -25°C, followed by immersion in liquid nitrogen. The survival rate of 84.8% was obtained after cryopreservation of 50% epiboly zebra fish blastomeres. However, the results obtained from a very small sample size and freezing rates were not controlled, rather tubes were allowed to equilibrate in the cooled alcohol baths. Lin et al. (2009) demonstrated the effect of cryopreservation on zebra fish blastomeres survival using the controlled slow cooling method. It was shown that DMSO was the most toxic to zebra fish blastomeres. However, DMSO was the best cryoprotectant in terms of survival of zebra fish blastomeres. Therefore, it is possible that the cryoprotective effect of DMSO may be greater than its toxicity effect. Although the survival rate in Lin's results progressed from 25% (Kopeika et al. 2005) to 70%, it was still lower than that obtained by using two step methods. The comparisons between these studies must take into consideration the different methodology.

Vitrification of zebra fish blastomeres was studied more recently and the highest blastomere survival was 93.4% (Cardona-Costa and Garcia-Ximénez 2007). Cryopreservation of blastomeres was also carried out in rainbow trout, carp and medaka after post-thawing. Rainbow trout blastomeres have been cryopreserved using the controlled slow freezing procedures with a survival of 95% (Calvi and Maisse 1998). It has been reported that the controlled slow freezing protocol adopted for rainbow trout was successfully applied to carp blastomeres with survivals of 94% and 96% (Calvi and Maisse 1999). Lower survival rates of cryopreserved blastomeres using controlled slow freezing have also been reported for other fish species such as whiting (20%), medaka (34%), pejerrey (67%) and chum salmon (59%) (Strussmann et al. 1999; Kusuda et al. 2002).



CONCLUSION

Cryopreservation of gametes and embryos are already routinely applied in the mammalian. Cryopreserved sperm, oocytes and embryos are used for artificial insemination and embryo transfer in the livestock industry. Cryopreservation also has enormous applications in the artificial propagation of widely diverse aquatic organism. Sperm and embryonic cells cryopreservaiton has

been successful in a number of teleosts and invertebrate species. However, cryopreservation of embryos and oocytes remain a major challenge. The practical application of cryopreservation in the aquatic species needs more vigorous research efforts in this area and the efforts may be prioritized on endangered, economical value and representative species from various aquatic habitats. Cryopreservation of gametes, embryos and embryonic cells has become of immense value in aquatic biotechnologies which provide an important tool for protecting the endangered species, genetic diversity in aquatic species. The establishment of cryobanks to utilize the cryopreservation worldwide would be a significant and promising task in the future.

****

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – V - AQUACULTURE – SBT1608

UNIT 5 FISH DISEASES AND CONTROL MEASURES

Disease diagnosis: Principles of disease diagnosis in finfish and shell fish. Microbial diseases: Diseases caused by bacteria (Vibriosis) - Fungi and viruses (WSSV). Parasitic diseases: Diseases caused by protozoa and metazoa (crustaceans, helminthes). Non–infectious diseases: Nutritional and environmental diseases. Aquafarm pollutants. Prevention and control of diseases: Symptoms, prevention, control and treatments (prophylactic and therapeutic).

Health Management

- **Chapter 1. Health Management**

12.1.1 Introduction

A health management program and a disease emergency plan are essential documents on farms. As many farmers have recognized, the frequency and severity of disease outbreaks in ponds seem to depend on a number of factors, not all of them were well understood. The first point to appreciate is that not all diseases are infectious. For example, some are caused by toxins, others by nutritional imbalances. The following section focuses on infectious diseases because their causes are usually complex and control and prevention can be more challenging. Infectious disease outbreaks in ponds depend on particular interactions between the host, the pathogen and the pond environment

12.1.2 Principles of fish health management

The principles of fish health management incorporates

i. Minimizing stress in cultivated fishes

ii. Confinement of disease outbreak to affected ponds

iii. Minimizing losses from disease outbreak.

This could be achieved through prophylaxis and positive treatment to the outbreak of epidermics. Because of the aquatic ambience, it is not easy to get aware of the activities of fish. It is difficult to conduct a correct diagnosis and timely treatment. This necessitates prevention of fish diseases

which is more important than control of fish diseases. This signifies the importance of the statement "Prevention is better than cure".

12.1.3. General preventive measures

Internal resistance: Increasing the internal resistance of fish is important in the prevention of diseases. Therefore, some additional points in fish culture are advisable.

1. Selection of healthy fish seeds.
2. Proper density and rational culture.
3. Proper pond management
4. Qualitatively uniform ratio and quality food
5. Good water quality
6. Prevention of fish body from injury

Chapter 2. Fish diseases

12.2.1. Medium for fish diseases

Water seems to be mainly responsible for various diseases since the vital functions of fish viz. movement, feeding, digestion, assimilation, growth, responses to stimuli and reproduction are dependent on water

12.2.2. Common symptoms of diseases

1. Unusual movements
2. Abnormal and unhealthy look
3. Discoloration
4. Film like covering on the skin
5. Swelling or spots on the skin
6. Pale gills, white and red spot on gills
7. Excess slime secretion

12.2.3 Sources of infectious diseases

1. Primary source: Sick fish serves as a primary infectious source and are the carriers of pathogens. The pathogen infects through direct contact or by discharge of disease causing agents into the water.
2. Secondary source: Water coming from diseased ponds, contaminated silt, feeds and gears.

12.2.4 Natural resistance of fish to infectious diseases

1. Surface texture of skin and mucous membrane of fish functions as a screen to keep the infectious micro-organisms out of it.
2. Lysozyme secreted from the cell can kill bacteria.
3. The pathogenic microbes entering into digestive tract will be under the influence of digestive enzymes which can kill pathogens
4. The phagocytotic function of white blood cells. Lymphoid cells, reticuto-endothelial cells of spleen, liver and blood vessel can eliminate foreign body as well as pathogenic micro-organisms.
5. Blood of fish contains bactericidin which can eradicate all kinds of pathogenic bacteria.

Chapter 3. Types of diseases

12.3.1 Types of diseases

Diseases which affect fish are parasitic and non-parasitic. The former includes those caused by bacteria, fungi, protozoans, worms, leeches and copepods and the latter comprise disorders associated with nutritional deficiency and sudden changes in abiotic and biotic factors. The common symptoms by which the affected fish are recognized are (a) changes from normal behavior, (b) signs of reduced vitality, (c) lack of appetite and failure to feed, and (d) presence of lesions or sores

12.3.2 Bacterial Disease

12.3.2.1. Disease: fin and tail rot
Causative organisms: Aeromonas, Pseudomonas and Vibrio
Symptoms: White line on the margin of the fin; fin rays become brittle and start breaking.
Treatments: 1 minute dip treatment in 500ppm copper sulphate solution.
12.3.2.2. Disease: Ulcer disease
Causative organisms: Aeromonas hydrophilla and Pseudomonas
Symptoms: Open sores or ulcers on the body.

Treatments: Dip treatment for 1 minute in 1:2000 copper sulphate solution for 3-4 days.

12.3.2.3. Disease: Dropsy

Causative organisms: Pseudomonas punctata

Symptoms: Accumulation of fluid inside the body cavity; scale protrusion; exophathalmic condition.

Treatments: Dip treatment in 5ppm potassium permanganate solution for 2 minutes.

12.3.2.4. Disease: Eye disease

Causative organisms: Aeromonas liquifaciens

Symptoms: Cornea of eye becomes vascularised and later becomes opaque eye ball gets decayed.

Treatments: Chloromycetin (8-10 mg/liter) bath for 1 hour for 2-3 day.

12.3.3. FUNGAL DISEASES

12.3.3.1. Disease: Water – mold disease (Saprolegniasis)

Causative organisms: Saprolegnia parasitica

Symptoms: Dies after ulceration or exfoliation of skin followed by haemorrhage blindness, tufts of white hair like out-growth in the affected region.

Treatments: Dip treatment for 3seconds in 1:10,000 solution of malachite green or for 5-10 minutes in 3% common salt solution or potassium permanganate

12.3.4. PROTOZOANS

12.3.4.1. Disease: Ichthyophthiriasis (White-spot disease)

Causative organisms : Ichthyophthirius multifilis

Symptoms: Small whitish-cysts of about 1mm diameter on the skin, gills and fins.

Treatments: 5 days bathing in 2ppm methylene blue, hourly dip treatment in 1:5,000 formalin solution for 7-10 days.

12.3.4.2. Disease: Boil disease

Causative organisms: Myxobolus pfeifferi

Symptoms: Large boils varying from the size of a nut to that of a hen’s egg on several parts of body.

Treatments: Bath in 3% common salt solution or in 1:2,500 formalin solution for 10 minutes.

12.3.4.3. Disease: Whirling disease

Causative organisms: Myxobolus cerebrails

Symptoms: Caudal bend, deformity of the oral region and blackening of tail region.

Treatments: Destroy all infected fish by applying quicklime (pond disinfectant) at the rate of 2t/ha.

12.3.4.4. Disease: Costiasis

Causative organisms: Costia necatrix

Symptoms: Bluish-coating on the skin, lesions as irregular patches.

Treatments: Bath in 3% common salt solution or in 1:2,500 formalin solution for 10 minutes.

12.3.5. TREMATODES

12.3.5.1. Disease: Gyrodactylosis

Causative organisms: Gyrodactylus sp.

Symptoms: Fading of colours, drooping of scales, peeling of skin.

Treatments: Dip treatment in 5% common salt solution or in 1:5,000 formalin solution for 5 minutes.

12.3.5.2. Disease: Dactylogyrosis

Causative organisms: Dactylogyrus sp.

Symptoms: Fading of colours, drooping of scales, peeling of skin.

Treatments: Dip treatment in 5% common salt solution or in 1:5,000 formalin solution for 5 minutes.

12.3.5.3. Disease: Diplostomiasis (Blacki-spot disease)

Causative organisms: Diplostomulum sp.

Symptoms: Small black nodules of about 1-5mm diameter in the affected region.

Treatments: Dip treatment in 3:1,00,000 picric acid for 1 hour, Di-n-butyl tin oxide at the rate of 250mg/kg fish.

12.3.6. CESTODES

12.3.6.1. Disease: Ligulosis

Causative organisms: Ligula sp.

Symptoms: Dull, sickly and with parts of alimentary canal swollen or completerly choked by cestoded cysts or worms.

Treatments: Dip treatment in 3:1,00,000 picric acid for 1 hour, Di-n-butyl tin oxide at the rate of 250mg/kg fish.

12.3.7. NEMATODES

Causative organisms: Philometra sp., Camallanus sp.

Symptoms: Dull, sickly and with parts of alimentary canal swollen or completerly choked by round worms.

Treatments: Dip treatment in 3:1,00,000 picric acid for 1 hour, Di-n-butyl tin oxide at the rate of 250mg/kg fish.

12.3.8. ACANTHOCEPHALA

Causative organisms: Acanthogyrus sp.

Symptoms: Yellowish white fibro epithelioma on lip, skin and fin.

Treatments: Quick lime.

12.3.9. HIRUDINEA

Causative organisms: Hemiclepsis sp.

Symptoms: Abnormal movements of the fish due to irritation as the parasites feed on the blood of host.

Treatments: Dip treatment in 1:1,00,000 solution of glacial acetic acid.

12.3.10. COPEPODS

Causative organisms: Argulus sp., Ergasilus sp., Lernea sp., Caligus sp., Pseudocycnus sp., Clavellisa sp.

Symptoms: Loss of scales and presence of red spots, damage of gills.

Treatments: Half an hour treatment in 500ppm formalin solution, mechanical removal by forceps followed by a bath in weak potassium permanganate solution for 2-3 minutes, bath in 1:1,000 glacial acetic acid solution for 5 minutes and subsequent bath in 1% common salt solution for 1 hour, pond may be disinfected by applying lindane at the rate of 8ml/1,000 litre.

12.3.11. VIRAL DISEASES

12.3.11.1. Disease: Lymphocystis

Causative organisms: Lymphocystis spp. (A DNA Iridovirus)

Symptoms: Lethargy, may affect balance and swimming control if along the lateral line.

Treatments: Frequent water changes and reduction of ammonia and nitrites in water may reduce stress to help the fish battle the infection and shrink tumors on its own.

12.3.11.2. Disease: Infectious Pancreatic Necrosis- (IPN)

Causative organisms: IPN virus

Symptoms: Darker in color, tail chasing, spiral swimming behavior. Treatments: uncontaminated water supply, providing optimum feed.

12.3.12. Miscellaneous Diseases

12.3.12.1. Gas bubble disease

When nitrogen of the water is in saturation, due to rapid temperature changes, gas bubble disease may result and fish fry particularly, die in large numbers. Fish affected by this disease often swim at an angle of 450 with head pointing down. Other symptoms are the presence of bubbles beneath the skin, on fins, around eyes in stomach and intestine or in blood capillaries. In such conditions, water should be well agitated to bring down the nitrogen saturation or affected fish should be transferred to other ponds. Besides, nitrogen, supersaturated levels of oxygen have also been reported to cause gas bubble disease in fishes.

12.3.13. Vitamin Deficiencies

• Scoliosis (Curved Spine)

• Reduced Growth

Anorexia (Lack or Loss or Appetite)

Symptoms

• Low fish weight

• Lethargy

Unit 13: Aquaculture Economics

- **Chapter 1. Aquaculture economics**

Aquaculture economics

As aquaculture has expanded, biological and technical problems have received most of the attention because biologists have by and large been the principal researchers. Biologists have focused and are continuing to focus on ways to overcome constraints to production and to modify and/or intensify traditional systems. Some biological issues such as induced breeding of fish in captivity, prevention of disease, nutritional requirements of indigenous and exotic species, and selective genetic improvement are being studied.

In spite of this ever-increasing biological research activity, inadequate attention has been devoted to other, equally important, problem areas and to the interplay among them. The viability of aquaculture technology involves more than the study of its biology and technology. Economics must be used to determine efficiency or resource allocation. Reliable information on the economics of existing aquaculture systems and the economic viability of the new technology is often lacking. In addition to the economics of production, evaluation of markets including demand, marketing infrastructure and marketing channels is important. Aquaculture Economics is a relatively new area of study. The economic study of aquaculture provides a basis for decision making for fish farmers and assists in the formulation of public policies. The potential scope for aquaculture economics research is wide. As aquaculture develops, economists will be called upon to analyse current production and marketing practices, particularly in the private sector and to evaluate improved husbandry techniques as they are developed. Economic studies can help appraise the current practices and potential of aquaculture by analyzing the production and marketing aspects of both experimental and existing culture systems, assessing the role and contributing of aquaculture as compared with other sectors in national economics and international trade and evaluating development projects and the institutional and cultural environment in which aquaculture development is expected to take place.

Chapter 2.Cost concepts

Cost

Refers to the total amount of funds used in production

Cost of production

Cost means the total efforts, sacrifices and exertion involved in the production of a commodity.

Categories of cost

Two major categories of cost namely (1) fixed cost and (ii) variable costs

Fixed cost

Fixed costs are those which do not change with the level of output and the incurred even when production is not undertaken.

Fixed cost items – DIRTI-5

Fixed costs are those which are not a function of output. Hence they do not vary with the level of output. Total Fixed Cost (TFC) is the sum of the fixed cost items like depreciation, interest on capital, repairs and upkeep, taxes and insurance.

Variable costs

Variable costs are the costs of using the variable inputs which vary with the level of production. Higher the production more will be the variable costs, lower the production, lower will be the variable costs. These costs include items such as cost of seed, feed, manures, fertilizers, fuel, electricity, wages paid to labour etc. It is this cost that affects production more then the fixed costs.

Total costs

Total costs = fixed cost + variable cost

Money value of all the inputs used on a farm during a given period, season or year is termed as the total cost. The sum of fixed and variable costs is total cost. Total cost is required for computing the net revenue.

Return concept

1.3.1 Total returns

Total returns are equal to total production times the price. $TR=Y \times P_y$

Net returns

Net returns are equal to the total returns less all costs. NR=TR-TC

Net returns (Variable cost basis)

Equal to the total returns over the total variable costs.

NR=TR – TVC

Chapter 3. Factors affecting the economics of aquaculture

Factors affecting the economics of aquaculture

The producers profit or net income per unit are (Y) is determined by production (Q), the cost of production and marketing (C) and the price received (P) as shown in the equation.

Y = QP – C

Increases in yield, reduction in costs and increases in price are the major means of increasing profits.

2.1 Increase in production

The major factors affecting the productivity per unit area are the stocking rate, survival rate and growth rate. σ

2.2 Increasing stocking rate

A fish pond can only support a certain quantity of fish because of its limited space and natural food called as the maximum stocking crop. Stocking rate can be increased by fertilization and feeding, polyculture, stock manipulation and aeration.

2.2.1 Fertilization and feeding

Production is usually much higher with fertilization and feeding than without. Although the total cost of production is higher with fertilization and feeding than without, the production cost per unit of fish may be lower and the additional revenue generated may be higher than the additional cost involved.

2.2.2 Polyculture

Significant increases in the stocking rate can be obtained through polyculture, which is rearing or several species together to make more efficient use of the growing space and the total pond environment composite fish culture in India.

Polyculture increases production per unit area and generate more profit than monoculture.

2.2.3 Stock manipulation

13.3.1.2.3.1 Multiple size stocking

Stocking of the same species is in different sizes to make more efficient use of water space. This continuous stocking method gives the farmer a constant income and a higher average price and also improves the growth rate as stocks are thinned out periodically.

13.3.1.2.3.2 Same size stocking

Involves stocking fish of one size in one pond and when more space is required, transferring them to a larger pond. E.g. Milkfish farming in the Philippines.

13.3.1.2.3.3 Double cropping

Stocking of two species is in the same pond in different seasons, thereby taking advantage of different requirements of the two species like temperature, salinity etc.

Aeration

Aeration increasing the dissolved oxygen content and can increase stocking density. But their economic feasibility depends on the additional revenues obtained over the additional cost involved in aeration.

Increasing survival and growth rates

Increase in survival and growth rates depend on 1) genetic improvement by selective breeding of hybridization 2) good pond management production. Genetic improvement is time taking and beyond individual farmers capacity. Good pond management includes correct stocking rate, right type and quantity of feed or fertilizer, proper water quality, Prevention of diseases and parasites and the elimination of predators and competitors.

Increase in farm prices

In a competitive market price is determined by the supply and demand for fish.

Improvement in the quality of fish

Fish is highly perishable. Poor quality, spoilage and waste reduce the average price that farmers receive. Quality of fish can be improved by proper preservation during transportation and marketing. Cost of preservation should be less than the additional revenue obtained. Graded fish usually command a higher price and live fish marketing increase the price.

Seasonability

Price fluctuates seasonally. Increase in price is possible by phased stocking and periodical harvesting.

Co-operative marketing and different markets and products

Individual farmers are in a weak bargaining positing and often receive a very low price. By formation of farmers co-operatives and farmers associations their bargaining position may be improved. Allocating fish to different markets and selling them in different forms (fresh, frozen, salted and smoked etc.) may increase the price and maximized revenue.

Chapter 4.Reduction in costs of production and marketing

Reduction in costs of production and marketing

Major production costs in aquaculture are cost of feed, fertilizer, seeds, labor, fuel, electricity, cost of marketing etc.

Cost of feed and fertilizers

Feed and fertilizer are most costly items in aquaculture. In many cases feed accounts for more than 50% of costs. Cost of feed can be reduced by an improvement in the conversion ratio or by lowering the unit price of feed or a combination of both. The amount of feed should be at a level where the additional cost of feed equals the additional revenue beyond which it is uneconomic. Integrated fish

farming increases the production of animal protein from the same unit area, reduces the cost of feed and or fertilizer.

Cost of seed

Seed accounts for a high percentage of total variable costs, particularly for species in which artificial hatching has not been successful. When the supply depends on natural sources, availability fluctuates and price increases. Ultimate solution appears to be breeding in captivity. Price of fingerlings is higher than that of fry, but the survival rate is also high. Cost of seed per unit of marketable fish would be lower if the growth period is less and if one stocks larger fingerlings than small fry.

Cost of labor

Labor cost is one of the major expenses in aquaculture because mechanization has not yet been developed to replace labor. Harvesting, feeding and maintenance are the major labor consuming tasks. Therefore efficient management of use of labor is essential in reducing the cost of product.