M.Sc. IN FISHERY SCIENCE
LAB MANUAL
3rd Semester

Prepared By
Biological Science Dept.
Fishery Science

MIDNAPORE CITY COLLEGE

FSC-395
GENERAL PRACTICAL
(Core course - 4 credits: 50 Marks)
[University Examination - 50]

1. Estimation of DNA, RNA and protein and enzyme by standard method.
2. Design and layout of different aquaculture farm.
3. Design and constructions of pond, pen, cage, Biofloc unit etc.
4. Study of physical and chemical properties of soil and water.
5. Study of various equipment's used in different hatchery.
6. Study of the heamatological parameters of fish.
7. Biochemical analysis of organs and fish immunity study.
8. Protein profiling study of different fish tissues.
9. Study of the principles & applications of instruments used in modern biology.
10. Collection of data and presentation of data. Testing of Goodness of fit; Chi squire (X^2) test and Student's t-test.
11. Operation of MS-Excel, tabulation of biological data, simple computation of different groups of data, making chart with MS-Excel, Bar-diagram, Line-diagram, Pie-diagram. Preparation of Power Point presentation on any topics on fisheries.
12. Seminar presentation.

LAB-1: Estimation of DNA

Introduction: The estimation of DNA concentration is an essential step in various molecular biology experiments and applications. Accurate measurement of DNA concentration ensures optimal sample preparation and proper quantification of DNA for downstream analyses. This lab manual provides a step-by-step guide to estimate DNA concentration using a spectrophotometer.

Materials:

1. DNA sample(s)
2. Spectrophotometer
3. UV-transparent cuvettes
4. Distilled water
5. Disposable micropipettes (appropriate volumes)
6. Micro centrifuge tubes
7. Calculator
8. Pen and paper
9. Safety goggles
10. Gloves

Procedure:

1. Prepare the spectrophotometer:
    a. Turn on the spectrophotometer and allow it to warm up according to the manufacturer's instructions.
    b. Set the spectrophotometer to the appropriate wavelength for DNA measurement, typically 260 nm (nanometers).
2. Prepare the sample:
    a. Obtain the DNA sample to be measured. Ensure the sample is adequately thawed if stored frozen.
    b. Using a disposable micropipette, transfer a small volume (e.g., 1-2 µL) of the DNA sample to a clean microcentrifuge tube.
    c. If the DNA sample concentration is expected to be high, dilute it using distilled water to bring it within the linear range of the spectrophotometer.
3. Measure the blank:
    a. Fill a UV-transparent cuvette with distilled water. This will serve as the blank/reference for subsequent measurements.
    b. Place the cuvette in the spectrophotometer's sample compartment, aligning it with the light path.
4. Measure the DNA sample:
    a. Pipette a small volume (e.g., 1-2 µL) of the DNA sample onto a clean cuvette.

b. Wipe any droplets or smudges from the cuvette exterior using a lint-free tissue.
c. Carefully place the cuvette in the sample compartment of the spectrophotometer, ensuring alignment with the light path.

5. Record the absorbance readings:

a. Read the absorbance value displayed on the spectrophotometer for the blank cuvette at 260 nm (A0). Note this value.
b. Read the absorbance value for the DNA sample cuvette at 260 nm (A).
c. Record the absorbance value.

6. Calculate the DNA concentration:

a. Subtract the absorbance of the blank from the absorbance of the DNA sample to obtain the corrected absorbance value (A260 - A0).
b. Use the following formula to calculate the DNA concentration:
DNA concentration (μg/μL) =
(Corrected absorbance value × Dilution factor) / DNA conversion factor, where the dilution factor accounts for any sample dilutions and the DNA conversion factor represents the conversion factor specific to your DNA source and unit of measurement.

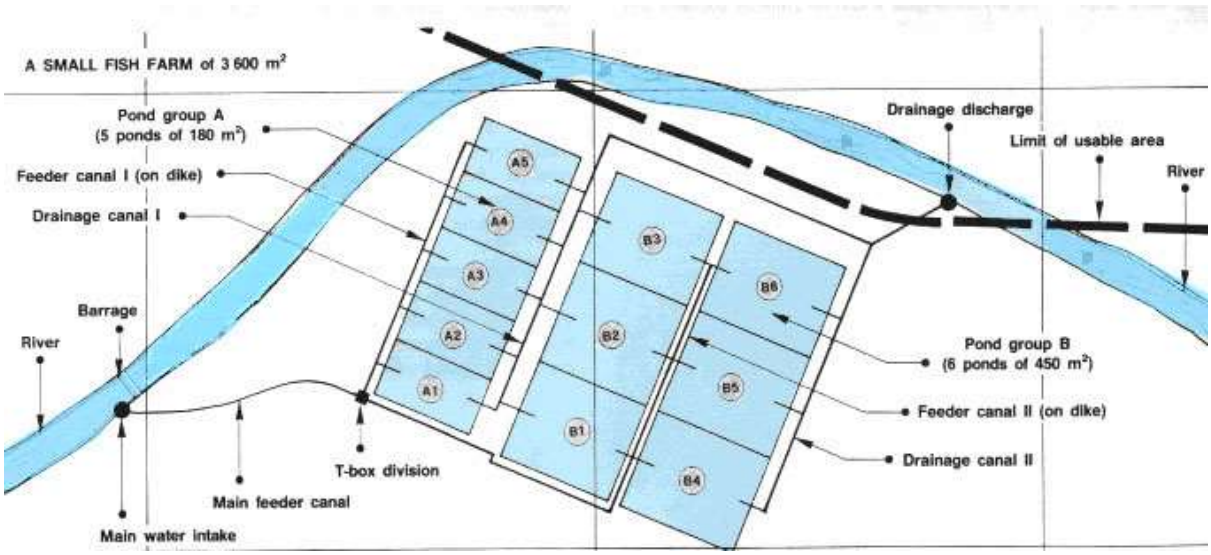
7. Document and analyse the results:

a. Record the calculated DNA concentration for each sample.
b. Analyse and interpret the results based on experimental objectives and experimental requirements.

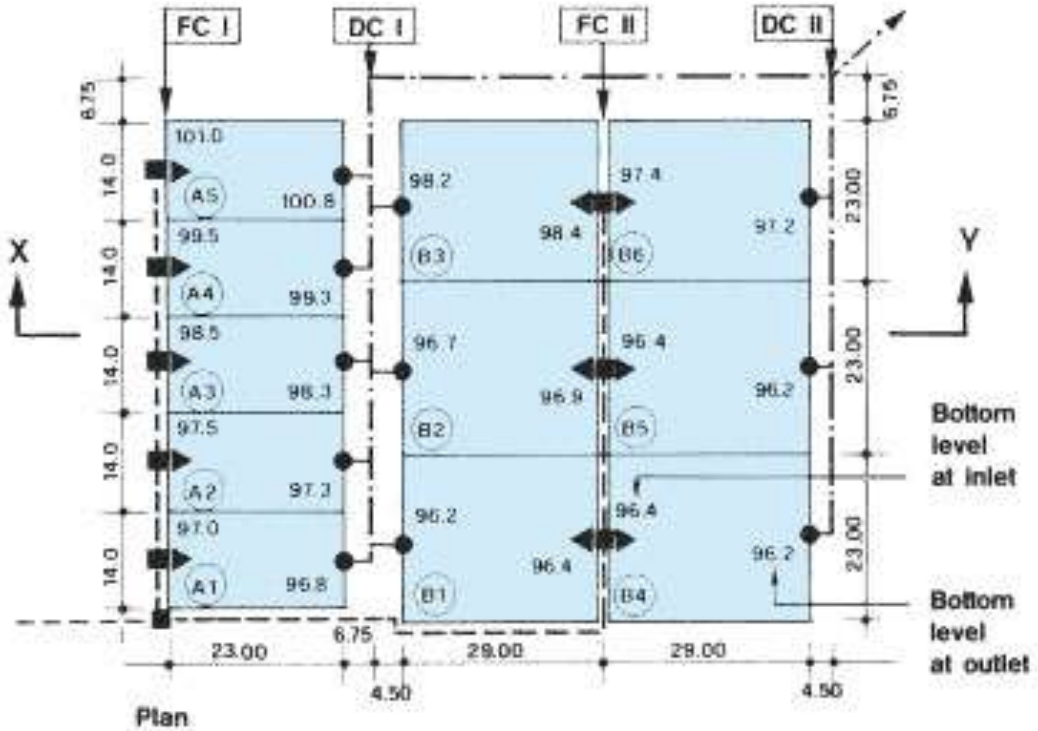
8. Clean-up: Dispose of the cuvettes, micro centrifuge tubes, and any other disposable items properly. Clean and wipe down the spectrophotometer according to the manufacturer's instructions.

Conclusion: Accurate estimation of DNA concentration is crucial for various molecular biology applications. By following this lab manual, you can successfully estimate DNA concentration using a spectrophotometer. This knowledge will help ensure accurate sample preparation and reliable quantification of DNA for a wide range of downstream experiments and analyses.

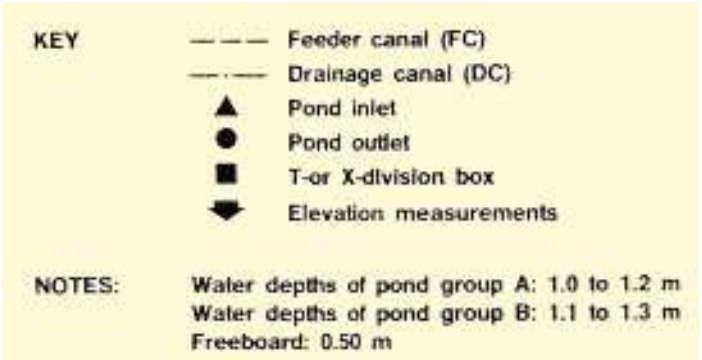
LAB – 2: Layout of an Aqua Farm

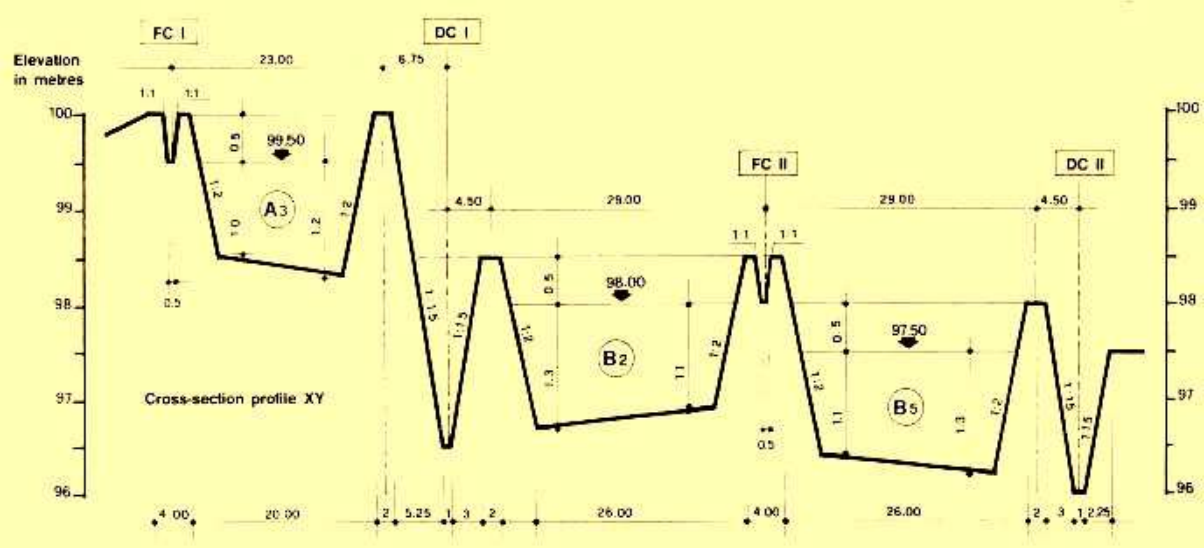


General Layout of the Farm

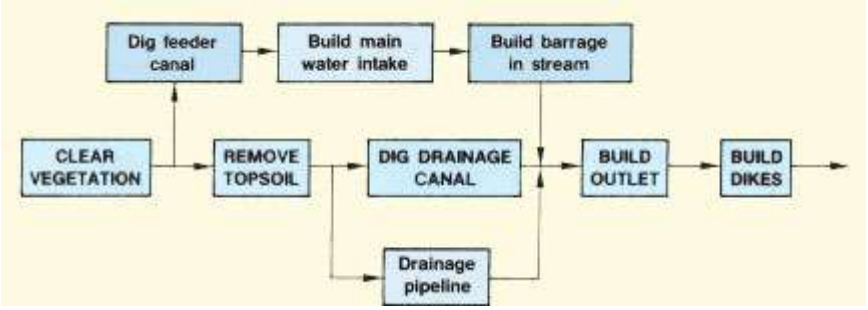


Detailed Layout of the Farm





Cross section Layout of the Farm



Schedule Activity

The farm having

1) Five small rearing pond of 180 m^3.
2) Six large pond of 450 m^3.
3) Feeder Canal – 200 m.
4) Dikes – 150 m^3

Working hours for the construction of the farm

1) Working hours for manual construction of rural ponds – 130 hrs.
2) Feeder canal – 50 hrs.
3) Excavation/dikes construction – 600 hrs.
4) Inlet/outlet pipes – 10 hrs.
5) Total working time – 785 hrs.

LAB – 3: Layout of an Eco-Hatchery System

The prototype carp hatchery, which can be developed for breeding of rohu consists of components such as

1. Overhead tanks,
2. Spawning pool,
3. Male broodfish cistern,
4. Acrylic jars for incubation of fertilized eggs,
5. Hatchling collection cisterns and
6. Circular hatching pool for mass scale incubation of fertilized egg

Overhead tanks - Two overhead tanks of 10,000 litre capacity each were constructed 5 feet above the ground level. Both the overhead tanks were joined together at the bottom by galvanized iron (GI) pipe. Water is supplied to the hatchery from a pond. The pond water is pumped to the overhead tank after filtering through bolting silk cloth to avoid plankton in the hatchery water. From the overhead tanks, water is supplied to different units through GI pipes.

Spawning pool - The Circular Chinese type of spawning pool was constructed for breeding/ spawning purpose. During selective breeding work, only female broodfishes were released in the spawning pool for full sib family production. However, for mass scale spawn production, both male and female broodfishes were released. The diameter of the spawning pool is 3.0 m. Water depth is maintained at 0.6-0.7 m. Showering system was also provided in the wall of the pool with plastic sieve tubes. Single water inlet with 60° tangent was fitted at the bottom of the sidewall. The water depth is controlled by outlet on the sidewall of the pool. Water current is produced in the spawning pool before two hours of calculated spawning time through the inlet pipe. The speed of the water is maintained at 3-4 m/ second. The volume of the spawning pool is 5 m^3. Broodfish @ 6 kg/m^3 is used on an average in the spawning pool for breeding purpose. For fullsib family production, 25-30 kg of female broodfishes are utilized; whereas for mass scale spawn production, average 16 kg female and 14 kg males (1:1 by number) are used for breeding in a single operation.

Male broodfish cistern - For the production of fullsib families in selective breeding study, male and female broodfishes are to be stripped separately to obtain gametes. So after hormone injection they have to be kept separately. One rectangular (3.5 m x 1.8 m x 1.0 m) male broodfish cistern was constructed for holding male broodfish. The water outlet was fitted at a height of 0.9 m in order to maintain the water depth at 0.9 m. The cistern was fitted with two showers from both the sides to supply oxygenated water. After hormone injection, male broodfishes are released to the breeding hapa fixed in the cistern.

Acrylic jar hatching unit - The transparent acrylic jars in which fertilized eggs are incubated get water supply from bottom through a flexible connecting pipe. There are seven acrylic jars in each unit. The hatchery complex consists of three such units; so, 21 acrylic jars are there in

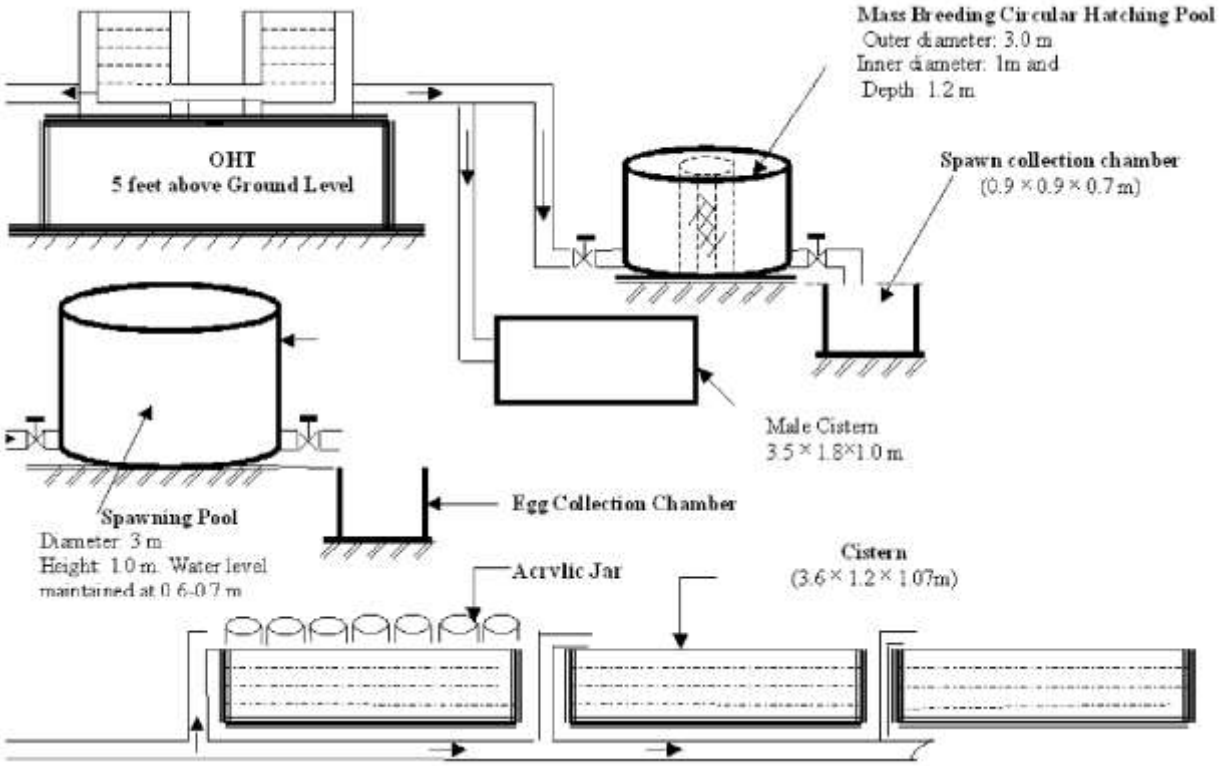
the hatchery that can incubate 21-fullsib families in a single operation. Each acrylic jar has the specifications as shown in Fig. 1b. The capacity of each jar is 15 l. At the bottom of the jar a sieved guard of 18 mm diameter and 5 mm thickness was fixed. A total 17 holes of 2 mm diameter were fitted in each jar to block the flow of eggs into the pipe. Only swollen eggs are loaded in the jars. In each jar 60,000-75000 numbers of fertilized eggs can be loaded @ 4000-5000 numbers/l. The outlet of the jar is connected to fibreglass channel, which opens to hatching hapa separately. After 14-16 hours of incubation, the eggs hatch and hatchlings start migrating with the water flow to the hatchling collection hapa. The migration normally gets completed by 20-24 hours.

Hatchling rearing unit - Each acrylic jar independently opens to hatchling collection hapa. The hatchling collection hapas are made up of bolting silk cloth having 1.2 m length, 0.5 m width and 1.0 m height. The hapas have tapes to join the four corners with the cistern. At the bottom side of the hapa small weights are tied to prevent floating. Each cistern receives spawn from seven acrylic jars separately into seven hatchling collection hapas. There are three cisterns in the hatchery and each cistern has the dimension of 3.6 × 1.2 × 1.07 m. Showers are provided from the top to each hatchling collection hapa. The water depth of the cistern is maintained at 0.85 m. Hatchlings are kept in those hapas for 60-72 hours (till complete absorption of yolk sac).

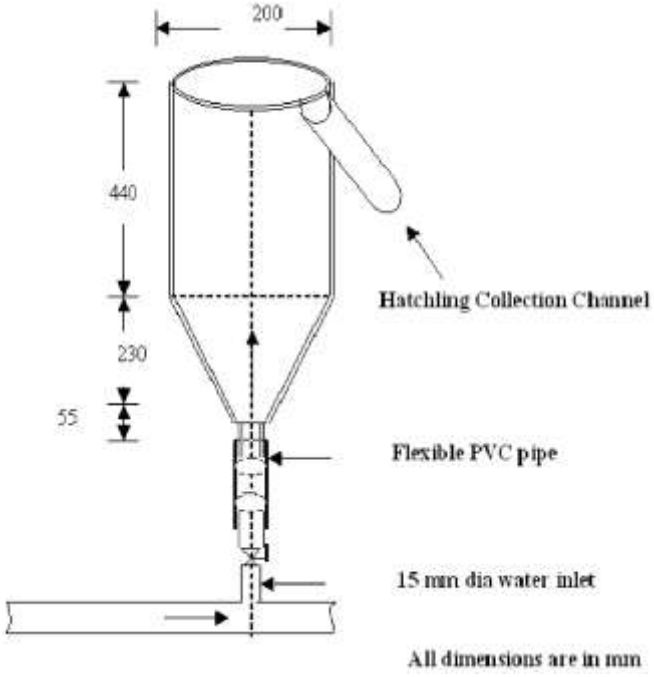
Circular hatching pool - One circular hatching pool was also constructed for mass scale incubation of fertilized eggs to spawn. The diameter of the pool is 3.0 m. It is a double circular smooth cistern made of brick and RCC material. The hatching pool has two chambers and water enters into the cistern by ten numbers of duck mouth inlets and expel out through a screen encircling the inner chamber. A delivery pipe was erected on the central outlet hole to maintain the water depth of the incubation chamber. The pool was also provided with spawn delivery outlet fixed at the bottom of the outer wall. The duck mouths were arranged in holes equidistant from each other and from both inner and outer walls of the chamber. The equidistant principle with unidirectional water flow allows the eggs to circulate without low oxygen and without touching the screen of the cistern. The depth of the pool is maintained at 1.0 m. The mesh size of the inner screen is 0.32 mm in order to prevent the escape of hatchlings. The eggs are loaded @ 7 lakhs/ m3. The regulated water flow in the hatching pool is maintained at 0.4-0.5 m/second for first 12 hours and then reduced to 0.1-0.2 m/second to avoid premature hatching. For rest of the period, it is maintained at 0.3-0.4 m/second.

Water requirement assessment - Water requirement study was conducted for fullsib family spawn production involving water used in spawning pool, male cistern, hatching acrylic jars and incubation in the cistern in one cycle i.e., from spawning to spawn. Whereas in mass scale improved rohu spawn production, water utilization in spawning pool and circular hatching pool were taken into account. Water requirement in each case was studied till complete absorption of yolk sac in the hatchlings. Water output was measured on hourly basis. Each hour was again divided into three observations of one-minute duration at every 20-minute interval. Finally, total water requirement for each full sib family and per million spawn production was estimated.

A Prototype Hatchery Model for Breeding of Indian Major Carps



Layout Diagram of Specialized Selective Breeding Hatchery



View of Single Unit Acrylic Jar

LAB-4: Design and Construction of Different Ponds in aquaculture

A. Nursery Pond:

The total fry (fish seeds above 8mm. and up to 40mm. in length) requirements for the presently cultivated waters have been estimated over 165.18 crores. If all existing readily available water areas for nursery production are to adequately stocked, the total requirements to meet the above demand would be 219.66 crores and this requirement will further increase the production of fish culture in our country.

The available sources of fry production in the country are

a) From riverine collection (contributes 5.90% of total seed production in country)

b) Production in different hatcheries

Nursery pond management is based on the principle of bringing suitable and proper ecological, physico-chemical and biological conditions in pond, where by protection from harmful external factors and promotion of optimum growth and survivality of the fry, will be ensure in natural conditions or through human manipulation.

Factors responsible for mortality of fish larvae in nurseries are –

a) Damage and injury to spawn collection and during transport.

b) Wide fluctuation of physico-chemical parameters of the container carrying the fry and pond water.

c) Defective method of fish seed stocking in pond.

d) Presence of unwanted organisms in nursery pond.

e) Macro and micro weed infestation.

f) Lack of sufficient natural food.

g) Rearing duration.

h) Improper feeding management and schedule.

i) Deterioration of soil and water quality in pond.

Nursey ponds are small, shallow ponds, perennial or seasonal, to rear the fishes from spawn to fry stage. In some larger production ponds may be large in size, though, generally, these types of ponds having 0.02-0.04 ha in size, 3-3.5 feet in depth. Cement riveted, stone, brick and even fiberglass tanks can also be used as nursery rearing tank with different management protocols than earthen pond. Culture period in nursery ponds is about 12-16 days.

The ecological condition – the inter-relationship of primary productivity, plant and animal life, which complete with each other and complete the food chain in a nursery environment

bears great significance in successful nursery management in earthen ponds. Ecological factors such as, abundance of macro-vegetations and higher animal life in the form of fish, insects and amphibian population hardly play any important and useful role in the rearing of the fry. An alternation of natural ecology through human agency is necessary to make a nursery is, there, to be limited to short cycle ending with zooplankton organism.

Time of the pond preparation: April to May.

Type of the pond: Seasonal (Dry type) and Perennial (Nevertheless, seasonal types are most common).

Pre-stocking Operation in Dry/Seasonal Pond:

1. Drying the pond still the cracks appear on the bottom.
2. Repair the dykes, berm, and bottom of the pond.
3. Ploughing of the pond bottom with raw cow dung and green manure by exposing the sub soil to the atmosphere to speeding up the oxidation process and release the nutrients.
4. Application of small volume water to the pond just to moist the pond bottom.
5. Application of manure – raw cow dung @ 1000 kg/bigha; single super phosphate @ 10 kg/ha.
6. Raking of pond soil after 7 days of manuring.
7. Application of lime @ 30 kg/ha.
8. Filling of pond with sufficient water inlet.
9. Testing of water parameters after 2 to 7 days. (The optimal condition should be like as follows)
    a) Colour of the water – turbid brown, light green, red,
    b) pH => 7.8 to 8.2
    c) Alkalinity => 120-170 ppm.
    d) Dissolved oxygen content => 5 to 8 ppm.
    e) Alkalinity – 70 to 150 ppm.
    f) Free ammonia – Nil.
    g) Phosphate => 0.2 to 0.4 ppm.
    h) Nitrite => 0.06 to 0.1 ppm.

10. Measurement of zooplankton density, when the density is found optimum, the pond is ready to be stocked.

11. Twenty-four hours before stoking, all the aquatic insects should be killed by using soap and oil emulsion, generally kerosene is used.

Pre-stocking Operation in Perennial Pond:

1. **Control of predatory and weed fishes -** In perennial pond predatory and weed fauna in the water body is to be eradicated by netting or using fish toxicants. The common fish toxicants are –

    a) Mohua oil cake - Mahua oil cake at 200-250 ppm is applied 15 days before stocking and pond effectively kills in predatory fishes. Mahua oils cake containing -6% saponin enters the blood stream of fishes causing haemolysis and subsequent mortality. The application of mahua oil cake has gained wide popularly due to its fertilizing effect after toxic effect.

    b) Derris root powder – It is a contact poisons with 5% rotenone, damages the respiratory system of the fishes and a connection of 4 ppm is effective against all forage fishes. The required quantity of derris root powder is applied 12 days prior to stocking. It is mixed thoroughly with water and sprayed over the water surface.

    c) Chlorinated hydrocarbons –

        i. Endrin (Tafadrin 20)

        ii. Organophosphate (Thiometon DDVP, Phosphamidon)

2. **Control of aquatic weeds -** Perennial water bodies is used for nursery operations may contain luxurious growths of various aquatic weeds which should be removed by following ways –

    a) Manual control – Nursery ponds are small and labours are cheap in India. Most of the ponds are cleared manually by hand pickling of aquatic weeds. Small floating weeds like *Lemna* and other algae can be removed by straw rope dragging towards the corner of the pond.

    b) Mechanical control – some weed cutting machine can be used for relatively large pond for larger production. But in nursery pond, mechanical weed cutters are hardly used.

    c) Chemical control - The manual removal of weeds from heavily infested large water bodies is difficult and time consuming. Under such conditions certain commercially available chemicals (herbicides) can provide an efficient means of eradication of undesirable aquatic plants. The common herbicide used in pond clearance is 2-4D (12 kg/ha). Water lettuce which often causes a serious problem

in fish ponds can be controlled with 0.1–0.2 kg of paraquat/ha. This infestation could also be controlled by foliar spray of aqueous ammonia (1%) at the rate of 50–75 kg/ha along with 0.2 % of any commercially available detergent as a wetting agent.

d) Biological control – biological control of aquatic weeds is not common in nursery pond. Grass carp is the most effective biological control agent against most of the submerged and floating weeds except the water ferns. Grass carp normally consumes some aquatic weeds, at least 50% of their body weight in a day.

3. **Fertilization -** Fertilization schedule involving both organic and inorganic fertilizers starts 10–15 days prior to stocking and is prepared on the basis of nutrient status and chemical environment of the pond soil and water.

   a) Organic fertiliser – Raw cow dung @ 1000 kg/bigha

   b) Inorganic fertiliser – single super phosphate @ 10 kg/bigha; urea @ 5 kg/ha.

   c) Maharashtra schedule of manuring consist of the following steps:

   i. **Stage-1:** Organic RCD @ 700kg/ha. and oilcake @ 700kg/ha, with inorganic superphosphate 150kg/ha, triple phosphate 80kg. /ha. the day before the expected date of stocking spawn.
   ii. **Stage-2:** Organic RCD @ 88kg/ha and peanut oil cake @ 350kg/ha on the day following the date of stocking.
   iii. **Stage-3:** Organic RCD 44kg/ha, peanut oil cake @ 175kg/ha on the second day following the date of stocking.
   iv. **Stage-4:** Organic RCD 22kg/ha, peanut oil cake @ 88kg/ha from 3rd day to 10th day following the date of stocking.

4. **Liming -** This is the first steps in the fertilization of a nursery pond. The quantity of lime to be applied depends on the pH of the soil. Liming is important to enhance the effect of organic fertilisers in pond. It can also disinfect the pond water and eliminates some harmful pathogens. It also maintains the optimum pH condition of water and soil.

PH range	Quantity of Lime kq/ha
4 - 4.5	1,000
4.5 - 5.5	700
5.5 - 6.5	500
6.5 - 7.5	200

5. **Control of aquatic insect** – A high survival in nursery pond can be expected only if the insect population is completely eradicated which otherwise will prey upon with spawn in addition to being competitors for the food. Among the insect beetles back

swimmers water bugs, water scorpions, dragonfly nymphs cause considerable harm to the span in nursery ponds.

a) Repeated drag netting using a fine method (1/16) on the previous day of commissioning the one can eradicate the insect population in nursery pond in to a considerable extend.
b) Most of aquatic insects utilize the atmospheric oxygen for respiration which if cut off from the water column will lead to its mortality by suffocation. This is achieved through. 'Oil emulsion' by producing and uniform oil firm over the water surface. For making soap-oil emulsion, the soap is mixed with oil and gently heated for some time with vigorous stirring. These emulsions are applied by spraying over the pond surface about 12–24 hours prior to stocking of spawn. It is the film of the emulsion which is important and hence care is taken not to disturb the film for a few hours. Windy days should be avoided as it will break the film.
c) Malathion application in nursery ponds also controls the predatory insects' population and hence subsequent treatment for control of insect is not required. However, if swarms of these predatory insects are seen in the nursery pond, treatment should be applied immediately.

6. **Water quality test** – (same as dry pond preparation mentioned above).

7. **Assessment of Food in Nurseries** – The quantitative and qualitative nature of standing crop of plankton following fertilization od nurseries is to be ascertained at short intervals by filtering 45 litres of pond water through plankton collection net made of No. 21 bolting silk. A rich production and dominance of Phyto-planktonic organisms in the nursery at the time of stocking suggests its immediate unsuitability for stocking and sediment volume of about 1.0ml. of zooplankters, consisting of rotifers, copepod nauplii and Cladocera is to be regarded as a good food reserve for the hatchlings.

Stocking of Nursery Pond:

Complete detoxification of the piscicide applied earlier should be ensured before stocking the nursery, rearing and stocking ponds. One or two days prior to stocking, a hapa should be fixed in the pond and some stocking materials should be put inside the hapa. Absence of distress and mortality after 24 hours confirm complete detoxification and the pond should be regarded as ready for stocking.

Carp spawn requires natural feed immediately after stocking and hence it is essential to have a minimum plankton value of 30–40 ml/m^3 in case of stocking at a moderate rate (1.5–2.5 million/ha). When a higher stocking rate is to be adopted, plankton population is also required to be increased accordingly. In case the stocking density is over 5 million/ha, the plankton volume should be around 100 ml/m^3. Self-produced or procured 3–4 days old spawn should be stocked in the morning at the rate of 4–6 million/ha. The stocking density must be according to the condition of the pond and the amount of fish food organisms available. The

rate of stocking in a well-prepared nursery pond with adequate fish food organisms can be as high as 10 million/ha.

Post-stocking Operation Nursery Pond:

1. **Feeding** - Soon after the entry into the pond the spawn starts voracious feeding on the plankton. Within a couple of days, the plankton population gets depleted. Hence a supplementary diet is resorted so as to keep up the plankton biomass. Nursery ponds are fed with rice bran and finely powdered ground nut oil cake in 1:1 by weight. The following feeding schedule is more economical and gives better survival. For better utilization half of the feed is given during the morning hours and half during the evening hours in every day.

    - ✓ First 5 days - Equal to the initial body wt. of spawn stocked.
    - ✓ Second 5 days - Double the initial body wt. of the spawn stocked.
    - ✓ Third 5 days - Thrice the initial body wt. of the spawn stocked.

The nursery feeds for higher stocking densities to get best survival rates should have the following qualities –

a) Ready acceptability to early fry,
b) Easy digestibility and
c) Higher conversion value

The daily ratio is estimated on the basis of fry population and their advancing growth with the approximate initial weight of each spawn as 0.0014 g. The total quantity to be fed as; first 5 days of stocking – double the weight, second 5 days of stocking – three times the weight, and third 5 days of stocking – four times the weight.

Broadcasting of feed on the surface water is the normal procedure. All the food broadcasted is not utilised by the early spawn and fry to full extent. A large quantity of feed drops down at the bottom of the pond, decomposed and forms organic manure.

Common carp is the best utiliser of artificial feed. Among Indian Carps, mrigal utilise artificial feed best, but Catla do not utilise well.

Mixture of dry finely powdered notonecta, small shrimps and cheap pulses in the ratio of 5:3:2 is also used as artificial feed at the same rate as that of grain barn and vegetable oil cake.

2. Harvesting - After 15 days of rearing the fry attains a size of 20-25 mms and the stock is ready for harvesting. Using 1/16 mesh cotton drag net pond is harvested repeated netting the survival range would be 60-85% with an average of 75%. The harvested fry required to be transferred to larger rearing ponds. Survival rates in nurseries have been registered as high as 88% and an average of about 50% with size of fry ranging between 20 and 30 mm.

B. Rearing Pond:

Rearing of the 15 days old fry (25 to 30 mm) to the fingerlings (100-150mm) size in a large pond with in shortest period of 3 months' time is called rearing pond management. A rearing pond should have an area of 0.8 to 0.1 ha preferably rectangular in shape with water depth ranging from 1.5 to 2 metres. The utility of the rearing pond culture is to provide more space to fry reducing stocking density and at the same time providing a larger pond than the nursery pond for their proper growth and development. On the other hand, the 15 days old fry are reared in the same small nursery pond, their growth will be retarded and hence production will be hampered. The time of rearing in rearing pond is normally 2-3 months and sometimes up to 6 months.

Pre-Stocking management –

1. **Eradication of Aquatic Weeds** - Being somewhat deeper and longer than nursery ponds, rearing ponds are more liable to get infested with weeds. Am overgrowth of weeds deprived the pond soil of nutritive elements, restrict movement of fish interferes with netting operations and harbours predatory and weed fishes and insects.

   a) Manual control - Floating weeds like *Eichhornia* and *Pistia* are best removed by manual labour.

   b) Chemical control - Chemicals like 2,4-D are quite effective and economical against *Eichhornia*. When mixed with common domestic detergent 2,4-D effectively against weeds like Pistia *Nymphaea* and *Nelumbo*. Taficide 80, at a dose of 2.2kg/ha is also effective against *Eichhornia*.

   c) Mechanical control - Marginal weeds like *Typha*, grasses, sedges, *Ipomoea*, *Sagittaria* and *Colocasia* are effectively controlled by ploughing and burning during dry season or repeated cutting by weed cutter.

   d) Biological control - Some of the better-known fishes that are used for biological control of weeds are the grass carp, *Ctenopharyngodon idella* and *Puntius javanicus.* Grass carp feeds most of the weeds like *Otelia valliharia, Utricularia, Trapa* and *Myriophyllum*.

2. **Eradication of Predatory and weed fishes** - Weed fishes (e.g. *Puntius* spp. *Amblypharyngodon mola, E. danricus etc.)* are those which compete with the culturable species of fishes for food, space and oxygen and causing serious problem to fish culture. Predatory fishes (e.g. *Channa* spp., *Clarias* sp., *Wallago attu* etc.) are those causing the above-mentioned problems and also directly prey upon the fry and fingerlings of the culturable species.

   a) These fishes may be controlled by repeated drag netting or by complete dewatering of the ponds.

b) However, when this is not possible and effective the unwanted fishes may be killed by the application of Mahua oil cake an effective fish toxicant a 2000-2500 kg/ha (at 1-meter water) to 200-250ppm/ Which kills toxicant fishes of the pond within 4-6 hours. The effect of the toxicant lasts for about 21 days after which it acts as 5% rotenone content at a dose of 4.20 mg/1 is perhaps the commonest pond toxicant used.

3. **Fertilisation** - The next step in rearing pond preparation is fertilization, the objective of which is to have sustained production of adequate quantities of zooplankton which forms the natural food of carp fry.

   a) Organic manuring: Organic manures raw cattle dung is generally used as 10,000 organic manure raw cattle before the anticipated of stocking.

   b) Inorganic Fertilization: Inorganic manures such as super phosphate can be used @ 250 kg/ha before stocking.

4. **Liming** - The advantage of liming pond is numerous enhances pond productivity and improves its sanitation. The commonly available lime for pond application are calcium carbonate, calcium hydroxide, calcium oxide and calcium sulphate. Lime can be applied to the pond bottom 200-250 kg/ha-added to water at inlets or uniformly broadcast on the water surface depending on the form of lime used.

Stocking Management:

In rearing ponds, the fry of IMC and Chinese are stocked in various combination at densities ranging from 2-3 lakhs/ha in the following rations.

Species Ratio - Some of the possible combinations are - catla, rohu, mrigal, common carp (3:4:1:3); silver carp, grass carp (1:1); silver carp, grass carp, common carp (4:3:3); catla, rohu, mrigal, grass carp (4:3:1.5); silver carp, grass carp, common carp, rohu (3:1.5:2.5:3), etc. Combination of too many species should be avoided as it invites excessive handling at the time of harvesting for species segregation. Fry are reared in ponds for about 3 months when they usually attain 100–150 mm in length and 15–40 g in weight. For healthy fry rearing it is recommended that the size of the fry at the time of stocking in the rearing pond should be as uniform as possible. This can be done by size grading at the time of fry harvesting from nursery ponds. Prior to stocking the rearing ponds, the pond waters must have a plankton level of about 30–50 ml/m^3.

Post stocking management –

1. **Feeding** - Supplementary feeding consisting of a mixture of ground nut /. Mustard oil cake and rice brain at 1: 1 ratio by weight in powders form broadcast every day in the pond during morning hours from the first day of stocking. The feeding schedule as shown below may be followed for 3 months rearing period.

Period	Quantity of Feed day/Lakh of Fry
First Month	6 kgs
Second Month	10 kg
Third Month	15 kg.

2. **Manuring** - During the culture period both organic and inorganic manures should be applied at 15 days interval in order to enhance zooplankton and phytoplankton respectively.

   a) Organic manure – 1000 kg/ha/month.

   b) Inorganic manure - 40kg/ha/ month.

3. **Liming** - During the culture period liming should be done 25kg/ha/ month liming should be followed on bright days and should be avoided on cloudy or rainy days.

4. **Netting Operations and Harvesting of fingerlings** - Netting should be done regularly at least once a month, the more the netting in a pond the better will be the yield of good sized fingerlings in pond. After 3 months the fingerlings can be harvested by which time they attain an average weight of 150-20gms. Supplementary feeding should be stopped a day before, harvesting. Harvesting should be done during cool morning hours.

C. Stoking Pond

Raising of fingerlings to table sized fish in large ponds (0.25-10.0 ha area and 0.8-3.0 depth) is referred to as the stocking pond management. Most of the following activities in the stocking pond management are similar to those of nursery and rearing ponds. To get maximum production of fish utmost care should be taken through the most economic management measures. The principles in the rational management of stocking ponds are increasing the carrying capacity or the maximum standing crop.

A pond can support a fish biomass up to only certain level or limit. This limit is called the carrying capacity or the maximum standing crop. Carrying capacity of ponds are increasing by fertilization and supplementary increasing by fertilization and supplementary feeding, optimum utilization of ecological riches in the pond by good management of water quality, the culture of first growing species and fish health monitoring. The management of stocking pond is broadly discussed in three stages as in rearing ponds.

Pre-stocking Management:

New ponds, pre-stocking operations starts with liming and filling of the pond with water. The first step for existing pond requiring development deals with clearing the unwanted weeds and fishes either by manual, mechanical or chemical means from the pond. Different methods are employed for this.

1. **Control of aquatic weeds -** Removal of weeds by Manual/Mechanical, Chemical or biological means.
    i. Manual/Mechanical – Clearance of weeds by means of hand pickling, mechanical cutter, burning etc.
    ii. Chemical means – herbicides like 2,4-D (2,4-dichlorophenoxy acetic acid), Paraquat or aqueous ammonia and Diuron/Karmex,
    iii. Biological means- introduction of Grass carp, Tilapia, Common carp, Pearl spot, Giant gourami
2. **Removal of unwanted and predatory fishes and other animals –** by repeated netting or using mahua oil cake @ 2500 kg/ha meters or by sun drying the pond bed. Other toxicants include tea seed cake with a dose of 15 ppm. in salinity less than 15ppt, tamarind seed powder with a dose of 175-200 ppm.
3. **Liming -** The soils/ tanks which are acidic in nature are less productive than alkaline ponds. Lime is used to bring the pH to the desired level. In addition, lime also has the following effects
    a) Increases the pH.
    b) Acts as buffer and avoids fluctuations of pH.

c) It increases the resistance of soil to parasites.

d) Its toxic effect kills the parasites.

e) It hastens organic decomposition.

The normal doses of the lime desired ranges from 200 to 250 Kg/ha. However, the actual dose has to be calculated based on pH of the soil and water as follows:

SoilpH	Nature	Lime(kg/ha)
4.0-4.5	Highly acidic	1000
4.5-5.5	Medium acidic	700
5.5-6.5	Slightly acidic	500
6.5-7.5	Near acidic	200
7.5-8.5	Alkaline	Nil

Liming materials- calcium carbonate, calcium oxide, calcium hydroxide etc**.**

4. **Fertilisation/ Manuring** - Fertilisation of the pond is an important means for intensifying fish culture by increasing the natural productivity of the pond. The fertilisation schedule has to be prepared after studying the quality of the pond soil. A combination of both Organic and Inorganic fertilisers also be used. The fertiliser programme has to be suitably modified depending on the growth of the fish, available food reserve in the pond, physicochemical conditions of the pond and climatic conditions.

a) Organic - Farm yard manure (FYM)- Cow dung @ 5000 kg/ha, Poultry, sheep manure.

b) Crop by-products - cotton seed meal, mustard oil cake.

c) Inorganic - Inorganic fertilisation to be undertaken after 15 days of organic manuring. Requirement of nitrogenous and phosphate fertilisers would vary as per the nature of the soil fertility indicated below. However, any one of the nitrogen and phosphate fertilisers could be used as per given rate.

Inorganic Fertiliser Application (kg/ha/month)

Soil fertility status	Ammonium sulphate	Urea
1. Nitrogen (mg/100 g soil)		
i) High (51-75)	70	30
ii) Medium (26-50)	90	40
iii) Low (upto 25)	140	60

2. Phosphorus (mg/100 gm soil)	Single super phosphate	Triple super Phosphate
i) High (7-12)	40	15
ii) Medium (4-6)	50	20
iii) Low (upto 3)	70	30

Stocking:

After proper preparation, the pond should be stocked with 100–150 mm long fingerlings of desired carp species. In case the fingerlings are not available, the pond can also be stocked with advanced fry or early fingerlings in absolutely predator-free ponds. The stocking rate depends primarily upon the volume of water and on the oxygen balance of the pond. Quality of available natural fish food in the pond and the capacity of the farmer to provide supplementary feed, are also matters for consideration. Usually a pond having average water depth of 1.5–2.5 m should be stocked at the rate of 5 000 fingerlings/ha. The volume of water available for fish in an undrainable pond should not be less than 2 m^3/fish if there is no provision of artificial aeration. In composite fish culture, rearing of six species of carps, viz. catla *(Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) is considered to be the ideal combination. However, depending on the availability of quality fingerlings of these carp species, three or four species combinations can also be taken up.

Species	3-species	4-species	6-species
Catla	4.0	3.0	1.5
Rohu	3.0	3.0	2.0
Mrigal	3.0	2.0	1.5
Silver Carp	-	-	1.5

Post stocking:

1. **Supplementary feeding** - Fishes need much more food than what is available naturally in the pond. Fishes can be fed with a mixture of rice bran and oilcakes in the ratio 4:1. Due to the high cost of Ground Nut Oil Cake (GOC) alternate sources like Cotton seed oil cake which is comparatively cheaper than GOC. GOC and cotton seed oil cake can be mixed in equal proportions and fed to the fish and is reported to give almost the same growth rate as that of GOC. The feed should be placed on a feeding tray or in feeding bags and lowered to the pond bottom or it can be dispersed at the corners of the pond. After some time, the fishes will get used to this type of feeding and aggregate at the same place at particular time for regular feeding thereby reducing the feed losses. The recommended feeding rate is 5 - 6 % of the body weight up to 500gm size of fish and then reduce to 3.5% of body weight from 500-1000gm size. The feeding is supplementary in nature.

2. **Manuring -**

a) Organic manuring may be done in monthly instalments @ 1000 kg/ha.

b) Inorganic fertilisation may be done at monthly intervals alternating with organic manuring. However, the monthly rate of fertilisation will depend on pond productivity and the growth of the fishes. It should be ensured that excess fertilisation does not take place which may result in eutrophication.

3. **Harvesting** - Harvesting is generally done at the end of first year, when the fishes attain average weight of 800 gm to 1.25 kg. With Proper management a production of 4 to 5 tons/ha can be obtained in a year. Harvesting is done by partial dewatering and repeated netting. In some cases, complete dewatering of ponds is resorted to. Some farmers resort to partial harvesting also depending on the season and demand for fish.

LAB-5: Study of the haematological parameters of fish

Lab Manual - Haematological Parameters of Fish

Introduction: Haematological parameters provide valuable insights into the health and physiological status of fish. The analysis of these parameters helps assess the fish's overall well-being, detect potential diseases or infections, and monitor the effects of environmental factors. This lab manual provides a step-by-step guide on how to measure and analyze the haematological parameters of fish.

Materials:

1. Fish samples
2. Anesthetics (appropriate for fish species)
3. Microscope slides
4. Microscope
5. Hemocytometer or automated cell counter
6. Pipettes (various volumes)
7. Eppendorf tubes
8. Centrifuge
9. Hematology stains (e.g., Wright's stain)
10. Hematology analyzer (optional)
11. Hemoglobinometer (optional)
12. Reagents and buffers for blood cell counting and staining
13. Distilled water
14. Safety goggles
15. Gloves

Procedure:

1. Sample Collection:

    a. Obtain fish samples, ensuring they represent a range of sizes and ages.
    b. Use appropriate methods to anesthetize the fish to minimize stress and ensure safe handling.
    c. Collect blood samples from the caudal vessels using a syringe or capillary tubes.
    d. Carefully transfer the blood samples into clean Eppendorf tubes.

9. Hematocrit Determination:

    a. Place a small volume of blood (approx. 1 mL) into a microhematocrit capillary tube.
    b. Seal one end of the capillary tube using a clay sealant or a hematocrit tube sealant.
    c. Insert the sealed capillary tube into a microhematocrit centrifuge, ensuring a balanced load.

d. Centrifuge the capillary tubes at a high speed for a predetermined time (e.g., 5 minutes).
e. After centrifugation, measure the ratio of the packed red blood cells (RBCs) to the total blood volume, which represents the haematocrit value.

10. Total Red Blood Cell Count:

a. Prepare a diluted blood sample by mixing a small volume of blood (e.g., 10 µL) with a known volume of diluent (e.g., 90 µL).
b. Load the diluted blood sample into the counting chamber of a haemocytometer or use an automated cell counter.
c. Count the total number of red blood cells in the designated counting area of the chamber.
d. Calculate the red blood cell count per unit volume using the appropriate formula.

11. Total White Blood Cell Count:

a. Dilute the blood sample using a suitable diluent in a predetermined ratio (e.g., 1:10).
b. Load the diluted blood sample into the counting chamber of a haemocytometer or use an automated cell counter.
c. Count the total number of white blood cells in the designated counting area of the chamber.
d. Calculate the white blood cell count per unit volume using the appropriate formula.

12. Differential White Blood Cell Count:

a. Prepare blood smears by placing a small drop of the blood sample on a clean microscope slide.
b. Spread the blood drop evenly using another slide, creating a thin blood film.
c. Allow the blood smear to air dry completely. d. Stain the blood smear using a suitable haematological stain, such as Wright's stain.
d. Examine the stained blood smear under a microscope using the appropriate magnification.
e. Identify and count different types of white blood cells, such as lymphocytes, neutrophils, monocytes, eosinophil, and basophils.
f. Calculate the percentage or absolute count of each cell type based on the total white blood cell count.

13. Haemoglobin Determination:

a. Collect a small volume of blood (e.g., 100 µL) in a clean, dry Eppendorf tube.
b. Add a suitable haemoglobin reagent or buffer to the tube and mix thoroughly.
c. Follow the manufacturer's instructions to measure the haemoglobin concentration using a hemoglobinometer or a hematology analyzer.

14. Data Analysis and Interpretation:

    a. Record all haematological parameters obtained from the analysis.
    b. Compare the results with reference ranges or previously established values for the specific fish species.
    c. Interpret the data to assess the health and physiological status of the fish.

15. Clean-up: Dispose of used capillary tubes, slides, and other disposable items appropriately. Clean and disinfect the microscope and other equipment as per laboratory protocols.

Conclusion: The analysis of haematological parameters provides valuable information about the health and physiological condition of fish. By following this lab manual, you can accurately measure and analyse key haematological parameters, including haematocrit, red and white blood cell counts, differential white blood cell count, and haemoglobin concentration. This knowledge will help you monitor fish health, detect abnormalities, and contribute to the overall understanding of fish biology and welfare.

LAB-6: Biochemical Analysis of Fish Liver

Introduction: Biochemical analysis of fish liver provides valuable insights into the metabolic activities and overall health of fish. The liver is a vital organ involved in various metabolic processes, including nutrient metabolism, detoxification, and protein synthesis. This lab manual provides a step-by-step guide to perform biochemical analysis of fish liver, including the measurement of enzyme activities and assessment of biochemical parameters.

Materials:

1. Fish liver samples
2. Ice or refrigerated storage for sample preservation
3. Mortar and pestle
4. Centrifuge
5. Eppendorf tubes
6. Microplate reader or spectrophotometer
7. Reagents for biochemical assays (specific to the parameters of interest)
8. Distilled water
9. Disposable micropipettes (appropriate volumes)
10. Test tubes and racks
11. Vortex mixer or shaker
12. Incubator or water bath (as required for specific assays)
13. Safety goggles
14. Gloves

Procedure:

1. Sample Preparation:

    a. Obtain fresh fish liver samples or thaw frozen samples if stored at low temperatures.
    b. Keep the liver samples on ice or refrigerate them to maintain their integrity during the analysis.
    c. Weigh and record the weight of each liver sample before further processing.

16. Tissue Homogenization:

    a. Using a clean mortar and pestle, grind the liver sample into a fine paste with the addition of a suitable buffer or reagent. Ensure the tissue is thoroughly homogenized.
    b. Transfer the homogenized liver sample into a labelled Eppendorf tube.

17. Centrifugation:

    a. Place the Eppendorf tubes containing the homogenized liver samples in a centrifuge.

b. Centrifuge the tubes at an appropriate speed and duration to separate the cellular debris and obtain a clear supernatant.
c. Carefully transfer the supernatant into new Eppendorf tubes, avoiding any transfer of debris.

18. Enzyme Assays:

a. Choose specific enzyme assays based on the metabolic pathways of interest (e.g., alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase).
b. Prepare the required reagents and buffers according to the assay protocol.
c. Add the appropriate volume of the liver supernatant to the respective reaction tubes or micro plate wells.
d. Follow the assay protocol for each enzyme, including the addition of substrates and necessary controls.
e. Incubate the reaction mixture at the specified temperature and duration as per the assay requirements.
f. Measure the enzyme activity using a micro plate reader or spectrophotometer at the appropriate wavelength.

19. Biochemical Parameter Analysis:

a. Determine the levels of biochemical parameters of interest, such as total protein, triglycerides, cholesterol, glucose, and glycogen.
b. Prepare the necessary reagents, standards, and controls for each biochemical parameter analysis.
c. Follow the assay protocol for each parameter, including the addition of reagents and standards to the reaction tubes or micro plate wells.
d. Incubate the reaction mixture at the specified temperature and duration as per the assay requirements.
e. Measure the absorbance or fluorescence using a micro plate reader or spectrophotometer at the appropriate wavelength.

20. Data Analysis and Interpretation:

a. Record the enzyme activities and biochemical parameter values obtained from the analysis.
b. Compare the results with reference ranges or established values for fish species.
c. Interpret the data to assess the liver function and overall health status of the fish.

21. Clean-up: Dispose of used tubes, tips, and other disposable items appropriately. Clean and disinfect the equipment and work surfaces as per laboratory protocols.

Conclusion: The biochemical analysis of fish liver provides valuable information about the metabolic activities and health status of fish. By following this lab manual, you can

accurately perform enzyme assays and analyse biochemical parameters to assess liver function and overall fish health. The obtained data will contribute to a better understanding of fish physiology, nutrition, and the effects of environmental factors on liver metabolism.

LAB-7: Protein profiling study of different fish tissues

Introduction: Protein profiling allows the comprehensive analysis of proteins present in fish tissues, providing insights into their functional roles and physiological processes. Protein profiling helps understand protein expression patterns, identify biomarkers, and study protein-protein interactions. This lab manual provides a step-by-step guide for performing protein profiling studies of fish tissues.

Materials:

1. Fish tissue samples
2. Liquid nitrogen (for tissue sample freezing)
3. Mortar and pestle (or tissue homogenizer)
4. Extraction buffer (appropriate for protein solubilization)
5. Protein quantification kit (e.g., Bradford assay, BCA assay)
6. SDS-PAGE apparatus (gel electrophoresis system)
7. Acrylamide and bis-acrylamide solutions
8. Protein molecular weight marker
9. Coomassie Brilliant Blue stain (or any suitable protein stain)
10. Western blotting system (if performing immunoblotting)
11. Primary antibodies (if performing immunoblotting)
12. Secondary antibodies (if performing immunoblotting)
13. Chemiluminescent substrate (if performing immunoblotting)
14. Transfer buffer (for Western blotting)
15. Blocking buffer (for Western blotting)
16. Tris-buffered saline (TBS) or phosphate-buffered saline (PBS)
17. Tween-20 or Triton X-100 (for wash buffers)
18. Electrophoresis power supply
19. Semi-dry or wet transfer system (if performing Western blotting)
20. X-ray film or chemiluminescence imaging system (for visualization)

Procedure:

1. Sample Collection and Preparation:

    a. Collect fish tissue samples of interest and freeze them immediately in liquid nitrogen or store them at -80°C until further use.
    b. Thaw the tissue samples on ice just before the extraction process.
    c. Prepare the extraction buffer by following the manufacturer's instructions or using a suitable recipe for protein solubilisation.

22. Tissue Homogenization:

    a. Cut the fish tissue samples into small pieces using sterile tools.
    b. Place the tissue pieces in a mortar or tissue homogenizer.
    c. Add the appropriate volume of extraction buffer to the tissue samples.

d. Grind the tissue samples thoroughly until a uniform paste or homogenate is obtained.
e. Transfer the homogenate to a clean micro centrifuge tube and keep it on ice.

23. Protein Extraction:

a. Centrifuge the tissue homogenate at a high speed (e.g., 10,000 × g) for 10-15 minutes at 4°C.
b. Collect the supernatant containing the protein extract and transfer it to a new micro centrifuge tube.
c. Determine the protein concentration using a protein quantification kit (e.g., Bradford assay, BCA assay) according to the manufacturer's instructions.

24. SDS-PAGE Analysis:

a. Prepare the SDS-PAGE gel by following the appropriate protocol and using the desired acrylamide percentage.
b. Load the protein samples onto the gel, along with a suitable protein molecular weight marker.
c. Perform electrophoresis using an appropriate electrophoresis power supply and running buffer until the tracking dye reaches the bottom of the gel.
d. Carefully remove the gel from the apparatus and proceed to protein staining.

25. Protein Staining:

a. Stain the protein gel using Coomassie Brilliant Blue or any suitable protein stain according to the manufacturer's instructions.
b. Immerse the gel in the staining solution and gently agitate for the recommended duration.
c. Destain the gel by rinsing it with distilled water until the background is clear and protein bands are visible.

26. Western Blotting (optional):

a. Transfer proteins from the SDS-PAGE gel to a nitrocellulose or PVDF membrane using a semi-dry or wet transfer system.
b. Prepare the transfer buffer and follow the recommended transfer conditions.
c. After transfer, block the membrane with a suitable blocking buffer to prevent nonspecific binding.
d. Incubate the membrane with primary antibodies specific to the proteins of interest, following the recommended antibody dilutions and incubation conditions.
e. Wash the membrane with TBS or PBS containing Tween-20 or Triton X-100 to remove unbound antibodies.
f. Incubate the membrane with secondary antibodies conjugated to an enzyme or fluorophore.

g. Wash the membrane again to remove unbound secondary antibodies. h. Develop the Western blot signals using a chemiluminescent substrate or suitable detection method.
h. Capture the signals using X-ray film or a chemiluminescence imaging system.

27. Data Analysis and Interpretation:

    a. Analyze the protein bands or spots on the stained gel using appropriate software or imaging tools.
    b. Compare the protein profiles among different fish tissues or experimental conditions.
    c. Identify differentially expressed proteins and potential biomarkers.
    d. Validate the protein profiling results using statistical analysis and additional techniques if required.

28. Clean-up: Dispose of used materials and chemicals appropriately. Clean and disinfect the equipment and work surfaces as per laboratory protocols.

Conclusion: Protein profiling of fish tissues provides valuable information about protein expression patterns, cellular processes, and potential biomarkers. By following this lab manual, you can perform a comprehensive analysis of fish tissue proteins using SDS-PAGE and optionally Western blotting. The obtained protein profiles will contribute to a better understanding of fish physiology, health, and the effects of various factors on protein expression.

LAB-8: Study of the principles & applications of instruments used in modern biology

Introduction: Modern biology heavily relies on various advanced instruments and technologies to study and analyse biological systems at the molecular, cellular, and organismal levels. These instruments provide researchers with precise measurements, imaging capabilities, and high-throughput analysis, enabling a deeper understanding of biological processes. This lab manual aims to familiarize students with the principles and applications of instruments commonly used in modern biology.

Materials:

1. Microscopes (light microscope, fluorescence microscope, confocal microscope)
2. Centrifuge
3. Spectrophotometer
4. Polymerase Chain Reaction (PCR) machine
5. Gel electrophoresis apparatus
6. Flow cytometer
7. Real-time PCR machine (optional)
8. Incubator
9. DNA sequencer (optional)
10. Plate reader (optional)
11. Pipettes (variable volume and fixed volume)
12. Consumables (microcentrifuge tubes, PCR tubes, pipette tips, etc.)
13. Biological samples or reagents (as required for specific experiments)
14. Safety goggles
15. Lab coats
16. Gloves

Note: Depending on the specific experiments or demonstrations, additional materials may be required.

Procedure:

1. Microscopy:

    a. Introduction to different types of microscopes: light microscope, fluorescence microscope, and confocal microscope.
    b. Demonstration of microscope parts and their functions.
    c. Preparation of microscope slides with biological samples.
    d. Adjusting and optimizing microscope settings for optimal imaging.
    e. Observation and documentation of samples under different microscope techniques.

29. Centrifugation:

    a. Introduction to centrifugation principles and types of centrifuges.
    b. Demonstration of proper loading and balancing of centrifuge tubes.

c. Setting appropriate speed and time for different sample types.
d. Collection and analysis of the supernatant and pellet after centrifugation.

30. Spectrophotometry:

a. Introduction to the principle of spectrophotometry and Beer-Lambert's law.
b. Demonstration of spectrophotometer operation and calibration.
c. Preparation of sample solutions with known concentrations.
d. Measurement of absorbance at specific wavelengths.
e. Calculation of concentration using standard curves.

31. Polymerase Chain Reaction (PCR):

a. Introduction to PCR principles and components.
b. Demonstration of PCR machine setup and optimization.
c. Preparation of PCR reaction mixtures with template DNA, primers, and enzymes.
d. Running PCR cycles and amplification of target DNA.
e. Analysis of PCR products using gel electrophoresis.

32. Flow Cytometry:

a. Introduction to flow cytometry principles and applications.
b. Demonstration of flow cytometer setup and calibration.
c. Preparation of single-cell suspensions and appropriate staining protocols.
d. Running samples through the flow cytometer.
e. Analysis of flow cytometry data using appropriate software.

33. Real-time PCR (optional):

a. Introduction to real-time PCR principles and applications.
b. Demonstration of real-time PCR machine setup and optimization.
c. Preparation of real-time PCR reaction mixtures with template DNA, primers, and fluorescent probes.
d. Running real-time PCR cycles and monitoring amplification in real-time.
e. Analysis of real-time PCR data using appropriate software.

34. Incubation:

a. Introduction to incubators and their applications in cell culture and microbial growth.
b. Demonstration of incubator setup, temperature, and humidity control.
c. Preparation of samples or cultures for incubation.
d. Incubation of samples for specific durations.

35. DNA Sequencing (optional):

a. Introduction to DNA sequencing principles and techniques.

b. Demonstration of DNA sequencer setup and operation. c. Preparation of DNA samples for sequencing.
c. Loading the samples into the DNA sequences.
d. Analysis and interpretation of DNA sequencing data.

36. Plate Reader (optional):

a. Introduction to plate readers and their applications in various assays.
b. Demonstration of plate reader setup and calibration.
c. Preparation of samples or assays in micro plate wells.
d. Reading and recording absorbance or fluorescence measurements.
e. Analysis of plate reader data using appropriate software.

37. Good Laboratory Practices and Safety:

a. Emphasize the importance of good laboratory practices and safety protocols.
b. Demonstrate proper handling of biological samples and hazardous materials.
c. Discuss waste disposal procedures and laboratory cleanliness.

Conclusion: Understanding the principles and applications of instruments used in modern biology is essential for conducting various biological experiments and analyses. By following this lab manual, students will gain hands-on experience and knowledge of the instruments commonly used in modern biology research. This will equip them with the skills necessary to apply these techniques in their future studies and contribute to advancements in the field of biology.

LAB-9: Analysis of Data through Computer

1) Make a bar diagram on inland fish production (2021-22) of different states of India using MS Excel and comment on it.

Table 1.6 : Year-wise Fish Seed Production in India		
S.N.	Year	Production (Lakh Fry)
1	1985-86	63,220
2	1986-87	76,010
3	1987-88	86,080
4	1988-89	93,250
5	1989-90	96,910
6	1990-91	1,03,320
7	1991-92	1,22,030
8	1992-93	1,25,000
9	1993-94	1,42,390
10	1994-95	1,45,440
11	1995-96	1,50,070
12	1996-97	1,58,520
13	1997-98	1,59,040
14	1998-99	1,51,560
15	1999-00	1,65,890
16	2000-01	1,56,080
17	2001-02	1,57,580
18	2002-03	1,63,330
19	2003-04	1,92,310
20	2004-05	2,07,910
21	2005-06	2,19,880
22	2006-07	2,36,480
23	2007-08	2,41,440
24	2008-09	3,21,770
25	2009-10	2,93,130
26	2010-11	3,41,095
27	2011-12	3,65,651
28	2012-13	3,49,202
29	2013-14	4,14,484
30	2014-15	3,93,487
31	2015-16	3,54,350
32	2016-17	3,57,439
33	2017-18	4,44,207
34	2018-19	4,81,974
35	2019-20	5,21,865
36	2020-21	5,40,690

2) Make a simple line diagram using year-wise fish production in India by MS Excel and comment on it.

Table 3.1: State wise Fish Consumption Data (Per Capita/Kg): 2020-21		
S. N.	States/UT	Yearly Fish Consumption (Per Capita/Kg.) 2020-21
1	Andhra Pradesh	8.91
2	Arunachal Pradesh	3.65
3	Assam	11.89
4	Bihar	9.6
5	Chhattisgarh	19.7
6	Goa	78
7	Gujarat	7.44
8	Haryana	0.3
9	Himachal Pradesh	2.22
10	Jharkhand	10.61
11	Karnataka	10.55
12	Kerala	17.93
13	Madhya Pradesh	4.87
14	Maharashtra	4.72
15	Manipur	18.25
16	Meghalaya	9.00
17	Mizoram	3.38
18	Nagaland	6.06
19	Odisha	16.34
20	Punjab	0.4
21	Rajasthan	0.01
22	Sikkim	1.16
23	Tamil Nadu	9.3
24	Telangana	8.7
25	Tripura	25.53
26	Uttarakhand	0.81
27	Uttar Pradesh	11.09
28	West Bengal	NR
29	A and N Islands	77.84
30	Chandigarh	NR
31	Daman and Diu, D & Nagar Haveli	1.2
32	Delhi	0.25
33	Jammu & Kashmir	6.00
34	Ladakh	0.05
35	Lakshadweep	125
36	Puducherry	18.88
ALL INDIA		6.31
NR: Not Received		
Source: Department of Fisheries, State Govt. / UT Administration		

3) Make a pie diagram on state wise fish consumption data by MS Excel and comment on it.

Table.5.1: Trend of Export of Fish and Fish Products (2010-11 to 2021-22)							
Year	Quantity (Tonnes)	Value (in ₹ Crore)	US Dollar (Million)	Unit value (Rs. /Tonnes)	Unit value Index*	Annual growth rate (%)	
						Quantity	Value
2010-11	813091	12901.47	2856.92	158671.89	12	19.85	28.39
2011-12	862021	16597.23	3508.45	192538.46	11	6.02	28.65
2012-13	928215	18856.26	3511.67	203145.42	10	7.68	13.61
2013-14	983756	30213.26	5007.70	307121.60	9	5.98	60.23
2014-15	1051243	33441.61	5511.12	318114.75	8	6.86	10.69
2015-16	945892	30420.83	4687.94	321609.99	7	-10.02	-9.03
2016-17	1134948	37870.90	5777.61	333679.62	6	19.99	24.49
2017-18	1377244	45106.89	7081.55	327515.69	5	21.35	19.11
2018-19	1392559	46589.37	6728.50	334559.46	4	1.11	3.29
2019-20	1289651	46662.85	6678.69	361825.42	3	-7.39	0.16
2020-21	1149510	43720.98	5956.93	380344.49	2	-10.87	-6.30
2021-22	1369264	57586.48	7759.58	420570.00	1	19.12	31.71

Source: Marine Products Export Development Authority(MPEDA), Kochi
* Descending order of unit value

4) Make a line diagram trend of
   a) export on fish and fish product in quantity
   b) export on fish and fish product in amount (Rupees)

FSC-396

SPECIAL PAPER PRACTICAL (Aquaculture)

(Core course - 4 credits: 50 Marks)

[University Examination - 50]

1. Identification of common aquarium fishes (Indigenous and exotic).
2. Different aquarium plants and decorative materials identification.
3. Identification of weed fishes and predatory fishes.
4. Construction of home aquarium and decoration and management.
5. Culture of live fish food organisms.
6. Identification of eggs/fry/life stages of important finfish & shellfish.
7. Methods of chromosome preparation and other cytogenetic techniques.
8. Identification of stages of fish oocytes/sperm cells
9. Study of hormonal manipulation in fish.
10. Study of different transgenic and hybrid fish.
11. Study of hybridization in fish
12. Identification of common fish feed ingredients.
13. Proximate analysis of feed ingredients.
14. Feed formulation in the laboratory.
15. Study the carotenoids in fish and fish feed for colour retention.
16. Estimation of the energy from fish feed ingredients and prepared feed.
17. Analysis of the growth, FCR, PER
18. Design and layout of different aquaculture system through computer.
19. Analysis of the aquaculture project: site, water supply, soil type, topography, drainage system, computations for water requirement, seepage and evaporation. Types of ponds and their designs.
20. Identification and working of various equipments used in aquafarm and hatchery.
21. Field visit.

LAB-1: Construction of Aquarium

Construction of an All-Glass Aquarium

Introduction: Building an all-glass aquarium is an exciting project that allows you to create a custom-designed aquatic habitat. Glass aquariums provide excellent visibility and durability for housing fish and other aquatic organisms. This lab manual will guide you through the step-by-step process of constructing an all-glass aquarium.

Materials:

1. Glass panels (bottom, sides, front, and back)
2. Aquarium-grade silicone sealant
3. Glass cutter or glass scoring tool
4. Glass cleaner and lint-free cloth
5. Measuring tape or ruler
6. Clamps or weights
7. Plastic or wooden spacers
8. Razor blade or scraper
9. Protective gloves
10. Safety goggles
11. Epoxy or aquarium-safe adhesive (optional, for attaching background or decorations)
12. Water-resistant marker or tape (for marking glass panels)

Procedure:

1. Design and Measurements:
    a. Determine the desired size and dimensions of the aquarium.
    b. Draw a blueprint or make notes of the measurements for the glass panels.
    c. Calculate the required thickness of the glass based on the size of the aquarium.
2. Glass Cutting:
    a. Place the glass panel on a clean and flat surface.
    b. Use a glass cutter or scoring tool to score a straight line along the measured mark.
    c. Apply even pressure to the cutter, ensuring it cuts through the entire thickness of the glass.
    d. Carefully snap the glass along the scored line, creating the desired panel size.
    e. Repeat the cutting process for all the necessary glass panels.
3. Glass Cleaning:
    a. Clean all the glass panels using a glass cleaner and a lint-free cloth.
    b. Ensure the surfaces are free from any dirt, grease, or residue that may affect adhesion.

4. Assembly:

    a. Begin by applying a thin, continuous bead of aquarium-grade silicone sealant along one edge of the bottom glass panel.
    b. Carefully position one side panel against the silicone bead, ensuring it aligns perfectly with the corner of the bottom panel.
    c. Apply gentle pressure to bond the two panels together.
    d. Repeat the process with the other side panel, front panel, and back panel, in the same manner.
    e. Use clamps or weights to hold the panels in place while the silicone cures.
    f. Add plastic or wooden spacers between the panels to ensure even spacing.
    g. Allow the silicone to cure for the recommended time, as per the manufacturer's instructions.

5. Silicone Reinforcement (optional):

    a. Apply an additional bead of silicone along the inner corners of the aquarium for added reinforcement.
    b. Smooth the silicone using a finger or a silicone smoothing tool.
    c. Remove any excess silicone using a razor blade or scraper.

6. Background and Decorations (optional): a. If desired, attach a background or decorations to the back panel using an aquarium-safe adhesive or epoxy. b. Follow the adhesive manufacturer's instructions for proper application and drying time.

7. Testing:

    a. Fill the aquarium with water outside or in an appropriate location.
    b. Check for any leaks or seepage. If leaks are found, drain the aquarium and reapply silicone to the affected area.

8. Clean-up:

    a. Dispose of any unused or excess silicone properly.
    b. Clean and wipe down the aquarium to remove any dust or debris.

Conclusion: Constructing an all-glass aquarium requires careful planning, precision, and attention to detail. By following this lab manual, you can successfully build a custom-designed aquarium using glass panels and silicone sealant. Ensure you follow safety precautions and allow sufficient time for the silicone to cure before adding water and aquatic life to the aquarium. Enjoy the process and the creation of your own unique aquatic habitat.

LAB-2: Daphnia Culture Process

Introduction: Daphnia, commonly known as water fleas, are small aquatic crustaceans widely used in ecological and toxicological studies. Culturing Daphnia in the laboratory allows researchers to maintain a steady supply of these organisms for experimentation. This lab manual will guide you through the process of establishing and maintaining a Daphnia culture.

Materials:

1. Daphnia magna or Daphnia pulex culture (starter culture)
2. Freshwater source (dechlorinated tap water or aged aquarium water)
3. Culturing vessels (aquariums, containers, or culture flasks)
4. Algae culture (Chlorella or other suitable freshwater algae)
5. Fish food or yeast (as supplemental food)
6. Fine mesh net or sieve
7. Microscope (optional, for observation and counting)
8. Culture maintenance tools (pipettes, droppers, and containers)
9. Water test kit (optional, for monitoring water parameters)
10. Lighting source (natural or artificial)
11. Temperature control (heater or room temperature regulation)
12. Timer or light control system

Procedure:

1. Setting up the Culture:
    a. Prepare the culturing vessels by thoroughly cleaning them with freshwater. Avoid using detergents or cleaning agents that may be harmful to Daphnia.
    b. Fill the culturing vessel with dechlorinated tap water or aged aquarium water, leaving enough headspace to prevent overflow during feeding and movement.
    c. Add a small amount of the starter culture (Daphnia magna or Daphnia pulex) to the culturing vessel. The number of individuals depends on the desired culture density.
    d. Allow the Daphnia to acclimate to the new environment for a few hours.
2. Feeding and Nutrition:
    a. Introduce an appropriate amount of freshwater algae culture (Chlorella or other suitable freshwater algae) into the culturing vessel. Start with a small quantity and adjust according to the growth and consumption rate of the Daphnia.
    b. Supplement the diet with fish food or yeast as needed. These can be provided in small amounts, considering the size of the Daphnia population.
    c. Observe the Daphnia to ensure they are actively feeding and exhibiting healthy behaviour.
3. Maintenance and Water Changes:

a. Regularly monitor the water quality parameters such as temperature, pH, and ammonia levels (using a water test kit, if available) to ensure optimal conditions for Daphnia growth.
b. Maintain a suitable temperature range (around 20-25°C) for Daphnia culture. Use a heater or regulate the room temperature accordingly.
c. Perform partial water changes at regular intervals (e.g., every 1-2 weeks) to maintain water quality. Replace a portion of the culture water with fresh dechlorinated water.
d. During water changes, use a fine mesh net or sieve to separate the Daphnia from the culture water and transfer them to a clean container before adding fresh water.
e. Avoid disturbing the sediment or bottom debris to prevent unnecessary stress to the Daphnia.

4. Monitoring and Observation:

a. Use a microscope to observe the Daphnia periodically. Look for signs of healthy individuals, including active swimming, feeding, and reproduction.
b. Count the number of Daphnia periodically to monitor population growth and density. Adjust the feeding regime and culture size accordingly to maintain the desired population level.

5. Culture Expansion and Subculturing:

a. As the Daphnia population grows, consider expanding the culture into additional culturing vessels to maintain a sustainable population.
b. To subculture, carefully transfer a portion of the Daphnia population from the existing culture to a new culturing vessel, ensuring to include sufficient water and food.
c. Repeat the feeding and maintenance procedures for the new culturing vessel.

6. Troubleshooting:

a. Monitor the culture for any signs of stress, disease, or contamination. If issues arise, consult with an experienced researcher or aquarist for guidance on troubleshooting and corrective measures.

Conclusion: Establishing and maintaining a Daphnia culture provides a valuable resource for ecological and toxicological studies. By following this lab manual, you can create and manage a successful Daphnia culture, allowing for continuous observation and experimentation. Regular monitoring, proper feeding, and maintenance of water quality will contribute to a healthy and sustainable Daphnia population.

LAB-3: Infusoria Culture Process

Introduction: Infusoria are microscopic organisms commonly found in freshwater environments. These organisms serve as a valuable food source for small aquatic organisms, such as fry and larvae of fish and invertebrates. Culturing infusoria in the laboratory provides a continuous and readily available food supply for raising these delicate organisms. This lab manual will guide you through the process of establishing and maintaining an infusoria culture.

Materials:

1. Freshwater source (dechlorinated tap water or aged aquarium water)
2. Culturing vessel (glass jar, container, or culture flask)
3. Plant matter (lettuce, spinach, or other leafy greens)
4. Infusoria starter culture (aquarium water or purchased culture)
5. Microscope
6. Pipettes or droppers
7. Culturing maintenance tools (sieve or filter cloth)
8. Lighting source (natural or artificial)
9. Temperature control (room temperature regulation)

Procedure:

1. Setting up the Culture:

    a. Prepare the culturing vessel by cleaning it thoroughly with freshwater. Avoid using detergents or cleaning agents that may be harmful to infusoria.
    b. Fill the culturing vessel with dechlorinated tap water or aged aquarium water. Leave enough headspace to prevent overflow during feeding and movement.
    c. Add a small amount of plant matter (lettuce, spinach, or other leafy greens) to the culturing vessel. This will serve as a food source and provide nutrients for the infusoria.
    d. Introduce a small amount of the infusoria starter culture (aquarium water or purchased culture) to the culturing vessel. The concentration of the starter culture depends on the desired infusoria density.

2. Maintenance and Feeding:

    a. Place the culturing vessel in a well-lit area, either under natural light or using artificial lighting. Avoid direct sunlight as it may cause temperature fluctuations and algal overgrowth.
    b. Maintain the culture at room temperature (around 20-25°C). Use a temperature control system or regulate the room temperature accordingly.
    c. Allow the culture to sit undisturbed for a few days, allowing the infusoria to reproduce and multiply.

d. After a few days, observe the culture under a microscope. If the infusoria population is visible, proceed to the next step. If not, allow more time for the culture to develop.
e. Feed the infusoria by adding small amounts of plant matter to the culturing vessel every 1-2 days. Remove any decomposed or moldy plant matter to prevent contamination.

3. Harvesting the Infusoria:

a. After a few days of culturing, the infusoria population should be visible under a microscope. Observe the water for a cloudy or greenish tint, indicating the presence of infusoria.
b. Use a pipette or dropper to collect a small sample of the culture water, ensuring to include some of the infusoria.
c. Transfer the sample to a separate container or feeding dish, ready for feeding to the desired organisms.
d. Repeat the process as needed, ensuring to maintain the culture by adding fresh water and plant matter.

4. Culture Maintenance and Expansion:

a. Monitor the culture regularly for water quality, including clarity and color. If the water becomes cloudy or foul-smelling, it may indicate overgrowth or contamination. In such cases, consider starting a new culture.
b. As the infusoria culture matures, you can expand the culture by transferring a portion of the culture to a new culturing vessel, along with fresh water and plant matter. This will ensure a continuous supply of infusoria.

Conclusion: Culturing infusoria provides a valuable food source for small aquatic organisms in a laboratory setting. By following this lab manual, you can establish and maintain a successful infusoria culture. Regular feeding, monitoring, and maintenance will ensure a healthy and abundant population of infusoria, ready for feeding to fry and larvae of fish and invertebrates. Remember to maintain optimal water quality and observe the culture under a microscope to assess population growth and quality.

LAB-4: Tubifex Culture Process

Introduction: Tubifex worms are freshwater oligochaetes commonly used as live food for various aquatic organisms, such as fish, turtles, and amphibians. Culturing tubifex worms in the laboratory provides a continuous and convenient food source for these organisms. This lab manual will guide you through the process of establishing and maintaining a tubifex culture.

Materials:

1. Tubifex starter culture (live tubifex worms)
2. Freshwater source (dechlorinated tap water or aged aquarium water)
3. Culturing vessel (aquarium or plastic container)
4. Fine mesh net or sieve
5. Substrate (sand or fine gravel)
6. Fish food or specialized tubifex food
7. Air pump and air stone
8. Thermometer
9. Lighting source (optional)
10. Temperature control (heater or room temperature regulation)
11. Culturing maintenance tools (pipettes or droppers)

Procedure:

1. Setting up the Culture:

    a. Prepare the culturing vessel by cleaning it thoroughly with freshwater. Avoid using detergents or cleaning agents that may be harmful to the tubifex worms.
    b. Add a layer of substrate (sand or fine gravel) to the bottom of the culturing vessel. This will provide a suitable environment for the tubifex worms to burrow.
    c. Fill the culturing vessel with dechlorinated tap water or aged aquarium water, leaving enough headspace to prevent overflow during feeding and movement. d. Introduce a small portion of the tubifex starter culture (live tubifex worms) to the culturing vessel. Start with a small number of worms and adjust as needed.

2. Maintenance and Feeding:

    a. Place the culturing vessel in a location with a stable temperature, preferably around 20-25°C. Use a temperature control system or regulate the room temperature accordingly.
    b. If desired, provide a gentle water flow using an air pump and air stone to maintain oxygen levels and prevent stagnant conditions.
    c. Feed the tubifex worms with fish food or specialized tubifex food. Start with small amounts and adjust the feeding quantity based on the population and

consumption rate. d. Monitor the tubifex culture to ensure there is enough food available without excess waste accumulation.

3. Harvesting the Tubifex:

    a. After a few weeks of culturing, the tubifex population should multiply and thrive.
    b. To harvest the tubifex worms, carefully pour the culture water through a fine mesh net or sieve. This will separate the worms from the substrate and excess debris.
    c. Rinse the harvested tubifex worms with clean freshwater to remove any remaining debris or sediment.
    d. Transfer the harvested tubifex worms to a separate container or feeding dish, ready for feeding to the desired organisms.

4. Culture Maintenance and Expansion:

    a. Regularly monitor the tubifex culture for water quality, including clarity, ammonia levels, and waste accumulation. Perform partial water changes as needed to maintain optimal conditions.
    b. Periodically clean the culturing vessel, removing excess waste and debris. Replace the substrate if it becomes overly contaminated.
    c. If desired, consider expanding the culture by transferring a portion of the culture to a new culturing vessel, along with fresh water and substrate. This will ensure a continuous supply of tubifex worms.

Conclusion: Culturing tubifex worms provides a reliable and convenient live food source for various aquatic organisms. By following this lab manual, you can establish and maintain a successful tubifex culture. Regular feeding, monitoring, and maintenance of water quality and temperature will ensure a healthy and abundant population of tubifex worms. Remember to harvest the worms as needed and adjust feeding quantities based on population size and consumption rate.

LAB-5: Lab Manual for Transgenic Fish

Creating transgenic fish involves complex laboratory techniques and requires expertise in genetic engineering. While I can provide a general outline of the process, it is essential to note that working with transgenic organisms requires specialized training and access to a well-equipped laboratory facility. Additionally, local regulations and ethical considerations may apply when conducting genetic modification experiments. Here is a basic overview of the steps involved in creating transgenic fish:

1. Experimental Design:
    - Define the purpose of the experiment and select the target gene or genes to be introduced into the fish.
    - Determine the appropriate fish species for the experiment and ensure access to a suitable breeding colony.
2. Gene Isolation and Modification:
    - Identify and isolate the desired gene from a donor organism or create a synthetic gene construct.
    - Modify the gene, if necessary, to optimize its expression or incorporate specific regulatory elements.
3. Vector Construction:
    - Prepare a suitable vector to carry the gene of interest. Commonly used vectors include plasmids or viral vectors.
    - Insert the modified gene into the vector, ensuring proper orientation and integrity of the DNA sequence.
4. Introduction of the Transgene:
    - Choose a suitable method for introducing the transgene into fish embryos or early-stage fish larvae. Common techniques include microinjection or electroporation.
    - Prepare the transgene delivery system, which may involve diluting the vector DNA and combining it with delivery reagents or buffers.
5. Transgenic Fish Screening and Selection:
    - Establish methods for identifying and selecting transgenic fish from non-transgenic individuals. This may involve visual markers or molecular assays, such as PCR or DNA sequencing.
    - Rearrange the selected transgenic fish in a controlled environment, ensuring appropriate conditions for growth and development.

6. Breeding and Line Maintenance:
    - Develop a breeding strategy to establish stable transgenic lines, which involves crossing transgenic fish with non-transgenic fish of the same species.
    - Conduct genotyping to confirm the presence of the transgene in subsequent generations and establish breeding protocols for maintaining the transgenic line.
7. Phenotypic Analysis and Characterization:
    - Observe and document any phenotypic changes or traits resulting from the transgene expression.
    - Perform molecular analyses to confirm gene expression levels and assess any functional changes associated with the transgene.
8. Ethical and Regulatory Considerations:
    - Ensure compliance with local regulations and ethical guidelines governing genetic engineering experiments.
    - Prioritize animal welfare and minimize any potential adverse impacts on the environment.

Advantages of Transgenesis in Fish:

1. Enhanced Growth and Productivity: Transgenic fish can be engineered to exhibit improved growth rates and feed conversion efficiency, leading to higher productivity in aquaculture operations. This can contribute to increased food production and potentially address global food security concerns.
2. Disease Resistance: Transgenic fish can be engineered to possess enhanced resistance to specific diseases or pathogens. By introducing genes with disease-resistant traits, such as antibacterial peptides or antiviral proteins, fish farmers can reduce the impact of infectious diseases and minimize economic losses.
3. Environmental Adaptation: Transgenesis can enable the introduction of genes that enhance fish tolerance to adverse environmental conditions, such as temperature extremes or low oxygen levels. This trait can help mitigate the effects of climate change and expand aquaculture operations to new regions.
4. Research and Biomedical Applications: Transgenic fish serve as valuable research models for studying gene function, developmental processes, and human diseases. They can also be used to produce specific proteins for biomedical purposes, including the production of pharmaceuticals or bioactive compounds.

Disadvantages of Transgenesis in Fish:

1. Ethical and Welfare Concerns: Genetic modification raises ethical considerations related to animal welfare and the potential impact on natural ecosystems. The welfare of transgenic fish must be carefully monitored to ensure they do not suffer from unintended physiological or behavioral consequences.

2. Environmental Risks: Transgenic fish that escape from aquaculture facilities may pose ecological risks if they reproduce in the wild. Interbreeding between transgenic and wild fish populations could lead to genetic contamination or alter the natural genetic diversity, potentially impacting ecosystem balance.

3. Regulatory Challenges: Developing and commercializing transgenic fish may face regulatory obstacles, including safety assessments, labeling requirements, and public perception. Compliance with regulations and public acceptance are critical factors in the successful adoption of transgenic fish in aquaculture.

4. Unintended Effects and Uncertainty: Despite extensive testing, unintended genetic effects or unpredictable interactions between transgenic fish and their environment may occur. It can be challenging to fully understand the long-term consequences of introducing foreign genes into the genome, emphasizing the need for thorough risk assessment and monitoring.

5. Potential for Genetic Escape: Maintaining containment and preventing the escape of transgenic fish can be challenging. If transgenic fish enter natural ecosystems, it may be difficult or impossible to reverse their introduction, potentially leading to irreversible ecological consequences.

LAB-6: Lab Manual for Moisture Content Analysis of Fish Muscle (Infrared moisture analyser)

Introduction: Moisture content analysis is a crucial parameter in assessing the quality and freshness of fish muscle. Determining the moisture content helps in evaluating the water-holding capacity and potential spoilage of fish samples. This lab manual provides a step-by-step guide for conducting moisture content analysis of fish muscle.

Objective: To determine the moisture content in fish muscle using an infrared moisture analyser.

Materials:

1. Fresh fish sample (muscle portion)
2. Infrared moisture analyser
3. Analytical balance
4. Sample containers
5. Desiccator
6. Spatula or scoop
7. Laboratory oven
8. Heat-resistant gloves

Procedure:

1. Sample Preparation:
    a) Obtain a representative sample of fish muscle.
    b) Remove any visible skin, bones, or scales from the sample.
    c) Cut the sample into smaller pieces to ensure uniformity and faster drying.
    d) Weigh the sample accurately using an analytical balance and record the weight.
2. Calibration of the Infrared Moisture Analyser:
    a) Switch on the infrared moisture
    b) Perform the calibration process as per the instrument's user manual.
    c) Ensure that the instrument is calibrated and ready for use before proceeding with the analysis.
3. Analysis with Infrared Moisture Analyser:
    a) Preheat the moisture analyser to the recommended temperature specified by the manufacturer.
    b) Open the sample container of the moisture analyser and place it on the sample pan.
    c) Tare the sample pan to zero.
    d) Using a spatula or scoop, transfer an appropriate amount of the prepared fish muscle sample into the sample container.

e) Close the sample container and place it back on the moisture analyser.
f) Start the moisture analysis program on the instrument, following the manufacturer's instructions.
g) Wait for the analysis to complete, and record the moisture content value displayed by the moisture analyser.
h) Remove the sample container from the analyser and clean it thoroughly before the next analysis.
i) Repeat the analysis for each sample if multiple samples need to be tested.

4. Calculation of Moisture Content:

a) Calculate the moisture content using the following formula:
Moisture Content (%) = [(Wet Weight - Dry Weight) / Wet Weight] × 100
Wet Weight: Initial weight of the fish muscle sample.
Dry Weight: Weight of the fish muscle sample after drying.

5. Drying the Sample in an Oven (Optional):

a) After completing the moisture analysis with the infrared moisture analyser, transfer the sample from the sample container to a pre-weighed drying dish.
b) Spread the sample evenly in the drying dish.
c) Place the drying dish in a laboratory oven preheated to the recommended temperature (usually around 105°C) for a specified duration (e.g., 2-4 hours).
d) After the specified drying time, remove the drying dish from the oven using heat-resistant gloves and place it in a desiccator to cool.
e) Once the dish reaches room temperature, weigh it using an analytical balance to obtain the dry weight of the sample.
f) Calculate the moisture content using the formula mentioned in Step 4.

Note: The optional step of oven drying provides a secondary method for moisture content determination and can be used for verification purposes or if the infrared moisture analyzer is not available.

Safety Precautions:

1. Follow standard laboratory safety protocols, including the use of appropriate personal protective equipment (PPE) such as gloves and lab coats.

2. Handle the fish samples carefully to avoid any contamination or injury.

3. Be cautious while operating the infrared moisture analyser to prevent burns or injuries from hot surfaces.

4. Use heat-resistant gloves when handling the drying dish from the oven.

LAB-7: Hormonal Manipulation in Fish

Introduction: Hormonal manipulation in fish refers to the use of exogenous hormones to induce specific physiological responses, such as reproduction, growth, and sex reversal. This lab manual will guide you through the process of conducting a study on hormonal manipulation in fish, focusing on the induction of reproductive behavior as an example.

Materials:

1. Live fish specimens (suitable for the study, depending on the specific objectives)
2. Hormone preparation (e.g., synthetic hormones such as gonadotropin-releasing hormone analogs or other suitable hormones)
3. Injection apparatus (syringes, needles, and injection mat)
4. Measuring equipment (graduated cylinders, balances, etc.)
5. Water tanks or aquaria with appropriate filtration systems
6. Thermometer
7. Stopwatch or timer
8. Recording tools (notebook, pen)
9. Analytical equipment (microscope, spectrophotometer, etc., depending on the study objectives)

Procedure:

1. Experimental Design:
    a. Define the objectives of the study and the specific hormonal manipulation you wish to investigate.
    b. Select the appropriate fish species for the study, considering their reproductive characteristics and feasibility for manipulation.
    c. Determine the number of treatment groups and control groups based on statistical considerations.
    d. Assign fish specimens randomly to the treatment and control groups.
2. Hormone Preparation:
    a. Obtain the synthetic hormone or hormone analogue suitable for the study. Follow manufacturer guidelines or consult with experts for the appropriate hormone and dosage.
    b. Prepare the hormone solution according to the recommended concentration and volume. c. Store the hormone solution appropriately to maintain stability and integrity.

3. Hormone Administration:

    a. Anesthetize the fish using a suitable anaesthetic, following recommended procedures and ensuring the fish's safety.
    b. Prepare the injection apparatus, including sterilizing the syringe and needle.
    c. Administer the hormone solution to the treatment group fish via intraperitoneal (IP) injection or other appropriate injection methods, following aseptic techniques.
    d. Administer a placebo or vehicle solution to the control group fish using the same injection procedure. e. Record the injection details, including the dosage administered to each fish.

4. Housing and Maintenance:

    a. Transfer the injected fish specimens to individual tanks or aquaria with suitable water quality parameters and temperature.
    b. Provide appropriate feeding and maintenance protocols to ensure the fish's well-being during the experimental period.
    c. Maintain consistent environmental conditions, including photoperiod, water temperature, and quality throughout the study.

5. Observation and Data Collection:

    a. Observe the fish daily and record any behavioural changes, such as courtship behaviour, spawning, or other reproductive activities.
    b. Monitor the fish closely for any adverse effects or health issues resulting from the hormonal manipulation. c. Record the frequency and duration of observed behaviours or responses in both the treatment and control groups.
    c. Collect additional data as per the study objectives, such as reproductive success, gamete quality, hormone levels, or other relevant measurements.

6. Data Analysis:

    a. Compile the data collected during the study, including behavioural observations and other measurements.
    b. Use appropriate statistical methods to analyse the data, such as t-tests, ANOVA, or non-parametric tests, depending on the nature of the data and study design.
    c. Interpret the results and draw conclusions based on the statistical analysis.

7. Conclusion and Reporting:

    a. Summarize the findings of the study, including the effects of the hormone manipulation on reproductive behaviour or other relevant parameters.
    b. Discuss the implications of the results and their significance in the context of fish reproductive biology.

c. Document the experimental details, observations, and results in a comprehensive report or scientific paper.

Note: Ensure compliance with ethical guidelines and obtain necessary approvals before conducting any studies involving animals.

Conclusion: The study of hormonal manipulation in fish provides valuable insights into their reproductive biology and allows researchers to induce specific physiological responses. By following this lab manual, you can design and conduct a study on hormonal manipulation, focusing on reproductive behaviour as an example. Remember to adhere to ethical guidelines and seek necessary approvals before conducting any experiments involving animals.

LAB-8: Feed Formulation for Fish in the Laboratory

Introduction: Proper feed formulation is crucial for maintaining healthy and thriving fish populations in the laboratory. This lab manual will guide you through the process of formulating a balanced and nutritionally complete feed for fish in a laboratory setting, considering their specific dietary requirements and nutritional needs.

Materials:

1. Ingredients for feed formulation (fishmeal, soybean meal, wheat flour, cornmeal, fish oil, vitamins, minerals, etc.)
2. Digital scale or balance
3. Mixing containers (buckets or mixing bowls)
4. Grinder or food processor
5. Sieve or mesh screen
6. Lab notebook and pen
7. Storage containers for the formulated feed

Procedure:

1. Determine Nutritional Requirements: a. Identify the species of fish for which the feed will be formulated. b. Research and gather information on the nutritional requirements of the target fish species, including protein, lipid, carbohydrate, vitamin, and mineral needs. c. Consult published literature, scientific papers, or established guidelines for reference values.

2. Ingredient Selection: a. Select appropriate ingredients based on the nutritional requirements and availability. b. Choose high-quality protein sources, such as fishmeal or soybean meal, to meet the protein needs of the fish. c. Include energy sources, such as carbohydrates from wheat flour or cornmeal, to fulfill the fish's energy requirements. d. Incorporate essential vitamins and minerals either through natural sources or commercial vitamin and mineral premixes. e. Consider the inclusion of fish oil or other lipid sources to provide essential fatty acids.

3. Ingredient Preparation: a. Weigh each ingredient using a digital scale or balance according to the desired feed formulation. b. Grind larger particles, if necessary, using a grinder or food processor to achieve a more uniform particle size. c. Pass the ground ingredients through a sieve or mesh screen to remove any larger particles or impurities.

4. Feed Mixing: a. Combine the weighed and prepared ingredients in a mixing container (bucket or mixing bowl). b. Thoroughly mix the ingredients together until a homogenous blend is obtained. c. Ensure that the feed mixture is evenly distributed, with no clumps or segregation.

5. Feed Storage and Handling: a. Transfer the formulated feed into suitable storage containers, such as airtight bags or containers. b. Label the containers with the feed

type, date of formulation, and any additional relevant information. c. Store the feed in a cool, dry place away from direct sunlight to maintain its nutritional integrity. d. Keep the feed containers sealed when not in use to prevent moisture absorption or contamination.

6. Feeding: a. Determine the appropriate feeding rate based on the fish species, size, and feeding habits. b. Feed the fish with the formulated feed according to their feeding schedule and observed appetite. c. Monitor the fish closely for feeding response, growth, and overall health.

7. Feed Evaluation and Adjustment: a. Monitor the growth performance, feed utilization, and health status of the fish over time. b. Evaluate the effectiveness of the formulated feed by assessing parameters such as weight gain, feed conversion ratio, and survival rate. c. Adjust the feed formulation as needed based on the observed performance, making necessary modifications to meet the fish's nutritional requirements.

Conclusion: Formulating a nutritionally balanced feed for fish in the laboratory is crucial for their growth, development, and overall health. By following this lab manual, you can develop a customized feed formulation specific to the nutritional needs of the fish species under study. Regular monitoring and evaluation will ensure that the formulated feed meets the desired performance criteria, allowing for optimal fish growth and well-being in the laboratory.

LAB –11: FCR Analysis of Fish

Introduction: Feed Conversion Ratio (FCR) is an important parameter used to assess the efficiency of feed utilization in fish. It measures the amount of feed required to produce a unit of fish biomass. This lab manual will guide you through the process of conducting FCR analysis on fish, providing insights into their feeding efficiency and feed management practices.

Materials:

1. Live fish specimens
2. Fish feed
3. Weighing scale or balance
4. Measuring containers
5. Stopwatch or timer
6. Notebook and pen
7. Calculator or spreadsheet software for data analysis

Procedure:

1. Experimental Design: a. Determine the objectives of the study and the specific fish species under investigation. b. Select appropriate fish tanks or aquaria with suitable water quality parameters for the study. c. Decide on the duration of the feeding trial and the number of replicates or treatment groups.
2. Pre-Feeding Preparation: a. Acclimate the fish specimens to the experimental conditions for a period of time to minimize stress. b. Measure and record the initial weights of each fish specimen using a weighing scale or balance. c. Prepare the fish feed following the recommended feeding protocol and ensure uniformity in feed quality and composition.
3. Feeding Trial: a. Distribute the pre-weighed fish specimens into individual tanks or aquaria, allocating equal numbers of fish to each treatment group. b. Feed the fish with the prepared feed at regular intervals and predetermined feeding rates. c. Record the amount of feed provided to each tank or individual fish specimen accurately.
4. Monitoring and Data Collection: a. Monitor the fish closely during the feeding trial, observing any changes in behavior, health, or feeding response. b. Regularly measure and record the weight of each fish specimen at specified time intervals during the trial. c. Keep detailed records of the amount of feed provided to each tank or individual fish specimen.

5. Calculating Feed Conversion Ratio (FCR): a. At the end of the feeding trial, calculate the total feed consumed for each tank or individual fish specimen by summing the recorded feed amounts. b. Calculate the weight gain of each fish specimen by subtracting its initial weight from the final weight. c. Calculate the FCR for each tank or individual fish specimen using the formula: FCR = Total Feed Consumed / Total Weight Gain

6. Data Analysis and Interpretation: a. Compile the recorded data, including feed amounts, fish weights, and calculated FCR values. b. Analyze the FCR data using statistical software or spreadsheet software to calculate means, standard deviations, and perform any relevant statistical tests. c. Compare FCR values among treatment groups or individual fish specimens to assess feeding efficiency and identify any significant differences. d. Interpret the results, considering factors such as feed quality, fish health, and environmental conditions that may influence FCR values.

7. Conclusion and Reporting: a. Summarize the findings of the FCR analysis, including average FCR values and any significant differences observed. b. Discuss the implications of the results in terms of feed efficiency, feeding management, and potential improvements in the feeding practices. c. Prepare a comprehensive report or presentation outlining the study objectives, methodology, results, and conclusions.

Note: Follow ethical guidelines and obtain necessary approvals before conducting any experiments involving animals.

Conclusion: FCR analysis provides valuable insights into the feed utilization efficiency of fish, aiding in the evaluation of feed quality and feeding management practices. By following this lab manual, you can conduct an FCR analysis on fish, allowing for a better understanding of their feeding efficiency and potential improvements in feed utilization in the aquaculture industry. Regular monitoring, accurate data collection, and sound data analysis will ensure reliable results and meaningful conclusions.

LAB –12: PER Analysis of Fish

Protein Efficiency Ratio (PER) Analysis of Fish

Introduction: Protein Efficiency Ratio (PER) is a measure used to evaluate the quality and utilization of dietary protein by fish. It assesses the growth performance of fish in relation to the protein content in the diet. This lab manual will guide you through the process of conducting PER analysis on fish, providing insights into their protein utilization and nutritional requirements.

Materials:

1. Live fish specimens
2. Fish diets with varying protein contents
3. Weighing scale or balance
4. Measuring containers
5. Recording tools (notebook, pen)
6. Timer or stopwatch
7. Water tanks or aquaria with suitable conditions
8. Fish nets or other appropriate tools for fish handling

Procedure:

1. Experimental Design: a. Determine the objectives of the study and the specific fish species under investigation. b. Select appropriate fish tanks or aquaria with suitable water quality parameters for the study. c. Decide on the number of treatment groups, each with a different protein content in the diet.
2. Diet Preparation: a. Prepare fish diets with varying protein contents by adjusting the proportions of protein-rich ingredients (e.g., fishmeal, soybean meal) and other components. b. Ensure that the diets are nutritionally balanced and meet the specific nutritional requirements of the fish species under study. c. Label and store the diets in appropriate containers, keeping them free from contamination and deterioration.
3. Fish Acclimation: a. Acclimate the fish specimens to the experimental conditions for a period of time to minimize stress. b. Weigh and record the initial weights of each fish specimen using a weighing scale or balance. c. Randomly distribute the fish specimens into individual tanks or aquaria, allocating equal numbers of fish to each treatment group.
4. Feeding Trial: a. Feed the fish with the prepared diets according to the feeding schedule and recommended feeding rates. b. Ensure that the diets are provided in

sufficient quantities to meet the nutritional requirements of the fish. c. Record the amount of feed provided to each tank or individual fish specimen accurately.

5. Growth Monitoring: a. Measure and record the weight of each fish specimen at regular intervals during the feeding trial. b. Ensure accurate measurements and avoid unnecessary stress to the fish during the weighing process. c. Monitor the growth performance of the fish over the designated period of the experiment.

6. PER Calculation: a. Calculate the weight gain of each fish specimen by subtracting its initial weight from the final weight. b. Determine the protein content of each diet using appropriate laboratory methods (e.g., Kjeldahl method). c. Calculate the PER for each diet by dividing the weight gain of the fish specimen by the protein content of the diet consumed.

7. Data Analysis and Interpretation: a. Compile the recorded data, including fish weights, diet protein content, and calculated PER values. b. Analyze the PER data using statistical software or spreadsheet software to calculate means, standard deviations, and perform any relevant statistical tests. c. Compare the PER values among different diets to assess protein utilization and identify any significant differences. d. Interpret the results, considering the nutritional requirements and protein utilization efficiency of the fish species under study.

8. Conclusion and Reporting: a. Summarize the findings of the PER analysis, including average PER values and any significant differences observed among diets. b. Discuss the implications of the results in terms of diet formulation, protein quality, and meeting the nutritional requirements of the fish species. c. Prepare a comprehensive report or presentation outlining the study objectives, methodology, results, and conclusions.

Note: Ensure compliance with ethical guidelines and obtain necessary approvals before conducting any experiments involving animals.

Conclusion: The PER analysis provides valuable insights into the protein utilization and nutritional requirements of fish. By following this lab manual, you can conduct a PER analysis to evaluate the growth performance of fish in relation to the protein content in their diet. Proper diet formulation and accurate data collection are crucial for obtaining reliable results and drawing meaningful conclusions about protein utilization efficiency in fish.