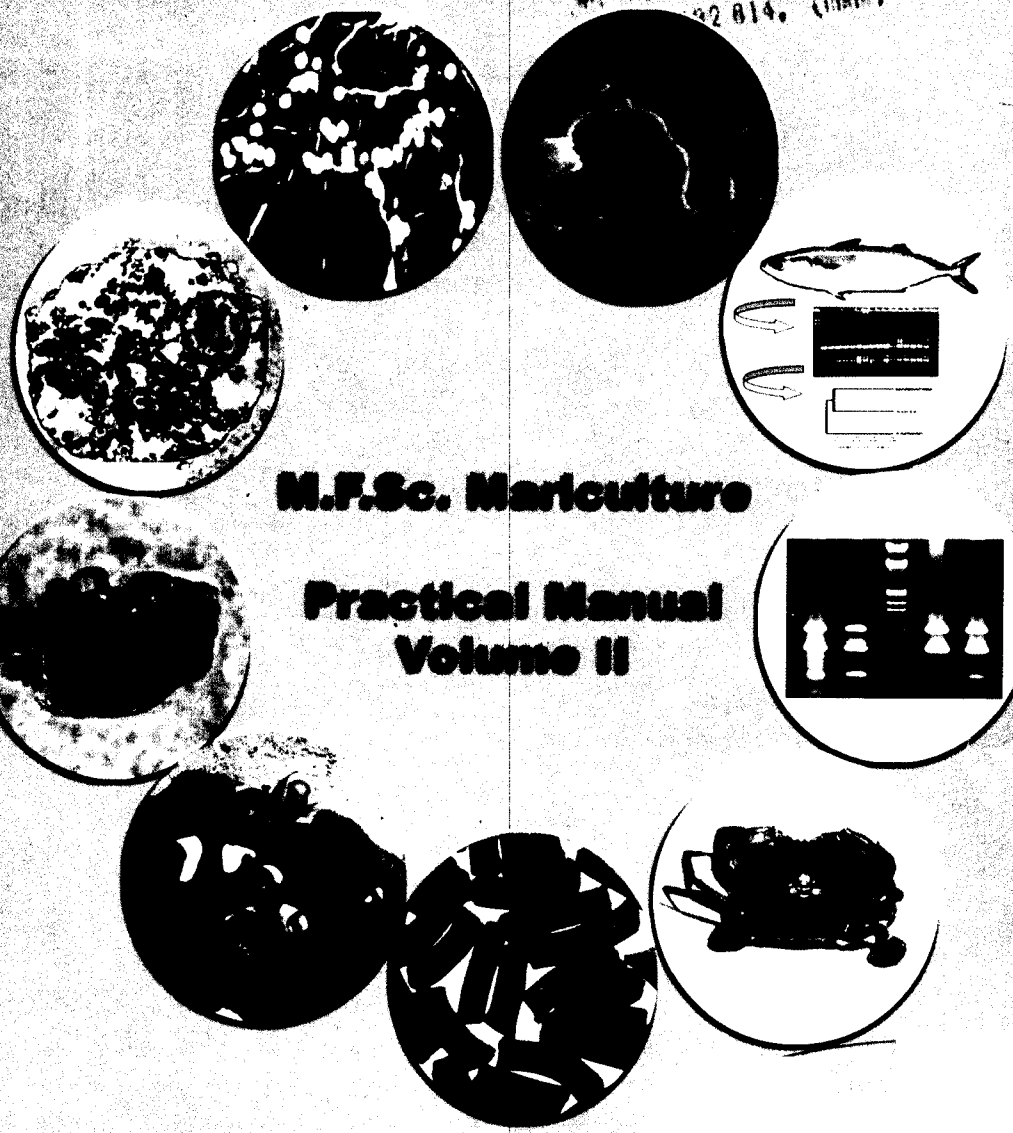
POSTGRADUATE PROGRAMME IN MARICULTURE



M.F.Sc. Mariculture

Practical Manual
Volume II

भाकृअनुप
ICAR

Central Marine Fisheries Research Institute
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INDIA

M.F.Sc - Mariculture
Fourth semester

PRACTICAL MANUAL

Course No. Mc-506 (1+1)

FINFISH AND SHELLFISH PHYSIOLOGY

Faculty

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Dissection, extraction and display of the pituitary gland from a fish

Objective: To understand the location, methodology of extraction and parts of the pituitary gland of a fish.

Principle: To understand the location and importance of the master endocrine gland.

Equipments/Reagents: Dissection board, dissection tools, fresh fish, alcohol, pipette, watch glass, microscope, magnifying glass, and sharp knife.

Procedure: The dorsal portion of the head of the fish is sliced out using the sharp knife, the cut progressing postero-anteriorly to expose the brain as instructed by the teacher. A little quantity of alcohol is squirted on the brain to harden the brain. Using a fine tipped forceps, the brain is lifted postero- anteriorly after severing the nerve behind medulla oblongata to expose the pituitary gland. The pituitary gland is then removed carefully detaching the connections with which it is attached to the brain, scooping it out of sella tursica. The gland is then placed in the watch glass with a little amount of alcohol and the different regions are studied under magnification.

Observations: The location of the gland is to be understood. The major regions of the gland are observed under high power.

Inferences:

Fig.

Practical-2 L.Krishnan

Title: Sub-sampling of ova diameter measurement of ova diameter

Objective: To learn the methodology of measurement of ova and calculate the average ova size.

Principle: Ova diameter data is extremely important in studies on maturity and spawning periodicity of fishes.

Equipments/ Reagents: Fresh fish, dissection tools, 1% formalin in salt water. Micro slides, pipette, microscope, micrometers (stage as well as ocular).

Procedure: First the method to calibrate the given microscope is explained. The given fish is dissected and the ovary removed. A portion of the ovary is teased out and immersed in a watch glass in 1% formalin in sea water. A few ova are removed using a pipette and spread along a slide. The ova are measured and data recorded from 100 ova.

Observations: The maturity stage of the ova is assessed vide the guidelines given by the teacher. The average ova diameter of the sample is calculated.

Inferences: The ova diameter data will throw light on the stage of maturity of the fish.

Charts, Tables, figs.

Practical:.3

K.K.Joshi

Title: Male Reproductive System of Fishes

Principle: The male reproductive system consists of pair of testes and seminal vesicles. The testes show variation in morphology and maturation in different groups of fishes.

Equipments/ Reagents: Fresh fish, dissection tools, Micro slides, Microscope.

Procedure: Take samples and measure total length and weight. Cut open and note down the colour, shape of the testes. Dissect and display important parts of the testes. Observe the shape and variation of testis under microscope. Draw neat-labelled sketches. For comparative study dissect different groups of fishes and study the variation in morphology and stages of maturation.

Observation: Morphology of testes with labelled sketches

Inference:

Practical 1 L.Krishnan

DISSECTION, EXTRACTION AND DISPLAY OF THE PITUITARY GLAND FROM A FISH

Objective: To understand the location, methodology of extraction and parts of the pituitary gland of a fish.

Principle: To understand the location and importance of the master endocrine gland.

Equipments/ Reagents Dissection board, dissection tools, fresh fish, alcohol, pipettes, watch glass, microscope, magnifying glass, and sharp knife.

Procedure: The dorsal portion of the head of the fish is sliced out using the sharp knife, the cut progressing postero-anteriorly to expose the brain as instructed by the teacher. A little quantity of alcohol is squirted on the brain to harden the brain. Using a fine tipped forceps, the brain is lifted postero- anteriorly after severing the nerve behind medulla oblongata to expose the pituitary gland. The pituitary gland is then removed carefully detaching the connections with which it is attached to the brain, scooping it out of sella tursica. The gland is then placed in the watch glass with a little amount of alcohol and the different regions are studied under magnification.

Observations: The location of the gland is to be understood. The major regions of the gland are observed under high power.

Inferences:

Fig.

SUB-SAMPLING OF OVA DIAMETER MEASUREMENT OF OVA DIAMETER

Objective: To learn the methodology of measurement of ova and calculate the average ova size.

Principle: Ova diameter data is extremely important in studies on maturity and spawning periodicity of fishes.

Equipments/ Reagents: Fresh fish, dissection tools, 1% formalin in salt water. Micro slides, pipette, microscope, micrometers (stage as well as ocular).

Procedure: First the method to calibrate the given microscope is explained. The given fish is dissected and the ovary removed. A portion of the ovary is teased out and immersed in a watch glass in 1% formalin in sea water. A few ova are removed using a pipette and spread along a slide. The ova are measured and data recorded from 100 ova.

Observations: The maturity stage of the ova is assessed vide the guidelines given by the teacher. The average ova diameter of the sample is calculated.

Inferences: The ova diameter data will throw light on the stage of maturity of the fish.

Charts, Tables, figs.

Practical:..3 K.K.Joshi

MALE REPRODUCTIVE SYSTEM OF FISHES

Principle: The male reproductive system consists of pair of testes and seminal vesicles. The testes show variation in morphology and maturation in different groups of fishes.

Equipments/ Reagents: Fresh fish, dissection tools, Micro slides, Microscope.

Procedure: Take samples and measure total length and weight. Cut open and note down the colour, shape of the testes. Dissect and display important parts of the testes. Observe the shape and variation of testis under microscope. Draw neat-labelled sketches. For comparative study dissect different groups of fishes and study the variation in morphology and stages of maturation.

Observation: Morphology of testes with labelled sketches

Inference:

Practical.4

K. K. Joshi

FEMALE REPRODUCTIVE SYSTEM OF FISHES

Principle: The female reproductive system consists of pair of ovary, oviduct and accessory structures. The ovary shows variation in morphology and maturation in different groups of fishes.

Equipments/ Reagents: Fresh fish, dissection tools, Micro slides, Microscope.

Procedure: Take samples and measure total length and weight. Cut open and note down the colour, shape of the testes. Dissect and display important parts of the ovary. Observe the shape and variation of ova under microscope. Draw neat-labelled sketches. For comparative study dissect different groups of fishes and study the variation in morphology and stages of maturation.

Observation: Morphology of ovary with labelled sketches.

CLASSIFICATION OF MATURITY STAGES

Principle: Classification of maturity stages is a common method for the determination of the cycle of maturity of gonads. The species which have prolonged breeding season are those inn which the ovaries include several batches of eggs destine to become mature and shed periodically. The population consists of fishes of variable stages of maturity. There are five stages of maturity. They are stage I: Immature, stage II: Maturing; stage III: Mature, stage IV: Ripe, stage V spent.

Equipments/ Reagents: Fresh fish, dissection tools, Micro slides, Microscope, Measuring scale, weighing balance.

Procedure: Measure the total length and weight of the specimens. Cut open and note the sex, colour and appearance of the gonads. Study the eggs under microscope. Classify each ovary into stage I, II, III, IV and V.

Observation:

Inference

Table

Graph

Practical :6 K. K. Joshi

OVA-DIAMETER FREQUENCY

Principle: The ova diameter frequency is another method used for determining the spawning periodicities in fishes. The size of the eggs is an important criterion for classifying them into distinct batches and ova diameter studies will give more accurate spawning periods.

Equipments/ Reagents: Fresh fish, dissection tools, Micro slides, Microscope, Measuring scale, Weighing balance, 5% formalin in salt water, slides, pipette, microscope, micrometers (stage as well as ocular).

Procedure: Measure total length and weight of the specimens. Cut open each specimen and note the sex, colour and appearance of the gonads. Then remove the gonads and preserve in 5 % formalin. For measurement of ova diameter take small pieces form anterior, middle and posterior regions of the ovary. Tease the ova on a micro slide and measure under a microscope with the help of an ocular micrometer.

Results: Tabulate the results and draw ova diameter frequency distribution graph

TECHNIQUES OF INDUCED MATURATION

Objective: To get an overall idea of the technique of induced breeding, hormones used, sampling ova from live fish and usage of hormones.

Principle: The practical will provide basic information on the methodology of induced breeding of fishes.

Equipments / reagents: Live fishes, catheter 1mm internal diameter , different hormones used in breeding such as HCG, ovaprim, pimozide; syringe and needle, slides, 1% formalin in sea water, microscopes.

Procedure: The method of live ovarian biopsy will be demonstrated and the students will practice the same. The different hormones used will be shown and their action explained. Sites of hormone induction will be shown, Methods of injection will be demonstrated and the students made to practice the same.

Observations: Methodology of live ova removal and examination, and assessment of the stage of maturity of the fish vide the standard stages of maturity. Sites of hormone injection. Methodology of preparation and injection of hormones.

Inference: Understanding the base principle of induced breeding.

L. Krishnan

EXAMINATION OF HISTOLOGICAL PREPARATION FROM GONADS OF GROUPER TO STUDY SEX REVERSAL

Objective: To understand the process of sex reversal in fishes.

Principle: To understand the physiology of hermaphroditism in fishes and how to achieve sex reversal through hormone usage

Equipments: Prepared slides on gonads of groupers undergoing sex reversal. Microscopes, Immersion oil, Methyl testosterone hormone sample.

Procedure: Examination of sections of gonads of groupers (from the prepared slides) under the microscope and see the histological changes in gonads undergoing sex reversal.

Observations: Actual observation of the change of ovarian tissue to testicular tissue through different slides.

Inferences: Understand the physiology of sex change and the use of this knowledge in aquaculture.

Practical: No. 9 K.K.Joshi

GONADOSOMATIC INDEX

Principle: The relative ovary weight or the gonadosomatic index will explain the stages of maturity and spawning periodicity in fishes. The gonads undergo a regular change during the year and when this is accompanied by large changes in females and their seasonal analysis will be the indicative of peak spawning activity.

Equipments/ Reagents: Fresh fish, dissection tools, Weighing balance

Procedure: Take samples, which represent wide range of length groups of fishes. Measure the weight of samples, cut open and remove the gonads. Take weight of the ovary.

$$\text{Gonadosomatic index} = \frac{\text{Wt. of Ovary}}{\text{Wt of fish}} \times 100$$

Observations:

Table:

Graph

Practical:10 K.K.Joshi

HISTOLOGY OF TESTIS

Principle: Different stages of testis growth can be studied by histological processing and based on the structure of nucleus and cytoplasm.

Procedure:

1. Dissect the fish and remove the testis
2. Cut the tissue into small pieces
3. Fix in 10 % Neutral buffered formalin or Bouins
4. Wash the tissue in running tap water after 24 hrs
5. Dehydrate in a series of alcohols from 30% to 100%
6. Clear the tissue in chloroform or xylene
7. Transfer the transparent tissues into the molten
8. Cut the sections at 5-7/um thickness
9. Spread sections over the slide
10. Deparffinise the sections in xylene
11. Hydration of slides in series of alcohols from 100% to 30%
12. Rinse in running tap water
13. EOSINE
14. Dehydration
15. Xylene
16. Mount in DPX

DISSECTION AND IDENTIFICATION OF REPRODUCTIVE SYSTEM OF MUSSELS AND IDENTIFICATION OF MATURITY STAGES

Objective: To observe the male and female reproductive organs and cells in mussel *Perna viridis*

Principle: Visual observation and microscopic examination reveals information on sex and maturity

Equipments: *Perna viridis* specimens, dissection instruments, microscope slides and cover slips, 5% formalin, stereozoom and compound microscope.

Procedure: Open the valves of the live mussels using a scalpel. Uncover the mantle and make gross observations of the different organs in the visceral mass. Identify the reproductive organs and record its texture and colour. Take a tissue smear and observe under a microscope. Make observations of at least 10 specimens.

Observations: Record the texture and colour of the reproductive organs to identify the maturity stage. Confirm with tissue smear observations of oocytes and sperms. Observe the size and density of the oocytes and sperms and classify into maturity stages such as immature, maturing, mature and spent.

Inferences: Record the sex and maturity stages of the observed specimens. Also record the sex ratio and percentage mature in the sub-sample. Make a table of the data obtained.

DISSECTION AND IDENTIFICATION OF REPRODUCTIVE SYSTEM OF CLAMS AND OYSTERS AND IDENTIFICATION OF MATURITY STAGES

Objective: To observe the male and female reproductive organs and cells in the clam *Villorita cyprinoides/ Paphia malabarica* and oyster *Crassostrea madrasensis*

Principle: Visual observation and microscopic examination reveals information on sex and maturity

Equipments: *Villorita cyprinoides/ Paphia malabarica* and *Crassostrea madrasensis* specimens, dissection instruments, microscope slides and cover slips, 5% formalin, stereozoom and compound microscope.

Procedure: Open the valves of the live clams and oysters using a scalpel. Uncover the mantle and make gross observations of the different organs in the visceral mass. Identify the reproductive organs and record its texture and colour. Take a tissue smear and observe under a microscope. Make observations of at least 10 specimens.

Observations: Record the texture and colour of the reproductive organs to identify the maturity stage. Confirm with tissue smear observations of oocytes and sperms. Observe the size and density of the oocytes and sperms and classify into maturity stages such as immature, maturing, mature and spent.

Inferences: Record the sex and maturity stages of the observed specimens. Also record the sex ratio and percentage mature in the sub-sample. Make a table of the data obtained.

DISSECTION AND IDENTIFICATION OF REPRODUCTIVE SYSTEM OF GASTROPODS AND IDENTIFICATION OF MATURITY STAGES

Objective: To observe the male and female reproductive organs and cells in gastropod *Babylonia spirata*

Principle: Visual observation and microscopic examination reveals information on sex and maturity

Equipments: *Babylonia spirata* specimens, dissection instruments, microscope slides and cover slips, 5% formalin, stereozoom and compound microscope.

Procedure: Cut open the shell of gastropod using a shell cutter. Uncover the mantle and make gross observations of the different organs in the visceral mass. Identify the reproductive organs and record its texture and colour. Take a tissue smear and observe under a microscope. Make observations of at least 10 specimens.

Observations: Record the texture and colour of the reproductive organs to identify the maturity stage. Confirm with tissue smear observations of oocytes and sperms. Observe the size and density of the oocytes and sperms and classify into maturity stages such as immature, maturing, mature and spent.

Inferences: Record the sex and maturity stages of the observed specimens. Also record the sex ratio and percentage mature in the sub-sample. Make a table of the data obtained.

DISSECTION AND IDENTIFICATION OF REPRODUCTIVE SYSTEM OF CEPHALOPODS AND IDENTIFICATION OF MATURITY STAGES

Objective: To observe the male and female reproductive organs and cells in squid *Loligo duvauceli*

Principle: Visual observation and microscopic examination reveals information on sex and maturity

Equipments: *Loligo duvauceli* specimens, dissection instruments, microscope slides and cover slips, 5% formalin, stereozoom and compound microscope.

Procedure: Observe the secondary sexual characters and identify the sex of the animal. Cut open the mantle of the squid ventrally to reveal its visceral organs. Uncover the mantle and make gross observations of the different organs in the visceral mass. Identify the reproductive organs and record its texture and colour. Take a tissue smear and observe under a microscope. Make observations of at least 10 specimens.

Observations: Record the texture and colour of the reproductive organs to identify the maturity stage. Confirm with tissue smear observations of oocytes and sperms. Observe the size and density of the oocytes and sperms and classify into maturity stages such as immature, maturing, mature and spent.

Inferences: Record the sex and maturity stages of the observed specimens. Also record the sex ratio and percentage mature in the sub-sample. Make a table of the data obtained.

Practical No. 15

Josileen Jose

SECONDARY SEXUAL CHARACTERISTICS, REPRODUCTIVE SYSTEMS, MATURITY STAGES, FECUNDITY, EGG DEVELOPMENT AND LARVAL STAGES IN SHRIMP

Objectives:

1. To study male and female reproductive systems of shrimp
2. To study different maturity stages in shrimp.
3. To select suitable spawners
4. To estimate fecundity and measurement of egg size in different species of penaeid shrimps.
5. To study the larval stages of shrimp
6. To learn induced breeding technique in shrimp - eye- talk ablation.

Principle:

A thorough knowledge of maturity stages is essential for the selection of ready spawners from wild for seed production. A fully mature shrimp with stage IV ovary ("diamond" shape in the 1st abdominal segment clearly visible through the exoskeleton against a light source) is dark olive green in colour with well defined anterior, middle and posterior lobes and oviduct.

Fecundity varies from species to species and within the species in relation to the size of the animal. If the fecundity is known one can roughly estimate the seed production rate. Fertilized eggs are spherical with distinct perivitelline space and sinks to bottom. The size of the perivitelline space varies from species to species.

Embryonic development is rapid and the nauplius larva hatches out 10-14 hrs. after spawning, depending upon the temperature of the sea water.The nauplius is pear shaped with 3 pairs of appendages and moults every 4-6 hrs. except the last sub stage. It passes through 6 nauplius sub stages before it metamorphoses into the protozoea.

The protozoea has 3 substages. First protozoea stage (PZ-I) has a broad 'head' and a narrow 'tail' comprising

segmented thorax and unsegmented abdomen with forked end. Eyes are sessile. In protozoea-II stage eyes become stalked, rostrum develops supra-orbital spine appears and abdomen become segmented. In protozoea - III stage: uropods develop, telson demarcated from the last abdominal segment, first abdominal segments with dorsal spine. Next stage is Mysis; it looks like a miniature shrimp. Carapace covers the thorax, 3rd maxillipeds and the 5 pereopods are functional with well developed exopods, the first 3 pereiopods have rudimentary chelae, pleopods if present rudimentary without setae. The second mysis stage has pleopod buds. The third mysis stage has two segmented pleopod buds. Mysis metamorphoses to postlarva. The first post larvae (PL-1) superficially resembles the 3rd mysis except for the development of setae on the pleopods.

The unilateral eyestalk ablation technique is used for inducing the shrimps to mature in captivity. In the normal shrimp, the development of the ovary is controlled by a hormone secreted by the endocrine gland situated in the eyestalk. The optic ganglia and the related neurosecretory centers which produce an ovary inhibiting hormone are removed by eyestalk ablation process.

Equipments/ Materials:

Microscope, occulometer, cavity slides, glass beakers, glass droppers, specimens of shrimp, dissection set, dissection board, electro-cautery apparatus, live shrimps, rearing tank.

Procedure:

Classification of different maturity stages – immature, early maturing, late maturing, fully mature and spent/recovering stages – observe the secondary sexual characteristics of male and female shrimp. Measure the egg diameter- study the differences between fertilized and unfertilized eggs – larval stages of penaeid shrimp Nauplius and its six sub stages- three protozoea stages – mysis stages- post larva.Select the healthy spawner and hold the animal gently but firmly without jumping out of the hand. One of the eyes is cut by passing the red hot loop of the cautery through the middle of the eyestalk.

Cauterisation seals the cut end and prevents bleeding. Release the animal gently back to the rearing tank..

Observations: Note down important characteristics of each maturity stage egg & stages of embryonic development and larval stages. Study the changes of ovary in eye-stalked animals and note down how many days each animal takes to reach the IVth stage ovary.

Figures: Draw figures of different maturity stages, egg & stages of embryonic development and larval stages.

SECONDARY SEXUAL CHARACTERISTICS, REPRODUCTIVE SYSTEMS, MATURITY STAGES, FECUNDITY, EGG DEVELOPMENT AND LARVAL STAGES IN CRAB AND LOBSTER

Objectives:

1. To study the reproductive systems and secondary sexual in crab and lobster.
2. To estimate the fecundity in crabs.
3. To study the different larval stages of the portunid crabs- *Scylla* spp and *Portunus pelagicus*
4. To study the larval stages of lobster.

Principle: In portunid crabs after spawning eggs are carried by the females in their abdominal chamber, attached to their pleopodal endopodites. This forms a compact berry and incubation period is around 10 days in tropical waters. Newly spawned eggs are bright yellow in colour and as the incubation progress the colour changes to dull yellow to deep grey. In advanced stages of incubation twitching of the developing zoea can be seen clearly through the egg membrane. Egg hatches out as zoea and passes through different zoeal stages and metamorphose to megalopa and then to crab stage. Thorough knowledge of embryonic development and larval stages is essential for the production of crab seeds. In lobster eggs hatches out phyllosoma and passes through several phyllosomal stages, which metamorphose to puerulus and then to juvenile lobster.

Equipments/ materials: Microscope, Glass beakers, glass droppers, cavity slides, specimens of berried crabs, larval stages of the crab and lobster

Procedure: Zoeae are with a long rostral and dorsal spines and a short lateral spine on the carapace. In first zoea, eyes are sessile, abdomen is 5 segmented and a forked tail. Inner margin of each fork bears three long serrated setae. In

the second zoea eyes become stalked and inner median margin of caudal furca develops a pair of short plumose setae. In the third zoea abdomen become six segmented. In the fourth zoea abdomen develops paired pleopod buds at the ventral posterior end of the second to fifth segments. In the fifth zoea, pleopod buds are biramous, elongated and well developed. Zoeal stages are attracted towards light and they often aggregate into groups along the sides of the rearing tanks. Megalopa is quadrangular in shape and with 6 segmented abdomen with dorso ventrally flattened tail. It usually clings to a substratum and with a peculiar 'whirling' movement. Megalopa stage often restricted to the sides and bottom of the rearing tank. Megalopa transforms to crab stage which resembles an adult crab. The margins are serrated with 9 anterolateral spines and periopods are well developed.
Take the morphometric details and total weight of the berried crabs. Take the berry weight separately and weigh three sub samples of 0.1 g and count the no. of eggs in each sample and take average number and calculate total no. of eggs in each berry.

Observations: Note the important identification characters of different zoeal stages, megalopa, crab stage and phyllosoma of lobster. Study thoroughly the different stages and compare between them. Study the pleopods in male and female crabs- estimate the fecundity in crabs.

Figures: Draw the diagrams of eggs, all zoeal stages, megalopa, crab instar and phyllosoma stage.

Practical No. 17

D. Noble

SPERM STRUCTURE IN FISH

Objective: The structural integrity of spermatozoa is important for cryopreservation and fertilizing ability.

Principle: The dilution of milt with cryodiluents, freezing and subsequent thawing causes structural changes. These damages ultimately result in reduced sperm motility and fertility. Hence the study of sperm structure is useful in selection of appropriate cryoprotectants, extenders and protocol for sperm freezing programme besides estimating the fertilizing ability of milt. Since the structural details of the fish spermatozoa is not visible under ordinary microscope, electron microscopic images are used for this purpose.

Equipments/ Reagents: Incubator, Ultratome, electron microscope

Procedure:

a. Processing of milt for electron microscopy

The samples have to be fixed in 3% gluteraldehyde buffered to pH 7.3 for 2 hours at 4 C following immersion fixation. Then the supernatant has to be decanted and pellets washed thrice in 15 mts each using sodium cacodylate buffer . The sampes are centrifuged for 10 mts at 500 rpm.

Post fix the pellets in 5% osmium tetraoxide for 1 hr at 4oC and centrifuged at 5000 rpm for 10 mts. After the fixation, the pellets are transferred to 2% agar. Then the agar blocks are trimmed to 1 mm3 size. The agar embedded samples are

transferred stepwise through a concentration series of acetone i.e. 30%, 50%, 70%, 90% and 100% v/v, following standard time schedule.

Following the dehydration steps, infilteration is carried out in Spurr's medium with acetone in 3 steps.

1. Spurr's / acetone at ratio 1:3 for 1 hr
2. Spurr's / acetone at ratio 1:1 for 1 hr
3. Spurr's / acetone at ratio 3:1 for 1 hr

a. Embedding is carried out in Spurr's medium in plastic mould and kept in incubator for 12 hrs at 70 C for polymerization.

b. Section cutting and staining : The polymerized blocks are cut into ultrathin sections in LKB Ultratome, NOVA. The thin sections were double stained in uranyl acetate and lead citrate for 10 mts.

c. The stained thin sections were mounted on the grid and observed in TEM mode , Hitachi Electron Microscope.

Observations:

Nucleus, plasma membrane, mitochondria, centioles and axoneme - normal structure and morphological changes.

Inference:

MILT COLLECTION AND EVALUATION

Objective: Captive breeding of marine animals is the basis of all sustainable sea farming technologies. The availability of quality gametes throughout the year is important to ensure constant supply of seed. Collection and assessment of sperm activity and viability is important to ensure fertility.

Principle: Oozing males can be stipped manually by applying gentle pressure on the abdomen.

Procedure: a. Collection of milt : All asceptic precaution should be taken and also care should be exercised to avoid contamination with blood, urine, scales and faeces while sripping. An intramuscular injection of 'Ovaprim'@ 0.3 ml/kg body weight can be given 8 hrs prior to stripping.

b. Evaluation of milt by motility test.

A drop of milt is placed in a glass slide and its motility checked in fresh/sea water. A cover slip is carefully placed over it and observed under a compound microscope at a magnification of 10 X.

Three main type of movements could be observed.

1. Progressive or shooting movement
2. Sluggish or lethargic movement
3. Vibration *in loco*

A quick eye estimation of the approximate percentage of spermatozoa belonging to each of the above categories can be made and accordingly a motility score can be given.

Samples with a motility score of 3 and above are only fit for cryopreservation motility score above 3 is only used

Criteria	Motility Score
• 90% or above of the sperms exhibiting rapid progressive or shooting movement	5
• 75% or more exhibiting rapid progressive, 10% sluggish and the rest immotile	4
• 50% exhibiting rapid progressive, 25% sluggish and 10% vibrating in loco and the rest immotile	3
• 25% exhibiting shooting movement, 50% moving sluggishly, 10% vibrating in loco and the rest immotile	2
• Occasional sperm shooting, 10% showing sluggish movement ,50% Vibrating in loco and the rest immotile	1
• Completely immotile	0

CRYOPRESERVATION OF FISH SPERMATOZOA

Objective: Long term preservation of fish sperm can be achieved by preservation in liquid nitrogen.

Principle: Supercooling of the cells causes the metabolism to near 'O' level in temperature below -125°C and can be preserved infinitely. Since the temperature of liquid nitrogen is -196 °C and has many advantages like ease of availability and transportability Ln is the commonly used medium for preservation and storage.

Equipments/ Reagents: Liquid nitrogen in cryocan, chemicals for extender preparation, Cryoprotectant, French straw, polyvinyl chloride for sealing the straw, goblet to hold the straw in Ln.

Procedure:

a. Preparation of cryoextenders:

Most extenders used in marine fish are saline (1-10%) or sugar -10%) based solutions, mimicking seminal plasma. Seminal plasma as such is not usually suitable for freezing. The molarity of the extender should be similar to that of the seminal plasma. As motility of sperm depends on internally stored ATP which can be resynthesised only at very slow rates, once utilized, the diluent must ideally inhibit sperm motility before freezing.

Chemical composition of different extenders

Extender / Chemical compostion (mg)	CC1 (Kuroku-ra *et al*, 1984)	Rana and Mc Andrew (1989)	Chao (1975)	Marine teleost ringer solution	mixture B (Elizabeth, 1987)	Mixture C (Elizabeth, 1987)	V_2E (Scott & Baynes, 1980)
NaCl	750	650	1350	1350	600	600	750
KCl	20	300	60	60	38	38	38
$CaCl_2$	20	30	-	-	-	23	-
$NaHCO_3$	20	20	20	20	200	100	200
$NaHPO_4.H2_O$	-	-	-	-	-	41	-
$MgSO_4.7H_2O$	-	-	35	35	23	23	-
$MgCl_2$	-	-	-	-	-	-	-
Na_2HPO_4	-	-	-	-	53	-	-
Glucose	-	-	5000	-	-	-	100
Egg yolk	-	-	-	-	-	-	20
Distilled water/sea water	100	100	100	100	100	100	100
pH	7.3	7.3	7.2	6.8	7.0	7.3	7.0

b. Cryoprotectants :

Addition of some chemicals (cryoprotectants) to extenders (cryodiluent) can minimize cell damage associated with ice formation or may suppress any ice formation. Cryoprotectants must be highly water soluble in order to alter the physiocochemical properties of water during freezing. Cryoprotectants can be divided into two groups: those permeable to the cell membrane and those not. Commonly used permeating cryoprotectants are DMSO, glycerol, methanol and 1,2-propanediol. Nonpermeating cryoprotectants include sugars (sucrose, glucose) polymers (eg. dextran, polyvinyl pyrrolidone

and proteins (egg yolk, serum and skimmilk). DMSO 10% is widely used because of its faster penetratability.

c. Cryopreservation Protocol:

Dilution: The milt is generally mixed with cryodiluent in the ratio of 1:3. All solutions to be maintained at 20°C

Equilibration: Equilibration time (time required for the cryoprotectant to diffuse through the cell membrane) of 10 mts over ice to be given including the time to fill the diluted milt into 0.5 ml French straws. Fill the straws and seal with poluvinyl alcohol powder.

Expose to liquid nitrogen vapour (-120°C) for 5 mts and plunge the straws into liquid nitrogen. The cryopreserved sperm can be stored indefinitely in Ln.

Post-thaw motility assessment:

The post thaw motility assessment of cryopreserved samples can be thawed by rapidly plunging the straws into water bath at 37C for 20 seconds. After thorough wiping the sealed ends of straws can be cut to expel the thawed milt. A small drop of milt can be taken in glass slide mix with sea water (salinity 35%) and immediately observe under microscope. Post thaw motility is judged by two variables: 1. Percentage motile spermatozoa estimated on 5 point scale and 2. Duration of sperm motility taken approx. 5 seconds after mixing the thawed milt.

Observation:

M.F.Sc. (MARICULTURE)

MC- 507

FINFISH AND SHELLFISH NUTRITION

PRACTICAL MANUAL

CONTENTS

PRACTICAL No. 1&2.

PROXIMATE COMPOSITION ANALYSIS OF FEEDS AND FEED INGREDIENTS

Introduction

The chemical composition of the feed gives its potential nutritive value and hence, in the assessment of quality of a feed the proximate principles are first determined. The Weende proximate analysis system for analyzing animal feed includes the determination of moisture (dry matter) ether extract (crude fat0 crude protein, ash, crude fibre and nitrogen free extract (NFE).

Collection and labeling samples for analyses.

The method used to collect samples for chemical analysis affects the values of the parameters obtained. The aim is to take a sample of the material that represents the total and to analyze it in such a way that it represents the material, which is consumed by the animal, or to sample other material that affects the objectives of the experiment.

Sampling of bagged ingredients is done with a spear probe. The probe is inserted diagonally and as horizontally as possible, from one corner of the bag to the other. In lots of 1-10 bags, all bags are sampled. In larger lots, 10% of all bags are sampled. Materials received in bulk are sampled using a scoop, according to the size of the consignment. For smaller than 10 tonnes consignments, two samples per tonne are taken. Larger consignments, up to 100 tonnes, require one sample per tonne or one sample for every two tones depending on the size of the consignment.

Oil cakes and other coarse materials are sampled by random selection of pieces from different parts of the entire consignment.

Grinding and sub-sampling

Samples taken in the above manner should be pooled, thoroughly mixed, and ground to pass through 1 mm screen in Wiley mill (large samples may be ground through hammer mill (3/8" screen), sub sampled, reground through 4 mm screen, sub sampled and ground through 1 mm screen). Combine all the materials left in mills with the ground portion before sub sampling; take precautions to mix ground samples well before sub sampling in a waring blender. Place samples in airtight containers. If possible preserve all samples high in moisture content by freezing.

Method of submitting samples for chemical analysis.

At the time a sample is collected, a tag is attached. The person collection the sample should fill in the project number, experiment number, date taken, a brief description of the sample and the name of the person doing the sampling.

Laboratory sample numbers

At the time the sample comes to the laboratory, it is given a laboratory sample number.

Teacher Dr Imelda Joseph

3. DETERMINATION OF FREE FATTY ACIDS

Objective: To determine the amount of free fatty acids in fish oil for its quality assessment.

Principle: Hydrolytic rancidity is caused by the hydrolysis or liberation of fatty acids from triglycerides and is detected by elevated levels of the fatty acids in a fat or oil. Free fatty acid levels in oils should be less than 3%.

Apparatus and reagents

a) Ethyl alcohol
b) Phenolphthalein (1% solution in alcohol)
c) Sodium hydroxide (0.25 N)
d) Stoppered flasks, 250 ml.

Procedure

Weigh oil or fat into a stoppered flask and add 50-ml alcohol previously neutralized by adding sufficient 0.25 N sodium hydroxide to give faint pinkish colour with phenolphthalein (2 ml). Titrate with sodium hydroxide and vigorously shake until a permanent faint pink colour appears.

Observations

Calculation

Free fatty acids % (as oleic acid) =

$$\frac{\text{g. Oil or fat}}{7.05} \times \text{volume of 0.25 N NaOH used in titration}$$

Inference:

Teacher Dr Imelda Joseph

4. DETERMINATION OF TRUE PROTEIN IN FEEDS

Objective: To determine the true protein content in feedstuffs.

Principle: By following this methodology only true protein nitrogen is determined, which will give the exact protein composition of the sample.

Reagents

1. Saturated solution of potash alum
2. Stutzer reagent

Stutzer Reagent Preparation: In a wide mouth stoppered bottle dissolve 40 g of pure copper sulphate into two litres of water. Add 10 ml of glycerine and sufficient quantity of 30% sodium hydroxide solution, which will give mixture just like alkaline reaction to litmus (dissolve 30 g of NaOH in 100 ml of water) and add the whole solution to the copper sulfate and shake the bottle thoroughly. Allow the precipitate to settle and siphon the supernatant liquid, pass the precipitate through filter paper into 15 cm diameter funnel and allow draining. Collect the precipitate with a horn spatula and transfer it into a glass mortar. Rub the precipitate thoroughly with a glass pestle after adding 3 ml glycerine and a little water. Transfer the whole thing with about 600 ml of water into a wide mouth stoppered bottle shake well and filter. The process is repeated till the filtrate is free from alkali and sulphate. Finally, collect the precipitate and add 30 ml of glycerol and enough water till the mixed emulsion comes to the level of 320 ml, which has previously been marked, on the container bottle.

Procedure

Weigh 1g of sample usually taken for crude protein estimation and transfer it into 250 ml beaker, add 100 ml of boiling water, mix the content in a hot water bath in such a way that the major portion of the beaker, inserted through the opening of the surface of the plate is submerged in hot water, leave the beaker thus for half an hour during which stir vigorously with a glass rod, take the contents in the beaker from the water bath and add 5 ml of saturated solution of potash alum and 10 ml Stutzer's reagent and stir thoroughly. Allow the mixture to stand for six hours or allow cooling down, filtering and washing with cold water several times till it becomes free from sulphate. Transfer the filter paper with the precipitate into a kjeldahl's flask and estimate nitrogen content as in the earlier experiment for crude protein determination.

Observation:

Calculation

Nitrogen content of sample (%)

$$= \frac{(\text{ml. acid} \times \text{normality of standard acid}) \times 0.014 \times 100}{\text{Wt. of sample (g)}}$$

True protein content (%) = Nitrogen content x 6.25

Inference:

Teacher Dr Imelda Joseph

5. DETERMINATION OF TOTAL NON-PROTEIN NITROGEN

Objective: To estimate the non- protein nitrogen in feedstuffs.

Principle: On subtracting the true protein value from crude protein, the non- protein nitrogen in feedstuff could be determined.

Procedure

Total non-protein nitrogen in any biological material may be estimated as below:

Total NPN= Total Crude Protein Nitrogen- True Protein Nitrogen

Observation

Inference:

Teacher Dr Imelda Joseph

6. ESTIMATION OF CHITIN CONTENT IN CRUSTACEAN MEAL/ WASTE

Objective: To estimate the chitin content in crustacean meals.

Principle: In prawn shell and other crustacean wastes, the total nitrogen is comprised of true protein and chitin nitrogen. For estimation of chitin nitrogen, the protein in the sample is removed by hydrolyzing with hot dilute alkali and the nitrogen in the residual material that is chitin is estimated.

Reagents

1. Distilled water
2. Sodium hydroxide (5%)
3. Conc. Sulphuric acid
4. Digestion mixture
5. Boric acid, 2%
6. 0.05N sulphuric acid
7. Mixed indicator

Apparatus

1. Beaker 250 ml
2. Standard flask
3. Kjeldahl digestion flasks
4. Kjeldahl distillation set

Procedure

1. Transfer quantitatively 0.5 to 1.0 g of the sample (Preferable in flake form) to a 250 ml beaker
2. Add 25 ml NaOH solution and keep on a boiling water bath for 15 min.
3. Decant the supernatant through a Whatman No. 1 filter paper and discard the filtrate.
4. Repeat the treatment once again and wash the residue with hot distilled water. Transfer the residue to the filter paper and the residue along with the filter paper is digested as mentioned above using Sulfuric acid and digestion mixture.
5. Make up the digested solution to 100 ml and estimate nitrogen.
6. Calculate the chitin content by multiplying the chitin nitrogen by 15.

Observation:

Calculation

Nitrogen content of sample (%)

$$= \frac{(\text{ml. acid} \times \text{normality of standard acid}) \times 0.014 \times 100}{\text{Wt. of sample (g)}}$$

Chitin content (%) = Nitrogen content x 15

Inference:

Teacher Dr Imelda Joseph

7. ESTIMATION OF PHOSPHORUS IN FEED BY TITROMETRIC METHOD

Objective: To estimate the phosphorus content in feedstuffs.

Principle: Phosphate ions react with phosphomolybdic acid and forms yellow ammonium phosphomolybdate precipitate. The precipitate is dissolved in known volume of 0.1 N sodium hydroxide and excess of alkali is determined by back titration with 0.1 N nitric acid using phenolphthalein indicator.

Reagents

1. Conc. HCl
2. Nitric acid
3. 20 % Ammonium molybdate solution
4. 2% Nitric acid
5. 3 % Nitric acid
6. 0.1 N Potassium nitrate
7. Phenolphthalein indicator.

Preparation of reagents: 250 ml of 20 % Ammonium molybdate solution – To 50 g of ammonium molybdate in 250 ml standard flask add about 100 ml of double distilled water and 40ml of Ammonium solution. Shake well to dissolve and make up to 250 ml with distilled water.

Procedure

Weigh 5 g of powdered sample in a silica crucible. Ash it at 550°C in muffle furnace for 2 h and cool to room temperature. Digest the sample in 25 ml of conc. HCl for 10 min. Cool, dilute and filter through Whatman No.1 filter paper. The contents of the crucibles along with washing are transferred into 250 ml volumetric flasks. Make up to 250 ml with double distilled water to get the HCl extracts. Measure 25ml of HCl extract in to 250 ml clean beakers, add 10 ml of concentrated Nitric acid followed by 10 ml of 20 % ammonium molybdate. Shake thoroughly to get the yellow precipitate of ammonium phospho-molybdate. Allow standing overnight to complete the reaction. The precipitate is filtered through Whatman No.42 filter paper. Sample is washed with 2 % nitric acid followed by 3 % nitric acid. To make it acid free, keep the precipitate along with the filter paper in a beaker. The precipitate is dissolved in measured volume of 0.1 N sodium hydroxide and excess of alkali is determined by back titration with 0.1 N nitric acid using phenolphthalein indicator. Difference between sodium hydroxide and nitric acid consumed gives the actual volume of sodium hydroxide to dissolve the precipitate.

Observations:

Calculation:

% of Phosphorus in the sample =

$$\frac{\text{Volume of sodium hydroxide X 0.00135 X aliquot factor (10) X 100}}{\text{Wt. of sample}}$$

Note:

1 ml of NaOH = 0.001325 gm of ' P'

Inference:

Teacher Dr Imelda Joseph

8. DETERMINATION OF CALCIUM IN FEED

Objective: To determine calcium content in feedstuffs, which is an essential mineral.

Principle: The calcium in the sample is precipitated as calcium oxalate using ammonium oxalate in acidic medium. The precipitated calcium oxalate is filtered out, washed with ammonium hydroxide to free ammonium oxalate from the precipitated and dissolved in hot sulphuric acid and the liberated oxalic acid is estimate by permaganimetric titration.

Apparatus/ Reagents

1. Silica crucible
2. Glass rod
3. Burette
4. Pipette
5. Whatman No.42 filter paper
6. Volumetric flask
7. Conical flask.
8. Conc. HCl
9. Methyl red indicator
10. Saturated solution of ammonium oxalate
11. Standard Potassium permanganate ($KMnO_4$)
12. N/10 ammonium oxalate (1+3)

Procedure

Weigh accurately 3 – 5 g of the dry material in a silica crucible, ignite at 550° C in muffle furnace, cool and add 5 ml of conc. HCl and 50 ml of water. Heat for half an hour and filter through Whatman filter paper No.42 in to a 100 ml volumetric flask with subsequent washing with water. Make the volume up to the mark. Take 25 ml of aliquot, dilute to 30 ml with water. Add 10 ml of saturated solution of ammonium oxalate and heat for 2-3 min, cool and add 2-3 drops of methyl red indicator. Neutralize with ammonium hydroxide till the color is slightly pink (i.e. pH 5.0) keep to settle for overnight and filter through Whatman No.42 filter paper. Wash with hot distilled water, add 100 ml of 10 % H_2SO_4 and heat the solution to about 50-70°C, Titrate it against N/10 $KMnO_4$. End point will be light pink color.

Observations:

Calculation:

$$\%\text{Calcium} = \frac{\text{Vol. in ml of N/10 } KMnO_4 \text{ x aliquot used (ml) x 0.1}}{\text{Weight of sample} \qquad 250}$$

Inference:

Teacher Dr Preetha Panikker

9. DETERMINATION OF IODINE VALUE OF OIL OR FAT

Objective: To determine the degree of unsaturation present in fat or oil.

Principle: Iodine value is expressed in number of gram iodine in 100 g oil. The excess of iodine is estimated by titrating against sodium thiosulphate.

Apparatus

1. Balance
2. Iodine flask
3. Burette
4. Conical flask

Reagents:

1. Iodine mono bromide (I Br): 13.2 gm of iodine in one litre of glacial acetic acid and 3 ml of bromine is added.
2. 10 % Potassium Iodide: 10 gm of potassium iodide dissolved in 100 ml of double distilled water.
3. Standard Sodium Thiosulphate (0.1N)
4. 1% Starch indicator (1 gm of starch dissolved in 100 ml of water)
5. Carbon tetrachloride

Standardization of Sodium Thiosulphate (0.1N)

0.1N potassium dichromate solution is prepared by dissolving 0.49 gm of potassium dichromate crystals in 100 ml of distilled water. Take 10 ml of solution of potassium dichromate solution; add 5 ml of 10 % potassium iodide, half test tube of sulphuric acid (14N). This is titrated against sodium thiosulphate using starch as an indicator. The end point is blue to colourless. The titration is repeated till two concordant values are obtained. Then the normality of sodium thiosulphate is calculated by using the formula

$$N_1V_1 = N_2V_2$$

Procedure

Weigh known amount of oil in a vial then transfer the oil along with the vials to a clean dry iodine flasks containing 10 ml carbon tetrachloride, shake well and add 25 ml of iodine monobromide. One of the iodine flasks is kept as a blank. All the flasks are shaken well and kept in a dark place stirring each for 15 minutes once and continued for 1-2 hrs. Add 50 ml of distilled water and 10 ml of 10% potassium iodide to each flask. Shake well and titrate against sodium thiosulphate solution using 1 % starch as indicator. The end point is blue to color less. The carbon tetrachloride layer at the flask is usually pink due to the presence of untitrated iodine, which is brought to aqueous layer by continuos shaking and the end point of both the layer becomes colorless.

Observations:

Calculation:

$$\text{Iodine Number} = \frac{V_1 - V_2 \times N_1 \times 129.6 \times 100}{W \times 1000.}$$

Where,

V1= Volume of sodium thiosulphate used for blank titration

V2= Volume of sodium thiosulphate used for sample titration

N1= Normality of sodium thiosulphate

W= Weight of the sample in grams.

Inference:

Teacher Dr Preetha Panikker

10. ESTIMATION OF TOTAL CAROTENOIDS

Objective: To determine total carotenoids in biological materials.

Principle: The method described by Olson (1979) was adopted to estimate the total amount of carotenoids present in different tissues like skin, muscle, liver and gonads of fish and crustaceans. Both males and females in different maturity stages were used in this method to extract the carotenoids.

Apparatus

1. Screw cap glass vial
2. Spectrophotometer

Reagents

3. Sodium sulphate, anhydrous
4. Chloroform

Procedure

1 g of tissue as above was weighed quickly and placed in a 10 ml screw cap clear glass vial. To this 2.5 g of anhydrous sodium sulphate was added and this sample gently mashed with a glass rod against the side of the vial until it reasonably mixed well with sodium sulphate. 5 ml of chloroform was added and the vial was sealed and placed at 0°C over night. When the chloroform formed a clear layer of 1-2 cm height above the caked residue, optical density is read at 380 nm, 450 nm, 475 nm and 500 nm, taking 0.3 ml aliquots of chloroform diluted to a column of 3 ml with absolute ethanol.

A blank prepared in a similar manner was used for comparison. The wavelength at which maximum absorption obtained was for calculation.

Observations:

Calculation:

The total carotenoids content was calculated as µg carotenoid/ g wet wt. of tissue as follows:

$$\text{Carotenoid content} = \frac{\text{Absorption at 475 nm X 10}}{\text{0.25 X sample weight (gm)}}$$

Where,

Dilution factor = 10

Extinction co efficient= 0.25

Inference:

Teacher Dr Imelda Joseph

11. DETERMINATION OF HYDROSTABILITY OF FEEDS

Objective: To determine the ability of pelleted diets to retain in water.

Principle: The quality of aquafeed mainly depends on its water stability for more than 1h which shows its capacity to retain in water without getting disintegrated, thereby making the nutrients unavailable to the cultured organisms as well as water quality deterioration.

Apparatus & Materials

1. Bolting silk pouches (80-100µ)
2. Hot air oven
3. Balance

Procedure:

1. Bolting silk of 80-100µ-mesh size is used, which is stitched into pouches of sufficient size to contain the feed samples for which the hydrostability is to be estimated.
2. Dry matter percentage of the feed sample is determined first.
3. Seawater of required salinity is taken by adjusting with fresh water using refractometer.
4. The bolting silk pouches are marked appropriately (eg. 30 min F_1, 1 h F_1, 2h F_1, 3h F_1 and 4h F_1) *etc.* where, 30 min, 1h, 2h, 3h and 4h represent the time of submergence of pouch with the feed in the seawater above and F1 is the sample identification mark.
5. Weigh empty pouches accurately and 5 g of feed sample is weighed into each pouch. The open end of the pouch is folded up, clipped and either hung on a rod using cotton thread or kept submerged in seawater for the required duration.

   30 min marked pouch should be removed after 30 min immersion in water. Likewise others are also removed at appropriate time.
6. In order to eliminate the salt, on drying in 30 minutes the pouches are kept 25 min in seawater and 5 min in fresh water. For 1h, it is kept 50 min in seawater and 10 min in fresh water.
7. After removing from freshwater, the pouches are kept in hot air oven at 70- 80oC overnight for removal of moisture. The dry weights are then determined.

1. The as such feed weights are converted into moisture free weights by multiplying them with dry matter percentage corresponding to each feed and dry weight of feed before experiment is determined.
2. Dry feed weight remaining after experiment is determined by subtracting the empty pouch weights from the dry pouch plus feed weights.

Observations:

Calculation

Hydrostability at each duration = $\frac{\text{Final Feed (dry) remaining}}{\text{Initial Feed (dry) weight}} \times 100$

A graph is plotted with duration of immersion in hours along the x- axis and hydrostability along the y- axis. It is assumed that at t_0, 100% feed is retained.

Inference:

Table

Name of sample	Wt. of empty pouch(W)	Wt. of pouch + feed W_1	Wt of feed W_1 -W	Wt of dry feed W_1 –W x DM/ 100 = W'	Wt. of pouch + feed after expt. W_2	Wt of feed remaining W_2- W	% Hydrostability W_2-W/ W' x 100

Chart:

Teacher Dr Preetha Panikker

12. DETERMINATION OF POLYUNSATURATED FATTY ACIDS

Objective: To determine polyunsaturated fatty acids (PUFA) from samples.

Principle: On extraction with chloroform methanol, lipid (bottom layer) is separated from the sample and is collected by filtering through anhydrous sodium sulphate. After saponification of the dried extract PUFA is determined using gas chromatograph

Apparatus/ Reagents

a) Chloroform methanol mixture (2:1)
b) Tenbrock or Potter Elvehjan tissue grinder
c) Anhydrous sodium sulphate
d) Potassium hydroxide (0.5%)
e) Nitrogen gas
f) BF3 MeOH
g) Petroleum ether
h) Steam bath
i) Gas Chromatograph (GC)

Procedure

Lipid extraction

10 g tissue together with chloroform methanol mixture (2:1) ratio is added to a large Tenbrock or Potter Elvehjan tissue grinder and homogenized. In the case of dry matter such as feed, distilled water is added to make the moisture content 80% and make it into a paste. Add the chloroform methanol mixture (15 times) and mix ($1/3^{rd}$ of the total volume). Filter the solution and the filtrate is collected. Repeat two more times with rest of the chloroform methanol mixture. To the filtrate, add distilled water (20% of the total volume of the filtrate) and leave overnight. The water-soluble residue diffuses away from the solvent and occupies the top position in the separating funnel. Solvent containing lipid (bottom layer) is collected by filtering through anhydrous sodium sulphate. Evaporate to dryness and make up the volume using chloroform.

Saponification

Accurately weigh 0.2 g made up sample. Add 5 ml alcoholic potassium hydroxide (0.5%) into a round bottom flask. Reflux for five minutes in an atmosphere of nitrogen (attach condenser and heat mix on steam bath until globules dissolves; about 5 to 10 minutes). Add specified amount of BF3 MeOH (6 ml) through condenser and proceed same as above. Let the flask and apparatus cool. To recover the dry ester extract 2-3 times with petroleum ether. Wash the extract with 25 ml distilled water three times (until acid free), dry with sodium sulphate and evaporate solvent under a stream of nitrogen in steam bath. Transfer into small vial and keep in freezer. Analyse fatty acid by GC.

Observation:

Inference:

Teacher Dr Imelda Joseph

13. DETERMINATION OF AFLATOXINS IN GROUNDNUT

Objective: To determine presence of aflatoxin in feed ingredients.

Principle: Aflatoxins are a group of highly substituted coumarins, of which aflatoxin B1 and G1 are most highly hepatotoxic to animals. The toxins are produced by some strains of *Aspergillus flavus* and other species of *Aspergillus* that develop in many foodstuffs, particularly groundnuts, oilcakes and flour. On chemical extraction and TLC plating, presence of aflatoxin can be determined in feed ingredients.

Apparatus

1. Mechanical Shaker
2. Separating funnel
3. Beaker
4. Conical flask
5. Thin layer chromatography plates, 20 x 20 cm
6. Qualitative standard Aflatoxin

Reagents

1. Acetone
2. Lead acetate
3. Chloroform
4. Anhydrous Sodium sulphate

Procedure

Method I

Weigh about 25 g of sample in to a conical flask or beaker mixed with 250 ml of acetone-water mixture (85:15); shake for 30 minutes in rotary shaker; sample extract is filtered through whatman No.1 filter paper. 125 ml filtrate is mixed with 20 ml of 20% lead acetate and 50 ml distilled water is taken in a separating funnel. Add 50 ml chloroform followed by thorough shaking. Lower most layer of chloroform is extracted and is decanted through anhydrous sodium sulphate and chloroform is evaporated to dryness in boiling water bath. Residue is dissolved in a known volume of chloroform and quantification is followed.

Method II

Weigh 10 g of material into a wide mouth bottle and thoroughly mix with 10 ml of water. If high fat material is used, a prior soxhlet extraction with petroleum ether will be necessary. Add 100 ml of chloroform, stopper with a chloroform resistant cork and shake for 30 min. filter the extract through 'Celite', take 20 ml of filtrate and make up to 25 ml (solution A). take another 20 ml of filtrate and concentrate to 5 ml (solution B).

Preparation of TLC plates

Prepare thin layer plates by shaking Kieselgel G (100 g) with water (220ml) for 20 min and applying the mixture to the plates with a spreader to a depth of 508 u. Leave for 1 h, then dry at 100oC. Spot 10 and 20 ul of solution B and 5 and 10ul of solution A on to a plate together

with a qualitative standard spot in a line 2 cm from the bottom of the plate and at least 2 cm in from each side. Carry out the spot application in subdued light.

Develop the plate in diethyl ether to a height of 12 cm. Allow to dry in subdued light then redevelop the plate in chloroform methanol (95/5, v/v) to a height of 10 cm from the base line. Examine the plate in a dark room, 30 cm from the UV source. The presence of a blue fluorescent spot at Rf 0.5-0.55 indicates aflatoxin B (check that the standard spot also lies in this range). The presence of a second spot at Rf 0.45 to 0.5 indicates aflatoxin G. the toxicity level of a sample can then be classified in terms of aflatoxin B and G according to the following table:

Volume applied	Conc. of aflatoxin (ug/kg)		Toxicity level of Fluorescence observed
	No fluorescence	fluorescence	
5µl (solution A)	<1000	>1000	very high
10µl (solution A)	<500	500-1000	High
10µl (solution B)	<100	100-500	Medium
20µl (solution B)	<50	50-100	Low

Observations:

Inference:

Teacher Dr Imelda Joseph

14. DETERMINATION OF FREE GOSSYPOL IN COTTONSEED MEAL

Objective: To determine the presence of free gossypol in cottonseed meal.

Principle: Gossypol is an anti nutritional factor present in cottonseed meal, which is growth depressive at 290 ppm in feeds. Permissible level is <100 ppm.

Reagents

1. Aqueous acetone; (7:3, acetone: distilled water)
2. Aqueous acetone- aniline solution: To 700 ml acetone 300 ml distilled water, add 0.5 ml redistilled aniline. Prepare solutions daily.
3. Aqueous isopropyl alcohol solution: 8 parts isopropyl alcohol, 2 parts distilled water (v/v)
4. Aniline: Distil reagent grade aniline over a small quantity of zinc dust, discarding the first and last 10% of the distillate. Store refrigerated in a brown glass stoppered bottle. Solution is stable for several months.
5. Standard gossypol solution: (a) Dissolve 25 mg of pure gossypol in aniline free acetone and transfer to 250 ml volumetric flask using 100 ml of acetone. Add 75 ml of distilled water, dilute to volume with acetone and mix.

   (b) Take 50 ml of the solution (a), add 100 ml pure acetone, 60 ml of distilled water, mix and dilute to 250 ml with pure acetone. Solution (b) contains 0.02 mg gossypol/ ml and is stable for 24 h in darkness.s

Apparatus

1. Mechanical shaker
2. Spectrophotometer
3. Conical flasks, 250 ml
4. Volumetric flasks, 25 and 250 ml
5. Water bath

Method

Grind sample to pass 1 mm sieve taking care not to overheat. Take approximately 1 gm of the sample and add 25 ml of pure acetone. Stir for a few minutes, filter, and divide filtrate into two. To one portion add a pellet of NaOH and heat it in a water bath for a few minutes. A deep orange red colour in the tube containing NaOH indicates the presence of dianilinogossypol and procedure (2) should be used. A light yellow extract which does not change colour with NaOH indicates that cotton seed meal and procedure(1) should be used.

Procedure 1

Weigh 0.5 to 1 g of sample depending on expected gossypol contents, into a conical s flask and add glass beads. Pipette into it 50 ml aqueous acetone solution, stopper the flasks and shake for 1 h. Filter, discarding the firat few milliliters of filtrate and then pipette out duplicate aliquots into 25 ml volumetric flasks (Take aliquots from 2- 10 ml, again depending on expected gossypol content).Dilute one of the alquots to volume with aqueous iso propyl

alcohol (solution A). While, to the other aliquot (Solution B) add 2 ml re distilled aniline and heat in a boiling water bath for 30 min. together with reagent blank containing 2 ml of aniline and a volume of aqueous acetone solution equal to the sample aliquot. Remove solution B and the blank, add sufficient aqueous iso propyl alcohol to effect homogenous solution and cool to room temperature in a water bath. Dilute to the volume with aqueous isopropyl alcohol.

Read samples at 400nm. Set instrument to zero absorbance with aqueous isopropyl alcohol and determine, absorbance of solution A and reagent blank. If the reagent blank is below 0.022 absorbance proceed as below, otherwise repeat the analysis using freshly distilled aniline.Determine the absorbance of solution B , with the reagent blank set at 0 absorbance. Calculate the corrected absorbance of the sample aliquot. Corrected absorbance (absorbance solution B absorbance solution A). Determine the mg free gossypol present in the sample solution using the calibration curve.

Procedure 2

Weigh out 1 g sample into a conical flask, add 50 ml aqueous acetone and shake and filter as above. Pipette duplicate aliquots of the filtrate (from 2- 5ml depending on expected free gossypol level) into 25 ml volumetric flasks. Dilute one of the aliquots to volume (solution A) with aqueous isopropyl alcohol and leave for at least 30 minutes before reading on the spectrophotometer. Treat the other aliquot (Solution B) as in procedure I, determine absorbances of solutions A and B as before and calculate the apparent content of gossypol in both solutions A and B using the calibration curve.

Preparation of calibration curve

Pipette duplicate 1, 2, 3, 4, 5, 7, 8 and 10 ml aliquots of the 0.02 mg/ml gossypol standard into 25 ml volumetric flasks. Dilute one set (Solution A) to volume with aqueous isopropyl alcohol and determines absorbance as previously. To other set (Solution B) add 2 ml of redistilled aniline and proceed as previously. Prepare, one reagent blank, using 2 ml aniline and 10 ml of aqueous acetone, heated together with the standards. Determine absorbance as in procedure I and calculate the corrected optical density for each standard solution.

Corrected absorbance = (absorbance solution B- absorbance solution A). Plot the standard curve, plotting corrected absorbance against gossypol conc. in the 25 ml volume.

Observations:

Calculation

Calculate free gossypol percent in normal meal as:

Free gossypol percent = 5G/ WV

Where, G = Graph reading

W = Sample weight

V = Aliquot volume used.

For chemically treated meal:

Free gossypol percent = 5(B-A)/WV

Where, A = mg apparent free gossypol in sample aliquot

B = mg apparent free gossypol in sample aliquot

W = Sample weight

V = Aliquot volume used.

Inference:

Teacher Dr Imelda Joseph

15. DETERMINATION OF UREASE ACTIVITY IN SOYBEAN MEAL

Objective: To determine the urease activity in soybean meal.

Principle: Urease is an enzyme found in raw soybean, which produces toxicity through the hydrolysis of urea to ammonia.

Apparatus

1. Water bath at 40°C, capable of maintaining temperature ±1°C, with shaking device
2. Conical flask 125 ml
3. Volumetric flasks, 25 ml and
4. Spectrophotometer

Reagents

1. Dimethylaminobenzaldehyde solution (DMAB): Dissolve 16 g DMAB in 1 litre 95% ethyl alcohol and add 100 ml concentrated hydrochloric acid (solution is stable for one month.
2. Pyrophosphate buffer: Dissolve 23.3 g $Na_4P_2O_7$ 10 H_2O in approximately 980 ml distilled water. Add 3 ml of concentrated HCl and then additional HCl until the pH of the buffer is 7.7- 7.8. Dilute to 1 litre.
3. Buffered urea solution: Dissolve 0.4 g urea in 1 litre pyrophosphate buffer (Solution is stable for one week)
4. Zinc acetate solution: Dissolve 22 g zinc acetate 2H2O in distilled water add 3 ml of glacial acetic acid, and dilute to 100 ml.
5. Potassium ferrocyanide solution: Dissolve 10.6 g potassium ferrocyanide in distilled water, and dilute to 100 ml.
6. Charcoal.

Procedure

Accurately weigh 1 g of soybean meal into a conical flask and 50 ml of the buffered urea solution. Incubate in water bath for exactly 30 minutes at 40°C with shaking. Remove from water bath and quickly add 0.5 ml each of concentrated HCl, ferrocyanide solution, zinc acetate solution and 0.1 g of charcoal. Shake for 15 min and filter. If the filtrate is coloured, repeat the procedure using more charcoal. Pipette 10 ml aliquots of the filtrate and the DMAB solution into a 25 ml volumetric flask and make up to volume with distilled water. Make up also a reagent blank (10 ml DMAB made up to 25 ml with water). Prepare a standard curve by pipetting aliquots of buffered urea solution from 2 to 12 ml into 25 ml volumetric flasks, adding 10 ml of DMAB and make up to volume). Mix flasks well, stand in water bath at 25°C for 10 min and then read at 430nm. Calculate urease activity as mg/ litre urea in urea blank less mg/ litre urea in sample.

Observations:

Calculation: Absorbance at 430nm =

Mg/L urea in blank =

Mg/L urea in sample =

Urease activity in sample (mg/L)= Mg/L urea in blank - Mg/L urea in sample

Table

Inference

Teacher Dr Imelda Joseph

16. DETERMINATION OF CHROMIC OXIDE IN FEED AND FAECES

Objective: To determine chromic oxide content in feed and faeces to determine digestibility of nutrients.

Principle: The well-mixed faecal matter containing organic waste and chromic oxide marker is digested with nitric acid to remove organic matter. The resultant solution is suitable for estimation of minerals in addition to chromium. On the addition of perchloric acid the chromium oxide is oxidized to dichromate, which is estimated by adding an excess of ferrous ammonium sulphate and titrating the mixture.

Reagents

1. Hydrochloric acid
2. Nitric acid
3. Perchloric acid, 60%
4. Sulphuric acid (6N; 167 ml of analar sulphuric acid diluted to 1 litre in distilled water)
5. Potassium dichromate (0.1) standard: Dissolve 4.90 g of analar grade solid previously heated to 100oC, cooled and stored in a dessicator in one litre of 2N sulphuric acid. Check the normality.
6. Ferrous ammonium sulphate (0.1N): Dissolve about 40 g ferrous ammonium sulphate in one litre of 2N sulphuric acid. Check the normality each day before use.
7. Indicator solution: Dissolve 0.1 g of N-phenylanthranilic acid in 2 ml of 5% sodium carbonate and dilute to 100 ml with distilled water.

Procedure

1. Weigh 50 to 100 mg of feed or faeces samples containing chromic oxide into a conical flask or kjeldahl flask.
2. Add 5 ml concentrated nitric acid.
3. After 5 min., boil the contents gently in a hot plate for about half an hour in a fume cupboard (alternatively samples can be digested in kjeltec system, if available). Additional acid may be added to prevent the content becoming dry.
4. Cool and add 3 ml of 60% perchloric acid. Heat by keeping inside a fume cupboard with plastic screen until no more fumes evolve and all nitric acid has been removed.
5. Cool and wash the digest into a 100 ml volumetric flask and make up the volume by adding distilled water.
6. Mix well and after allowing a minimum of 5 minutes. Measure the OD at 350 nm in a spectrophotometer. The percent chromic oxide is read from a standard curve where 'Y' is the OD at 350nm and 'X' the chromic oxide content of the sample in mg/ 100 ml.

Observation:

Calculation:

If 't' ml of 0.1 N potassium dichromate solution is required, then the dichromate in the digest has reached with 5 't' ml of 0.1 ferrous ammonium sulphate. 1 ml of 0.1 N dichromate is equivalent to 2.53 (5-'t') mg of chromic oxide can be present in the digest.

Inference:

Table

OD of the standard

OD of the sample

17. DETERMINATION OF DIGESTIBILITY BY CHROMIC OXIDE INDICATOR METHOD

Objective: To determine the digestibility of feed ingredients as a tool in biological evaluation of feedstuffs.

Principle: Chromium oxide mixed with prepared diets and measured in the faeces provides a general comparison of the overall digestibility of a feed. When the percentage of nutrient in feed and faeces is analysed as well as the corresponding percentage of the indicator substance the digestibility percentage can be calculated by the given formula:

Observation:

Calculation

Apparent digestibility coefficient

$$= 100 - \frac{\text{\% chromic oxide in feed}}{\text{\% chromic oxide in faeces}} \times \frac{\text{\% nutrient in faeces}}{\text{\% nutrient in feed}}$$

Inference:

Teacher Dr Imelda Joseph

18. DETERMINATION OF RNA AND DNA RATIOS IN SINGLE CELL PROTEINS

Objective: To determine the nucleic acid ratio in yeast cell *saccharomyces cerevisiae*.

Principle: The growth rate in animals is directly proportional to the nucleic acid ratio. By determining the ratio, it is possible to analyze the feed quality in terms of growth rate.

Reagents

1. TE Buffer
   10 mM Tris- HCl } pH –8.0
   1 mM EDTA
2. Breaking buffer
   2% v/v Triton x 100
   1% (w/v) SDS } pH- 8.0
   100mM NaCl
   10mM Tris HCl
   1 mM EDTA
3. 1 mg/ ml RNAse A
4. 4mM ammonium acetate
5. 100% ethanol

Procedure

1. Grow a 10 ml culture of yeast in YPD overnight to stationary phase in a suitable sterile glass culture tube.
2. Centrifuge the culture for 5 min in a microfuge at 3000 rpm at room temperature. Remove the supernatant and resuspend in 0.5 ml sterile double distilled water.
3. Centrifuge at 3000 rpm for 5 min at room temperature and remove the supernatant. Disrupt the pellet by vortexing briefly.
4. Add 200 ml of breaking buffer and 200 ul of phenol chloroform isoamyl alcohol and vortex vigorously for 3 min. Add 200ul of TE and vortex briefly. Centrifuge for 5 min at room temperature and transfer the supernatant (aqueous layer) to a clean microcentrifuge tube.
5. Add 1 ml of 100% ethanol and mix by inversion. Centrifuge for 3 min at high speed at room temperature and remove the supernatant. Resuspend the pellet in 0.4 ml of TE buffer.
6. Add 30ul of 1mg/l RNAse, mix and incubate 5 min at 37oC.
7. Add 10ul of 4M-ammonium acetate and 1 ml of 100% ethanol. Mix by inversion. Microcentrifuge for 3 min at high speed, room temperature. Discard supernatant and dry pellet. Resuspend DNA in 100ul of TE buffer.

Observations:

Inference:

Teacher Dr. Preetha Panikker

19. DETERMINATION OF VITAMIN C IN FEEDS

Apparatus

a) Dessicator

b) Rrfrigerator

c) Pulverizer

Reagents

a) Metaphosphoric acid-acetic acid stabilizing extracting solution: Dissolve, with shaking, 15 g glacial HPO_3 pellets or freshly pulverized stick HPO_3 in 40 ml acetic acid and 200 ml water; dilute to Ca 500 ml and filter rapidly through fluted paper into glass stoppered bottle. HPO_3 slowly changes to H_3PO_4, but if stored in a refrigerator this solution remains satisfactory for 7-10 days.

b) Ascorbic acid standard solution: Reference ascorbic acid should be kept cool, dry, and out of sunlight.

c) Indophenol standard solution: Dissolve 50 mg 2.6 dischloroindophenol Na salt (Eastman No.3463), that has been stored in dessicator over soda-lime, in 50 ml H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously, and when dye dissolves, dilute to 200 ml with H_2O. Filter through fluted paper into amber glass-stoppered bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. Decomposition products that make end point indistinct occur in some batches of dry indophenol and also develop with time in stock solution. Add 5 ml extracting solution containing excess ascorbic acid to 15 ml dye reagent. If reduced solution is not practically colorless, discard, and prepare new stock solution. If dry dye is at fault, obtain new specimen.

Weigh accurately (0.1 mg) about 100 mg of the reference standard ascorbic acid, transfer to 100 ml glass-stoppered volumetric flask, and dilute mark with the HPO_3-HOAC reagent. Standardize indophenol solution at once as follows: Transfer three 2.0 ml aliquots of the ascorbic acid solution to each of three 50 ml Erlenmeyer flasks containing 5.0 ml of the HPO_3-HOAC reagent. Titrate rapidly with the indophenol solution from 50 ml burette until light but distinct rose-pink colour persists at least 5 sec. (Each titration should require Ca 15 ml of the indophenol solution, and titrations should check within (0.1 ml). Similarly titrate 3 blanks composed of 7.0 ml of the HPO_3-HOAC reagent plus volume H_2O equivalent to volume indophenol solution used in direct titrations. After subtracting average blanks (usually Ca 0.1 ml) from standardization titrations, calculate and express concentration of indophenol solution as mg ascorbic acid equivalent to 1.0 ml reagent. Standardize indophenol solution daily with freshly prepared standard ascorbic acid solution.

Preparation of sample and determination

Prepare a juice from sample as follows: Mix thoroughly by shaking to insure uniform sample, and filter through absorbent cotton or rapid paper. Prepare fresh juices by pressing well-pulped fruit and filtering. Express juice of citrus fruits by one of common devices used

for squeezing oranges or lemons, and filter. Add aliquots of at least 100 ml prepared juice to equal volumes of the HPO3-HOAC reagent. Mix, and filter rapidly through rapid folded paper (Eaton- Dikeman No.195, 18.5 cm, or equivalent). Titrate 10 ml aliquots, and make blank determinations for corrections of titration as described previously, using proper volumes of acid reagent and H_2O. Express ascorbic acid as mg/100 ml original juice.

Observations:

Calculation

Inference:

Teacher Mini, K.G.

20. LINEAR PROGRAMMING AND ITS APPLICATION IN FISH FEED FORMULATION

Objective: To formulate a catfish diet using limited available feedstuffs while satisfying nutrient and feed ingredient constraints.

Ingredient	Cost,	Bags/ kg	% Protein,	%Digestible energy (DE), Meal/kgCalcium,
Maize	2.15	9	1.10	0.02
Fishmeal	8.0	65	3.90	3.7
Soymeal	6.0	44	2.57	0.3
Ricebran	2.0	12	1.99	0.1
Limestone	0.4	0	0	38.0

Constraints:

1. Total weight of mix =100kg
2. Total protein, at least 30kg
3. Total digestible energy, at least 250 Meal
4. Total calcium, at least 0.5kg
5. Total calcium, not more than1.5kg
6. Amount of fishmeal, at least 8kg
7. Amount of rice bran, not more than 20kg
8. The total cost of the blend to be minimal

Principle: Optimization using linear programming. Linear programming is applied to get the solution of the problem in which objective function and the constraints appear as linear functions of the decision variables. The optimal solution is found out by using simplex algorithm.

Procedure: Write down the decision variables of the problem

Formulate the objective function to be optimized (maximized or minimized) as a linear function of the decision variables.

Formulate the other conditions of the problem such as resource limitations, market constraints, inter-relation between variables etc., as linear equations or inequalities in terms of the decision variables.

Add the 'Non-negativity' constraint from the consideration that negative values of the decision variables do not have any valid physical interpretation.

Find out the optimal solution.

Observations:

Graph: Graphical Method for the solution of the linear programming problem

Equations: Express the constraints in a series of linear equations:

Figures: Optimal solution

Inferences:

Teacher Dr Imelda Joseph

21. PREPARATION OF A COMPOUNDED FEED USING TWIN SCREW EXTRUDER

Objective: To formulate and prepare a compounded extruded diet for shrimp.

Principle: during processing in an extruder, the feed mix is subjected to high temperature and pressure in combination with shear force. This results in physical and chemical modification of the ingredients. For *eg* the chemical composition of starch can be changed by gelatinization and breakdown. This results in the mixture being made into dough like consistency, which is then forced at high pressure through the die. When the pellet leaves the die, the pressure drop will cause evaporation of trapped water, which is in liquid form, and expansion of gelatinized ingredient mixture leading to formation of air pockets. After cooling the density will be so less about 0.25 to 0.3 gm/m^3 that the pellets float or drink. Hence by adjusting the ingredient combination and cooking condition/ process variables, a floating or sinking type feed can be prepared.

Procedure:

Operating points for production of sinking pellet

a) Low rpm of about 200 rpm
b) High moisture
c) Low temperature
d) Large number of holes and higher total die cover
e) Oil content <8%.

Materials required

a) Trays
b) Pulverizer with sieve of 100 micron size
c) Weighing balance
d) Mixer
e) Measuring cylinder
f) Pressure cooker
g) Plastic mug
h) Porous cloth
i) Extruder machine tools
j) Die cleaner
k) Sieve
l) Hot air oven
m) Feed ingredients (fish meal, shrimp meal, groundnut oil cake, gingelly oil cake, wheat flour, oil *etc.*)

Feed Formula

A formula is to be developed for extruded shrimp feed with about 35% protein and 7% lipid.

Steps in Production of extruded shrimp feed

1. Procurement of raw materials: Good quality raw materials are to be obtained and stored in proper storage conditions.
2. Grinding, batching and mixing: The raw materials are to be ground into uniform particle size with pulverizer having 60 micron mesh size sieve. Label the ingredients. Weigh the ingredients as per the feed formula. Mix the ingredients in a mixer.
3. Equilibration: Add a pre- determined quantity of water drop-wise to the above blended mixture, sieve and keep packed for 15-20 minutes in a closed container (to avoid moisture loss).
4. Extrusion: Before extrusion, set the process parameters at the required levels (temperature, rpm, feeder speed). After setting the process parameters, put on the motor drive and introduce the feed mixture to feeder and collect the extruded feed at the die in a tray.
5. Cooling and drying: Reduce the temperature and moisture by cooling it for few minutes in room temperature. Then dry in a hot air oven at 40oC for 5 hours to get a final moisture of less than 10% in feed.s
6. Crumble the feed to required size depending on the species by hand and crumblers. Remove dust or fines and oversized product.
7. Packing: Pack in airtight polythene bag and label.

Observations

Inference

Teacher Dr Imelda Joseph

A. DETERMINATION OF MOISTURE AND DRY MATTER

Objective: To determine the moisture level in the feed ingredient/ feed, which is essential in bulk purchase of feed ingredients and storage. The safe limit for storage is 15% moisture and those containing more than 15% moisture should not be stored as it develops undesirable moulds.

Principle: The moisture of the sample is lost by volatilization caused by heat. The amount of material left after the removal of the moisture is the dry matter.

Apparatus

a) Oven, 105^0C

b) Covered aluminium dishes 50 mm diameter.

c) Dessicator

Procedure

a) Wash the dishes with a detergent. Dry the dishes in 105^0C oven overnight. Place in dessicator, cool, and weight. Handle dishes with meta tongs.

b) Weight by different 20.g. of sample into a weighed dish. Place it in 105^0C oven overnight. Remove dishes; put cover on top and place in dessicator and cool. Remove from dessicator and weight as quickly as possible.

Observation:

Calculation

Dry matter (%)

$$= \frac{\text{(Wt. of dish + Wt. of dried sample) – Wt. of dish}}{\text{Wt. of sample before drying}} \times 100$$

$$= \frac{\text{Wt. of dry sample}}{\text{Wt. of sample before drying}} \times 100$$

$$\text{Moisture content (\%)} = \frac{\text{(wt. of fresh sample - Wt. of dry sample}}{\text{Wt. of fresh sample}} \times 100$$

Inference:

Teacher Dr Imelda Joseph

B. DETERMINATION OF ASH

Objective: To determine the total content of mineral matter *ie.* Non combustible portion of feedstuff .

Principle: The sample is ignited at 600°C to burn off all organic material. The inorganic material, which does not volatilize at that temperature, is called ash.

Apparatus

a) Muffle furnace

b) Silica crucibles

c) Dessicator, with magnesium perchlorate desiccant.

Procedure

a) Place clean crucibles in a muffle furnace at 600°C for one hour. Transfer crucibles from furnace to a dessicator and cool to room temperature. Weigh as quickly as possible to prevent moisture absorption. Use metal tongs to move the crucibles after they are ashed or dried.

b) Weigh by difference 2.0 g of sample into tared silica crucibles. Place in a muffle furnace and hold the temperature at 600°C for 6 h.

c) Transfer the crucibles to a dessicator and cool to room temperature. When cool, weigh the crucibles as quickly as possible to prevent moisture absorption.

d) Save the ash sample if mineral determinations are to be made.

Observations:

Calculation

$$\text{Ash (\%) on partial dry or as fed basis} = \frac{\text{Wt. of ash}}{\text{Wt. of sample}} \times 100$$

$$\text{Adjusting to dry basis} = \frac{\text{as \% on as fed sample}}{\text{Dry matter \% of as fed sample}} \times 100$$

Inference:

Teacher Dr Imelda Joseph

C. DETERMINATION OF ACID INSOLUBLE ASH

Objective: To determine the sand and silica content in feed ingredient/ feed.

Principle: When the total ash is dissolved in dilute acid, all minerals except sand and silica go into solution. The solution is filtered and the residue left behind is ignited cooled and weighed to get the acid insoluble ash.

Apparatus and reagents

a) Hydrochloric acid (1-2.5 v/v)
b) Whatman filter paper, ash less (No.42)
c) Dishes, porcelain
d) Volumetric flasks
e) Dessicator
f) Funnel
g) Muffle furnace

Procedure

Use the residue obtained from the ash determination. Boil with 25 ml HCl, taking care to avoid spattering, filter through ashless filter paper, and wash with hot water until acid-free. Place filter paper and residue into a dry, tared porcelain dish and place in a muffle furnace at 600°C for 2 h or until carbon free.

Observations:

Calculation

$$\text{Acid insoluble ash (\%)} = \frac{\text{Wt. of acid-treated ash} \times 100}{\text{Wt. of sample}}$$

Inference:

Teacher Dr Imelda Joseph

D. DETERMINATION OF CRUDE FIBRE

Objective: To determine the insoluble carbohydrates and lignin as crude fibre.

Principle: A moisture free and ether extracted sample is digested first with a weak acid solution, then a weak base solution. The organic residue is collected in a filter crucible. The loss of weight on ignition is called crude fibre.

Apparatus

a) Beakers, 600 ml tall-sided
b) Round-bottom flask condenser unit
c) Buchner flasks, 1 liter
d) Buchner funnels, Hartley 3 section pattern
e) Crucibles, silica with porous base, and
f) Rubber cones to fit above.

Reagents

a) Sulphuric acid solution (0.25 N)
b) Sodium hydroxide solution (0.313 N)
c) Antifoam reagent (octyl alcohol)
d) Ethyl alcohol/ Acetone
e) Hydrochloric acid 1% v/v

Procedure

Weigh about 2 g of the dried, fat-free sample into a 600 ml beaker. Add 200 ml of hot sulphuric acid, place the beaker under the condenser, and bring to boiling within 1 min. Boil gently for exactly 30 min., using distilled water to maintain volume and to wash down particles adhering to the sides. Use antifoam reagent if necessary. Filter through Whatman No.541 paper in a Buchner funnel, using suction, and wash well with boiling water. Transfer residue back to beaker and add 200 ml hot sodium hydroxide solution. Replace under the condenser and again bring to boiling within 1 min. After boiling for exactly 30 min., filter through porous crucible and wash with boiling water; 1% hydrochloric acid and then again with boiling water. Wash twice with alcohol or acetone, dry over night at 100°C, cool, and weigh. Ashes at 500oC for 3 h, cool, and weigh. Calculate the weight of fibre by difference.

Observations:

Calculation

Crude fibre (% of fat-free DM)

$$= \frac{(\text{wt. of crucible + dried residue}) - (\text{Wt. of crucible + ash residue})}{(\text{Wt. of sample})} \times 100$$

Inference:

Teacher Dr Imelda Joseph

E. DETERMINATION OF CRUDE FAT (Soxhlet Method)

Objective: To determine the ether soluble fat materials (a combination of simple fat, fatty acids, esters, sterols, waxes, fat soluble vitamins, carotene, chlorophyll *etc.*) in feedstuff.

Principle

Ether is continuously volatilized, then condensed and allowed to pass through the sample, extracting ether soluble materials. The extract is collected in flask. When the process is completed, the ether is distilled and collected in another container and the remaining crude fat is dried and weighed.

Apparatus and reagents

a) Soxhlet extraction apparatus
b) Extraction thimbles
c) Petroleum ether (b.p. 40-60°C)

Procedure

Weigh by difference 2 to 3 g. of the dried sample (residue from dry matter determination can be used). Place the thimble inside the soxhlet apparatus. Connect a dry pre-weighed solvent flask beneath the apparatus and add the required quantity of solvent and connect to condenser. Adjust the heating rate to give a condensation rate of 2 to 3 drops and extract for 16 h. on completion, remove the thimble and reclaim ether using the apparatus. Complete the removal of ether on a boiling water bath and dry flask at 105° C for 30 min. cool in a dessicator and weigh.

Observations:

Calculation

$$\text{Crude fat (\% of dry matter)} = \frac{\text{wt. Of fat} \times 100}{\text{Wt. Of sample} \times 1}$$

Inference:

F. DETERMINATION OF CRUDE PROTEIN (Kjeldahl Method)

Objective: To determine the total nitrogen in feedstuff for estimation of crude protein.

Principle: Sample is digested in sulphuric acid using copper sulphate as catalyst and by adding potassium sulphate the boiling point is elevated converting organically bound nitrogen to ammonium sulphate which when heated with excess alkali (40% NaOH), ammonia is liberated which is distilled into known excess of standard sulphuric acid. The unreacted acid is back titrated with standard acid. From the titre value, the nitrogen content and crude protein are calculated.

Apparatus

a) Kjeldahl digestion and distillation units
b) Kjeldahl flasks (500 ml. capacity or larger), and
c) Conical flasks, 250 ml

Reagents

a) Sulphuric acid (98%), nitrogen free
b) Potassium sulphate, reagent grade
c) Mercuric oxide, reagent grade
d) Paraffin wax
e) Sodium Hydroxide, 40% solution
f) Sodium suiphide, 4% solution
g) Pumice chips,
h) Boric acid/indicator solution. Add 5 ml of indicator solution (0.1% methyl red and 9.2% bromocresol green in alcohol) to 1 litre saturated boric acid solution.
i) Hydrochloric acid standard solution (0.1N)

Procedure

Accurately weigh 1 g of sample into a digestion flask. Add 10 g potassium sulphate, 0.7g mercuric oxide and 20 ml sulphuric acid. Heat the flask gently at an inclined angle until the solution clears. Continue boiling for an additional half hour. If the frothing is excessive, a small amount of paraffin was may be added. On cooling, add about 90 ml distilled water, recool, add 25 ml sulphide solution, and mix. Add a small piece of boiling chip to prevent bumping and 80 ml of sodium hydroxide solution while tilting the flask so that two layers are formed. Connect rapidly to the condenser unit, heat, and collect distilled ammonia in 50 ml boric acid indicator solution. Collect 50 ml of distillate. On completion of distillation, remove the receiver (wash condenser tip) and titrate against standard acid solution.

Observations:

Calculation

Nitrogen content of sample (%)

$$= (\text{ml. acid} \times \text{normality of standard acid}) \times 0.014 \times 100$$
$$\text{Wt. of sample (g)}$$

Crude protein content (%) = Nitrogen content x 6.25

Inference:

Teacher Dr Imelda Joseph

G. DETERMINATION OF NITROGEN FREE EXTRACT

Objective: To determine the soluble carbohydrates in feed ingredient/ feed.

Principle: The nitrogen free extract is determined by difference of percentage moisture, crude protein, crude fat, crude fibre and crude ash from 100.

Procedure

Nitrogen Free Extract (NFE) of a feed is determined by difference after the analyses have completed for moisture crude ash, crude fibre, crude fat and crude protein.

Calculation

NFE (%) on dry basis

= 100- (% ash on dry basis + crude fibre on dry basis+ crude fat on dry basis + crude protein on dry basis)

Inference:

PRACTICAL MANUAL

COURSE NO. MC-508

(FINFISH AND SHELLFISH PATHOLOGY)

FACULTY

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(CREDITS : 2+1)

Practical 1. (2 Sessions) **K.C.George**

NECROPSY OF FISH

Principle and objectives: Necropsy is the systematic examination of dead specimen in order to record the lesions caused by the disease encountered during the life of the organism. It will give the health history of the organism. Samples are collected for histopathological studies, microbiological, toxicological and parasitological studies from properly conducted necropsies, which will yield valuable information on disease and health status.

Common features of necropsy are (a) the fish is examined from outside in, (proceeding from outer tissues to inner tissues) and (b) each tissue is observed intact *in situ* before it is dissected. **Necropsy must be completed with in the same day of sample collection, otherwise the observations are not valid**.

Materials: Live fish, dissection set including scissors, forceps, needle, knife, bone cutter, BP blades and BP blade holder. Wooden board and metal tray. Microscopes, slides watch glasses, petri dishes and magnifying glass. Formalin, rectified sprit vials of pepsin etc.

Procedure: Kill live fish without undue tissue damage by a blow on head. Take forks length and weight of fish and record it. Place the fish on a wooden board on its right side, head-pointing left and with the ventral side to the examiner.

Step 1. The skin, fins anus eye and nose. Look for lesions/ abnormal signs or parasites. The position of abnormalities should be recorded. The parasites are removed, identified and stored. Smears of mucus and pus from pustules are prepared, stained and examined microscopically. Scrapings of suspected tissue should be examined microscopically. Gross abnormalities should be excised and examined microscopically. Examine perianal region, watching for flushing of skin, prolapsed intestine and other lesions. Examine the left eye by rotating it to stretching fold of skin. Cut the skin around the orbit with the tips of sharp fine scissors and pull eye towards you with forceps. Cut the eye muscles and optic nerve, remove the eye and place it in a petri dish. Make an incision around the eyeball in the equatorial plane and remove the anterior chamber. Take out the lens and vitreous humor from the posterior chamber. Examine separately the inner contents of

eyeball. With a hand-held magnifying glass examine the nares. If the nasal openings are large enough, insert a blunt pin to test for unusual quantities of mucus. It may be necessary to remove the skin in the region of the nose, to expose the nasal cavity. Examine the olfactory tissues for protozoa. Parasites of the skin and fins can be removed from the fish by placing it in 1: 4000 formalin solution for 20 minutes and examining the container bottom. This method does not permit the recording of the exact location of each parasite on the skin. The fins can be individually examined under a dissecting microscope.

Step 2. Cut off the left operculum, exposing the gill cavity. Place the operculum inner surface up in a petri dish and examine under dissecting microscope for lesions.

Step 3. Excise the first left gill arch by cutting its ventral and dorsal ends. Remove arch to a petri dish, cover with water, and examine under a dissecting microscope by holding it with forceps and running a blunt needle along the filaments. Watch for parasites, disclourations, mucus coating or other lesions. A wet mount of entire gill or cut off portion of filaments may be prepared and squash gently to spread the filaments. Examine for lesions, protozoa, glochidia or helminth eggs. Examine individual gill arches separately. Observe the wall of the gill cavity exposed after the removal of the fourth arch.

Step 4. Turn fish on to left side, so that its dorsal side is now towards you. Repeat step 1, except for examination of the anus

Step 5. Cut away the right operculum, repeat step 2.

Step 6. Examine the right gills, repeat step 3

Step 7. Return the fish to its previous position, with its left side up. Open the moth by pushing the lower jaw with forceps and examine the surface of the buccal cavity, beginning with the roof. Take note of possible parasites or lesions behind the vomer and along the inner margin of teeth. Search around the tongue, particularly in the fold obscured under its rim, and look into the pharynx. Scrape some tissue from the roof of the mouth and examine it under the compound microscope.

Step 8. Open the visceral cavity. Using sharp-pointed scissors pierce the abdominal wall in the mid-ventral line just behind and between the pectoral fins, taking care not to stab too deeply. Cut in a posterior direction about 2 cm. Lift the upper side of the cut with the

forceps and check the position of blades, keeping them close to the abdominal wall and away from the viscera. Continue to cut along the mid- ventral line until the anal region is reached, then the scissors blades are directed towards dorsal side and continue the cut till roof of abdominal cavity is reached. Arch the scissors anteriorly along the margin of abdominal cavity. When the cut is completed, remove the left belly flap and expose the viscera. Examine them *in situ* for abnormal position, enlargement, discolouration, and parasitic cysts on their surfaces, lesions and other pathological signs. Pay attention to size, colour and consistency of liver. Abnormal fluid in the cavity should be noted and sampled for bacteria.

Step 9. Remove the entire alimentary canal and associated organs as a unit by making a transverse cut through the oesophagus close to the buccal cavity, snipping the hepatic ligament and severing the rectum at the anus. Place the entire complex in a large dish and separate its components: intestinal tract, liver and gall bladder, pancreas and spleen. Examine these organs one by one, beginning with liver and gall bladder. Separate gall bladder from the liver, weigh the liver and records its colour. Take a slice of liver tissue for histological studies. Cut the liver into thin slices, examining each surface before the next cut is made. Open gall bladder, examine its inner surface, scrape it and examine the scraping and fluid as a squash at high power under compound microscope.

Step 10. Cut the spleen in half, make impression smear. Observe size, texture, and possible lesions. Macerate tissues and search for parasites. Examine a smear under median power of a compound microscope. Reserve a part for histological studies.

Step 11. Divide the alimentary canal into sections: stomach and oesophagus, pyloric region and intestine. The intestine should be cut in several parts, depending on its length; three sections usually suffice and cutting must be careful so as not to damage the gut contents. Use fine scissors and cut with their tips, making short shallow cuts. Place the oesophagus and stomach in a petri dish with the bottom scored to divide it into squares. Incise the stomach wall longitudinally, exposing its contents. Remove the contents and wash the inner surface of the stomach. Under a dissecting microscope examine both inner and outer surfaces for lesions or parasite cysts. Remove the stomach from the dish. Place it between two glass slides. Squash until the tissues appear translucent and examine under dissecting microscope for parasite cysts. When thicker portions are examined, the inner

surfaces should be scraped carefully with a scalpel and the scraping examined as wet mount for protozoa. Stomach contents should be distributed evenly over the bottom of the dish. Examine them, square by square, identifying type of food and parasites. Repeat this procedure for entire gut recording the parasite found for each section separately. Examine the mesenteries and pancreatic tissue. *Parasite can be easily collected from intestine by sedimentation technique, which will be dealt separately.

Step 12. Identify the sex and examine the gonads superficially. Record the state of maturity. Remove and weigh the gonads; open and examine them under dissection microscope. Macerate some of the tissue, and examine under dissection microscope. Prepare and examine a wet mount.

Step 13. Examine outer wall of the swim bladder, remove the swim bladder, open it and examine the inner surface. Sample and examine any unusual contents. Incise and tear off peritoneum, exposing the kidneys.

Step 14. Note the general appearance of kidneys. Carefully dissect them away from the dorsal wall of the cavity and transfer into petri dish. Cut transversely through the head kidney and make an impression smear. Retain a tissue sample for histological study. Prepare a wet mount and examine it. In large fishes remove the urinary tract and bladder along with the kidneys. Open the bladder; examine the contents and inner surface. Examine a wet mount also.

Step 15. Open the pericardial cavity and remove the heart. Examine its surface for lesions and parasitic cysts. Open the ventricle and examine inner surface. Slice ventricular muscle, examining the cut surface. Open and examine other parts of heart.

Step 16. Open the cranium by cutting the dorsal muscles at the point of attachment to the skull and pry the skull roof away with a strong forceps or cranium can be opened with a sharp knife, making a horizontal incision beginning just above the eyes. Observe the dorsal surface of the brain. Cutting the cranial nerves, remove the brain and as much of the spinal cord as possible and examine them externally under a dissecting microscope. Retain a tissue sample for histological study. Make several wet mounts and examine under the medium power objective of a compound microscope. If brain parasites are indicated the entire brain tissue is digested in pepsin- hydrochloric acid digestion medium (2 g pepsin in 400 ml and 0.5% HCl). Parasites are recovered by centrifugation.

Step 17. The auditory capsules have been exposed by the removal of the cranial roof. If the cut proved to be too high to open them make another cut, parallel to, and slightly below, the first. The semicircular canals are likely to be damaged in this process. Larger fish, with strong bones, may have to be treated differently. Make a transverse incision in the dorsal wall of the cranium at the otic region and apply pressure to break the skull in the plane of the cut and to expose the ears. Examine the cartilage and bone of the ear and its surrounding for discolouration, inclusions or damage, particularly for whitish concretions irregularly distributed in the bone or cartilage (Possibility of myxozoan spores). Sample abnormal tissues or substances and examine as wet mounts. Examine the endolymph and fragments of ear tissue in the same way. Remove otoliths for age determination

Step 18. To examine the body musculature, remove it from the skeleton by filleting. A sharp knife with a blade about 15 cm long and a fine tip is used. Slice the muscles behind the head. Slide the blade in a posterior direction, holding it at sharp angle to the body, the tip of the blade close to the dorsum. The blade should remain in contact with the spine and ribs throughout the incision. Cutting with one hand, hold up the severed slab of muscle with the other, so that the exposed skeleton can be seen and the incision guided accordingly. When incision is complete, turn the fish over and make a similar incision on the other side. When both incision have been made, it should be possible, by holding the by head with one hand, to pull away all musculature together, leaving only the head, skeleton and one belly flap attached to the ribs. Spread the fillets, skin downward and linked together in the mid-dorsal line, and examine the cut surfaces. Slice each side transversely into very thin strips. Look for abnormalities, internal foci of necrosis and parasites. If fillets are thin, they can be examined under candling. Retain pieces of suspect tissue for histology. Examine the belly flaps. To recover nematodes from muscle, a digestion technique can be applied. Prepare a stock solution of 1-% hydrochloric acid. Add 1 g of 1:2500 pepsin powder to 100 ml of acid solution and heat to 52° C. Pieces of muscle are added, shaken stirred at this temperature until completely dissolved (about1-3 hours). The solution is then washed through a 2 mm mesh sieve and nematode recovered.

Step 19. Remove scraps of tissue adhering to the skeleton. Examine the spinal column for abnormalities, fusion of vertebrae, telescoping or twisting.

Step 20. Cut the spinal column into several parts sever the spinal nerves close to the cord. Using strong scissors or bone clippers cut the neural arches and remove sections of spinal cord. Examine as in step 16.

Note: collection of samples for bacteriological and virological studies must be done aseptically. This is difficult in field, as contamination occur from wind. Hence, it is better to take the sample to laboratory, where samples are taken from internal organs or central layers of lesion using a sterile swab or an inoculating loop. Loops are sterilized by dipping in ethanol followed by flaming. Generally cultures from kidneys are preferred, when septicaemia is suspected.

Record your necropsy observations in the format provided

Observations:

<table>
<tr><td colspan="2">Host ..... Host No.
Locality
Fork Length..... Weight ..... Sex ..... Mature (...) Immature (...)
Host condition: Fresh (...) tank held (...) Refrigerated (...) Frozen (...) Fixed (...)
Collection date ..... Collected by .....
Examination date ..... Examined by .....</td></tr>
<tr><td>Blood smear</td><td>Urinary bladder smear</td></tr>
<tr><td rowspan="2">External surface:
(1: 4000 formalin treatment (..)</td><td>Kidney:
Smear ( ):</td></tr>
<tr><td>Gall bladder:
Smear (0:</td></tr>
<tr><td rowspan="2">Fins:</td><td>Body cavity:</td></tr>
<tr><td>Mesenteries:</td></tr>
<tr><td rowspan="2">Nasal cavities:</td><td>Liver:</td></tr>
<tr><td>Smear ( ):</td></tr>
<tr><td rowspan="2">Operculum: Left
Right</td><td>Spleen:</td></tr>
<tr><td>Smear:</td></tr>
<tr><td>Mouth:</td><td>Stomach</td></tr>
<tr><td>Eyes: Left:
Right</td><td>Pyloric caeca</td></tr>
<tr><td>Brain and spinal cord:</td><td rowspan="3">Intestine: Anterior:
Posterior:</td></tr>
<tr><td>Heart and aorta:</td></tr>
<tr><td>Swim bladder:</td></tr>
<tr><td>Gonads:</td><td>Intestinal smear:</td></tr>
<tr><td colspan="2">Notes and preservation methods</td></tr>
</table>

Musculature	Right	Left

Quad I II III IV V VI		
Gills Arch I II III IV	Right	Left

Inferences: write briefly about the health status of fish you have autopsied

Practical 2. (4 SESSIONS) **K.C.George.**

HISTOPATHOLOGICAL TECHMIQUES

Objective: To impart basic knowledge in histological techniques.

Principle: The study of pathology requires through knowledge of micro-anatomical changes taking place due to the action of etiological factors. Knowledge in histopathological techniques is essential for a diagnostician

Materials and reagents: 1. Fixatives. 2. Knife, BP blades and scalpels; bottles, coupling jars, syringes with hypodermic needles etc. 3. Hot air oven, paraffin wax of 58°C melting point, cups for keeping wax, metal or plastic capsules for keeping tissues, water bath of 45° C to 60° C, and electrical dryer plate for fixing sections. 4. Rotary microtome and dispoable blades with holders. 5. Hari's haematoxylin, Mayer's haematoxylin, Eharlich's haematoxylin or any haematoxylin stain and eosin stain. 6. xylol, chloroform, cedar wood oil, ethyl alcohol/ isopropyl alcohol, DPX mountant and cover glass.

Preparation of fixatives and fixation of fish and shrimp tissues.

Prepare following fixatives. 1. Fixatives for fish-- ten- percent formalin, formol saline, buffered formalin, acetic formalin acohol fixative and Bouin's solution. 2. Fixative for crustacesns—Davidson's fixative.

Fixatives for fish tissues.

Procedures:

Ten percent formalin—add 100 ml (37-40%) formaldehyde solution to 900 ml of water and mix.

Formol saline—prepare 1000 ml of normal saline by dissolving 90 g of sodium chloride in 500 ml of distilled water and then make up the volume to 1000 ml by adding distilled water. Take 900ml of the above saline solution. Add 100ml (37-40%) formaldehyde solution to the saline and mix.

Acetic formalin alcohol fixative—10% formalin 20 ml; ethanol or propanol 40 ml; glacial acetic acid 10 ml and distilled water 30 ml.

Buffered formalin

40% formaldehyde 100 ml

Tap/ distilled water 900 ml

$NaH_2PO_4 H_2O$ 4 g

Na_2HPO_4 6 g

Bouin's fluid

Saturated aqueous picric acid 75 ml.

100% formalin (37-40%) 25 ml.

Acetic acid 5 ml.

Fixative for crustaceans.

Davidson's fixative

Ethyl alcohol 95% 330 ml.

Formalin 100% 220 ml.

Glacial acetic acid 115 ml.

Distilled water 336 ml.

Proper fixation is fundamental to satisfactory histological preparations. The objective of fixation is to preserve the morphology of tissue in a condition as near as possible to that existing during life. It is better to fix the whole animal for histological studies. When it is not possible to fix the whole animal, remove samples of tissues exhibiting gross lesions from freshly killed animals and make small pieces and suspend in 10-20 times their volume of fixative. Never use samples collected from frozen samples or animals dead for some time.

Fixation of fish tissues: Select a live fish and kill it humanely by giving blow to head or pithing. Slit open the abdomen give cuts to liver and spleen. Slit the stomach and intestine at several points. Prick the genital organs at several points. Open the peritoneum over kidney. Make 2-3 transverse cuts on the kidney. Slit open the pericardium to expose heart. Slit the heart. Put fish on a wooden board; make transverse parallel cuts on both sides of body musculature starting from the posterior part of operculum and proceeding towards tail. Each parallel cut is made 3 mm apart. Remove the operculum and expose the gills. Slit open the cranial bone by making a transverse cut just above the posterior broader of eye with a strong knife or scalpel. Take a wide mouthed bottle layer bottom and sides of the bottle with absorbent cotton or filter paper. Pour 10-20 times of volume of the fish the fixative and put the fish in it. Small fish (Fry and fingerlings) can be put in

the fixative directly after giving several needle pricks on the body. In small and medium sized fishes needle pricks can be made on visceral organs instead of cuts. In formalin fixatives the tissue will be ready for processing after 18 hrs. In Bouin's fluid tissue must not be kept after 72 hrs. The tissue has to be stored in 70% alcohol.

Fixation of shrimp: Collect live shrimps. Take 10 ml Davidson's fixative in a syringe; inject the fixative at following positions; a) laterally in hepatopancreas proper, b) in the region anterior to the hepatopancreas, c) in the posterior abdominal region and anterior abdominal region. Inject an equivalent of 5-10% 0f shrimps body weight; all signs of life should stop. Immediately following injection, slit the cuticle with dissecting scissors, from the sixth abdominal segment to the base of the rostrum, paying particular attention not to cut deeply into the underlying tissue. The incision in the cephalothroax should be just lateral to the dorsal nidline, while that in the abdominal region should be approximately mid- lateral. Shrimp larger than 12 grams, should then be transversely slit once at the abdomen/cephalothroax junction or again mid-abdominally. Put the shrimp in 10 times the volume of the fixative and allow it to remain in the fixative at room temperature for 24 to 72 hr depending on the size of shrimp. Following proper fixation the specimens are transferred to 50% ethyl alcohol.

Preparation of tissue for processing: the tissues of fish are cut with razor blade in 3 mm thick pieces and processed. Other wise each organ as such is processed. Each tissue is put in a tissue capsule and labels written by lead pencil accompanied each tissue. Small shrimps are processed as such. Larger shrimps are removed from 50% alcohol. Place the shrimp on a wooden board. Using a razor blade, bisect shrimp transversely at the junction of the cephalothroax and abdomen. Longitudinally bisect the cephalothroax just lateral of mid-line. Take the half of the cephalothroax without mid-line and remove, with diagonal cut starting at the distal surface, the brachio-stegal region containing the gills. Remove the distal 80% of head appendages if these are not to be studied. Separate abdominal segments # 1, 3 and 6 from the remainder of the abdomen, remove the distal ends of uropods. Longitudinally bisect the 6^{th} abdominal segment as in the manner of the cephalothroax. Place the tissue blocks into histological embedding cassettes. 1) Half of complete shrimp with mid-line, cut-side down (in case of small shrimp). 2) Half of cephalothroax with mid-line, cut-side down. 3) Brachio-stegal region cut-side up. 4)

Other half of complete shrimp. 5) Other half of the cephalothroax without the mid-line placed with the cut-side up. 6) Transverse blocks of abdominal segments. 7) Longitudinal block of 6^{th} abdominal segment placed with cut-side down.

Decalcification of chitin

Put the tissues in 8% formic acid in distilled water for 8-12 hr and check for decalcification. Solution must be renewed every 12 hr.

Processing: Processing of fixed tissue involves; dehydration through ascending grades of alcohol; clearing in a wax miscible agent such as xylene or chloroform and finally impregnation with wax.

Schedule

1. Wash the fixed tissue in running water for 12 hr.
2. 70% alcohol 4-8 hrs.
3. 90% 4 hrs.
4. Absolute alcohol I 3 hrs.
5. Absolute alcohol II 3 hrs.
6. Absolute alcohol III 3hrs
7. Chloroform I* 1 hr.
8. Chloroform II 1hr.
9. Wax (molten) I 2 hrs.
10. Wax (molten) II 2 hrs.
11. Wax (molten) III 2 hrs.
12. Place in embedding molds to form blocks

*In case of shrimp tissues xylene is preferred to chloroform.

Make serial sections of 5μ thick in a rotary microtome. Float sections on water bath held at 48°C and mounted on a clean slide coated with glycerin-albumin solution (1:1 glycerin &egg albumin). Keep on dryer bench for heat fixing.

Staining.

1. Xylene I	5 min.
2. Xylene II	3 min.
3. Absolute alcohol	3 min.
4. .90% alcohol	3 min
5. 70% alcohol	3 min.
6. 50% alcohol	3 min.
7. Distilled water	1-2 min.
8. Haematoxylin stain	5-8 min.
9. Tap water	0.5 to 1 min.
10. Acid alcohol	a few dip.
11. Tap water	0.5-1 min
12. *Scott's tap water	1-2 min.
13. Eosin stain@	1-3 min.
14. Absolute alcohol	2 min.
15. Absolute alcohol	2 min.
16. Xylene I	5 min.
17. Xylene II	3 min.

18. Mount in DPX mountant with clean cover glass.

Observations: Observe the slides under compound microscope.

Inferences: Comment about fixation, dehydration, section thickness and staining characters of your preparation and submit 5 slides along with your observations. *Scoott's tap water—3.5 g 0f sodium bicarbonate and 20 g of magnesium sulfate in 1000 ml distilled water. @1 g of eosin and 5ml of glacial aceic acid in 1000 ml of 70% alcohol.

Practical 3. (1 Session): **K.C.George**

OBSERVATION OF APPARENTLY NORMAL TISSUES.

Objective and principle: To prepare a record of histological preparations from apparently normal animals and understand the microanatomy of important vital organs of fish and shrimp

Materials: Compound microscope, stained histological sections

Procedure: examine sections of liver, spleen, kidney, heart, gills, skin, brain and intestine of fish. Examine sections of hepatopancreas, gills cephalothrax and intestine of shrimps.

Observations and inferences: While examining each section, prepare colour diagrams of each section. Write briefly about histological structure of each section, you have observed.

Practical 4. (1 Session) **K.C.George**

STUDY OF DEGENERATION AND NECROSIS

Objective and principle: To understand different type cellular degenerations and necrosis.

Materials: Histological preparations depicting cellular degenerations and compound microscopes.

Procedure: examine sections of cloudy swelling, hydropic degeneration, hyaline droplets, fatty degeneration, coagulative necrosis and liquefactive necrosis.

Observation and inferences: Prepare colour diagrams depicting these changes and write brief description of each degeneration.

Practical 5. (1 Session)

STUDY OF INFLAMMATION.

Objective: To understand different stages of inflammation and to gain expertise in recognizing inflammatory lesions

Principle: Inflammation is a major pathological process involved in microbial and other diseases. A good knowledge is essential requirement for diagnosis.

Materials: Compound microscope, stained histological sections depicting different stages of inflammation.

Procedure: Examine the slides showing inflammation.

Observation and inference: Identify hyperaemia, Margination of leucocytes, emigration of leucocyte, accumulation of leucocytes in tissue, epithelioiod cells, giant cells, fibrinous, inflammation and granulomatous inflammation in fish tissues. Prepare colour diagrams of the slides observed depicting different stages of inflammation

Practicals No.6

Teacher:Dr.K.S.Sobhana

Isolation of bacterial pathogens from fish

Objective :

To isolate bacterial pathogens from blood/kidney of diseased fish from coastal aquaculture systems.

Principle :

The bacterial pathogens causing diseases in fish can be isolated from the blood/kidney using a general purpose medium and then separated on selective media or can be directly isolated using selective media.

Equipments and Reagents:

Autoclave
Hot air oven
Drier
Laminar flow hood
Bunsen burner
Bacteriological incubator
Sterile syringe
Sterile scalpel, scissors and forceps
Micropipette
Inoculation loop
Petridishes
Test tubes
Triptic Soy Agar
Tryptic Soy Broth
TCBS agar
Rimler Shotts Agar
Pseudomonas Isolation Agar

Procedure:

Live/moribund fish should be used for isolation of bacteria. Blood sample is collected using a sterile syringe from the heart or from the caudal vein after anaesthetizing the fish. For sampling from kidney, the abdomen is opened

by aseptic dissection and cutting open the peritoneal membrane exposes the kidney. The samples of blood/kidney may be directly streaked on to Tryptone soy agar (TSA) and other selective media or in to Tryptone soy broth under aseptic conditions. In the latter case, after incubating the broth for 18-24 hrs at 25-30°C, loop full of inoculum is streaked on to selective media.

The selective media used for the most common bacterial pathogens of fish (mostly gram negative) are : TCBS agar, Rimler Shott's agar and Pseudomonas isolation agar. The inoculated media are then incubated at 25 – 30 °C for 18 – 24 hrs. Representative numbers of the most numerous colony type are isolated, purified on a general purpose medium and then transferred to Nutrient agar/TSA slants.

Observations and Inference :

Practicals No.7

Teacher:Dr.K.S.Sobhana

Identification of bacterial pathogens Isolated from diseased fish

Objective :

To identify the bacterial pathogens isolated on selective media from blood/kidney of diseased fish to species level.

Principle :

The bacterial pathogens isolated and purified are identified to the species level based on colony morphology, gram staining, motility test and biochemical reactions.

Equipments and Reagents:

Microscope
Autoclave
Hot air oven
Drier
Laminar flow hood
Bunsen burner
Bacteriological incubator
Micropipette
Inoculation loop
Petridishes
Test tubes
Slides and cover slips
Gram staining reagents
Media for testing biochemical reactions

Procedure :

The purified bacterial isolates are then tested for the following aspects

1) Colony morphology
2) Gram staining
3) Motility test
4) Biochemical reactions

The biochemical tests to be carried out for identification :

1)Cytochrome oxidase test

2)Oxidation-Fermentation reaction (Hugh-Leifsons's test)

3)Methyl red and Voges – Proskauer test (MR-VP test)

4)Indole test

5)Fermentation of sugars

6)Amino acid utilization

7)β-galactosidase test (ONPG test)

8)Catalse activity

9)Citrate utilization

10)Nitrate reduction

11)Gelatinase activity

12)H_2 S production

13) Sensitivity to Novobiocin and O/129 discs (in the case of Aeromonas and Vibrios)

The bacterial pathogen is identified to the species level based on the above tests using identification keys.

Observations and Inference:

Practicals No.8

Teacher: Dr.K.S.Sobhana

Pathogenecity testing of bacterial isolates in fish/shrimp

Objective :

To test the pathogenecity of bacterial pathogen isolated from fish/shrimp

Principle :

The pathogenecity of the bacterial isolate may be confirmed by challenge experiments in healthy animals.

Equipments and Reagents :

Autoclave
Hot air oven
Drier
Laminar flow hood
Bunsen burner
Bacteriological incubator
Refrigerated centrifuge
Micropipette
Inoculation loop
Petridishes
Test tubes
Nutrient agar (NA)/TSA slants
Phosphate buffered saline (PBS)
Experimental tanks for maintaining animals
Live, healthy fish/shrimp acclimatized to the experimental conditions

Procedure :

The bacterial isolate to be tested is subcultured on NA/TSA slants and incubated for 18-24 hours. The culture is then harvested and suspended in PBS. The bacterial cells are washed three times in PBS by centrifugation. The cells are then resuspended in PBS to the required cell density by measuring OD in a spectrophotometer. 0.1 ml of the suspension is then injected intramuscularly in to the fish/shrimp. A control group is also

maintained and are injected with 0.1 ml of PBS. The animals are then observed for a period of minimum 10 days for mortality or disease conditions.

Observations and Inference :

Practicals No.9 **Teacher:Dr.K.S.Sobhana**

Agglutination tests for identification of bacterial pathogens

Objective :

To identify bacterial pathogens based on agglutination reactions using polyclonal rabbit antisera

Principle :

Bacterial pathogens can be identified based on formation of antigen-antibody complexes in agglutination reactions using antisera raised in rabbits against the bacterial pathogens

Equipments and Reagents:

Autoclave
Hot air oven
Drier
Laminar flow hood
Bunsen burner
Bacteriological incubator
Micropipette
Inoculation loop
Test tubes
Nutrient agar (NA)/TSA slants
Phosphate buffered saline (PBS)
Microscope slides
Microtitre plates
Polyclonal rabbit antisera

Procedure :

Slide agglutination :

The slide agglutination test is one of the simplest methods for rapid identification of bacterial pathogens. However, auto agglutination and cross reactivity can be a problem particularly in gram negative bacteria and therefore suitable controls are essential. This method can also be used for detecting the presence of specific antibody in the serum and to quantify them.

Two drops of saline are placed on a clean microscope slide . A loop full of 18 – 24 hr old bacterial culture to be detected is suspended in the saline. One drop of

the antiserum is then added to one of the suspensions in the slide. The other suspension serve as a control to check autoagglutination. In separate slides, suspensions of unrelated organisms are also prepared and atiserum is added, which would serve as negative controls. The slides are then gently rocked to mix the antigen and antibody. Generally visible agglutination occurs within 5 minutes.

Though slide test is rapid and convenient, the serum has to be used in low dilutions or without dilutions. Therefore non-specific agglutinations might occur. Therefore, slide agglutinations should be confirmed by tube/ microtitre agglutination methods.

Microtitreplate agglutination :

This a modification of tube agglutination method. By this method, the level of antibody present in the antiserum can be quantified.

50µl saline is taken in each well of 96 well microtitre plates. Serial doubling dilutions of the serum are prepared by adding 50µl serum to the first well, mixing and transferring 50µl to the second well and continuing to the 3rd well and so on. A saline control is also kept to check for autoagglutination. 50µl of the bacterial suspension is then added to each well and the plates are incubated overnight. The plates are then examined for agglutination. Formation of a mat at the bottom of the well indicates positive reaction, whereas button formation at the bottom indicates negative reaction. Highest dilution of the antiserum causing complete agglutination of bacterial cells is taken as the titre.

Observations and Inference:

Practicals No.10 **K.S.Sobhana**

Isolation and Identification of fungal pathogens from fish

Objective :

To isolate and identify fungal pathogens from infected tissue of fish

Principle :

Fungal pathogens causing diseases in fish can be isolated from the infected tissue of fish using suitable fungal media and can be identified based on morphology and sporulation characteristics

Equipments and Reagents:

Autoclave
Laminar flow hood
Bunsen burner
Sterile scalpel
Inoculation loop
Petridishes
Test tubes
Czapek Dox agar/Saboraud Dextrose agar/Rosebengal agar/Glucose Peptone (GP) agar

Procedure:

Live/moribund fish should be used for isolation of fungi. The lesion area is surface sterilized using a red-hot scalpel. Using aseptic techniques, pieces of infected tissue (appox. 2 mm^3) are exiced from the lesion area. The tissue pieces are then washed in sterile water and aseptically transferred to a suitable fungal medium supplemented with antibiotics to prevent bacterial growth. General fungal media used are Czapek Dox agar, Saboraud Dextrose agar, Rosebengal agar, Glucose Peptone (GP) agar etc. The plates are then incubated at 25 C. The incubated plates are observed for growth of fungus from the second day onwards. Once the fungal colony develops, from the margin of the colonies, aseptically transfer bits of mycelium to fresh nutrient media plates.

Emerging hyphal tips may be repeatedly transferred to fresh agar plates until cultures are free of bacterial contamination. The fungus is subcultured by aseptically

cutting a block of agar, 3 –4 mm in dia. from the periphery of the colony and placing them upside down on to a petridish of fresh agar.

The leading hyphae of the mycelium on the surface of the agar can be examined directly under microscope to study the morphology and sporing characteristics. Bacteria free leading hyphae are cut in a small block of agar on which they are growing and transferred to agar slopes in test tubes and preserved by pouring sterile liquid paraffin covering the entire slope.

Observations and Inference :

Practicals No.11 **K.S.Sobhana**

Examination of slides of various disease conditions in fin fish

Objective :

To observe and understand the various disease conditions of finfish using histopathological preparations.

Principle :

Various infectious and non-infectious disease conditions in fish can be identified based on the tissue level alterations in comparison with normal histological features of vital organs and tissues.

Equipments and Reagents:

Compound Microscope

Immersion oil

Histological slides

Procedure :

Histological preparations of the infected tissues of various diseases conditions will be observed under microscope. The presence of the pathogen involved in the disease condition, if present, and the histopathological changes in the affected tissues will be observed and recorded. The changes present shall be analyzed in comparison with normal histological features of the corresponding tissues in order to understand the tissue level changes occurred due to the disease condition.

Observations and Inference:

Practicals No.12 **K.S.Sobhana**

Examination of slides of various disease conditions in crustaceans

Objective :

Tto observe and understand various disease conditions of crustaceans using histopathological preparations.

Principle :

Various infectious and non-infectious disease conditions in crustaceans can be identified based on the tissue level alterations in comparison with normal histological features of vital organs and tissues.

Equipments and Reagents:

Compound Microscope

Immersion oil

Histological slides

Procedure :

Histological sections of the infected tissues of various diseases conditions in crustaceans will be observed under microscope. The presence of the pathogen involved in the disease condition, if present, and the histopathological changes in the affected tissues will be observed and recorded. The changes present shall be analyzed in comparison with normal histological features of the corresponding tissues in order to understand the tissue level alterations occurred due to the disease condition.

Observations and Inference:

Practical No 13

N. K. Sanil

Examination of fishes for the presence of parasites

Principle: Examination of the fish sample for the presence of parasites/larval stages in a systematic way.

Objective: Examining the fish for obtaining information on the disease/ health status of the organism.

Materials: Live fish, dissecting instruments, formalin, microscope, slides and cover slips, hydrochloric acid, methanol.

Procedure: Normally the fish is examined from outside first followed by the inner organs/tissues in detail. Kill the live fish without undue tissue damage by a blow on head or by cervical rupture. Take forks length and weight of fish and record it. Place the fish on a wooden board on its right side, head-pointing left and with the ventral side to the examiner. The examination is to be done in the following manner.

The external surface is examined for the presence of parasites, cysts, larval stages or lesions under a stereozoom dissection microscope. Any parasites/stages if present are to be carefully removed using needles/forceps/brush, transferred to a drop of saline and studied in detail. Smears of mucus and scrapings are taken, for microscopic examination. Some scales are removed at random and scale pockets examined. Fins are also examined. External nares, mouth and anal region were examined. Eyes are removed into a petridish, dissected and examined.

Cut off the left operculum and examine the under surface. Excise the first left gill arch, and carefully examine under the dissection microscope. Watch for parasites, disclourations, mucus coating or other lesions. A wet mount of entire gill or cut off portion of filaments may be prepared and squashed gently to spread the filaments. Examine for lesions, crustacean parasites, protozoans, cysts/eggs, encysted larval stages etc. Examine individual gill arches separately. Observe the wall of the gill cavity exposed after the removal of the fourth arch. The same procedure is repeated for the right gills.

Open the mouth by pushing the lower jaw and the surface of the roof and buccal cavity examined.

Using a scissors, remove the left belly flap and expose the viscera. Examine the visceral organs *in situ* for abnormal position, enlargement, discolouration, parasitic cysts on their surfaces, lesion and other pathological signs. Pay attention to size, colour and consistency of liver.

Remove the entire alimentary canal and associated organs as a unit. Place the entire complex in a large dish and separate its components: intestinal tract, liver and gall bladder, pancreas and spleen. Examine these organs one by one, beginning with liver and gall bladder. Separate gall bladder, cut open and examine its contents on a slide for the presence of myxosporean stages under a compound microscope. The liver surface is examined, slices cut and examined and impression smears prepared. Spleen is cut and impression smear made.

Divide the alimentary canal into sections - stomach and oesophagus, pyloric region and intestine. The intestine is further cut into several parts and transferred to separate petri dishes. Incise the stomach wall longitudinally, exposing its contents. Remove the contents and scrape/wash the inner surface of the stomach using a fine brush and scrapings examined as wet mount. Under a dissecting microscope examine both inner and outer surfaces for lesions or parasite cysts. Squash until the stomach tissues appear translucent and examine under dissecting microscope for parasite cysts. Stomach contents at the bottom of the petri dish examined for parasites. Repeat this procedure for entire gut sections, recording the parasite found for each section separately. Gut contents can be subjected to sedimentation techniques for separation of eggs if present.

Identify the sex and examine the gonads superficially, open and examine them under dissection microscope. Macerate some of the tissue, prepare a wet mount and examine under microscope.

Examine outer wall of the swim bladder, open it and examine the inner surface. Expose the kidneys, note the general appearance, dissect them and transfer into petri dish. Cut transversely through the head kidney and make an impression smear. Prepare a wet mount and examine it. In large fishes remove the urinary tract and bladder along with the kidneys. Open the bladder, examine the contents and inner surface. Prepare a wet mount and examine.

Remove the heart, examine its surface for lesions and parasitic cysts, open the ventricle and examine inner surface for cysts/any abnormalities.

Remove the brain and as much of the spinal cord as possible and examine them externally under a dissecting microscope. Make wet mounts and examine under a compound microscope. If brain parasites are observed, the entire brain tissue is digested in pepsin- hydrochloric acid digestion medium (2 g pepsin in 400 ml and 0.5% HCl). Parasites are recovered by centrifugation.

Examine the cartilage and bone of the ear and its surroundings for discolouration, inclusions or damage, particularly for whitish concretions irregularly distributed in the bone or cartilage. Abnormal tissues or substances are examined as wet mounts.

Muscles are removed and observed for abnormalities, internal foci of necrosis and parasites. Examine the belly flaps. Nematodes if present, are recovered from muscle by digestion technique. (Prepare a stock solution of 1% hydrochloric acid. Add 1 g of 1:2500 pepsin powder to 100 ml of acid solution and heat to 52° C. Pieces of muscle are added, shaken stirred at this temperature until completely dissolved (about1-3 hours). The solution is then washed through a 2 mm mesh sieve and nematode/larvae recovered).

Examine the spinal column for abnormalities, fusion of vertebrae, telescoping or twisting.

Observation : Record the necropsy observations in the format provided.

Host ..... Host No. Locality Fork Length..... Weight ..... Sex ..... Mature (...) Immature (...) Host condition: Fresh (...) tank held (...) Refrigerated (...) Frozen (...) Fixed (...) Collection date ..... Collected by ..... Examination date ..... Examined by	
Blood smear	Urinary bladder smear
External surface:	Kidney:
Mucus/scrapings:	Smear ( ):
Scales:	Gall bladder: Smear ( ):
Fins:	Body cavity:
Nasal cavities:	Liver:
	Smear ( ):
Operculum: Left Right	Spleen:
	Smear:
Mouth:	Stomach:
Eyes: Left: Right	Intestine:
Brain and spinal cord:	Anterior:
Heart and aorta:	
Swim bladder:	Posterior:
Gonads:	Intestinal smear:
	Muscles:
Notes and preservation methods	

Practical No 14

N.K. Sanil

Examination of prawns for the presence of parasites

Principle: Examination of prawn for the presence of parasites/larval stages in a systematic way

Objectives: . Examination of prawn for obtaining information on the disease/ health status of the organism.

Materials: Live prawns, dissecting instruments, formalin, microscope, slides and cover slips.

Procedure: Normally the prawn is examined from outside first followed by the inner organs/tissues in detail The animal is taken in a petridish or suitable container and examined under a under a dissection microscope. The examination is to be done in the following manner.

General Gross Examination: Look for : Deformities, general discolorations, thin shells, blisters, melanised areas, cysts/attached larval forms, fouling organisms /ciliates on the tip of appendages.

Gross examination of gills: Look for discolouration from fouling organisms, crustacean parasites, cysts/larval stages.

Gill /appendage examination: Place the gill process in a drop of sea water and examine under a cover slip for the presence of fouling organisms, metacercarial cysts, melanisation etc.

Hepato pancreas: Remove, place on a clean slide, bisect, prepare an impression smear. Observe discolouration, melanisation etc. Examine the squash preparation for gregarine trophozoites and metazoan cysts.

Midgut: Place on a slide, strip to exude contents, add a drop of clean sea water, put cover slip and examine for gregarine trophozoites and / or gametocytes.

Muscle/gonads: Remove a tissue sample, prepare a smear and examine for the presence microsporidean spores if muscle /gonad is white in colour. Smear fixed and stained in geimsa and examine.

Take haemolymph samples, prepare smear, stain and examine for any parasites.

Observations: Record the findings.

Practical No 15

N.K. Sanil

Examination of snails for the presence of trematode larval stages

Principle: Systematic examination of the snail host (gastropod molluscs) for the presence of trematode larval stages.

Objectives: To identify the various trematode larval stages present in gastropod molluscs.

Materials: Live snails, dissecting instruments, formalin, microscope, slides and cover slips, stains.

Procedure: The snails collected are transferred to glass beakers and kept covered with a net. Water exchange is to be carried out and the snails fed daily. Observe the snails as well as the water in the container for the presence of cercarial emission. Examine the water under a dissection microscope periodically. Observations are to be carried out at least 3 times a day.

Isolate the cercariae if present, take on a slide, stain with neutral red and / or nile blue sulphate and observe under a phase contrast microscope. Collect the cercariae, take measurements using a micrometer and make drawings. Collect the details regarding the emergence pattern, time, photoperiodicity etc. Identify the cercarial group based on the details.

Isolate the gastropod mollusc, which has shed the cercaria, and dissect to find out the site of parasitic development. Remove the hepatopancreas/infected organs, tease and examine in detail under a microscope. Isolate the developing larval forms – sporocyst/redia/other larval stages and examine under the microscope after staining. Make drawings, take measurements and collect details.

Observations : Record the findings

Practical No 16 N.K. Sanil

Examination of slides / specimen of parasites

Principle: Examination of the given specimens / slides of parasites.

Objectives: For the identification of the specimen, making observations/comments regarding the various features/adaptations and making drawings of the item.

Materials: Specimen/slides, microscope.

Procedure:

Specimen / slides are examined.

Identification of the specimen

Observations and comments on the various parasitic adaptations exhibited by the specimen and/or pathological changes caused .

Make drawings of the given specimen

The following specimens are to be examined:

Trematode – adult

Cestode – scolex

Cestode - immature body segments

Cestode - gravid segments

Gregarines in the midgut

Zoothamnium in the gills

Microsporidean infected tissues

Observations: Draw the diagrams of the parasites, label them and make notes

M.F.Sc - Mariculture

PRACTICALS : OUTLINE

Course No. Mc-509
(MARICULTURE GENETICS)

Faculty

Dr.P.C.Thomas
Dr. P.Jayasankar

Technical Assistance
Shri.M.P.Paulton

Practical No.1 **Prepared by Dr. P.C. Thomas**

Title: Preparation of chemicals and buffers for molecular biology

Objectives : To prepare stock solutions of chemicals and buffers for molecular biology.

Principle : Working solutions can be quickly prepared by diluting the stock solutions.

Procedure/composition:

1. 0.5 M Tris-Cl (pH 8.0):

   Tris base - 3.028g
   Distilled water - 40ml

   Adjust the pH to 8.0 using conc. HCl
   Makeup final volume to 50 ml
   Autoclave and cool it down to room temperature
   Store at 4°C

2. 0.5 M Tris-Cl (pH 8.3):

   Tris base - 3.028g
   Distilled water - 40ml

   Adjust the pH to 8.3 using conc. HCl
   Makeup final volume to 50 ml
   Autoclave and cool it down to room temperature
   Store at 4°C

3. 0.5 M EDTA (pH 8.0):

   EDTA - 9.34g
   Distilled water - 40ml

   Adjust the pH to 8.0 using NaOH pellets
   Makeup final volume to 50 ml
   Autoclave and cool it down
   Store at room temperature

4. 10 mM Tris-Cl (pH 7.5):

   Tris base - 0.3028g
   Distilled water - 20ml

   Adjust the pH to 7.5 using conc. HCl
   Makeup final volume to 25 ml
   Autoclave and col it down to room temperature
   Store at 4°C

5. RNAase Buffer:

10 mM Tris-Cl (pH 7.5)	- 10 µl
15mM NaCl (o.8475 g in I ml)	- 30 µl
Distilled water	- 960 µl

Autoclave it
Cool it down to room temperature
Store at 4°C

6. 5X TAE:

Tris base	- 12.10 g
0.5 M EDTA (pH 8.0)	- 5.0 ml
Glacial Aceti c acid	- 2.85 ml

Makeup the solution to 500 ml with distilled water
Store at room temperature

7. Bromophenol Blue dye:

0.25% Bromophenol blue	- 2.5 mg
40% Sucrose	- 40.0 mg

Dissolved in 1 ml distilled water
Autoclave
Store at 4°C

Working Solutions:

1. High TE:

Stock 0.5 M Tris-Cl (pH 8.0)	- 20 ml
Stock 0.5 M EDTA (pH 8.0)	- 8 ml

Makeup the solution to 100 ml with distilled water
Autoclave it
Cool it down to room temperature
Store at 4°C

2. Lysis Buffer:

Stock 0.5 M Tris-Cl (pH 8.3)	- 2 ml
Stock 0.5 M EDTA (pH 8.0)	- 0.2 ml
400 mM NaCl	

Makeup the solution to 100 ml with distilled water
Autoclave and Cool it down to room temperature
Store at 4°C

3. Proteinase K:

Proteinase K	- 10 ml
Autoclaved distilled water	- 500 µl

Dissolved Proteinase K in distilled water and store at -20°C

4. Saturation of Phenol with Tris-HCl (pH 8.0):

Reagents required:

Water saturated Phenol (1 bottle)	- 500 ml
0.5 M Tris-HCl (pH 8.0) (60.56g of Tris base in 1000 ml)	- 1000 ml
0.1 M Tris-HCl (pH 8.0) (For 300 ml of 0.5M Tris-HCl (pH 8.0) add 1300 ml of Distilled water)	- 1500 ml

1. If Phenol is transparent, add 0.1% (50 mg) 8-hydroxyquinoline to 500 ml of water saturated phenol)
2. Cover flask containing phenol with aluminium foil to avoid light reaction
3. Add 500 ml 0.5 M Tris-HCl
4. Stir the solution using magnetic stirrer for 15 minutes
5. Keep the solution for 30 minutes to allow the phenol to settle
6. Decant the supernatant
7. Add 500 ml of 0.1 M Tris-HCl
8. Repeat the steps 4,5,6.
9. Repeat step 8 once more
10. Check pH of decanted supernatant using pH paper, which shosuld be 8.0
11. Add 500 ml of 0.1 M Tris-HCl to phenon
12. Store at 4°C in dark bottles covered with aluminium foil

5. Chloroform: Isoamyl alcohol (24:1 V/V):

Chloroform	- 96 ml
Isoamyl alcohol	- 4 ml

6. 3M Sodium acetate (pH 5.2):

Sodium acetate	- 12.4 g
Distilled water	- 20 ml

Adjust the pH to 5.2 using glacial acetic acid
Makeup the solution to 50 ml
Autoclave and Cool it down to room temperature
Store at 4°C

7. TE buffer:

Stock 0.5 M Tris-Cl (pH 8.3)	- 2.0 ml
Stock 0.5 M EDTA (pH 8.0)	- 0.02 ml

Makeup the solution to 100 ml with distilled water
Autoclave and cool it down to room temperature
Store at 4°C

8. RNAse:

RNAse	- 10 mg
RNAse buffer (autoclaved)	- 1 ml

Dissolved RNAse in RNAse buffer
Heat at 100 °C for 15 minutes in boiling water
Allow to cool at room temperature
Store at -20 °C

9. 0.5X TAE (Running buffer):

5X TAE	- 25 ml
Distilled water	- 225 ml

Keep at 4 °C until required

10. 0.7% Agarose Gel:

Agarose	- 210 mg
5X TAE	- 3 ml
Distilled water	- 27 ml

Boil Agarose
Add 1.5 µl of Ethidium bromide before cooling (Approximately 50 °C)
Pour it in gel plate with already adjusted gel comb
Let it cool at room temperature for ½ hr for the gel to set

Observations: Label all the stock solutions and store.

Inferences:

Practical No.2 Prepared by Dr. P. Jayasankar

Title: **Extraction of DNA from fish tissue**

Objective: To isolate total genomic DNA from fish tissue for PCR amplification.

Principle: When the frozen tissues are ground in a blender, the cells are broken and the molecules inside the cells are released. A detergent solution along with an enzyme would break down the proteins. The cell lysate is treated with phenol to remove proteins that have escaped enzymatic digestion. This step is followed by chloroform extraction to remove residuals of phenol. Isoamyl alcohol mixed with chloroform acts as a defoaming agent. Finally DNA is precipitated with ethanol in presence of a salt. A single band without smear indicates good DNA preparation. The smear appears due to degradation of DNA.

Equipments & reagents: Mortar & pestle, centrifuge tubes, water bath, micropipettes, refrigerated centrifuge, refrigerator, digestion buffer, SDS, Proteinase-K, buffered phenol, chloroform, isoamyl alcohol, ethanol and sodium acetate.

Procedure:

1) Mince approximately 200 mg of tissue, homogenize in 1600 µl digestion buffer (10 mM Tris HCl, 25 mM EDTA, 100 mM NaCl, 0.5% SDS and pH 8.0) and transfer to 5.0ml volume centrifuge tubes
2) Add 400 µl of 10% SDS (1.0 g/ml) and 20 µl proteinase-K (10 mg/ml) solution to the homogenized sample
3) Gently mix and incubate in a water bath at 55^0C for 2 to 2 ½ hrs
4) After incubation, purify DNA by successive extraction with buffered phenol, phenol: chloroform: iso-amyl alcohol (25:24:1) and chloroform: iso-amyl alcohol (24:1) respectively
5) Precipitate DNA with ice-cold ethanol and 3M sodium acetate (pH 5.2)
6) Keep the tubes overnight at -20^0C
7) Next day pellet the DNA by centrifugation at 9K for 10 min at 4^0C and wash with 70% ethanol
8) Air dry the Pelleted DNA and resuspend in 50 µl TE buffer (1M Tris HCl, pH 8.0; 0.5 M EDTA, pH 8.0)
9) Store the DNA at -20^0C.

Observations: Electrophorese the DNA in 0.8% agarose, stain with ethidium bromide and observe under UV. Check whether band is intact without smear.

Inference:

Charts, Tables & Figures:

Practical No.3

Prepared by Dr. P.C.Thomas

Title : Agarose Gel Electrophoresis of DNA

Objective : To separate DNA molecules according to molecular size.

Principle: The DNA molecules are resolved in agarose gel on the basis of their molecular size. Since DNA molecules are negatively charged they migrate to the positive electrode under the electric field. The frictional force exerted by the gel vary with the size of the molecule, resulting in differential migration, thus separating the molecules depending upon their size.

Requirements:

1. Chemicals

   a) Agarose : (general purpose agarose is type II)
   b) Bromophenol (Dye)
   c) Ethidium bromide

   d) TBE 10X (Gel and Tank buffer)

Tris	54 g
EDTA	4.6 g
Boric acid	27.5 g
Make up to 500 ml (pH 8)	

   e) Loading buffer (10 ml)

Glycerol	3ml
Dye (BPB)	1 ml from 0,5% stock
Buffer (1X TEB)	6ml

2. Equipments

   Electrophoresis unit
   Power pack
   UV Transilluminator

Procedure :

(For casting a 0.6% gel : volume 30 ml).

- Seal edges of a clean dry glass mold plate with autoclave tape and form a mold.

- Add 180 mg of powdered agarose to 3 ml of 10 X TBE of electrophoretic buffer.
- Add 25 ml water & heat the slurry in boiling water bath until agarose dissolves.
- Make it up to 30 ml.
- Add 1 µl of ethidium bromide from a stock of 10 mg/ml.
- Pour warm agarose solution (around 50°C) into the mold and immediately clamp the comb into position near one end of the gel.
- After the gel is completely set (30-45 min at room temperature) carefully remove the comb and autoclave tape and mount the gel into electrophoresis tank.
- Add just enough tank buffer (1X TBE) to cover the gel to a depth of about 1 mm.
- DNA samples are mixed with 2 ul of loading buffer and loaded into the slots of the submerged gel using a micropipette.

Run conditions : Carry out electrophoresis at constant voltage of 80 till dye front reaches other end of the gel.

Visualization of DNA in gel:

The most convenient method of visualizing DNA in Agarose gel is by use of fluorescent dye viz. ethidium bromide. This substance contains a planar group that intercalates between the stacked bases of DNA. The fixed position of this group and its close proximity to the bases causes dye bound to DNA to display and increased fluorescent yield compared to dye in free solution. UV rays absorbed by the DNA at 260 nm or 302 nm and transmitted to the dye, is emitted at 590 nm in the red-orange of the visible spectrum. The DNA in the gel is visualized using a UV transilluminator. DNA is seen as distinct bands in the gel.

Observation:

Observe the DNA in the gel as distinct bands under UV light. Document the bands.

Inference:

Charts, tables & figures:

Practical: 4 Prepared by Dr. P.C.Thomas

Title : Quantitative and qualitative evaluation of DNA

Objective: To quantify and to check the purity DNA in the sample.

Principle: The absorption of light by a compound is measured using a spectrophotometer. UV light of 260 nm and 280 nm wave length is made to fall on a DNA solution. The absorption is measured and is used to quantify DNA in the sample, since the absorbence is proportional to the concentration of DNA. OD_{260} of 1 indicates 50 µg/ml double stranded DNA or 40 µg/ml RNA. A ratio of OD_{260} to OD_{280} of 1.8 indicates very pure double stranded DNA. Value greater than 1.8 indicates RNA contamination and lower than 1.8 indicates protein contamination.

Requirements: UV -Vis spectrophotometer, Quartz cuvette, micropippete, DNA sample, tissue paper.

Procedure:

A) Spectrophotometric estimation

1) Dilute 1 µl DNA with 999 µl of TE or double distilled water and mix well
2) Set up a blank and adjust the absorbance to zero.
3) Measure the absorbance of the DNA sample at 260 nm and 280 nm.
4) Calculate the conncentration of DNA
5) Calculate ratio of OD_{260} to OD_{280} and find out the purity of the sample.

B) Electrophoretic quantification.

Carryout agarose gel electrophoresis with the sample DNA as well as known quantity of marker DNA. Stain with ethidium bromide and check the integrity and quantity of DNA by comparing the intensity of both sample and marker.

Observation:

Record the values of absorbance of the DNA sample at 260 nm and 280 nm. Calculate ratio of OD_{260} to OD_{280}.

Inference :

Charts, tables &figures:

Practical No.5 Prepared by Dr. P. Jayasankar

Title: **RAPD analysis**

Objective: To study genetic variations among individuals of the same species (intraspecific DNA fingerprinting) and to develop genetic profiles of different species (interspecific DNA fingerprinting) for ratification of their taxonomic status

Principle: Random Amplified Polymorphic DNA (RAPD) is a method of revealing DNA polymorphisms by PCR amplification of the genomic DNA using arbitrary primers. The major advantage of the method is that it does not require any prior information on the target sequence for PCR amplification. RAPD PCR contains a cocktail comprising of a buffer, primer, dNTPS, and *taq* DNA polymerase, which is added to the DNA template. Invariably the annealing temperature of the PCR would be lower (such as 36°C), thereby allowing low stringent annealing of primer with the target DNA. The amplified products appear as bands of varying sizes as determined by comparing the DNA size standard. The bands data are used to calculate distance matrix which in turn would be used to generate dendrograms in cluster analysis. Genetic variability can be inferred from the clustering patterns of individuals/populations of fish species.

Equipments & Reagents: Microfuge tubes, micropipettes, laminar hood, PCR themocycler, horizontal electrophoretic apparatus, power pack, buffer, primers, dNTPS and *taq* DNA polymerase.

Procedure:

1) Prepare total reaction volume 25 µl - 10 to 15 ng template DNA, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.2mM each of dATP, dCTP, dGTP and dTTP, 10 picomoles primer (Arbitrary decamer primers from kits A and F of Operon Inc), 1U *Taq* DNA polymerase (Bangalore Genei, India) and deionized water to make up volume to 25 µl
2) Tap the tubes gently and spin for few seconds and place them in a thermal cycler, programmed for an initial denaturation of 30s at 94°C followed by 45 cycles each consisting of 30s at 94°C (Denaturation), 30s at 36°C (Annealing) and 120s at 72°C (Extension)
3) Set up a final extension (72°C) for 7min
4) Resolve the PCR products in 1.5% agarose gels containing 1x TBE buffer at 100V for about 3h in a horizontal slab electrophoretic unit
5) Stain the gels in ethidium bromide and document using a gel documentation system.

Observation: Score the bands by binary method, i.e. '0' for no band and '1' for a band and prepare binary matrix for each individual fish.

Inference:

Charts, Tables, & Figures:

Practical No. 6 & 7 Prepared by Dr. P.C.Thomas

Title : Plasmid DNA isolation

Objective: To isolate and purify plasmid DNA from bacterial cells.

Principle: Bacteria are harvested by centrifugation. Cell wall and outer membranes are broken down by lysozyme. The covalently closed circular plasmid is isolated by selective denaturation of genomic DNA by alkaline lysis, followed by standard phenol chloroform extraction.

Requirements:

High speed refrigerated centrifuge, Micropippete, Microfugetubes, Nutrient broth, ice crystals, Lysis buffer, Alkaline lysis mix, Pot.acetate, Neutral phenol, Chloroform ,Isoamyl alcohol, Sod.acetate and ice cold Ethanol.

Procedure:

Culture

1. Culture the Vibrio in nutrient broth.
2. Sub culture into nutrient broth with 2 % Nacl
3. Isolate plasmid DNA from overnight culture, which is in log phase of growth

Protocol for plasmid DNA extraction:

1) Collect 10 ml bacterial culture in log phase into a capped centrifugation tube.
2) Centrifuge at 10,000 rpm for 5 minutes.
3) Dry the pellet at the bottom on blotting paper
4) Transfer the pellet into an eppendorf tube
5) Add 0.5 ml of lysis buffer.
6) Vortex on a cylomixer
7) Keep the tube in ice for 10 minute
8) Add 1 ml of alkaline lysis mix.
9) Incubate the contents on ice for 15 minutes shaking by hand in between
10) Add 0.75 ml of ice cold, 3M potassium acetate, keep it on ice for 10 minutes.
11) Centrifuge at 10,000 rpm for 10 minutes
12) Collect the supernatant into another eppendorf tube.

13) Add equal volume of neutral phenol and mix by hand for 5 minutes.

14) Centrifuge at 10,000 rpm for 10 minutes

15) Collect the aqueous phase into an eppendorf tube using micropipette.

16) Repeat the phenol extraction (Steps 13-15)

17) Add equal volume of chloroform : isoamyl alcohol(24:1) to the supernatent

18) Centrifuge at 10,000 rpm for 15 minutes.

19) Collect the supernatant carefully

20) Add $1/10^{th}$ volume of 3M sodium acetate and mix well.

21) Add twice the volume of ice cold ethanol and mix

22) Keep at –20 ° C overnight

23) Centrifuge at 10,000 rpm for 15 minutes

24) Discard the supernatant carefully, retaining the bottom pellet.

25) Suspend the pellet in 70 % ethanol.

26) Centrifuge at 10,000 rpm for 5-10 minutes and pour of the ethanol

27) Dry the pellet in speed vacuum or air dry it.

28) Dissolve the pellet in minimum volume of TE buffer

Carrying out a submarine agarose gel electrophoresis, followed by ethidium bromide staining. Visualize the DNA over a UV transilluminator.

Observations:

Observe the plasmid DNA in the gel as distinct bands under UV light and document the same.

Inferences:

Charts, tables &figures:

Practical No.8&9 Prepared by Dr. P.C.Thomas

Title : Detection of White Spot Syndrome Virus
(Duplex PCR Kit of CMFRI)

Objective : To detect the presence of WSSV DNA through PCR amplification of viral genome.

Principle: Oligonucleotide primers will anneal to the complementary regions on both strands of the viral DNA and will amplify the portions intervening between the primers. PCR cycles consisting of denaturing, primer annealing and extension are effected through temperature manipulation using an automatic thermal cycler.

Requirements:

Microfuge tube, sterile forceps, homogenizer, parafilm, thermocol float, micropipette, centrifuge, PCR thermocyler, agarose gel electrophoresis unit, UV transilluminator, PCR pre-mix, Taq DNA polymerase, homogenizing solution, loading dye ,ethidium bromide and shrimp for testing.

Procedure:

I. Template DNA preparation

1. Thoroughly wash the shrimp with distilled water
2. Wear gloves and carefully remove about 30 mg gill tissue using a sterile forceps into a sterile microfuge tube supplied.
3. In case of larvae and post larvae, aseptically transfer about 25 numbers of larvae (30 mg) / 10 to 15 numbers of post larvae (30 mg), into the microfuge tube.
4. Homogenize the sample using 1 ml of homogenizing solution and disposable pestle supplied as described below. Initially the sample may be crushed in 200 µl (0.2 ml) of the homogenizing solution and make up the volume to 1 ml and thoroughly homogenize. Discard the used homogenizer after autoclaving.
5. Tightly close the tube containing the homogenate, cover the lid with parafilm and insert pin prick hole on the lid.
6. Arrange the tubes on a hard thermocol float and boil for 10 minutes.
7. Allow the sample to cool to the room temperature.
8. Centrifuge the tube at 10000 rpm for 15 minutes in a centrifuge.
9. Carefully recover the supernatant containing the DNA template using a micropipette, transfer into another sterile microfuge tube and store at –20° C till used for PCR.

II. Polymerase Chain Reaction (PCR)

1. Arrange sterile 0.2 ml PCR tubes supplied in aseptic environment and label properly.
2. Take out the PCR pre-mix and Taq DNA polymerase supplied as well as the sample supernatant containing the DNA template and keep on ice.
3. Carefully pipette out **23.6 µl** of PCR mix into a PCR tube using micropipette (aerosol tips are ideal)
4. Add **0.4 µl** of Taq polymerase and **1 µl** of the sample supernatant into the PCR tube.
5. Tightly close the PCR tubes and ensure that no air bubbles are trapped inside the mix. Mix the contents of the tubes by gently tapping with fingers or by spinning in a mini microfuge tube at 6000rpm for 15-20 seconds. (Similarly arrange for the PCR with the positive and negative DNA samples also)

Note: If more samples are to be screened, then prepare a master mix as follows.

* Determine the toal number of PCRs to be performed by adding **one** positive control and **one** negative control **plus one** to the **number of samples to be tested**.(ie, Number of sample to be screened + three)
* Calculate the volume of ingredients to be added for the master mix as follows.
  Volume (µl) of pre mix = 23.6 x total number of PCRs to be performed.

  Vol. (µl) of Taq Polymerase = 0.4 x total number of PCRs to be performed.
* Prepare the Master mix by carefully pipetting out the above volumes into a 2 ml sterile microfuge tube.
* Aliqout 24 µl from the above master mix into each of the PCR tubes . Add 1µl template DNA into respective PCR tube.
* Add 1µl of the Positive control DNA into the Positive control PCR tube.
* Add 1µl of the negative control DNA into the negative control PCR tube

6. Load the PCR tube into the thermocyler and set the PCR as follows.
   - Initial denaturation: 95°C for 3 miutes
   - Followed by 35 cycles of:

     95°C for30 seconds

     58°C for 60 seconds

     72°C for 60 seconds
   - Followed by final extension at 72°C for 5 minutes

III. Detection of the PCR product:

Detection of PCR product is carried out by 1.5% agarose gel electrophoresis.

a) Mix 5µl of the PCR product and 2µl of the loading dye supplied. (Prepare for each of the PCR sample, positive control and negative control)

b) Mix 5µl of the DNA marker supplied and 2µl of the loading dye

- Cast a 1.5-% agarose gel and load 7µl of the above sample + dye mixture into the well of the gel. Load each sample into a separate well. Load the marker, positive control and negative control in separate wells and carry out electrophoresis at a constant voltage of 80.After the run is over, stain the gel with ethidium bromide(1µg /ml) and visualize the bands on UV transilluminator.

Observations:

Observe for amplified DNA bands in the gel on UV transilluminator. Presence of virus in the sample will be indicated by two bands of 0.9Kb and 0.6Kb resolved in the gel.

Inferences :

Charts, tables & figures:

Practical No.10 Prepared by Dr. P. Jayasankar

Title: **Preparation of chemicals and buffers for cytology**

Objective: To prepare the reagents and other chemicals required for studying metaphase chromosome and Chromosome banding of fish

Principle: The chemicals and buffers are used to subject the cells to hypotonic conditions followed by fixation and staining.

Equipments & Reagents: Microscope with photographic attachment, BOD incubator, Water bath, Electronic balance, Hot-air oven, Table top centrifuge, pH meter, Magnetic stirrer, Disposable syringes, Microscopic slides (1 mm thick), Cover slip (No.1), B/W film (100 ASA), pasture pipettes, graduated centrifuge tubes, coplin jars, tissue grinder, filter paper, test tube stand, spirit lamp, 0.05% colchicines, RPMI-1640 (10.39 g/l), a fixative, Giemsa stain, Barium Hydroxide, Disodium hydrogen orthophosphate, Potassium dihydrogen orthophosphate, 2X SSC, potassium chloride, Hydrochloric acid, Colloidal developer and silver nitrate.

Procedure:

1) Reagents for chromosome preparation – 0.05% colchicines, RPMI-1640 (10.39 g/l), fixative (methanol, 3 parts: acetic acid, 1 part)
2) Reagents/buffers for chromosome banding - Giemsa stain (0.5 g Giesma powder+33 ml Glycerol+33 ml methanol), 5% Barium Hydroxide, 0.5 M Disodium hydrogen orthophosphate, 0.5 M Potassium dihydrogen orthophosphate, 2X SSC (0.3 M Sodium chloride; 0.03 M Tri-sodium citrate), 0.56% potassium chloride, 0.2 N Hydrochloric acid, Colloidal developer (2% Gelatin+1% Formic acid) and 50% silver nitrate.

Observations: Prepare all the chemicals and buffers required for chromosome preparation and banding.

Inference:

Charts, Tables & Figures:

Practical No.11 Prepared by Dr. P. Jayasankar

Title: **Preparation of metaphase chromosome**

Objective: To observe the number and other characteristics of chromosomes in fish cells.

Principle: Theoretically chromosomes can be prepared from any tissue, which is actively dividing. Kidney and gills in fishes are a good source of dividing cells which can be arrested at metaphase stage, using spindly inhibitor, cochicine. For obtaining chromosomes, the cells are subjected to hypotonic treatment followed by fixation and staining. Staining is necessary because without it, the colourless chromosomes are difficult to distinguish from equally colourless cytoplasm.

Equipments & Reagents: Microscope with photographic attachment, BOD incubator, Water bath, Electronic balance, Hot-air oven, Table top centrifuge, pH meter, Magnetic stirrer, Disposable syringes, Microscopic slides (1 mm thick), Cover slip (No.1), B/W film (100 ASA), pasture pipettes, graduated centrifuge tubes, coplin jars, tissue grinder, filter paper, test tube stand, spirit lamp, 0.05% colchicines, RPMI-1640 (10.39 g/l), a fixative, Giemsa stain, Disodium hydrogen orthophosphate, Potassium dihydrogen orthophosphate and potassium chloride.

Procedure:

1) Take kidney tissue from live/freshly dead fish and homogenize to prepare cell suspension in 8 ml RPMI 1640 culture medium
2) Add 50 µl of 0.05% colchicine and incubate the cell suspension in BOD incubator for 30-50 min at 27-32°C
3) Centrifuge the cell suspension at 1200 rpm for 10 min and decant the cell supernatant
4) Add 8 ml hypotonic solution (0.56% KCl) to collect the pellet and keep the cells in solution for 20-25 min
5) Stop the action of isotonic solution by adding 1.0 ml freshly prepared chilled Carnoy's fixative (methanol:acetic acid in 3:1 ratio) slowly and mix it gently with pasture pipette
6) Centrifuge the cell suspension at 1,200-1,500 rpm for 8-10 min to get cell pellet
7) Remove supernatant and add slowly freshly prepared chilled fixative
8) Keep the cell in the refrigerator for half an hour for thorough fixation
9) Repeat steps 8-10 thrice till clear transparent cell suspension is obtained
10) Take small quantity of cell suspension in pasture pipette and drop it from a height of 1-1.5 feet onto grease free, precleaned slide
11) Allow to air/flame drying and keep the slide for ageing for 1-3 days
12) Stain it with 4-5% giemsa in phosphate buffer (pH 6.8) for 15-20 minutes

Practical No.12 & 13 Prepared by Dr. P. Jayasankar

Title: **Chromosome banding**

Objective: To observe banding patterns of chromosomes.

Principle: Chromosome banding is variation in staining pattern along the length of the chromosome. Chromosome banding allows greater insight into the structure and organization of chromosomes. One of the most important applications of banding technique is unequivocal identification of chromosome in karyotype and to study heteromorphism between and within the species. Banding technique can also identify chromosomal rearrangement that has taken place between specie during the course of evolution. The important banding techniques used are G-, C-, Q-, R-, NOR, DA-, DAPI, RE banding, etc.

Equipments & Reagents: Microscope with photographic attachment, BOD incubator, Water bath, Electronic balance, Hot-air oven, Table top centrifuge, pH meter, Magnetic stirrer, Disposable syringes, Microscopic slides (1 mm thick), Cover slip (No.1), B/W film (100 ASA), pasture pipettes, graduated centrifuge tubes, coplin jars, tissue grinder, filter paper, test tube stand, spirit lamp, Giemsa stain, Barium Hydroxide, Disodium hydrogen orthophosphate, Potassium dihydrogen orthophosphate, 2X SSC, potassium chloride, Hydrochloric acid, Colloidal developer and silver nitrate.

Procedure:

(A) *C-banding*

1) Place one-week old, air dried slides in 0.2N HCl solution for 1 h at room temperature followed by rinsing with double distilled water
2) Place the rinsed slides in coplin jar containing 5% aqueous solution of barium hydroxide at 50°C for 5 min and rinse with double distilled water
3) Incubate the slides for 1 h at 60°C in 2X SSC (0.3M sodium chloride; 0.03M tri-sodium citrate) followed by rinsing them with double distilled water
4) Stain the slides with 2% giemsa in phosphate buffer (pH 6.8) for 45 min and rinse with double distilled water and air dry
5) Clear the slides in xylene and mount in DPX mount (avoid air bubble formation)

(B) *Nucleolar organizer regions banding*

1) Take four drops of developing solution and 8 drops of silver nitrate solution
2) Mix it thoroughly and place it on unstained slide with the help of pasture pipette and cover it with cover glass
3) Incubate the slide at 50°C for 3-5 min till solution takes golden yellow colour and rinse with DD water
4) Dry the slide in air, mount in DPX and observe under the microscope.

Observations: Observe C- banding and NOR- banding in the chromosomes.

Inference:

Charts, Tables & Figures:

13) Wash with double distilled water thoroughly and keep it for air drying .

14) Observe the metaphase spreads in bright field microscope to ascertain the quality of staining and make the slides permanent by mounting in DPX/Canada Balsm (Avoid air bubble formation)

15) Screen the slides for good spreads and take photographs of metaphase spreads under the oil immersion objective

16) Cut individual chromosomes from the photo prints and group the individual chromosomes into four categories, metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t)

17) Paste the chromosomes on ivory sheet in decreasing order of the size within the group.

Observations: Observe the chromosomes in metaphase stage of dividing cells.

Inference:

Charts, Tables & Figures:

Practical No. 14 Prepared by Dr. P.C.Thomas

Title: Measures of Central tendency/dispersion of quantitative trait.

Objective: To describe a population with respect to a quantitative trait.

Principle: Population is the unit of study for a quantitative trait. It is described using statistical parameters like measures of central tendency and dispersions.

Procedure/ formulas:

1. Mean Performance:

$$\bar{X} = \frac{\Sigma X}{n}$$

2. Variance (σ^2) = Mean of the squared deviations of individual values from the mean value of the population

$$\sigma^2 = \frac{\Sigma (\bar{X}i - X)^2}{n-1}$$

Computational formula:

$$\sigma^2 = \frac{\Sigma X^2 - ((\Sigma X)^2 / n)}{n-1}$$

3. Standard deviation of phenotypic values:
More accurate measure of variation in a population than is range and can be used effectively with mean,to describe a population.

$$S.D = \sqrt{\frac{\Sigma X^2 - ((\Sigma X)^2 / n)}{n-1}}$$

3. Coefficient of variation:

$$CV = \frac{\sigma^2 \times 100}{\bar{X}}$$

Problem ;

Body weight measured in live fish are given below. Estimate the measures of Central tendencies and dispersion

Body Weight (Xi)	Deviations from the mean $(Xi - \overline{X})$	Squares of deviation $(Xi - \overline{X})^2$	Sum of each observation Xi^2
47	8.50	72.25	2209
38	-0.50	0.25	1444
39	0.50	0.25	1521
32	-6.5	42.25	1024
34	-4.5	20.25	1156
37	-1.5	2.25	1369
31	-7.5	56.25	961
43	4.50	20.25	1849
41	2.50	6.25	1681
43	4.50	20.25	1849
Sums:385	0	240.50	15.063

Observation:

Estimate and the following parameters

1. Mean body weight
2. Variance
3. SD
4. Coefficient of variation

Inferences:

Charts, tables &figures:

Practical No.15 Prepared by Dr. P.C.Thomas

Title : Coefficient of correlation among traits

Objective: To estimate the magnitude of correlation between quantitative traits.

Principle : The magnitude of association between two traits can be quantified statistically as the coefficient of correlation. Coefficient of correlation indicate the degree of association between two traits.

Procedure/formulas:

Live body weight and rate of gain in 10 fish are given below. Estimate the coefficient of correlation among them.

Number	Body weight (X)	Rate of Gain(Y)	Cross Products (X times Y)
1	47	2.0	94.0
2	38	1.7	64.6
3	39	1.8	70.2
4	32	1.7	54.4
5	34	1.8	61.2
6	37	1.8	66.6
7	31	1.7	52.7
8	43	1.6	68.8
9	41	1.4	57.4
10	43	1.8	77.4
	$\sum X$ 385 $\sum X^2$ 15063	$\sum Y$ 17.3 $\sum Y^2$ 30.15	$\sum XY$ 667.3
	$\bar{X}$ 38.50	$\bar{Y}$ 1.73	

Observations:

Estimate and record the coefficient of correlation between body weight and rate of gain .

Inferences:

Charts, tables &figures:

Practical No.16 & 17 Prepared by Dr. P.C.Thomas

Title : Half-sib analysis to partition components of variance

Objective : To estimate components of variance and heritability from half-sibs.

Principle : Variance components from the analysis of variance provide estimates of various genetic parameters. The mean sum of squares(MS) between progeny within sires consist of only environmental variance whereas the between sires MS consist of the sire component of variance also in addition to environmental variance. Therefore, the difference between the two will give the sire component of variance which is ¼ of the additive genetic variance.

This design is useful mainly for uniparous animals. The reference population is the population from which the sires and dams were obtained. Each sire is mated to several dams and each dam produces one progeny. The measurement is taken on the progeny.

Analysis of Variance Table

Source of Variation	d.f	SS	S	EMS
Between sires	S-1	SS_S	MS_S	$\sigma^2_w + k\,\sigma^2_s$
Progeny within sires	n.-S	SS_W	MS_W	σ^2_w

S = number of sires
n_i = number of individuals with the i^{th} sire = number of dams mated to the i^{th} sire
k = n_i in expected mean squares
n. = total number of individuals

Procedure: Computation formulae

Source of Variation	Sums of Squares	Mean Square	Variance components
Correction term (C.T.)	$\frac{Y^2_{..}}{n.}$	---	
Between sires	$\Sigma_i \frac{Y_{i.}^2}{n_i} - C.T.$	$SS_S/(S-1) = MS_S$	$\sigma^2_w + \sigma^2_s$
Progeny within sires	$\Sigma_i\Sigma_k\, Y^2_{ik} - \Sigma \frac{Y^2_{i.}}{n_i}$	$SS_W / (n.- S) = MS_W$	σ^2_w

Estimating variance components

$$\sigma^2_S = \frac{MS_S - MS_W}{k}; \qquad \sigma^2_W = MS_W$$

Estimating genetic and phenotypic variances with sex linkage effects zero

$$\sigma^2_S = COV_{HS} = \tfrac{1}{4} VA + 1/16\ VAA + 1/64\ VAAA$$

$$4\sigma^2_S = 4\ COV_{HS} = V_A + \tfrac{1}{4} V_{AA} + 1/16\ V_{AAA}$$

$$\sigma^2_S + \sigma^2_W = V_P \text{ (individual basis)}$$

Estimating heritability

$$h_S^2 = \frac{4\sigma^2_S}{\sigma^2_S + \sigma^2_W}$$

Standard error of h^2

$$S.E.\ (h^2_S) = 4\sqrt{\frac{2(1-t)^2[1 + (k-1)t]^2}{k(k-1)(s-1)}}$$

where "t " is the intra class correlation

$$t = \frac{\sigma^2_S}{\sigma^2_S + \sigma^2_W}$$

The reference population is a large non-inbred stock of groupers. Five series were mated to eight dams each. The 8 week body weight of one male progeny each from every dam is recorded and tabulated below.

Sires				
A	B	C	D	E
687	618	618	600	717
691	680	687	657	658
793	592	763	669	674
675	683	747	606	611
700	631	678	718	678
753	691	737	693	788
704	694	731	669	650
717	732	603	648	690

Observations:
Estimate and record the components of variances and heritability ,viz.

σ^2_S =

σ^2_W =

h_S^2 =

S.E. (h^2_S) =

Inferences:

Charts, tables & figures:

Practical No. 18 & 19 Prepared by Dr. P.C.Thomas

Title : **Full - sib analysis of variance (Single pair matings)**

Objective : To estimate variance components and heritability from full sib data.

Principle : This design can be utilized with multiparous animals like fish where pair mating can produce large number of progenies, which are full sibs.Pairs of individuals are chosen at random from the reference population and mated together. Each mating produces several offspring.

The variance component, σ^2_S is due to the fact that the mating groups differ. The members of these groups are composed of full sibs and therefore the variance component is equivalent to covariance of full sibs.

$\sigma^2_S = COV_{FS} = 2/4\ V_A + 1/4\ V_D + 1/2\ V_A + 1/4\ V_D$

Therefore, sire component of variance represent half of the additive genetic variance.

Analysis of Variance Table

Source of Variation	d.f.	SS	MS	EMS
Between matings	S -1	SS_S	MSs	$\sigma^2_W + K¼\ \sigma^2_S$
Between progeny, within matings	n.- S	SSw	MSw	σ^2_W

S = number of matings
n_i = number of individuals within the i^{th} mating
K = n_i in expected mean squares
n. = total number of individualss

The variance component σ^2_S estimates ½ of the additive genetic variance, ¼ of the dominance variance and various amounts of epistatic variances.

The component σ^2_W estimates the remainder of the genetic variance plus the environmental variance.

Procedure: Computation formula:

The analysis of variance formulas are the same as the previous case for half sibs. However, the σ^2_S is half the additive genetic variance.

The heritability (h^2) = $\dfrac{2\sigma^2_S}{\sigma^2_S + \sigma^2_W}$

The numerator contains an estimate of ½ the dominance variance and all the maternal effects variance. If the dominance and maternal effects variance equals zero, then this heritability will be the same as the previous heritability except for additive interactions.

Because the numerator estimates ½ dominance variance this ratio approaches the genotypic heritability which is the fraction of the total genetic variance to the total phenotypic variance.

The standard error is (equal numbers)

$$S.E.\ (h^2_S) = 2\sqrt{\frac{2\,(1-t)^2\,[1+(k-1)\,t]^2}{k\,(k-1)\,(S-1)}}$$

For unequal numbers

$$S.E.\ (h^2_S) = 2\sqrt{\frac{2\,(n.-1)\,(1-t)^2\,[1+(k_1-1)\,t]^2}{k_1^2\,(n.-S)\,(S-1)}}$$

Observations:
Estimate and record the components of variances and heritability ,viz.

σ^2_S =

σ^2_W =

h^2_S =

S.E. (h^2_S) =

Inferences:

Charts, tables & figures:

Practical No. 20 Prepared by Dr. P.C.Thomas

Title : **Selection differential estimation**

Objective: To estimate actual and effective selection differentials .

Principle : The effective selection differential vary with the number of progeny produced by each parent. Therefore, the selection differential is weighted by the number of progeny to adjust the selection differential and arrive at effective selection differential. Agreement of actual selection differential and adjusted selection differential indicate natural selection complimenting artificial selection.

Procedure / formulae:

A. Actual Selection differential

Mean of the selected individuals minus the poopulation mean

S D = $\bar{X}$- selected - $\bar{X}$ population

B. Standardized Selection differential

Actual selection differential divided by standard deviation

SD / σ

C. Adjusted Selection differential

The actual selection differential may be weighted for the number of progeny produced by each sire and dam.

Problem: From a population of fish, six males were selected on the basis of their 24-week body weight as sires for the next generation. The population mean was 10,867 grams.

Rank	Weight in grams	No. Progeny at 24 weeks (Male + female)
1	12,460	76
2	12,060	62
3	11,440	116
4	11,100	85
5	10,600	83
6	10,360	91

Observations:

Estimate the selection differential and adjusted selection differential .

Actual Selection differential =

Standardized Selection differential =

Adjusted Selection differential =

Inferences:

Charts, tables & figures:

Practical No.21 Prepared by Dr. P.C.Thomas

Title : Prediction of genetic gain from selection

Objective: To predict the gain in a quantitative trait from individual selection.

Principle: Selection differential quantify the mean phenotypic difference between the selected parent and the population from which it has been selected. This consists of variation due to both genetic and environmental factors. Only the genetic portion of the variation will be transmitted to the progeny. Since the heritability is an estimate of the genetic fraction of the phenotypic variation, it can be used to predict the selection gain.

Procedure:

Estimate of the expected genetic gains from selection is obtained by multiplying the SD with heritability estimate.

Gain = SD x h^2

Problem : A reference population consists of a large non-inbred fish population. Individuals were weighed to the nearest gram at 12 months of age. When mature, seventeen males were selected and they randomely mated each to several selected females and their progeny weighed at 12 month of age. The various estimates are given below.

Dams	Sires
$\overline{X}_D$ = 772	$\overline{X}_S$ =1,880 grams
h^2 = 0.3	h^2=0.4
Selected F average = 815	Selected M average = 1952

Observation:

Estimate the expected gain from the selection.

Inference:

Charts, tables & figures:

M.F.SC. MARICULTURE

PRACTICAL MANUAL

COURSE No. MC-510

BIOSTATISTICS AND COMPUTER APPLICATIONS

Course Teacher : T. V. SATHIANANDAN
Faculty Members : M. SRINATH
SOMY KURIAKOSE
WILSON MATHEW
K.G. MINI

Course Title: **Bio-statistics and computer applications**
Course No: **MC-510** Course Credit: **2+1**

Wilson T Mathew

Practical No:1 Title: Disk Operating System

Objective: To get familiar with various commands in Disk Operating System

Principle: Microsoft Disk Operating System.

Equipments/Reagents: Use PC loaded with Windows 98 or higher version.

Procedure: Let the students open Windows operating system and let they Double click at the MS DOS prompt so that they will be at the DOS command prompt. Each student is asked to create a new directory of their choice in C: or D: drive, using MD or MKDIR (directory name).To understand the concept of DIR command let them go to c:\mydocuments directory and use DIR command. To clear the screen let they use CLS command. They are asked to open any text file using TYPE (filename). Let them rename a file using REN (old file name) (new file name). If they are at C:\mydocuments directory they are asked to move to the parent directory viz. C:\ by using CD .. command at c:\mydocuments directory. They are asked to delete a file using DEL command. That is, DEL Filename.extension. Let them format a new or existing floppy disk using FORMAT B: command. Let them copy a few files from hard disk to floppy disk using the syntax:
COPY filename B: and for copying multiple files by using COPY *.DOC B:
To copy files from floppy disk to hard disk use the following syntax, viz.
COPY b:\filename C: or COPY b:\Filename. To copy multiple files from floppy to hard disk use the following syntax:
COPY B:*.XLS c: or COPY b:*.XLS.
To move files from one location to another use the following syntax:
MOVE source location\file(s) target location\file(s)
To remove directory use the following syntax: RD (directory name).
To exit MS DOS command prompt type EXIT.

Observations: NIL

Inferences: Nil.

Charts, Tables, Fig etc.: NIL

Course Title: **Bio-statistics and computer applications**
Course No: **MC-510** Course Credit: **2+1**

Wilson T Mathew

Practical No:2 Title: Windows operating system

Objective: To get familiar with Windows operating system.

Principle: Windows operating system

Equipments/Reagents: PC loaded with Windows operating system.

Procedure: Open any program located on the start-program. Close the program you opened. Identify icon, task bar, system tray and drive. Search for Windows help to see how to create a folder. Create a folder in D: or C: in your name using windows. Create a notepad file and save it in your folder as myfile1. Create second file in notepad and save it in your folder as myfile2. Create third file in notepad and save it in your folder as myfile3.
Open all the three files. Identify the active window, control box, title bar, menu bar, tool bar, work area, status bar, close button, minimize button, maximize button, restore button. Cascade the windows. Switch between the windows. Move windows from one location to another. Minimize all the windows.
Copy one of the files in your folder to floppy disk- (a) by copy and paste method (b) by drag and drop method. Copy all the files in your folder to floppy disk (a) by copy and paste method (b) by drag and drop method. Delete one of your files in the new folder. Create a desktop shortcut for your folder. Create a desktop shortcut for any program, if it does not exist. Change the wallpaper to a different one. Set a screen saver, which will activate after 1 minute. Deactivate the screen saver. Set the monitor such that it will turn off after 2 minutes. Create a shortcut for the floppy drive on your desktop, if it does not exist already. Find particular file starting with **a** and having an extension DOC. Find the file/ files containing the text " CMFRI" in your c: or d: drive. Find files created/ modified between two dates. Locate your recent files in the hard disk without going to windows Explorer or my computer. Arrange icons on the desktop by (a) name, (b) by size (c) by date and (d)by type. Arrange icons in your folder by (a) name, (b) by size (c) by date and (d) by type. Delete the files and folders you created.

Observations: NIL

Inferences: NIL

Charts, Tables, Fig etc.: NIL

Course Title: **Bio-statistics and computer applications**
Course No: **MC-510** Course Credit: **2+1**

Wilson T Mathew

Practical No:3 Title: Excel

Objective: Using Excel how to enter data, save the file, insert a row/column, delete a row/column, insert a worksheet, copy and paste contents of cells to another location, rename worksheet, edit contents of cell, align the contents using standard tool bar. Use of find and replace, use of AutoFill, hide/unhide a row/column, adjust height of rows/width of columns, format a cell using number format, fill the cells using pattern and other formatting tools. Use of format painter. How to give/ delete background to the worksheet. How to clear the format and contents of a cell.

Principle: Microsoft Excel

Equipments/Reagents: PC loaded with Microsoft Excel.

Procedure:

1. Enter the following data in the worksheet with column A has label Sl. No, column B has label Year, Column C has label Species, Column D has label 1 Qr. and so on.

Sl. No	Year	Species	1Qr	2Qr	3Qr	4Qr	Total
1	1996	Oil sardine	12500	24012	18203	50145	
2	1997	Mackerel	8318	42674	27391	38805	
3	1998	Prawns	14752	2634	10105	50873	
4	1999	Cephalopods	3985	1270	402	2641	
5	2000	Crabs	821	130	105	736	

a) Save the data with a file name "landings" and close the file
b) Reopen the file "landings" from file menu - open
c) Rename the sheet 1 as "year"
d) Insert a row between 5th and 6th row of the current work sheet
e) Insert a range of rows (eg 5rows)
f) Insert a column between label name
g) Insert a range of columns (eg 3 columns)
h) Delete the inserted row or range of rows
i) delete the inserted column or a range of columns
j) Insert a new work sheet
k) save the file and close

2. Reopen the file "landings" and do the following exercises

a) Copy/paste cell B1 to cell B10
b) Copy/paste a range of cells A1:G1 to A10
c) Copy/paste the range A2:G6 to A11
d) Undo/redo the copy commands
e) Copy and paste range A1:G6 to a new work sheet
f) Rename the new sheet as "data'
g) Copy / move the range to a new location
h) Copy / move the data to n new work book
i) Save the new work book with a new file name " Catch"

3. Reopen the new work book " Catch"

a) Edit the contents of the cell D1 as Quarter
b) Align the contents using standard tool bar
c) Find and replace " Qr." with " Quarter"
d) Find and replace all zeros (0) with "1"
e) Undo the replacement
f) Fill the series 1,2,3 .. 20 using AutoFill command with starting number 1 and increment 1
g) Repeat the same with different increment
h) Fill the series with drag and drop method
i) Hide / unhide a row / range of rows
j) Hide/ Unhide a column or range of columns
k) Adjust the height of rows
l) Adjust the width of the column
m) Format the cell using number format
n) Format the cells using format, alignment, Font, Border
o) Add bulletin or number to the text
p) Fill the cell with pattern and other formatting tools
q) Copy the format using format painter
r) Give / delete background to the work sheet
s) Clear the format and contents of a cell

Observations: NIL

Inferences: NIL.

Charts, Tables, Fig etc.: NIL

Course Title: Bio-statistics and computer applications
Course No: MC-510 **Course Credit: 2+1**

Wilson T Mathew

Practical No:4 Title: Excel

Objective:
To get familiar with the following concepts of EXCEL:
Wrap text in a cell, merge cells, align text using centre across, change the font size, color, bold and italic. Use of AutoSum, change formula in to values, transpose columns into rows. Add different graphic objects in the worksheet, preview the worksheet, select print area and clear print area, adjust margins, format headings, page numbers etc. How to print worksheet. Use of relative and absolute addressing. Sorting, filtering and autofiltering. How to protect and unprotect sheet.

Principle: Microsoft Excel

Equipments/Reagents: PC loaded with Microsoft Excel.

Procedure:
1. In cell A1 type " Central Marine Fisheries Research Institute, Cochin-14"

i) Wrap the text in cell A1, merge cells
ii) Align the text using center across
iii) Change the font size, Color, bold, italic

2.What would be the results of the following format

=2*3+4, =12/4*4, =12/4+4, =8+3*4, =(8+3)*4

4. Using the following data

Sl. No	Year	Species	1Qr	2Qr	3Qr	4Qr	Total
1	1996	Oil sardine	12500	24012	18203	50145	
2	1997	Mackerel	8318	42674	27391	38805	
3	1998	Prawns	14752	2634	10105	50873	
4	1999	Cephalopods	3985	1270	402	2641	
5	2000	Crabs	821	130	105	736	

i) Find the total landings of 4 quarters using formula
ii) Find the average landings of one quarter using formula

iii) Find grand total landings of 5 years and average landings using function
iv) Find the percentage of landings using formula
v) Copy and paste the formula and function in other cells also
vi) Change the formula into values
vii) Transpose the Columns "Qr." into rows"

4. Add different types of graphic object in the work sheet using drawing tool bars

5. Using the above work sheet

a) Preview the work sheet
b) Select print area and clear print area
c) Adjust the margins, format headings, page numbers etc.
d) Print 1 copy

6. Enter the following details in to a new work sheet

Sl. No	Name	Age	Sex	Basic Pay
1	RAJAN	22	M	10000
2	ABDUL	24	M	6500
3	MINI	23	F	7750
4	KARIM	28	M	8000
5	BINDU	24	F	5250
6	KIRAN	27	M	4500
7	MANOJ	25	M	5500
8	DEEPA	23	F	7800
9	DEEPAK	29	M	6500

a) Create another column with label DA and calculate the DA of each person.

The current DA is 52% of basic pay. Use relative and absolute referencing

b) Sort the data-using Name in ascending and descending order
c) Sort the data using other labels also
d) Filter the data using auto filter
e) Protect and unprotect sheet.

Observations: NIL

Inferences: NIL.

Charts, Tables, Fig etc. : NIL

Course No: MC-510 **Course Title: Bio-statistics and computer applications**

Course credit: 2 + 1

T.V. Sathianandan

Practical No: 5 Title: Problems in Probability Theory

Objectives: Computing probability of events.
Principle: Theory of probability, conditional probability and Baye's theorem
Equipments: None
Procedure: Specific to the problem
Observations:

1. There are three identical boxes each having two drawers. The first box contains a gold con in each drawer. The second contains a silver coin in each drawer; but the third contains a gold coin in one drawer and a silver coin in the other. A box is chosen at random what is the probability that it contains coins of different metals? A box is chosen at random, one of its drawers opened and a gold coin was found. What is the probability that the other drawer contains a silver coin?
2. There are three urns containing black and read balls. The first urn contains 10 black and 20 red balls (all marked 1), second urn contains 15 black and 15 red balls (all marked 2) and the third urn contains 15 black and 25 red balls. These balls were mixed well and one ball selected at random was found to be red. What is the probability that the chosen ball was belonging to the second urn?
3. In a tank there are 200 fishes belonging to two species out of which 125 are males and 75 females. The first species consists of 50 males and 30 females and the second consists of 75 males and 45 females. It is assumed that all the animals have equal chance of infection by a particular disease. A fish infected with the disease is found to be male. What is the probability that it belongs to the first species?
4. A laboratory blood test is 95% effective in detecting AIDS when applied to a diseased person. The test also gives a false result for 1% of the healthy persons tested for HIV infection. In a village it is known that 0.5% of the population are actually infected with AIDS. What is the probability that a person from this village is actually HIV positive when his test result is positive?
5. A family has two children. What is the probability that both are boys when it is known that at least one of them is a boy?
6. Suppose that brown color of eye is a trait controlled by a pair of genes C,c of which C is dominant over c. A person with the genotype CC is dominant homozygous and that with genotype cc is recessive homozygous and those with Cc are heterozygous. Both the dominant homozygous and the heterozygous individuals will have brown coloured eyes. Two hybrid (heterozygous) parents have four children. What is the probability that exactly 3 of them will have brown coloured eyes?

Inferences:
Charts, tables, Fig. etc:

Course No: MC-510 Course Title: Bio-statistics and computer applications

Course credit: 2 + 1

T.V. Sathianandan

Practical No: 6 Title: Fitting Binomial Distribution

Objective: Fit a suitable probability distribution to represent the data on the frequency distribution of number of fishes infested with a disease in 25 tubs with 5 fishes each assuming that the chance of infection remains the same through out the period of experiment. Also examine the suitability of the fitted probability model by a suitable statistical test.

Principle: Probability distribution of a Binomial random variable

Equipments/Reagents: Calculator

Procedure:

- Identify the suitable probability distribution to be fitted for the given data.
- Estimate the parameters of the distribution from the given data.
- Calculate probabilities for each class using the estimated parameters and the identified distribution.
- Calculate the expected frequencies for each class by multiplying the calculated probabilities with the total frequency.
- Compute the test statistic for testing goodness of the fitted data using the observed and expected frequencies for each class.
- Calculate the degrees of freedom for the test statistic.
- Compare the calculated value of the test statistic with the critical value from table and make the conclusions.

Observations:

Infection	0	1	2	3	4	5
Frequency	5	8	6	4	1	1

Inferences:

Charts, tables, Fig. etc: Table of observed and expected frequencies.

Course No: MC-510 **Course Title: Bio-statistics and computer applications**

Course credit: 2 + 1

T.V. Sathianandan

Practical No: 7 **Title: Fitting Poisson Distribution**

Objective: Fit a suitable Poisson distribution for the data and test goodness of the fit.

Principle: Probability distribution of a Poisson random variable

Equipments/Reagents: Calculator

Procedure:

- Identify the suitable probability distribution to be fitted for the given data.
- Estimate the parameter of the distribution from the given data – the mean of the distribution.
- Calculate probabilities for each class using the estimated parameter and the identified Poisson distribution.
- Calculate the expected frequencies for each class by multiplying the calculated probabilities with the total frequency.
- Compute the test statistic for testing goodness of the fitted data using the observed and expected frequencies for each class.
- Calculate the degrees of freedom for the test statistic.
- Compare the calculated value of the test statistic with the critical value from table and arrive at a conclusion.

Observations:

Class	0	1	2	3	4	5	6
Frequency	1	2	7	12	31	42	64
Class	7	8	9	10	11	12	13
Frequency	73	70	68	52	34	21	15
Class	14	15	16	17	18	19	20
Frequency	7	7	3	2	1	1	1

Inferences:

Charts,tables,Fig. etc: Table of observed and expected frequencies.

Course No: MC-510 **Course Title: Bio-statistics and computer applications**

Course credit: 2 + 1

T.V. Sathianandan

Practical No: 8 **Title: Fitting Normal Distribution**

Objective: Fit a suitable probability distribution and examine the suitability of the fitted distribution through a statistical test for the data from a culture pond.

Principle: Probability distribution of a Normal random variable

Equipments/Reagents: Calculator

Procedure:

- Identify the suitable probability distribution to be fitted for the given data.
- Estimate the parameters – mean and standard deviation of the distribution from the given length frequency data.
- Standardize the length classes to work out the probabilities for each class
- Calculate probabilities for each class using the table on cumulative distribution of standard normal variate.
- Calculate the expected frequencies for each class by multiplying the calculated probabilities with the total frequency.
- Compute the test statistic for testing goodness of the fitted data using the observed and expected frequencies for each class.
- Identify the degrees of freedom for the test statistic.
- Compare the calculated value of the test statistic with the critical value from table corresponding to the degrees of freedom and a desired level of significance.
- The fit is suitable when the calculated value is less than the critical value.

Observations: A sample of 250 fishes were collected and their lengths were measured. The following table gives the classification of the length sample.

Length-Class	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45
Frequency	1	3	10	35	77	71	41	9	3

Inferences:

Charts, tables, Fig. etc: Table of observed and expected frequencies.

Course No: MC-510 Course Title: Bio-statistics and computer applications

Course credit: 2 + 1

T.V. Sathianandan

Practical No: 9 Title: Statistical test of significance – z and t distributions

Objectives:

1. Test the hypothesis that the length at maturity of the species is 100 mm.
2. Test the hypothesis that the life length of the item is 700 hours.
3. To examine whether there is any difference in nicotine content of two kinds of cigarettes based on an experiment performed.

Principle: Formulation and testing of statistical hypothesis based on standard normal distribution and Student's t distribution.

Equipments/Reagents: Calculator

Procedure:

- Formulate the null hypothesis and the alternate hypothesis.
- Select the suitable test statistic based on the situation.
- Compute the test statistic using the estimates of sample means, sample standard deviations and sample sizes.
- Decide on the level of significance to be used and calculate the degrees of freedom of the test statistic.
- Compare the calculated value of the test statistic with the critical value in statistical tables corresponding to the degree of freedom and the level of significance.
- Arrive at a conclusion based on the above comparison.

Observations:

1. A sample of 25 fishes belonging to a single spawning that were measured to examine their length at maturity and it was found that the average length is 120 mm. The standard deviation of length is known to be 12 mm.
2. A manufactured item has lasted on the average for 643 hours as revealed by a sample of size 120 with a known standard deviation of 48 hours.
3. A sample of 50 cigarettes of the first kind had an average nicotine content of 2.61 milligrams with a standard deviation of 0.12 milligram where as the other kind had an average nicotine content of 2.38 milligrams with a standard deviation of 0.14 milligrams. Arrive at a conclusion based on a suitable statistical test.

Inferences:

Charts, tables, Fig. etc:

Course No: MC-510 **Course Title: Bio-statistics and computer applications**

Course credit: 2 + 1

T.V. Sathianandan

Practical No: 10 Title: Statistical test of significance – Chi-square and F distributions

Objectives:

1. To test whether the observed data on throat colour and filament colour in tobacco follow the Mendalian ratio of segregation of *F2* generation under dominance.

2. To examine whether the preference over a feed is the same for all the species.

3. Test the null hypothesis that the variance in growth is the same for two feed compositions.

Principle: Formulation and testing of statistical hypothesis based on Chi-square distribution and *F* distribution.

Equipments/Reagents: Calculator

Procedure:

- Formulate the necessary hypothesis – the null hypothesis and the alternative.
- Identify the suitable test criterion for performing the test.
- Calculate the expected frequencies under the assumption that the null hypothesis holds good for the given data.
- Using the observed and expected frequencies compute the test statistic and its degree of freedom.
- Compare the critical value of the test statistic obtained from statistical table for a desired level of significance and the degree of freedom of the statistic with the calculated value of the test statistic.
- Reject or accept the null hypothesis based on the evidence provided by the test.

Observations:

1. To study the segregation of genes that cause throat colour and filament colour in tobacco an experiment was conducted. Given below is the observed segregation in the four phenotypic classes from cross of red-pigmented *Kelieu* (*F1* generation). Here the allelic pair (A,a) represent green and pale throat colour respectively and the allelic pair (B,b) represent green and white filament colour with green dominant over white.

Phenotype	AB	Ab	aB	ab	Total
Frequency	123	30	27	20	200

2. Given below in the table is the data on feed preference by three species to examine whether the preference over a feed is the same for all the species.

	Favoring Feed A	Favoring Feed B	Total
Species-1	232	168	400
Species-2	260	240	500
Species-3	197	203	400

3. Details of an experiment conducted to study the difference in growth due to two types of feed compositions yielded are given below.

$n_1 = 13, \quad n_2 = 16, \quad s_1^2 = 19.2, \quad s_2^2 = 13.5$

Inferences:

Charts, tables, Fig. etc:

Course Title: **Bio-Statistics and Computer Applications**
Course No.: **MC: 510** Course Credit: **2+1**

Practical No.:11 *. Somy Kuriakose*

Title : NON-PARAMETRIC STATISTICS

1. Chi-square Test for Independence of Attributes

Objectives: To examine whether the sampling techniques adopted by the two researchers are independent using chi-square test.

Principle: Testing of statistical hypothesis for independence of attributes based on Chi-square distribution.

Equipments: Calculator, Computer, Microsoft Excel, Systat statistical software

Procedure:

- Set the null and alternate hypothesis
- Identify the suitable test criterion
- Compute the expected frequencies under the assumption that null hypothesis holds for the data.
- Compute the test statistic.
- Specify the level of significance
- Determine the degrees of freedom
- Obtain the critical value of the test statistic at specified level of significance from the statistical tables and compare it with the calculated value of the test statistic.

Observations

Two researchers adopted different sampling techniques while investigating the same group of fishes to find the number of fishes in different length groups. The results are as follows:

Researcher	No. of fishes in different length groups				Total
	10 – 15	15-20	20-25	25-30	
A	75	60	50	15	**200**
B	50	28	20	2	**100**
Total	**125**	**88**	**70**	**17**	**300**

Inferences:

Charts, tables ,fig.etc:

2. Sign Test

Objectives: To examine the joint decision making between husbands and wives in a purchase decision

Principle: Formulation and testing of statistical hypothesis based on non-parametric sign test

Equipments:

Procedure:

- Set the null and alternate hypothesis
- Identify the suitable test criterion
- Determine the significance level
- Find out the sampling distribution
- Make the appropriate inferences by rejecting or accepting the null hypothesis based on the test

Observations: In studying the joint decision-making between husbands and wives in a purchase decision, the following data were obtained from a sample of 10 couples

Sl No.		1	2	3	4	5	6	7	8	9	10
Rating Influence	**Husband**	5	4	6	6	3	2	5	3	1	4
	Wife	3	3	4	5	3	3	2	3	2	3

Inferences:

Charts, tables ,fig.etc

Course Title: **Bio-Statistics and Computer Applications**

Course No.: **MC. 510** Course Credit **2+1**

Practical No.:12 *Somy Kuriakose*

Title: NON-PARAMETRIC STATISTICS

1. Run test-

Objectives: To examine whether the sequence of wins and losses of certain basketball team is random

Principle: Formulation and testing of statistical hypothesis based on non-parametric run test

Equipments: Calculator, computer, Microsoft Excel

Procedure:

- Set the null and alternate hypothesis
- Identify the suitable test criterion
- Determine the number of runs
- Calculate the mean and variance of the number of runs.
- Compute the test statistic.
- Specify the level of significance
- Obtain the critical value of the test statistic at specified level of significance from the statistical tables and compare it with the calculated value of the test statistic.
- Reject or accept the null hypothesis based on the test

Observations: The win-lose record of a certain basketball team for it last 50 consecutive games was as follows:

W W W W W W L W W W W W W W L W L W W W L L W W W W
L W W W L L W W W W W W L L W W L LL W W L W W W

Inferences:

Charts, tables ,fig.etc

2. Kolmogrov Smirnov two sample test

Objectives: To test whether there is any difference in the proportion of errors made by the two groups of students .

Principle: Formulation and testing of statistical hypothesis based on Kolmogrov Smirnov two sample test

Equipments: Calculator, Computer, SYSTAT statistical software

Procedure:

- Set the null and alternate hypothesis
- Find X_{min} and X_{max} for 2 samples and lay out a column of class categories.
- List the cumulative frequencies of the two samples in respective columns.
- Determine relative expected frequencies by dividing by sample sizes.
- Compute the absolute differences (d) between relative expected frequencies.
- Identify largest d
- Compute the test statistic
- Compare the calculated value of test statistic with critical value in table
- Make the appropriate inferences by rejecting or accepting the null hypothesis based on the test

Observations The following table gives the percentage of total errors by two different groups of students

Group I	35.2	39.2	40.9	38.1	34.4	29.1	41.8	24.3	32.4	-
Group II	39.1	41.2	45.2	46.2	48.4	48.7	55.0	40.6	52.1	47.2

Inferences:

Charts, tables ,fig.etc

Course Title: **Bio-Statistics and Computer Applications**

Course No.: **MC. 510** Course Credit: **2+1**

Practical No.: 13 *Somy Kuriakose*

Title : ANALYSIS OF VARIANCE - ONE WAY CLASSIFICATION

Objectives: To examine whether the four feeds have the same effect on the growth of the fish using the data collected in a feeding trail with four feeds

Principle: Test of significance for the difference in means using oneway analysis of variance

Equipments: Calculator, computer, Microsoft Excel, SYSTAT statistical software

Procedure:

- Set the null and alternate hypothesis
- Compute the grand mean and mean of different groups
- Compute Total sum of squares
- Compute Between group sum of squares
- Compute within group (Error) sum of squares
- Determine the Total, Between groups and Error degrees of freedom
- Calculate Between groups mean sum of squares and Error mean sum of squares
- Compute the Test Statistic , F-ratio as the Ratio of Between Group Mean Square to Error Mean Square
- Determine the degrees of freedom for test statistic
- Compare the critical value of the test statistic at specified level of significance obtained from the statistical tables and compare it with the calculated value.
- Comparisons Among Treatment Means using The Least Significant Difference (LSD) and Duncan's Multiple Range Test.

Observations:

Treatments	Net gain in Weight				
Feed I	0.56	0.58	0.51	0.59	0.60
Feed II	0.59	0. 60	0.62	0.55	0.57
Feed III	0.91	0.95	1.00	0.97	0.92
Feed IV	0.65	0.70	0.68	0.71	0.74

Inferences:

Charts, tables ,fig.etc

- Analysis of variance table
- Charts indicating the comparison of means of the four feeds

Course Title: Bio-Statistics and Computer Applications

Course No.: MC. 510 Course Credit: 2+1

Practical No.:14 *Somy Kuriakose*

Title : ANOVA – TWO WAY CLASSIFICATION

Objectives: To determine whether there is any significant difference the total number of euphausiids/1000m^3 of water during three different seasons and five depth ranges

Principle: Test of significance for the difference in means using two way analysis of variance

Equipments: Calculator, computer, Microsoft Excel, SYSTAT statistical software

Procedure:

- Formulate the General Model
- Set the null and alternate hypothesis.
- Compute the grand mean and mean of different groups
- Compute Sum of Squares
- Determine the degrees of freedom
- Calculate Mean Sum of Squares
- Compute the test statistic as the ratio of between group mean square to error mean square
- Determine the degrees of freedom for test statistic
- Prepare the analysis of variance table.
- Compare the critical value of the test statistic at specified level of significance obtained from the statistical tables and compare it with the calculated value.
- Make the appropriate inferences by rejecting or accepting the null hypothesis based on the test.
- If the null hypothesis is rejected, compare the difference among treatment means

Observations:

The data collected onboard FORV *Sagar Sampada* from the Exclusive Economic Zone off the west coast of India on total number of euphausiids/1000m^3 of water during three different seasons and five depth ranges (two samples each) are as follows.

	Depth I	Depth II	Depth III	Depth IV	Depth V
Pre-monsoon	106, 110	95, 100	94, 107	103, 104	100,102
Monsoon	110,112	98,99	100,101	108,112	105,107
Post-monsoon	94,97	86,87	98,99	99,101	94,98

Inferences:

Charts, tables ,fig.etc

- Analysis of variance table
- Charts comparing the means of the two variables

Course Title: **Bio-Statistics and Computer Applications**

Course No.: **MC. 510** Course Credit : **2+1**

Practical No.:15

Mini, K. G

TITLE: CORRELATION ANALYSIS

Objective: To study the degree of association or relationship existing between two variables and to test the significance of association.

Prinicple : By using Karl Pearson's product moment correlation coefficient. Significance of correlation coefficient is tested using t test.

Equipment : Calculator, Computer - Microsoft Excel, Statistical software- SYSTAT

Procedure :

- Compute Σx, Σy, Σx^2 , Σy^2 , Σxy, $\bar{x}$, $\bar{y}$, $\frac{1}{n}\Sigma xy$, $\bar{x}$ $\bar{y}$
- Work out Covariance of (x, y)
- Compute $\frac{1}{n}\Sigma x^2$ $\bar{x}^2$, V(x), $\frac{1}{n}\Sigma y^2$, $\bar{y}^2$, V(y)
- Calculate correlation coefficient
- For testing the significance of correlation coefficient, set the null and alternate hypothesis
- Identify the suitable test criterion
- Compute the test statistic.
- Specify the level of significance
- Determine the degrees of freedom
- Obtain the critical value of the test statistic at specified level of significance from the statistical tables and compare it with the calculated value of the test statistic.

Observations : The total length and standard lengths of 10 fishes of a particular species were measured and given below.

Fish number	1	2	3	4	5	6	7	8	9	10
Total length (mm)	120	114	181	134	126	145	177	124	170	145
Standard length (mm)	93	90	140	104	95	112	135	95	131	112

Inference :

Charts, Tables ,Fig. Etc. : Draw scatter diagram, Prepare the following table

Fish Number	**Total length (mm) x**	**Standard length (mm) y**	**xy**	x^2	y^2
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
Total	Σx	Σy	Σxy	Σx^2	Σy^2

Course Title: **Bio-Statistics and Computer Applications**

Course No.: **MC. 510** Course Credit **2+1**

Practical No.:16 *Mini, K. G*

TITLE: REGRESSION ANALYSIS

Objective : i) To assess the relationship between fish yield and stocking density, obtain a regression equation from the data given below.
ii) To estimate fish yield for stocking density of 5,200/ha.
iii) To Test whether the regression coefficient is significant.

Principle : By fitting the regression equation of fish yield on stocking density. Parameters are estimated using least square technique. T-test is used for testing the significance of regression coefficient.

Equipment : Calculator, Computer - Microsoft Excel, Statistical software- SYSTAT

Procedure :

- Compute Σx, Σy, Σxy, Σx^2, Σy^2
- Work out $b = \dfrac{\Sigma xy - \dfrac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \left(\dfrac{\Sigma x}{n}\right)^2}$
- Calculate $a = \bar{y} - b\bar{x}$
- Substitute the values of a and b in the regression equation

ii)

- To obtain the estimate of fish yield for stocking density of 5,200, Substitute the value of x as 5.2 in $y = a+bx$

iii)

- Set the Null hypothesis to be tested $Ho: \beta=0$
- Alternate hypothesis

- Compute $S^2 = \frac{1}{n-2}\Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2}$
- Work out the Test statistic $t = \frac{b}{\sqrt{S^2/\Sigma x^2}}$
- Determine the degrees of freedom
- Obtain the critical value of the test statistic at specified level of significance from the statistical tables and compare it with the calculated value of the test statistic.

Observations :

The data on fish yield tested under 5 stocking densities are given below.

Sl.no.	1	2	3	4	5
Fingerlings (1000/ha)	3	4	5	6	7
Fish yield (*t*/ha)	3.5	4.6	5.4	6.0	6.4

Inference :

Charts, Tables ,Fig. Etc. :

Sl.no	Fingerlings ('000/ha) x	Fish yield y *t*/ha	xy	x^2	y^2
1	3	3.5			
2	4	4.6			
3	5	5.4			
4	6	6.0			
5	7	6.4			
Total	**Σx**	**Σy**	**Σxy**	**Σx^2**	**Σy^2**

Course Title: **Bio-Statistics and Computer Applications**

Course No.: **MC. 510** Course Credit **2+1**

Practical No.:17

Mini, K. G

TITLE: CHARTS IN EXCEL

Objective : To create different types of charts in Microsoft Excel

Principle : Pictorial representation of data using charts and diagrams . They are visually appealing and make it easy for users to see comparisons, patterns, and trends in data.

Equipment : Computer, Microsoft Excel

Procedure : Use the Chart Wizard to step through the process of choosing the chart type and various chart options – how worksheet data to be represented in a chart – Standard types – Column chart – Sub-types – Clustered column – Stacked column – Clustered column with 3D effect – Stacked column with 3D effect – 3D column chart – Bar chart – Clustered bar – Stacked bar – Clustered bar with 3D effect – Stacked bar with 3D effect – Line graph – Stacked line – Line with markers – Stacked line with markers – 3D line – Pie chart – 3D pie chart – Exploded pie – Exploded pie with 3D effect – Bar of a pie – Scatter diagrams – Scatter with data points connected – Area chart – Stacked area – Stacked area with 3D effects – Surface diagrams – Contour diagrams – Column charts with cylindrical shape – Stacked column with cylindrical shape – Bar with cylindrical shape – Column with conical shape – Stacked column with conical shape – Column with conical shape.

Define data range – Series – Axis values – Category names – Chart data series names – Data markers – Legend – Placement – Output range – Embedded charts and chart sheets – Format chart area – Patterns – Border – Area – Font – Font style – Font size – Effects – Object positioning – Changing of chart types – Changing of source data – Changing of location Other formatting options like cut, copy, paste and clear.

Observations :

1. All India marine fish landings (in t) during 1991 to 2000 is given below

1991	1992	1993	1994	1995	1996	1997	1998	1999	20
2251259	2309696	2276142	2359525	2258832	2414649	2726230	2669480	2435510	26996

Employ an appropriate pictorial representation for the above data

2. Draw a pie chart for the data given below.

Year	Mechansied Landings	Outboard Landings	Non-mechansied Landings	Total Landings
2000	179651	131095	8318	393332

Inference :

Charts, Tables ,Fig. Etc. :

Course Title: **Bio-Statistics and Computer Applications**

Course No.: **MC. 510** Course Credit **2+1**

Practical No.:18 *Mini, K. G*

TITLE: DATA ANALYSIS IN EXCEL

Objective :

1. To work out the mean, variance, covariance, and coefficient of correlation for the data below.
2. Find out the regression equation of fish yield on stocking density for the data given below.
3. To get acquainted with other options in Data analysis using Excel

Principle : Building Mathematical and Statistical functions in Excel

Equipment : Computer , Microsoft Excel

Procedure:

1. To compute mean and variance – Go to Tools menu – Data analysis option – Select descriptive statistics – define Input range – define output options – Summary statistics – covariance and coefficient of correlation – Go to Tools menu – Data analysis option – define input range – Grouping of data – define output range
2. Go to Tools menu – Data analysis option – Select descriptive statistics – define Input range of independent and dependent variable – Labels – Value of constant is zero – Output range – Residuals – Standardized residuals – Residual plots – Line fit plots
3. F-test two sample for variances – Formula – Input range for the two variables – Labels – Alpha – Output range – Fourier analysis – Input range – Labels – Output range – Histogram – Input range – Bin range – Labels – Output range – Cumulative percentage – Chart output – Moving Average – Basic concept – Input

range – Labels – Interval – Output range – Chart output – Standard errors – Random Number Generation – Number of variables – Number of random variables – Distribution – Uniform – Normal – Bernoulli – Binomial – Poisson – Discrete – Parameters – Random seed – Output range – Rank and percentile – Input range – Labels – Output range — Sampling – Input range – Sampling method – Periodic – Random – Output range – t-test – paired t-test – Input range – Hypothesised mean difference – Labels – Alpha – Output range – z-test – Input range – Hypothesised mean difference – Labels – Alpha – Output range

Observations :

1. The total length and standard lengths of 10 fishes of a particular species were measured .

Fish number	1	2	3	4	5	6	7	8	9	10
Total length (mm)	120	114	181	134	126	145	177	124	170	145
Standard length (mm)	93	90	140	104	95	112	135	95	131	112

2. The data on fish yield tested under 5 stocking densities are given below.

Sl.no.	1	2	3	4	5
Fingerlings (1000/ha)	3	4	5	6	7
Fish yield (*t*/ha)	3.5	4.6	5.4	6.0	6.4

Inference :

Charts, Tables, Fig etc. :