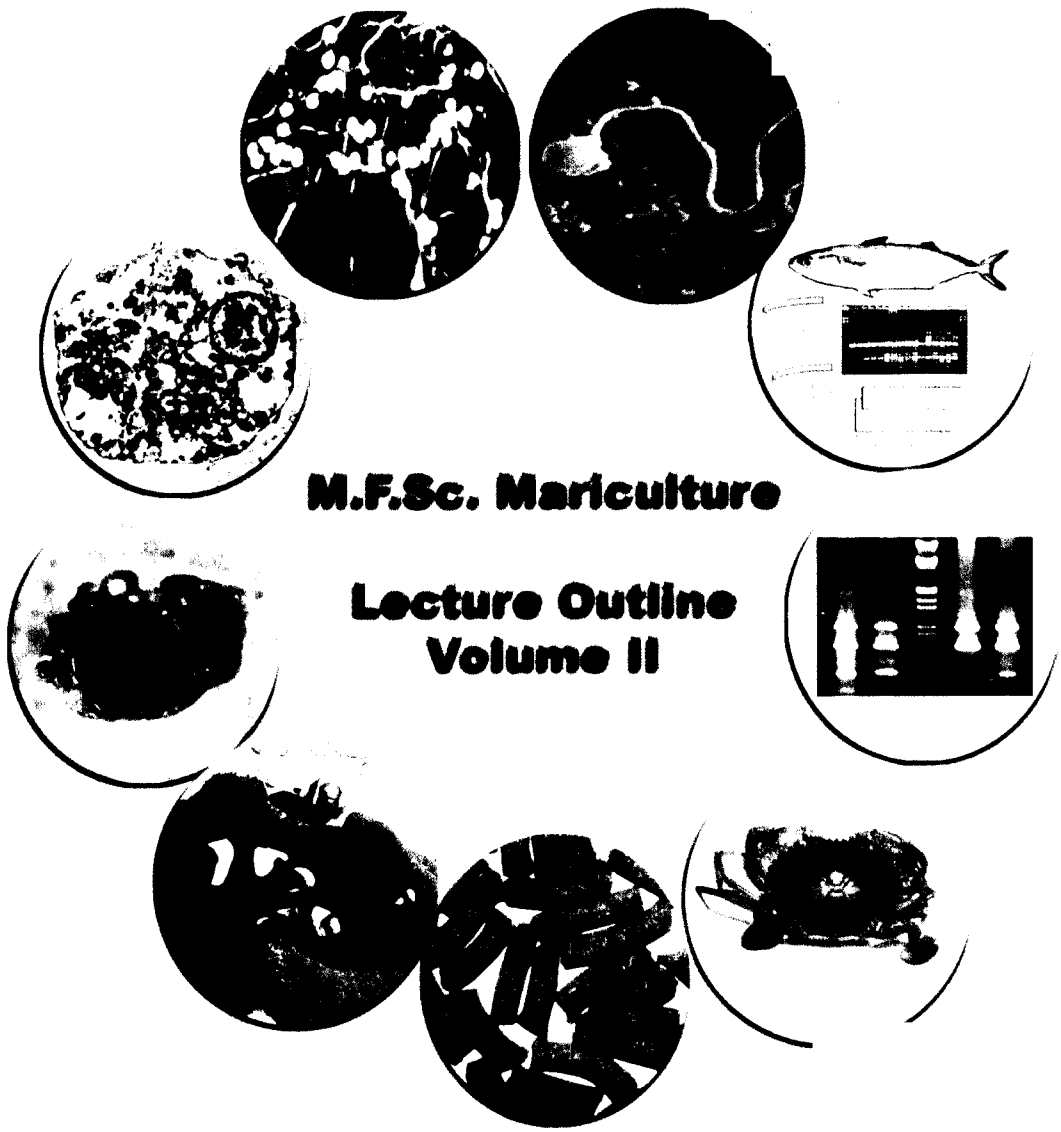
POSTGRADUATE PROGRAMME IN MARICULTURE



M.F.Sc. Mariculture

Lecture Outline
Volume II

भाकृअनुप
ICAR

Central Marine Fisheries Research Institute
P.B. No. 1603, Tatapuram P.O., Kochi-682014
INDIA

M.F.Sc - Mariculture
Fourth semester

LECTURE OUTLINE

Course No. MC-506 (1+1)

FINFISH AND SHELLFISH PHYSIOLOGY

Faculty

Dr.D. Noble
Dr.L. Krishnan
Dr. K.K. Joshi
Dr. Josileen Jose
Dr. K.S. Mohammed

Lecture No.1 L. Krishnan

TOPIC: NEUROENDOCRINE ORGANS IN FISHES

Various neuroendocrine organs in fishes – an overview- their location-structure and function. Short descriptions of pituitary gland, caudal neurosecretory system, thyroid gland, endocrine pancreas, the ultimobranchial gland and the corpuscles of stannous, interrenal bodies and chromaffin cells, juxtaglomerular cells- The hormones produced by these organs etc.

Description on the hormones produced by the above organs and their function. Hormones helping in osmoregulation-migration.

Hormones associated with physiology of reproduction in finfishes-their functions-mode of action. Gonadal hormones, sex steroids and their role-Endocrine tissue in gonads- corpora lutea, sertoli cells- their structure – function.

Pheromones and their role in reproduction.

Lecture No.2 L. Krishnan

TOPIC: PHYSIOLOGY OF REPRODUCTION IN FISHES

Reproduction and its importance- Pituitary gland in fishes – position, structure – division into neurohypophysis and adenohypophysis. Types of cells distributed in the various regions of the pituitary and their function.

Pituitary function in relation to reproduction – Hormones produced by pituitary – their functions.

Gonadal function in relation to pituitary and environmental stimuli. Feedback mechanism. Vitellogenesis.

Pituitary control on gonadal maturation – pathway of pituitary action resulting in ovulation.

The sequential steps involved in maturation, ovulation and spawning in relation to endocrine mechanism – hormones involved.

Lecture No: 3 .K.K.Joshi

TOPIC: REPRODUCTIVE BIOLOGY OF FISHES

Male reproductive organs of fishes – Structure of testis-seminal vesicles- modification of sperm ducts-Seasonal variation- Maturation-stages of maturity- Comparison between elasmobranchs and teleosts-Description of Testes- Morphology-variations Discussion on the Evolutionary significance.

Description of different stages of maturation in testis leading to production of sperm- Definition of gonadosomatic Index- method for estimation of Ganado somatic index- Uses – applications of GSI.

Lecture No: 4 K.K.Joshi

TOPIC: REPRODUCTIVE BIOLOGY OF FISHES

Female reproductive system- Morphology of Ovary- Types of ovary- Structure of ovary- Oviduct- modification of oviducts-Stages of maturation of ovary-Details of maturational stages- Criteria for determining different maturity stages-

Description of ovulation –spawning. Seasonal changes in maturation- its significance- Definition of fecundity- Methods of estimation of fecundity- Ova diameter and related studies-Significance of ova diameter frequency studies- Comparison of female reproductive structures in elasmobranchs and teleosts.

Lecture No:5 K.K.Joshi

TOPIC: REPRODUCTIVE BIOLOGY OF FISHES

Definition of sexual dimorphism- examples- secondary sexual characters- examples- definition of oviparity- ovoviviparity- viviparity in fishes- examples -Significance-examples from teleosts and elasmobranchs.

Sex differentiation and Sex determination in fishes- Spawning - Fertilization- formation of fetilisation membrane- significance- process involved in sex differentiation- environmental influence - role of hormones.

Lecture No.6 K.K.Joshi

TOPIC: REPRODUCTIVE BIOLOGY OF FISHES

Reproductive biology of mullets- Description of gonads- Morphology and structure -stages of maturation- fecundity- spawning- spawning season- Spawning grounds- influence of environment.

Reproductive biology of groupers- Description of gonads-Morphology and structure- stages of maturation- fecundity-spawning- spawning season- Spawning grounds - Importance to mariculture- influence of environment.

Reproductive biology of sea bass - ornamental fishes- pearl spot.

Lecture No : 7 L. Krishnan

TOPIC : INDUCED MATURATION IN FISHES PARENTAL CARE

Definition of induced maturation Role of environmental factors in maturation. The pathway of action as to how the environmental factors trigger maturational process - -Methods employed in inducing maturation–

Use of steroids/ hormones in inducing maturation

Use of testosterone through diet in inducing females to change sex to get males. Examples

Environmental manipulation in attainment of maturation of fishes-methods adopted.

Importance of nutrition in broodstock, reproduction and survival of larvae-Parental care in fishes-examples- Different adaptations

Lecture No:8 L.Krishnan

TOPIC: SEX REVERSAL IN FISHES

Definition of sex reversal- Hermaphroditism - types of hermaphroditism- examples.

Process of sex reversal in fishes-sex reversal in groupers and sea bass. Cellular changes during sex reversal - description of the process.

Structural changes of gonads in relation to sex change -histological details of gonads during sex reversal.

Use of steroids in sex reversal. Practical application of sex reversal in aquaculture. - Examples.

Lecture No:9 K.K.Joshi

TOPIC: GAMETOGENESIS IN FISHES

Definition of spermatogenesis- steps in spermatogenesis- Process of mitosis and meiosis. Changes in chromosomal numbers and its significance.

Detailed structure of sperm- Variations in structure of sperms.

Polyspermy- motility of sperms behaviour of sperms during fertilization.

Lecture No:10 K.K.Joshi

TOPIC: GAMETOGENESIS IN FISHES

Definition of oogenesis-steps in oogenesis- Process of mitosis and meiosis. Changes in chromosomal numbers and its significance.

Structure of egg- polarity - different egg membranes- classification of eggs.

Definition and the process of vitellogensis- yolk synthesis- auto synthesis- heterosynthesis- mobilization of yolk.

TOPIC: MALE AND FEMALE REPRODUCTIVE SYSTEMS IN CRUSTACEANS

Male and female reproductive systems - shrimps, crabs and lobsters – stages in maturity – secondary sexual characteristics – sex differentiation – sexual dimorphism – external characteristics – sex differentiation – sexual dimorphism – external characteristics – gonopores – thelycum and petasma in penaeid shrimps – pleopods in male crabs and their structure and functions – pleopods in female crabs and their structure and functions – maturation - maturation moult / puberty moult in crabs – pre-copulatory embrace – mating – sperm transfer – spawning - embryonic development – duration and egg size – incubation in crabs and lobsters – changes during incubation – hatchuing mechanisms – hatching rhythms – larval stages.

Physiology of reproduction – in females – oogenesis – oviposition - reproduction and moult - reproduction and regeneration - accessory sex glands and their functions – in males - spermatogenesis – seminal plasma – male accessory sex glands – spermatophore and its formation – nurse cells – sperm storage in females - moulting and spermatogenesis – mating and transfer of sperms – factors influencing reproduction – genetic - neural – hormonal – conclusions.

Induced maturation in crustaceans – eye stalk ablation – hormones associated with reproduction – environmental manipulation – effect of nutrition - rematuration system – brood stock development – artificial insemination – conclusions.

Lecture 12 Josileen Jose

TOPIC: GROWTH IN CRUSTACEA

Introduction – the process of moulting - different steps involved in moulting – moult cycle – stages of the moult cycle – ecdysis – metecdysis – stages A - B – C1 to C3 – Anecdysis - C4 - pro ecdysis - D0 – D1 - D2 – D3 – D4 – role of astaxanthin – problems associated with moulting – effect of limb loss and regeneration – terminal anecdysis – moult inhibiting hormone – natural stimuli – effect of eye stalk ablation on moulting – moulting during different stages of growth – autotomy – Y-organs – structure – changes associated with moult cycle – functions – degeneration of Y-organs – effect of exogenous ecdysteroids – regeneration.

Absolute growth – growth format - moult increments during successive moultings – effects of size – effects of sex and maturity – loss of appendages – food supply – light – salinity – temperature – intermoult period – effect of size - sex - maturity – loss of appendages – food supply - effect of light – salinity – temperature - effect of parasitism – methods of determining age and growth – polymorphism – heterochely – egg production and growth rate – sexual difference in moulting – conclusions.

Lecture 13 K.S. Mohamed

TOPIC: REPRODUCTIVE PHYSIOLOGY OF MOLLUSCS

Reproductive Methods – Gonochorism – Sexual dimorphism and proportion of sexes

Hermaphroditism – Bivalvia - Gastropoda

Self-fertilization – Parthenogenesis

Gametes – Maturation – Biochemical changes – Environmental influences - Nutrients

Spermatozoa – Types of sperm – spermatophores

Eggs – Size and fecundity – Covering

Fertilization – Spawning – Effect of temperature and lunar periodicity – Courtship and copulation – Cephalopods – Oviposition – Spawn and capsules – Incubation – Ovoviviparity – Viviparity

Neuroendocrine control of reproduction

TOPIC: PHYSIOLOGY OF GROWTH IN MOLLUSCS

Absolute and relative growth – instantaneous relative growth rate

Length-weight and dimensional relationship - Allometry

Methods of growth measurement – Growth rings – Linear and weight measurements – Tetracycline as marker

Population sampling

Influence of environment – nutrients

Variability and abnormality in growth

Metabolic aspects of shell formation – uptake of calcium – carbon dioxide – organic compounds – inorganic ions

Conditions affecting rate of shell formation

TOPIC: PHYSIOLOGY OF DIGESTION IN MOLLUSCS

Digestive system stomach digestive gland phasic activity Digestive enzymes

Food intake through siphons –trapping of food particles – labial palps

Function of Ctenedia in feeding

Function of cilia control of gill cilia

Filtration rate ingestion rate change with size – temperature – salinity

Particle size and concentration – pseudofaeces production - absorption efficiency

TOPIC: ENVIRONMENTAL PHYSIOLOGY OF MOLLUSCS – RESPONSE TO METALS

Susceptibility to metal bioaccumulation and implications of bio monitoring

Metal turnover in oysters and mussels

Cellular mechanisms of metal sequestration – Granular deposits – lysosomes - metallothioneins – other metal binding proteins

Toxic effect of metals on molluses

Tributyltin and oysters

Public health implications of metal bioaccumulation

TOPIC: CRYOPRESERVATION OF GAMETES

A. Spermatozoa

Definitions – cryogenics, cryobiology, cryopreservation.

Brief history of cryopreservation – initial efforts to preserve fish spermatozoa – present status – physiological changes in cells connected with cooling, ice crystal formation and salt accumulation, consequent physiological and chemical injuries – slow and fast freezing – cryoprotectants – penetrating and non-penetrating types – quality of cryoprotectants – mechanism of prevention of cryoinjuries – role of extender – various extenders. milt collection – quality assessment and evaluation of sperm fitness – androgammon and K +ions in milt – concept of suspended animation – motility of spermatozoa. Extender : cryoprotectant : milt ratio – dilution – media used for cryopreservation – liquid nitrogen - dry ice – liquid helium etc. cryopreservation protocol – French straw/pellets – sperm storage – testing the viability of cryopreserved milt – cryopreservation of crustacean and molluscan spermatozoa .

TOPIC: CRYOPRESERVATION

B. Egg and embryo, artificial insemination and *in itro*fertilization

Egg and embryo cryopreservation – problems and prospects in teleosts and shellfishes. Ice seeding, programmed slow cooling, vacuum equilibration and super cooling – freezing and transfer of nucleus – viability of gametes-future prospects.

Artificial insemination and *in vitro* fertilization – activation of ova – closure of micropylar apparatus – post-thaw motility – insemination temperature – insemination medium – optimum sperm concentration for high fertility.
Role of cryopreservation in mariculture and genetic conservation – research activities in India and abroad – future prospects.

TOPIC: EXCRETION AND OSMOREGULATION

Excretory end products of metabolism – participation of liver, gill, kidneys and certain other tissues in their synthesis and selective handling – patterns of excretory end products - ammonia – urea – trimethylamine oxide and other non-protein nitrogenous products – inorganic products – conjugation – detoxification- deamination and transamination.

Classification of animals according to the major end products of excretion – excretory products in teleosts, cartilagenous fishes etc.

Carbon dioxide excretion , role of gills – pattern of carbon dioxide excretion in elasmobranches and teleosts.

Concept of '*milieu interior*' – cell membrane separating the extra cellular environment from the internal environment – difference in concentration of ions and electrical charges – maintenance of differential distribution – passive diffusion and active transport. Homeostatic mechanisms to changes in external ionic concentration in teleosts.

Lecture No. 20 D. Noble

TOPIC: HORMONAL CONTROL OF OSMOTIC AND IONIC REGULATION

Developments in cellular, molecular, tissue culture, biochemical and electrophysiological approaches in the areas of ion regulatory mechanism endocrinology of teleost osmoregulation and adaptive responses – role of prolactin – cortisole – growth hormone and other rapid acting hormones and neurotransmittors – effect of second messengers of rapid acting hormones – ion transport by urinary bladder, intestinal cells, renal proximal tubules, rectal gland epithelial cells in sea water teleosts.

Chloride cell function, calcium transport – hormonal regulation – cortisole, growth hormone – insulin like growth factor – prolactin – thyroid hormones and sex steroids.

Lecture No. 21 D. Noble

TOPIC: ENVIRONMENTAL STRESS ON CULTIVABLE FISHES, CRUSTACEANS AND MOLLUSCS, BIOTIC AND ABIOTIC FACTORS, AMELIORATIVE MEASURES

Fish's environment – response of fishes, crustaceans and molluses to environmental changes – basic concepts of stress response – definition of stress – classification of stress – biotic and abiotic factors – lethal and sub lethal – acute and chronic stress in culture systems – manifestations of stress – endocrine effects – primary and secondary effects – stress and diseases – immune responses – specific and non specific responses.

Monitoring of stress – biological, hormonal, biochemical, immunological, histological and other indices. Fishery environment management – maintenance of optimum water quality, proper stocking densities, nutritional strategies. Amelioration of stress – immunostimilants, probiotics, anaesthetics, stress tolerant strains for aquaculture.

M. F. Sc.- (MARICULTURE)

MC- 507

FINFISH AND SHELLFISH NUTRITION

Course Teacher: Dr. Imelda Joseph

Faculty Members: Dr. R. Paul Raj
Mrs. K.G. Mini

CONTENTS

25. Conventional Feed Ingredients
26. Non-conventional Feed Ingredients
27. Anti- Nutritional Factors
28. Additives in Feeds I
29. Additives in Feeds II
30. Nutritional Enrichment of Feedstuffs and Live Feeds
31. Feed Formulation
32. Linear Programming and its application in feed formulation
33. Feed types used in aquaculture
34. Feed Manufacturing Process I
35. Feed Manufacturing Process II
36. Extruded Feeds
37. Feed handling and Storage
38. Quality control and Quality Assurance
39. Feeding Strategies
40. Feed Dispensing Methods
41. Factors Affecting Feed Performance
42. Feeding Management

Principles of Nutrition and Historical Background

Introduction to finfish and shellfish nutrition- difference between nutrition of terrestrial animals (livestock/ poultry) and aquatic organisms – accuracy of methodologies; basic and applied nutrition – overview of nutrition and feed development.

Nutritional characteristics of fish and other aquatic animals (crustaceans/ mollusks) – zoological - large inter specific diversity - variability of requirements; Biological- larval stages- continuous growth- stomachless species; Physiological - poikilothermy- ammonotelism- high net utilization of protein energy; Ecological- temperature- oxygen content- relatively high environment viscosity- high density of environment- pH - salinity- unique aquatic food chains- herbivores- omnivores- carnivores.

Diet and growth; diet and environment interaction; principal waste output and associated stress.

Historical background- advances in fish and shellfish nutrition in the 20^{th} century- proteins- essential amino acids- lipids- fatty acids- sterols- energy- protein energy ratios- vitamins- minerals- carotenoids- advances in aquafeed technology- hard pellets, soft pellets, floating pellets, microcapsules, flakes; development of extruded feeds- linear programming for feed formulation; principles of chemical and biological evaluation of feeds- quality assessment and quality assurance; aquafeed demand projections.

Lecture 2 Prepared by Dr. Imelda Joseph

Nutritional physiology

Introduction-

Nature of fish feeds- Feeding behavior and its importance in nutrition- predators- grazers- strainers- suckers –parasites;

Ingestion- definition- morphological and physiological characters to match food and feeding habits- organs involved- mouth and buccal cavity- teeth- stomach and intestine- chemoreception-stimuli for feeding- External signals- environment- feed; Internal signals- hormonal- nervous- metabolic (nutrients); Food intake- Appetite- arousal and search- location and identification- capture- orobranchial manipulation and testing- swallowing or rejection- satiation- drive reduction.

Techniques for measuring voluntary intake of feed- manual feeding until satiation- supply of feed in excess- containing a proportion of substance identifiable by X-ray or a radioactive label- demand feeders.

Factors affecting voluntary intake of feed- nutritional factors- complete in requirement; environmental factors- temperature – thermal optimum stage; chemical characteristics- dissolved oxygen- ammonia- nitrites etc.; behavioral factors- inhibition in feeding due to various forms of stress- handling- presence of predators- low density- high heterogeneity in growth rates- high density- reduced living space- competition.

Diurnal pattern in feeding- examples- adaptability.

Digestion

Classification based on food ingested; herbivores – detritivores – omnivores – carnivores; groupers based on feeding conditions; pelagic plankton feeders – benthos feeders; surface and column feeders; functional adaptations related to nature of food – moult, buccal cavity and pharynx – predigestive processing of food – oesophagus/ gullet' – structure – stomach – stomachless fishes – fish with stomach – structure – anterior and pyloric regions – intestine – structure – Rectum – pyloric caecae - Digestion – process – digestive fluids and enzymes – gastric secretions – HCl; function; gastric glands – zymogen – trypsinogen, pepsinogen, HCl, pepsin – functions; Pancreas – bicarbonate – functions; proteases – (trypsin – chymotrypsin – carboxy peptidases) – esterase functions; Bile (liver) – bile salts – organic anions – cholesterol – functions; Intestinal enzymes – aminopeptidases (alkaline/acid) polynucleotidase – lecithinase – functions; Digestive enzymes –hydrolases – water soluble proteins

Digestion – extracellular – lumen of alimentary canal – protein digestion – zymogens – acid hydrolysis/proteolytic action; peptides link breakdown – exopeptidases – endopeptidases; pepsin hydrolyses band on amino side; - Trypsin on peptide bands between arginine & lysine; exopeptidases – carboxypeptidases – aminopeptidases – dipeptidases; all three exhibits specificity; functions; Fat digestion – liver major role – bile activity on food; lipase activity – little substrate specificity – (7.0-7.5 pH carbohydrate diestion; - in pancreatic juice –α amylase optimum pH 6-8; Microbial digestion – cellulose digestion and protein synthesis – cellulase enzymes – abundant in macrophyte feeders – exhibit proteolytic and amylolytic activity chitinase and lecithinase activity – conclusion.

Lecture 4 Prepared by Dr. Imelda Joseph

Digestive enzymes and their kinetics

Enzyme kinetics – definition – factors affecting enzyme catalyzed reactions – enzyme concentration – pH – ionic strength – temperature – substrates – products – inhibitors – activators

Kinetic equation – principles of enzyme kinetics – principle of mass action – enzymatic reaction – concentration of substrate – products – active enzyme – conversion of substrate (A) to product (P) Rate of inversion – First order reaction – (A) decreases – (P) increases – second order reaction. Two substrates –(A) +(B) P – rate of substrates decreases proportionately – Zero order reaction – enzyme limited concentration of substrate determines rate of reaction – substrate concentration and enzyme reaction E+A=EA E+P – Michelis-menten equation – four commonly used transformations of michelis equation – Line weaver Burk equation. Eadic- hofstee equation; Hanes- woolf equation; Eisenthal and cornsh-Bowden equation-Direct linear plot.

Kinetics of enzyme inhibition – complete inhibition - Bixon plot-non-competitive-inhibition-non-competitive anticompetitive

Lecture 5 Prepared by Dr. Imelda Joseph

Absorption and assimilation of feed

Definition- mechanism of absorption- Products of digestion diffusion – active transport-eg: active transport-uptake-uptake of glucose involving a carrier- energy requiring process-moves glucose across a membrane to epithelical cells-irrespective of glucose concentrators in cells-Diffusion facilitated – carrier system to move across impermeable membrane- eg: Fructose absorption – no energy required-movement based on concentrator gradient-simple diffusion require no carrier or energy-*eg*: fatty acids absorption-micelles or droplets fatty acids and bile salts-epithelic cell surface lipids released call membrane-lipids reform droplets called chylomicrons move through cell-circulation – transportation to liver.

Antibiotics influence absorption – physiological alterion in gut-physical/chemical interaction between drugs ingested material-some increase absorption unsaturated fatty acids- absorption by endocytosis-absorption by diffusion or transport- non- symptotic kinetics- Amino acid and peptide absorption- sodium dependent process- absorption of proteins- lipid absorption via micelles- transport of plasma lipids-absorption of carbohydrate- vitamin absorption- mineral absorption.

Transport and Nutrient exchange;

Definition of Requirements for Growth and Reproduction

Macronutrients/ Energy Nutrients

Major components of body tissue- carbon- hydrogen- oxygen- nitrogen- sulphur- supplied through diet- these are essential for maintenance- activity- growth- reproduction.

Protein and Amino acids I

Protein - Definition- Structure- Major functions-protein synthesis- production of other compounds-Requirement for fish and shellfish; Amino acids- amino acid metabolism- important amino acids- essential amino acids (EAA) - free amino acids and those linked to proteins (peptide chains)- synthesis of amino acids- utilization of amino acid- dietary amino acid requirements of fish- determining amino acid requirements- expressing amino acid requirements- amino acid amino acid interactions- meeting amino acid requirements- utilization of crystalline amino acids- - EAA balance in the diet- limiting amino acids- supplementing diets with amino acids- synthesis of other nitrogenous compounds- amino acids as precursors of other nitrogenous compounds- oxidation of amino acids- excretion.

Lecture 7

Prepared by Dr. R. Paul Raj

Protein and Amino acids II

Factors influencing protein metabolism- dietary protein requirement- quantitative requirements- factors influencing protein requirement- age- abiotic factors like temperature- nutritional factors- biotic factors- requirement for proteins and EAA- experimental diets- nutritional quality of proteins- digestibility- digestible energy to protein ratio; biological value (BV)- net protein utilization (NPU)- evaluation criteria- protein efficiency ratio (PER)- productive protein value (PPV)- Dietary protein composition- chemical score- EAA index- dietary non protein nitrogen and its utilization by some fishes- protein sources- plant origin- animal origin- Non-protein nitrogenous constituents formed from amino acids in animals- biological compound- amino acid precursor- physiological functions- Protein retention and excretion.

Lecture 8

Prepared by Dr. R. Paul Raj

Lipids

Lipids- definition – carbon atoms in chain-ring forms-substances of biological origin-soluble in organic solvents sparingly soluble in water-high energy storage molecules-components–cell-memberanes-classes-fattyacids-triglycerides-phospholipids-sterols-sphingolipids Types-classification- non-polar lipids- polar lipids- sterols- waxes- fats-phospholipids- sphingomyelins; Functions of lipids- source of dietary energy-gross and digestible energy levels of lipids and stored energy-calorific value of lipids- digestibility of lipids- Lipid metabolism

Fatty acids: fatty acids long chain hydrocarbon side groups- carbocyclic acids-nomenclature- *eg*: C_{18}: 3 (n6) – C; 18 = no: carbon atoms- 3 number of double bands- 6- the carbon at which first double bond-non-carboxyl COOH end- fatty acid occur esterified- triacylglycerols(TAG) triesters-glycerol- TAG- less oxidized than carbohydrates or proteins-stored anhydrous form-six times metabolic energy provided.

Saturated- mono unsaturated- poly unsaturated- highly unsaturated- fatty acid nomenclature - fatty acid metabolism- synthesis of saturated, monounsaturated and poly unsaturated fatty acids; bioconversion of oleic, linoleic and linolenic series fatty acids in fish and shellfish -.

Protein sparing action of lipids in fish and crustaceans; natural lipid sources- plant, animal and microbial sources; negative aspects-cyclopropenoic acid; oxidation, rancidity.

Lecture 9 Prepared by Dr. R. Paul Raj

Essential fatty acids (EFA)

Definition; Functions- Carriers of fat soluble vitamins ADE&K- lipid transport- digestive utilization and lipid metabolism in fish- lipid digestibility- storage- transport- mobilization- functions-lipid and fatty acid requirements- estimated by using optimal dietary concentrations of other energy yielding nutrients- lipid sources of high digestibility-

EFA- endogenous synthesis and bioconversion of fatty acids- main fatty acids present in fish lipids- role of EFA- deficiencies- requirements for EFA and meeting the requirements- dietary liid composition and reproduction- influence of dietary lipid composition on fish health- problems of balance and excess.

Protein sparing action of lipids- role of fatty acids in growth promotion- brain development- prostaglandin- cellular and sub cellular membranes.

Phospholipids – glycerophospholipids -major lipid components Cellular and sub cellular biomembranes- Glycerophospholipids- similar structure to TAGS – re fatty acid chain-replaced by phosphate attached to another organic molecule; Phosphatidylcholine- Phosphatidylethanolamine- Phosphatidyl serine and phosphatidyl inositol- phosphatidyl choline- Functions- Role of phospholipids in aquaculture diets; Sphingolipids – membrane component – nerve cells –charged 'head' region –a po_4 group as in glycerophospholipids;

Cholesterol- sterols – four fused carbon rings -Functions- role in moulting- growth- and reproduction- dietary requirement – precursor – steroid hormones – bile acids-Relationship between dietary lipids and tissue lipids- effect of dietary lipids on fish quality- synthesized in fish-not in crustaceans -Lipid as dietary energy source- marine and non- marine sources in feeds- effect of lipid on growth and body composition- anti oxidants on storage of feeds and PUFA rich oils- Negative aspects of lipids- lipid oxidation- rancidity.Fatty acid oxidation- Libertaes energy-in-two steps-priming/activation; β oxidation reactions-cyclic process-four steps-each cycle liberate two carbon atom-fatty acid-produce acetyl CoA-oxidized in citric acid cycle-release energy.

End product fatty acid synthesis-palmittic acid (C16:0) precursor- longer chain saturated fatty acids.

Regulation – fatty acid metabolism-peptide hormones insulin-stimulates fa synthesis –glucagon-stimulates degradation-secreted by pancreatice tissue.

Carbohydrates

Classification of carbohydrates- monosaccharides; trioses- tetroses- pentoses- hexoses- oligosaccharides; disaccharides-trisaccharides- tetrasachharides- pentasaccharides- polysaccharides; homopolysaccharides- heteropolysaccharides- others- chitin- n-acetyl glucosamine; . Nutritive value – cheaper source of energy- Digestion- digestibility- utilization efficiency of carbohydrates- carnivores- herbivores- omnivores-hydrothermal treatment for better digestibility- starch gelatinization- cooking extrusion- flaking- toasting- protein sparing effect- glucose- starch- amylose- amylopectin- stored carbohydrate- glycogen- glucose metabolism- intolerance phenomena when >20% simple sugar added in diet- apparent digestibility of carbohydrate- 20- 75%- species dependent- hyperglycemia and control by insulin- blood glucose levels- glucose phosphorylation- conversion of glucose to glucose d phosphate by hexokinases- carbohydrate catabolism- glycolysis-transformation of glucose to pyruvate with ATP formation- lactate formation in anaerobic conditions- synthesis - gluconeogenesis- formation of glucose from non-carbohydrate substrates- amino acids and gluconeogenesis- alanine- serine- enzyme activities and protein content in diet- glycogen reserves- hepatic reserve- brain- red muscle and white muscle- glycogenolysis- and mobilization of glycogen reserves under stress- hypoxia- exercise- importance and limits of the energetic role of carbohydrates- crude energy content- fibre and filling agents- definition- cellulosic compounds that cannot be hydrolyzed by higher vertebrate enzymes- lignin a polyphenol- assays- weende method- evaluation of cellulose and lignin- van soest method- nutritive roles- partial digestion in stomach by HCl and microbial enzymes- digestibility more in herbivores- negligible in fish- other roles- as binders- alginates- agar- guar gum- carrageenans- carboxymethyl cellulose- chemically transformed cellulose.

Lecture 12 Prepared by Dr. Imelda Joseph

Vitamins

Definitions- term coined by Funk (1910)- amines necessary for life- organic compounds – catalytic compounds required in minute amounts- classification of vitamins-group neither chemically or functionally homogenous- water soluble vitamins- B group and C – B group fundamental to intermediate metabolism- coenzymes- fat soluble vitamins- A, D, E and K- has varied roles- simple coenzyme vitamins- thiamine- functions- carbohydrate metabolism-thiamine mono, pyro and triphosphates- role in neurotransmission- Pyridoxine- function- amino acid metabolism- pyridoxal phosphate- neuromediators- synthesis- pancreatic enzymes- glycogen metabolism- immuno functions- Biotin- function- carboxylation- decarboxylation- folic acid and polyglutamates- function- monocarbon transfer reactions-cyanocobalamine- metabolism of folates- vitamin A- retinal- retinal- functions- vision- as growth hormone- transfer and oxidation reduction coenzyme vitamins-riboflavin- functions- FMN- FAD-oxidation reduction reactions of catabolism like beta oxidation- L or D amino acid- degradation of purine bases (adenine and guanine)- metabolites entering TCA cycle- glutathion reduction- Niacin- functions- NAD and NADP- hydrogen transporters- pantothenic acid- Coenzyme A and Acyl carrier protein- cholestrol biosynthesis- K vitamins- phylloquinone- menaquinone- menadione- anti-oxidant vitamins-Ascorbic acid (vitamin C)- functions- hydrogen donor- collagen maturation- neutralisation - free radicals-reproduction- immune response ractions- tocopherols (Vitamin E)- functions- alpha tocopherol- anti oxidant vitamin- reproductive functions-Calciferol (vitamin D)- prohormone- ergocalciferol- cholecalciferol- steroids- quasi vitamins -choline- part – phosphatidyl choline- inositol- meso and myo inositol- functions- structural role- constituent- phospholipid- growth factors- orotic acid- ubiquinone- lipoic acid- carnitine- stability and availability of vitamins-stable forms of vitamins.

Lecture 13 Prepared by Dr. Imelda Joseph

Vitamin Requirements, Sources and Available Forms

Vitamin requirement- based on performance- tissue storage -specific deficiency signs- Recommended supplementation level in commercial shrimp and fish feeds - Thiamine – Riboflavin- Pyridoxine- Pantothenic acid- Niacin- Biotin- Inositol- Choline- Folic acid- Cyanocobalamine- Ascorbic acid- Vitamin A- Vitamin D- Vitamin E- Vitamin K- Vitamin E and flesh quality - Factors affecting Vitamin C bioavailability- L ascorbic acid (most common form) - diketogluconic acid- degradation- Leaching- Processing factors- Use of coated or protected form of ascorbic acid - stable derivatives- Ascorbyl 2- monophosphate (AMP)-Ascorbyl polyphosphate (APP)- Factors influencing dietary vitamin requirements- Species- age- size- rearing conditions- maturation-dietary components - leaching from feeds in the water -manufacturing condition - natural food supplies-Exposure to stress –Sources- forms and activity of vitamins added to fish feeds- production by industrial processes- chemical synthesis- fermentation- Forms- Fat soluble vitamins- Vitamin A and Vitamin D- beadlet- Vitmain E -Vitamin K as menadione (K3) - Menadione sodium bisulphate (MSB, K3)- Menadione nicotinamide bisulphate (MNB, K3)- Menadione sodium bisulphate complex (MSBC, K3)- Menadione dimethylpyrimidinol bisulphate (MPB, active K3).Water-soluble vitamins- Thiamine- thiamine monotitrate-Riboflavin (B2)- Pyridoxine (B6) as pyridoxine hydrochloride- Pantothenic acid as calcium d pantothenate (90% PA). Niacin as niacinamide or nicotinic acid-Biotin as d biotin- Folic acid -dry dilution- Choline - chloride salt-Inositol as Myoinositol - Additional factors influencing vitamins in feeds- Processes used- manufacture (encapsulation, spray drying, coating, adsorption, compaction, and high shear granulation)- formulation processes - Simple rules for vitamin preservation - Measuring vitamin levels in feeds- A, D3, E, K3, thiamin, riboflavin, niacin and ascorbic acid by HPLC- Pyridoxine, B12, pantothenic acid, biotin, and folic acid by microbiological assays.

Lecture 14 Prepared by Dr. Imelda Joseph

Minerals

General- inorganic elements- dietary minerals and structural role- catalytic role- 40 elements in body- essential and non- essential minerals- classification of minerals- macro minerals-seven- calcium-component in hard tissues- physiological roles- absorption- requirement levels- phosphorus- limiting in water- component – nucleotides- phospholipids- coenzymes- DNA-RNA- buffer role- absorption- excretion- eutrophication effect- magnesium- diverse roles- essential for respiration and neuromuscular transmission- source- plant source rich in magnesium- sodium- primary electrolyte- extracellular fluids- chlorine- anion- extracellular- potassium-intracellular cation- sulphur- organic molecules- trace minerals- chromium-functions- cofactor- protein and lipid metabolism- cobalt-cyanocobalamine component- copper-blood cell formation- copper dependent enzymes- collagen linking- haemocyanin- crustaceans- iron-haemoglobin- other enzyme systems- iodine-thyroid gland- manganese- urea synthesis- amino acid- fatty acid – glucose oxidation- selenium-enzyme component- biological anti-oxidant- zinc- nutritional interactions of minerals- functions- toxicity of minerals- dietary supplementation of minerals- sources and bio availability of minerals- nutritional supply and requirements- Summary and conclusion.

Lecture 15 Prepared by Dr. R. Paul Raj

Carotenoids

General- chemical structure- 40-carbon polyene chain- hydrocarbon carotenoids- carotenes- xanthophylls-beta carotene (Carot) leutine (marigold petals)- structure related anti oxidant properties- forms in biological systems- esterified- unesterified- protein complexes- biological significance- intracellular anti oxidant action- intracellular respiration- boost immune system- pro vitamin A activity-

Pigmentation efficiency- widespread carotenoids- specific colors- metabolic conversion- specific affinity for certain tissues- carotenoid pigments in fish and shrimp- astaxanthin- canthaxanthin- natural pigments in salmonids- physiological functions of astaxanthin- criteria for assessment of pigmentation efficiency-

Carotenoid - has to be supplied through diet- Sources- antartic krill- algae- pink yeast- astaxanthin content of selected natural materials- xanthophylls content of selected plant materials- carotenoid in crustaceans- carotenevitellin of crab- major carotenoids- concentration-synthesis of astaxanthin from beta carotene.

Chemical analysis- colorimetric measurement- definition of color- sensory analysis- Dietary factors- carotenoid sources- carotenoid stability- pigment quantity- animal related variability- role of carotenoid in animals.

Nutrition and Body composition

Influence of feed on the body composition of fish- protein and mineral composition- body fat and dietary fat in cultured and wild fish- its relation with dietary energy supplied as fat-

Composition of wild fish- dependent on environment and physiological factors- diversity of feed- seasonal diet variations- locality- stage of growth- protein as amino acid- carbohydrate as glycogen in body composition-

Dietary protein and energy/protein ratio- dietary carbohydrate- dietary fat- pigmentation- carotenoid- astaxanthin- synthetic canthaxanthin- dietary growth hormones- anabolic steroids- 17 alpha methyl testosterone- conclusion.

Lecture 17 **Prepared by Dr. R. Paul Raj**

Energetics

Study of energy requirements – flow of energy – energy – capacity to do work – measured as it is converted from one from to another – energy in food converted to heat – Heat energy expressed – caloriec -joule (J) kilojuole – mega joules. Gross energy (E) – substance oxidized to CO2-N2O or water – Deternmination of E – Bomb calorimeter – Intake energy (IE) carbohydrate/protein/lipid – Faecal energy (FE) gross energy of faeces –PE = energy of undigested food and energy of metabolic origin – digested energy (DE) – DE = IE-FE; Urinary energy (UE) Gill excretion energy (ZE) – surface energy (SE) Metabolizable energy (ME) ; ME=IE-(FE+UE+ZE). Total heat production (HE) – energy lost as heat – balas metabolism (HeE) – Heat of activiuty (HjE) – Heat of thernmal regulation – heat of waste formulation – heat increment or specific dynamic action (SDA) Retained energy (RE) Consumption energy (C)- growth/ production energy (P)- Energy for respiratory metabolism (R) – Non faecal excretion (U)- faecal energy (F)

C=F+U+R+F Gross conversion efficiency (K) – indicator – bioenergetic physiology – factors affecting BMR – body size – oxygen vailability – temperature –stress –osmoregulation – factors affecting non-BMR – Gonadal growth.

Nutritional Bioenergetics

Energetics and feeding – diet composition – CH – lipid – proteins – Determining quality of diet – Dietary sources of energy —Food conversion ratio (FCR)- assimilation efficiency/ absorption efficiency-Net conversion efficiency- true digestibility- digestibility of a nutrient- total digestibility or assimilation- trophic coefficient- partial growth efficiency- ecological efficiency- body weight gain- nutrient retention- optimum ratio of dietary energy to protein- metabolizable energy- specific growth rate (SGR)- nitrogen balance (NB)- Protein efficiency ratio (PER)- protein conversion ratio (PCR)- net protein retention- gross protein value (GPV)- optimum ratio of dietary energy to protein- apparent biological value (ABV)- biological value (BV)- physiological fuel value of protein- chemical score (CS)- EAA index- energy units- methods- gastric evacuation- use of food markers-Hydrolysis Resistant Organic Matter- Hydrolysis Resistant Ash- physiological method- rate of filtration- satiation- critical cell density in bivalves- superfluous feeding in crustaceans- assimilation- assimilation efficiency- total energy- digestible energy- metabolizable energy- metabolism- scope for activity- scope for metabolism- specific dynamic action- energy spent on activity- urinary loss- endogenous- exuvia- growth and over all budget- ration vs. growth-scope for growth- budget- energy budget: energy retained in growth ratio- indirect calorimetry from oxygen consumption- oxycalorific coefficient (energy equivalent calculation)- carbon dioxide and ammonia measurements- energy lost in nitrogenous excretory products- ammonia excretion through gills- other nitrogenous wastes- factors affecting ammonia excretion- dietary quality and quantity- Nitrogen carbon balance after Brody- Herbivores- carnivores- Measurement of RNA: DNA ratio as an index for growth- Energy lost as heat.

Nutritional Deficiencies

Deficiencies in an organism and nutritional requirement independent of food intake -Environment stress-Altered gastro-intestinal activity-diseases-physiological needs- Drug induced anorexia- Metabolic defects & Food contaminants -Impaired nutritional status - defective host resistance–Malnourished fish- Nutritional problems -decreased appetite-non-specific– Starvation

Protein deficiency- Fatty liver (amino acid imbalance)- Tryptophan deficiency- Methionine deficiency-

Lipid deficiency

Deficiency results in growth retardation (protein will be used up for basal metabolism rather than for protein synthesis)- EFA deficiency - Lipoid liver degeneration - Oxidation of fatty acids- Ceroid formation -Sekoke disease -Deficiency in broodstock.

Carbohydrate deficiency/ excess

Hyperglycemia- glycosuria

Vitamin deficiency

Avitaminoses- Hypovitaminoses- diseases, which are non-specific-Thiamine-Riboflavin- Pyridoxine- pantothenic acid -Niacin- Inositol- Biotin-Folic acid- Choline- Nicotinic acid - Vitamin C -Vitamin A- Vitamin D-Vitamin E- Vitamin K-

Mineral deficiency

Calcium- Phosphorous- Magnesium- Sodium-sulphur- Chlorine-Iron-Copper- Manganese- Cobalt- Iodine- Selenium- Molybdenum- Chromium-Fluorine-Excess of minerals leads to other disorders and toxicoses.

Conclusion.

Larval Nutrition

Introduction- Potential sources of diets for larvae- Live or formulated feed- live feeds used in aquaculture- unicellular organisms- yeasts- bacteria phytoplankton- diatoms- green algae- flagellates- - zooplankton- Artemia- copepods- rotifers-

Nutritional requirements of larvae- nutritional quality - culture- enrichment methods- energy- lipid, EFA and protein composition- minerals- vitamins;

Formulated feed and feeding- factors affecting nutrient availability- digestibility- nutrient requirements;

Practical feeding- feeding behavior of larvae- size and type- quantitative aspects- Larval feeds- micro bound feeds- classes of micro bound feeds- crumbled feeds- pelleted feeds- flaked feeds- cake feeds- on-size feeds- micro extruded feeds- particle assisted rotationally agglomerated feeds- spray beadlets- complex particles;

Wet micro particulate diets- custard diet; dry micro-particulate diet- water stable matrix of dry ingredients- freeze drying- vacuum drying- or oven drying is followed- most widely used diet;

Micro encapsulation- micro bound diets- micro coated diets- Characteristics of artificial feed for larvae- objectives and constraints- physical characteristics- size- manufacturing methods- composition of feed particles- nutritional requirements- larval feed difficulties.

Nutritional quality of natural food for larvae- formulated larval diets.

Lecture 21

Prepared by Dr. R. Paul Raj

Broodstock Nutritional Requirements

Nutritional importance in reproduction- nutrient requirements of broodstock animals- factors affected by maturity fecundity-egg-size-egg-quality-feed composition- fresh feed vs prepared feed- fatty acid profile of ovary vs that of feed- importance of fatty acids- use of marine animal source as protein- dietary levels of phospholipids and sterol in shrimp broodstock- effect of nutritional quality of broodstock diets on egg development.

Broodstock performance measurement- total number and nature of spawnings- egg fertility- average hatching rate- Protein- level- source- energy- lipids- linoleic acid- arachidonic acid- n3/ n6 fatty acid ratio and improved performance Nutritional importance in Normal maturation of gonads- fecundity- egg size- fertilization- hatchability- viability of larvae- carotenoids and broodstock performance- Gonad Index (GI) in shrimp- vitamins- role of vitamin A in shrimp broodstock -vitamin E- minerals- hormones- steroids.

Lecture 22 Prepared by Dr. Imelda Joseph

Chemical Evaluation of Feeds

Quality of feed -quality of ingredients-Ingredient quality –adulteration- poor quality raw material- inadequate or excessive processing-deterioration associated with storage /microbial contamination- Quality by chemical evaluation- various factors- improper /non-representative sampling-mistake in analysis- lack of replicates- imprecise lab techniques- errors in calculations - lack of understanding of the values and limits- Fishmeal Processing-Raw fish-Cooker- Press -Presscake- Decanter- Stickwater-Separator- Evaporator- Oil -Solubles- Drier; The factors affecting fishmeal quality - Raw material freshness -Presscake drying- -Solubles-Freshness indices of fishery products- Fresh- product having composition identical to that of a live fish- Spoiled- chemical and microbial changes - Factors contributing to the rate of decomposition- Sp. of fish Handling-sorting of the product ; Tests for detecting freshness - Total Volatile Nitrogen (TVN)- Total Volatile Bases (TVB)- Biogenic amines (eg. Histamine)- Microbial profiles- Nucleotides formed from the breakdown of ATP- K1 Value; Degree of lipid oxidation- Chemical tests to measure FM quality in aquatic feeds- Pepsin digestibility (Torry)- In vivo ADC (protein); Soybean products- tests for detecting under-heated soybean meal-Measurement of urease activity- Trypsin inhibitor activity -Water solubility-Other oil seed products- Glucosinate in canola/ rapeseed meals -Gossypol in cottonseed meal -Fats & oils - rich in PUFA - oxidation- produces free radicals- peroxides - other potentially toxic or reactive compounds-Specifications for fish oils used in aquatic feeds- Peroxide value (PV) - Anisdine value (AV) -Proximate composition-Moisture- Dry matter- Crude Protin- Kjeldahl technique-Lipid- Extracted with ether -Crude fiber- Ash-NFE -Energy content- Average energy values for major nutrients -Specific nutrient content- Amino acid- Chemical score, Indispensable AA index and Essential AA index - Other methods for feedstuff evaluation- Near infrared spectroscopy (NIRS).

Biological Evaluation of Feeds

Feeding trials - Measurements of performance Weight gain or growth -% Weight gain (relative growth -Absolute growth rate (AGR) - Relative growth rate (RGR)- Instantaneous growth rate (g)- Specific growth rate (G)- Feed conversion ratio (FCR)- Feed efficiency ratio (FE)- % Survival -- digestibility of feed ingredients determination with inert markers- Apparent digestibility- True digestibility - In vitro digestibility- pH drop method and pH stat method- problems and sources of error with digestibility measurements- tracer studies- in vitro digestibility- nutrient retention or deposition- electrical conductivity- protein quality- protein efficiency ratio (PER)- protein conversion efficiency (PCE)- biological value (BV)- apparent net protein utilization (ANPU)- Other condition measurements- RNA/ DNA ratio- formulating and reformulating balanced diets in combination with several ingredients- laboratory evaluation- measuring feed intake and weight gain- calculating feed efficiency- calculating nutrient retention efficiencies (NRE)-growth performances.

Growth Rates and Models

Description of growth-monitoring growth- prediction of growth and nutrient and energy gains- prerequisite for estimation of energy or feed requirement- specific growth rate (SGR)- main coefficients used to estimate growth in fish and crustaceans-daily growth rate- specific growth index- daily growth index- growth coefficient.

Growth model- degree of external control over the growth process- Putter-von Bertalanffy anabolism- catabolism model or the balanced energy equation- Ration (x absorption factor)= metabolism + growth

Fundamental relationship between growth / production and metabolism/ respiration- organizational energy cost- Brady (1942)- post feeding metabolic rates- recovery growth- correlation between rates of weight gain and metabolism- specific dynamic action (SDA).

Lecture 25

Prepared by Dr. R. Paul Raj

Conventional Feed Ingredients

Ingredients –10 categories –grasses-legumes-fruits – vegetables-root crops-cereals-oil-bearing seeds-oilcakes-animal products- miscellaneous feed stuffs- additives; Feedstuff classification- conventional source- definition- Non- conventional source- definition;

Protein sources- animal origin- 27- 85%- marine organisms- conventional sources- fish meal- fish solubles- condensed or dried- shrimp head meal and crab meal- squid meal- trash fish/by catch- mussel meal-
Rendered by-products- meat and bone meal- meat meal- blood meal- blend of all the above; poultry wastes- poultry by-product and meal- poultry feather meal, hydrolyzed;

Plant origin- 15- 55% protein- legumes- oil seeds-
Conventional plant sources -oil cakes-soybean meal- cottonseed meal- peanut meal- sunflower meal—copra meal - rapeseed meal and canola meal- contain toxic anti-nutritional factors-

Lipid sources- cod liver oil- Pollack oil- sardine oil- squid liver oil- plant sources- corn- cocnut- soybean- sun flower- peanut- cotton seed- linseed.

Carbohydrate sources- cereals- source of vitamin B- root crops- bread- wheat flour- starches- corn – potato- cassava- sago palm- rice bran- and other rice products.

Vitamin sources;
Mineral sources.

Lecture 26 Prepared by Dr. R. Paul Raj

Non-conventional Feed Ingredients

Definition: ingredients with potential of either partially or wholly replacing fish meal.

Non- conventional- crabs- clams- scallops- earthworm meal- artemia- silkworm pupae- milk and by products- poultry feather meal- golden apple snail- frog.

Non- conventional- brewer's yeast- leaf meals- ipil ipil- cassava leaf meal- papaya- acacia- alfalfa- lupin meal- corn gluten meal- plant protein concentrates.

Single cell proteins- microalgae- yeast; silages and fermented products;

Lecture 27

Prepared by Dr. R. Paul Raj

Anti- Nutritional Factors

Definition- prevent utilization of nutrients- reduce nutritional value of feedstuffs- referred as 'toxic factors'- Major gropus – proteins-protease inhibitors-haemagglutinins, Glycosides-goitrogens- cyanogens- minerls-anti-vitamins-anti-enzymes-food altergrens-microbial plant carcinogens-toxic amino acids; divided into three groups based on physico-chemical nature- Those depressing metabolic utilization of protein- protease inhibitors- legume seeds- adverse effects-inhibitors of trypsin-chymotrypsin- enzymes for protein digestion- growth retardation- lower survival- poor reproductive performance- effect of processing and quality control; lectins (haemagglutinins)- nature- affinity to sugar molecules-toxicity-mode of action; saponins or glycosides- significant characteristics-biological effect- biochemical role; polyphenolic compounds-tannins-adverse effect on growth; Those interfering with mineral nutrition- phytic acid or phytate- plant phosphorus- gut microbes- phytase; oxalic acid- free state and as salts- glucosinolates or glucosinolates- characteristics-thyroid suppressing activity; gossypol- polyphenolic gossypol pigments-cotton seed-physiological effects- toxicity- unabsorbable complex with metals;Those interfere with vitamin functioning- anti fat soluble vitamins A,D,E,K- anti B-complex vitamin factors- thiamine- nicotinic acid-pyridoxine- cyancobalamine; Cyanogens- amygdalin- dhurrin- linamarin: heat labile- easily destroyed by cooking – effects on organisms-death due to growth inhibition-decrease in food efficiency – pancreatic hypertrophy-hypoglycernia-liver damage- extent of manifestation – antimolabite type-species – age- size- physiological condition - lectins- goitrogens- anti vitamin D- anti vitamin E- thiaminases- adverse actions of these compounds- occurrence- removal or remedy.Heat resistant substances-estrogens (isoflavons)- gossypol- tannins- cyanogens- adverse actions of these compounds- occurrence- removal or remedy.

Lecture 28

Prepared by Dr. R. Paul Raj

Additives in Feeds I

Feed additives- Definition- role in diets- nutritional quality- pelleting- ingestion- acceptability- essential nutrient supply in pure form- Binders: Role in diet preparation- stability of feeds- storage- transport- Examples of natural and synthetic binders- level of incorporation-Antioxidants- Role- rancidity of fat- vitamin deterioration- natural and synthetic anti oxidants- level of incorporation in feeds- Humectants- Maintain low moisture level in feeds- sodium chloride- propylene glycol-Mold inhibitors/ Fungistats- Why is it necessary to add mold inhibitors (MI) in feed? Moisture level and mold growth- mycotoxicity-level of incorporation of MI- examples of commonly used MI- Attractants and Phagostimulants -Role in diet preparation- consumption- nutrient leaching- weaning of fish larvae- examples of commonly used attractants- natural and synthetic attractants- Pigments- Importance/ necessity- imparting color- natural pigments- crustacean- artemia- algae- beta carotene- astaxanthin- lutein- synthetic pigments.

Lecture 29

Prepared by Dr. R. Paul Raj

Additives in Feeds II

Anabolic agents

Additives that affect fish performance and quality- enzymes- hormones- antibiotics.

Zeolite

Hydrated sodium alumino silicates- control ammonia- supplementation level- source of trace mineral.

Miscellaneous Additives: anti- stressors- anti-viral factors; Olaquindox- Bay-o-nox- growth stimulant; Thyroprotein- increase thyroxine activity growth; Bile acids- assimilation of lipids- lipid solubles- improve absorption; Enzymes- proteolytic and amylolytic acids.

Fillers: Role in diets- commonly used fillers.

Probiotics- Introduction- What are probiotics? Types of aquatic probiotics- bacteria- yeast. -microbial flora in the gut- Modes of action of probiotics- of biocontrol agents- source- protects from- fungal/ bacterial attack;- Probiotics in live feed bio encapsulation-

Lecture 30 Prepared by Dr. Imelda Joseph

Nutritional Enrichment of Feedstuffs and Live Feeds

What is enrichment? Factors to be considered prior to enrichment- techniques of enrichment- indirect method- direct method- preparation of enrichment media- enrichment- feeding of enriched feed to larvae.

Enrichment of Artemia- Nutritional value of Artemia and EFA content in it- Artemia cannot synthesize EPA or DHA- essential EFA for immunity development- larval survival- profeeding Artemia with HUFA rich feeds.

Unicellular algae- omega yeast- emulsification- preparation of emulsifiers- self imulsifying concentrates- enrichment diet- self dispersing marine oil sources- vitamins- carotenoids.

Feed Formulation

Basic informations required for feed formulations- nutrient requirement- species cultivated- feeding habits- local availability- cost- nutrient composition- ingredients- ability- cultured organism- utilize nutrients- from various ingredients- prepared diet- digestibility – nutrient availability- expected feed consumption- feed additives needed- binders- anti oxidants- nutrient interactions- mineral- mineral interaction- vitamin- mineral interactions- vitamin- vitamin interactions; Diet formulation and preparation- combining feed ingredients –

Practical considerations- anti- nutritional factors- pelletability of mixture- storage and handling requirements-

Balancing a ration- Peterson square technique –

algebric equation method - different steps followed for balancing protein- feed formulation - protein computation- energy level adjustment.

Nutritionally balanced- economical- palatable- water stable- minimize waste output- safe-

Lecture 32

Prepared by Smt. Mini K.G.

Linear Programming and its application in fish feed formulation

Introduction – History of linear programming – Linear programming model and the underlying assumptions – Mathematical formulation of the problem – A prototype linear programming problem – General linear programming formulation – Understanding the Problem – Collection of information and data – Identification and definition of the decision variables – formulation of the objective function – Isolation and formulation of the constraints – linear functions – linear equations – linear inequality – Geometry of linear programming problems- Graphical Method for the solution of two variable linear programming problem – Limitations of Graphical Method – Feasible regions of two variable linear programming – The solution space of a single equality constraint – The solution space of a single inequality constraint – Representing the objective function in the linear programming solution space – Graphical solution of the prototype example: a two variable Linear programming with a unique optimal solution – Two variable Linear programming with many optimal solutions – Infeasible two variable linear programming – Basic Feasible solutions: An algebraic characterization of extreme points for Linear programmming in standard form – Unbounded two variable Linear programming.

The Simplex Algorithm – Motivation to the simplex method – The basic simplex iteration through an example: – Two phases of the simplex method – Writing with slack variables – Basic variables – Non basic variables – Basic solutions – Feasible solutions – Optimal solutions – Applications of linear programming in Fish feed formulation – Illustrative examples

Feed types used in aquaculture

Starter feeds- crumbles- flakes- fry/larvae- nutritionally complete- easily digestible- appropriate particle size- live food; fry feeds- unmetamorphosed young- high protein- highest relative weight gain- flakes/ crumbles; fingerling feed- 10- 20 g fish- crumble to pellets- lesser protein; grow out feeds- weight increase uniform- requirement uniform- protein for growth-s- broodstock feeds- somatic growth less- gonadal growth more; product quality feeds- to increase market quality- fed near to harvest- carotenoid supplementation;

Wet feeds- moisture level 45- 70%- ingredients- trash fish- fishery waste- slaughterhouse waste – feeds are farm made on day to day basis- mainly for fishes; Moist feeds- moisture level 25- 45%- high moisture ingredients- dry pulverized ingredients; Semi-moist diets- 15- 25% moisture. Disadvantages of these feeds- transportation and storage- irregular raw material availability- pathogen introduction- nutrient loss- deteriorate water quality when wasted.

Dry feeds- moisture 7- 13%- easy to transport- store- and dispensing to culture systems- different size feeds for different stages of fish can be prepared- possible to produce specialized feeds- medicated feeds- nutrient rich feeds –maturation feeds – Preparation- dry ingredients or a mixture of wet and dry ingredients mixed together- it can be made as meals- pellets- flakes; Meals- dough or paste- possibility of water polluting is there; Pellets- sinking pellets for bottom feeders- floating pellets for surface or column feeders;

Lecture 34 Prepared by Dr. Imelda Joseph

Feed Manufacturing Process I

The market value of the species to be fed- dietary nutrient requirements of the species to be fed (or closest relative) - dietary protein, amino acid, fatty acid, mineral, vitamin and energy requirements for each phase of the culture cycle (i.e. for larvae/fry, juveniles, production and broodstock, if known); natural feeding habits of the species in question -preferred food items and feed size - feeding station -feeding behaviour - determining dietary food preferences- analysis of the natural feeding behaviour of the target species --determine the physical characteristics of the artificial diet to be produced (i.e. feed size and shape, texture, palatability, buoyancy and water stability);Available feed ingredient sources, composition and cost - seasonal availability- current usage, proximate composition, quality control, digestibility and nutrient availability, and additional ingredient handling and processing cost prior to mixing or pelleting; intended feed manufacturing process to be used - grinding, mixing, cold pelleting, steam pelleting, expansion pelleting, flaking, and/or microencapsulation - manufacturing process employed- diet is to be produced as a mash, crumble, paste, ball, moist pellet or dry pellet - intended stocking density and farm production unit - extensive, semi-intensive or intensive fish/shrimp production systems

Lecture 35 Prepared by Dr. Imelda Joseph

Feed Manufacturing Process II

Feedstuff Processing

Why is processing of feedstuff essential? Removal of anti-nutritional factors- moisture balance- feed particle size reduction- improve palatability- digestibility- nutrient availability- optimum processing is made possible

Processes- soaking- heating- cooking- extraction with organic solvents- chemical treatments- dehulling- Common processes in feed manufacture- grinding- reduce particle size- increase surface area- facilitate mixing- equipments- mills for grinding- plate mills- hammer mill- impact grinders- attrition or shattering mill- cutting mills- premix preparation;

Sieving- vibrating table with sieve-dosing or proportioning or weighing- homogenizing- mixing- homogenous blend- Mixers- horizontal ribbon type mixer- batch and continuous mixing- vertical boot loading mixer- oscillating screw mixer- structuring of feed- cooking – pelleting- compacting of feeds- durability- pelleting plant- supply bin- pellet mill- cooler- pellet crumbler- sifter- collector- types of pellets- granules- fines;

Lecture 36

Prepared by Dr. Imelda Joseph

Extruded Feeds

Applications of dry extrusion- processing of oil seeds and pulses- other feed ingredient processing- industrial products- complete feeds- human foods- reclamation of wet organic by-products- effects of extrusion process on feed components- effects on starches- proteins- fibre- fats- minerals and vitamins- feed additives- extrusion process- raw material processing area- extrusion processing area- post extrusion process- drying and cooling processes- post drying/ cooling processes- Advantages of extrusion cooking.

Complementary processes- drying- cooling- crumbling- coating.

Methods of characterizing textured feeds- Geometrical and physical features- particle size- mass/ volume or densities- mechanical characteristics in air- durability- hardness- behavioral characteristics in the aquatic environments- aptitude for rehydration- water stability- static method- dynamic method- displacement of liquid in relation to solid- displacement of solid in relation to liquid.

Lecture 37

Dr. Imelda Joseph

Feed handling and Storage

Deteriorative effects during storage- oxidative- microbial- insect/ rodent- damage- infestation- other chemical changes- Aquafeeds - highly perishable nutrients - adequate handling and storage procedures - to protect the finished feed – moisture- light, heat, humidity, air and water-

The economic- nutritional consequences of poor feed handling and storage -profound and immediate- a reduction in the nutritional performance of the feed - increased FCR and reduced fish or shrimp growth- mechanical damage -increased "fines" content- oxidative damage- lipid peroxidation -vitamin destruction- microbial/pest infestation- nutrient destruction -mycotoxin production-

Negative effects of feed handling and storage -minimized by the use of dietary anti-fungal compounds- higher dietary antioxidant/ vitamin fortification -

Quality control and Quality Assurance

Raw materials- odour- freshness- microscopic examination- proximate analyses- microbiological tests-

Feed- factors affecting feed quality -Moisture- crude protein- non-protein nitrogen- biogenic of amino acids- iodine number- mineral profile- mycotoxins- microbial flora– feed mill sanitation- management and maintenance.

Chemical characteristics- lipid- hydrolytic rancidity- liberation- fatty acids- triglycerides- moisture- microorganism- lipase to oil- free fatty acids release- digestibility- water stability- performance – cultured organism- growth- carcass quality- <3%- oxidative rancidity- reaction of oxygen- double bonds of unsaturated fatty acids- hydroperoxides- peroxides- to aldehydes- ketones- peroxide value; <1 in 1000g- thiobarbituric acid test- measure- malonaldehyde- <50 in 1000g – anisidine value- measure oxidative rancidity of oil- not feed- Kries test-

Feeding Strategies

Maximum benefit from an artificial diet will only be achieved

Factors affecting feed quality in storage- moisture content in feed- relative humidity- temperature- oxygen supply- - vitamin potency- lipid peroxidation- insect infestation- fungal proliferation- bacterial contamination-

Why feeding strategies are important? Feeding strategy and temperature- dietary energy level and total feed requirements- rations- rationing and its effects on growth and feed conversions- feeding to satiation- Calculation of energy requirements of fish- calculation of feed conversion ratio (FCR)- calculation of feeding rate- specific growth rate (SGR).

Feeding strategies are based on -Biomass assessment- stocking density- no fertilizer or feed input- fertilizer application levels- supplemental feeding- complete feeding- Relative importance of natural productivity and artificial feed- feeding rate and frequency- Factors affecting feeding rate-

How to choose feeding levels? Feeding schedules- feeding records- pond details- species- source- density of stocking- stock size- fertilizer details- feed details- feeding rate- feeding frequency-feeding records- time- growth rate- survival rate- water exchange details- water quality parameters .

Lecture 40

Prepared by Dr. Imelda Joseph

Feed Dispensing Methods

Ensures that feed is distributed evenly- dry pellets- granules- crumbles- broadcasting from bund or by using small canoe- moist feeds dispensed in trays placed at peripheral areas- 30-40 trays/ hectare is used-
Manual distribution- automatic dispensing- demand feeding (pendulum)- automatic dispensing (mechanical means)- Check trays- advantages of check trays.

Broadcasting – feed trays – in shrimp farms – Feeders - cost cutting measure – less labour – mechanicl feeders – Non-demand feeders – hopper – regulator – dispenser – controller.
Drop feeders – Auger feeders
Disc feeders – dispense > 3mm pellets -) wide area – design varies.
Pneumatic feeders – use high or low-pressure air stream regulator – blow feeder – common in shrimp farms. - Wide area coverage
Demand feeders – neither activated by the stock –not controlled *ad libitum* feeding – accurate knowledge on biomass nor required.
Feeders – useful tools for replacing hand feeding.
Non-demand feeders – predetermined quantity of feed pre-determined time intervals – quantity adjusted by motor power-pressure of air stream - size of pellet –
Drop feeders – belt dispensers/rotating discs – adjust speed – controlled feed delivery – no regulator – mainly for crumbles/starter feeds –
Auger feeders – an auger is used – regulated by the period of operation – broadcast up to 4-5m.
Advantages and disadvantages of common feeding practices in aquaculture.

Factors Affecting Feed Performance

Feed dependent factors- Nutrients balance- hydrostability of feed- feed particle size- bioavailability of nutrients- presence of growth promoters- absence of anti-nutrients- attractability of the feed- palatability of feed- shelf life.

Animal dependent factors- Size of the animal- moulting stage in the case of crustaceans- health- age.

Environment dependent factors- temperature of the water- salinity- hydrogen ion concentration (pH)- dissolved oxygen- hydrogen sulphide level- ammonia- nitrites- Biological oxygen demand (BOD)- Chemical oxygen demand (COD)- phytoplankton blooms- toxic blooms- dinoflagellates- cyanophyceae – blue green algae.

Feed quality and environmental pollution – uneaten feed – partially digested feed – faecal matter –excess phosphates/nitrates – other additives – phosphorus content – effluents – minimized – variable leaching of p from faeces – feed ingredients with high level of P low water solubility – use of easily digestible ingredients – well balanced feed formulation.

Lecture 42

Prepared by Dr. Imelda Joseph

Feeding Management

Introduction- feeds and feed management essential for profitable – sustainable aquaculture-

Feed management based on production targets- environmental conditions- socio-economic consideration-

Why feed management is essential?

Ecofriendly feed management affecting feed performance

Introduction- Methods and technology- principal approaches- sources of environmental pollution in aquaculture- genetic and ecological pollution;

Minimum dietary requirements of nutrients for commercial size fish- digestibility and availability of nutrients in feed ingredients- phosphorus- dry matter- other minerals- approaches to increasing digestibility of dietary nutrients- dietary level- phytase- acidification- heating chelation;

Formulation of environment friendly feeds- concept of sustainable feeds- ingredient selection- balanced formula- protein levels- fat levels- carbohydrate levels- finishing feeds;

Need for ecofriendly feed and feed management systems- characteristics of ecofriendly feeds- nutrient balance- antinutrients- additives- quality of raw materials- processing- feed particle size- digestibility- shelf- life

LECTURE OUTLINE

COURSE NO. MC-508

(FINFISH AND SHELLFISH PATHOLOGY)

FACULTY

DR. K.C. GEORGE

DR. A.P. LIPTON

N.K. SANIL

DR. K.S. SOBHANA

(CREDITS : 2+1)

K.C.George

Lecture-1

Finfish and Shellfish Pathology - Introduction

What is health? Definition of health. Relation between homeostasis and status of health.

Importance of maintaining homeostasis to get optimum production

Deviation from homeostasis results in disease.

Factors favouring or resulting in the disturbance of homeostasis

Role of temperature on control and rate of metabolism, excretion of metabolic waste material------growth and reproduction of microbes.

Role of light in productivity and metabolism of aquatic species.

Chemical composition of water.

Biological content of water.

Space availability.

Availability of food.

Frequency of fright stimuli like moving shadows.

Physio-chemical composition water. Buffering action. Carbon dioxide-carbonate alkalinity and hardness.

Presence of ammonia- ammonia production from nitrogenous material. Toxicity of ammonia. Symptoms of ammonia toxicity.

Hydrogen sulphide production from organic material. Toxicity due to hydrogen sulphide.

Anthropogenic pollution.

Biotic environment of water body.

Algae present in water body. Productivity of algae. Role of algae in providing food. Blooms produced by algae and oxygen depletion resulting in mortality. Causes of blooms-species of algae responsible for blooms and control of algal blooms.

K.C.George

Lecture2

Introduction

Definition of term pathology. How the pathology is the basis of medical science. What is lesion? Explain the process pathogenesis.

Definition of stress. Physiological basis of stress.

How pituitary is controlled by nervous system?

Environmental stimuli reach nervous system via nerves.

Changes in pituitary hormone profile affects the secretion of hormones by other endocrine glands especially thyroid and adrenals.

The reaction occurring in neuro-endocrine axis lead to physiological changes viz. General Adaptation Syndrome (GAS).

Stress is intimately connected to GAS.

Thyroxin, adrenaline, corticosteroids oxytocin, and aldeosterone effect increased metabolism, hypertension, retention of minerals like sodium, calcium etc. and hyper-glycemia.

Glucocorticoids increase blood sugar level and cause lysis of lymphoid cells---affect the immunity.

The lysosomes of phagocytes are stabilized---affect phagocytosis.

The facultative microbes in the environment and gut cross the natural barriers and grow inside without resistance from host.

Methods to monitor stress—biological indicators, hormonal indicators-catacholamines, and plasma lactate. Specific biochemical indicators—metallothioneins. Heat shock proteins. Enzymatic indicators. Immune indicator assays. Histological indicators. Condition related indices. Organ related indices. Growth indicators. Reproductive indicators. Community indicators

The frequent stress occurring in aquaculture and aquatic environment are

1) Handling and transport

2) Overcrowding

3) Change in water temperature

4) Excess organic load

5) Fright stimuli and a number of other conditions

Lecture 3. K.C.George

General Pathological processes

Cellular responses to injury- Structure of a typical cell. Nucleus- nuclear membrane, nuclear pores, euchromatin and heterochromatin, and nucleolus. Tri-lamellar structure of cell membrane. How does cell membrane maintain osmotic gradient with in a cell? Receptors for chemical or biochemical molecules. Cytoskeleton of the cell.

Structure and function of mitochondria.

The rough endoplasmic reticulum with ribosomes for synthesis of proteins for export.

Free ribosomes in cytoplasm and synthesis of proteins for endogenous purpose.

Function of Golgi apparatus.

Lysosomes breaking down unwanted products, broken-down components of organelles and particulate matter taken into the cell: and the generation of lipofucins and ceroid pigments.

Smooth endoplasmic reticulum and its role in detoxification and production of new compounds

Microtubules and control of cell movements.

Degenerations –General intracellular changes.

Alterations in cell membrane appearance-----folds, blebs and whorls-separation from junction or by the formation of holes.

Osmotic swelling.

Peroxidations of unsaturated lipids in all membranes by free radicals and consequent changes in cell structure and function.

Exocytosis or extropy. Endocytosis or estropy.

Injury to sodium pump and cell volume.

Mitochondrial changes----loss of mitochondrial granules, condensation, swelling, fluffy deposits, division, calcium deposition, paracrystalline deposits, formation of megamitochondrion etc. Endoplasmic reticulam--- degranulation, dilation, vesiculation, fragmentation, peroxidation, proliferation etc. lysosomal injury--- leakage of enzymes, autophagic vacuoles and autophagia. Nuclear changes--- pyknosis (condensation of chromatin)-formation of heterochromatin. Karyorrhesis---formation of chromatin clumps. Karyolysis and chromatolysis.

K.C.George

Lecture 4.

Degenerations

Specific types of intracellular degenerative changes—Cell swelling—Acute cellular swelling-light microscopic level; cloudy swelling, hydropic degeneration ballooning degeneration, vacuolar degeneration.

Electron microscopic level--- changes in RER and SER, mitochondrial damages membrane changes.

Role of sodium pump, role of lysosome, changes in pH.

Nuclear changes.

Fatty degeneration and fatty infiltration.

Explain the mechanism of fat transport from intestine. Phospholipid formation and lipoprotein formation in liver. Transport of lipid from liver to fat depots and back. Role of choline and methionine in phospholipid synthesis.

How does cell injury lead to accumulation of neutral fat in cell?

Organs affected.

Gross appearance. Microscopic appearance. Demonstration of fatty degeneration by special stains. Lipoid liver degeneration in fish. Role of rancid fat and antioxidants-vitamin E .Fatty infiltration. Difference between intracellular fatty degeneration and fat storage/ fatty replacement.

Intracellular inclusions—Hyaline droplets—protein endocytosis-myelin bodies, heavy metal toxicity, viral infections, and parasitic infections.

Extra-cellular hyalinization, hyaline casts.

Fibrinoid and Amyloid. Calcification---how does calcium deposit in soft tissue?

Type of pathological calcification—dystrophic and metastatic calcifcation.

Role parathormone and vitamin D in metastatic calcifiction.

Necrosis/degeneration and dystrophic calcification. Microscopic and gross appearance of calcification. Special stains to demonstrate calcium deposition in tissues.

Lecture 5 **K.C.George**

Necrosis, pigmentation, and other vascular changes

Necrosis-definition of necrosis-cellular characteristics-types of necrosis-coagulation necrosis, caseation necrosis, liquefactive necrosis.

Reaction to necrosis.

Leakage of enzymes from necrosed cells.

Gangrenes.

Differentiation between autolysis and necrosis.

Pathological pigmentations.

Exogenous pigmentation. Caroteinoids.

Endogenous pigmentation-melanin, lipid pigments—ceroid, lipofuscin-vitamin E deficiency. Hemoglobin and prophyrin derivatives—hemoglobin, hemosiderin, hematins, bilirubins, jaundice or icterus; types of jaundices-Vanden-Berg test.

Circulatory disturbances--haemostasis, hyperemia, venous congestion,

Hemorrhage—diapedesis, petechial and echymotic hemorrhages.

Thrombosis and post-mortem clots.

Formation of thrombus, types of thrombi and causes of thrombosis.

Fate of thrombi

Embolism and infraction, cause and pathogenesis.

Ischemia and anoxia.

Oedema--mechanism of its formation.

The role of capillary hydrostatic pressure and osmotic pressure.

Permeability changes.

Lymphatic obstruction.

Difference between inflammatory exudate and oedema transudate.

Recognition of oedema

Transudate in oedema- composition of tranudate. Reabsorption of tranudate.

Fluid in body cavities -hydropericardium—ascites.

Lecture 6. **K.C.George**

Inflammation

Inflammation-explain what is inflammation. Definition of inflammation. Explain the role of humoral and cellular components involved in inflammation.

Inflammation causing irritants-biological, chemical, physical causes.

Cardinal signs of inflammation -*rubor et tumor cum calore et dolore.*

Vascular changes—vasoconstriction followed by dilation—hyperaemia---axon reflex-chemical mediators cause vasodilatation and hyper permeability. Role of capillary and venule blood pressure. Exudation of colloids and emigration of leukocytes.

Damage to endothelium and expression lectins like selectin cause leukocytes to adhere to endothelium.

Change in the rate of blood flow—retardation due to loss of fluid and increase in viscosity; stickiness of leukocytes (lectins&integrins) and dilatation of vessels. Stasis favours exudation

Change in blood stream-- heavy elements marginate

Margination and pavementing of leukocytes—stickiness of endothelium.

Emigration of leukocytes and diapedesis of erythrocytes

Exudation of serum—composition of exudate.

Leukocytes. Neutrophils shape of cell, nucleus and type of cytoplasmic granules. Origin of neutrophils. Type of different enzymes. Description of oxygen dependent system and oxygen independent system. Phagocytic function. Role in purulent inflammation.

Eosinophils shape and appearance in fish. Enzymes present. Antagonistic role in inflammation. Origin of eosinophils in fish.

Lymphocytes. Origin of lymphocytes. Type of lymphocytes. Their function in identifying antigens. Lymphocyte modulation of inflammation through lymphokines.

Plasma cell—formation from lymphocyte and its function.

Monocyte. Origin of monocytes. Structure and morphology; migration to tissues and conversion to mononuclear phagocytes—Phagocytic and antigen processing function. Presentation of antigens and stimulation of B-lymphocytes. Receptors of macrophages. Enzyme profile of phagocytes and conversion of macrophages into epithelioid and giant cells

Lecture 7. **K.C.George**

Inflammation-contd.

Basophils in fish; mast cells and their role in inflammation—Eosinophilic granular cells and their analogy to mast cells. Chemical mediators of inflammation---histamine, serotonin, kinins, prostaglandins, cytokines, epinephrine, DOPA, Dopamine and glucocorticoids. Origin of mediators of inflammation—cell products---mast cells and EG cells. Lymphocyte products. Neutrophil products. Mediators originating from plasma. Products of plasma coagulation—fibrinopeptides—complement activation products. Immunologically mediated inflammation

Classification of inflammation—serous, fibrinous, purulent, phelgmonous, fibrino-prulent, henorrhagic, catarrhal, lymphocytic and granulomatous inflammations.

Healing—removal of products of inflammation, repair of damaged tissues and regeneration---epithelial tissues, muscle, nervous tissue, connective tissues—cartilage, bone, collagenous tissue etc.

Purpose of inflammation----How does inflammation brings the elements specific immunity in close contact with irritants?

Lecture 8 **K.C.George**

Classification of diseases.

Classification of diseases. Infectious and non-infectious diseases

Infectious diseases- contagious infections and non-contagious infections. Epidemic (Epizootic) diseases and endemic (enzootic) diseases, existing diseases, exotic diseases, emerging diseases. Viral, bacterial and protozoan diseases, parasitic diseases.

Zoonotic diseases.

Non-infectious diseases. Metabolic diseases Genetic defects, congenital diseases nutritional diseases, diseases due to physical and chemical causes.

Etiology- multi factor etiology of diseases—role of environment in fish disease out breaks. Intrinsic factors—genus, species, race, strain, genetics, age sex etc.

Extrinsic factors----Physical factors—environmental temperature, sunlight, depth of pond, atmospheric pressure, rainfall, ionizing radiation, dissolved gases etc. Chemical factors—pH, exogenous poison, heavy metals, pesticides and endogenous toxins. Organic load. Biological factors—flora and fauna co-existing. Nutritional factors—deficiencies and excesses.

Idiopathic jaundice in pike. idiopathic bloat.

Lecture 9. **K.C.George**

Epidemiology.

Epidemiology of diseases--- population approach-- definition---epizootiology-importance of this branch.

Descriptive measurement of disease- necessity for uniform nomenclature.

Measures of disease occurrence-discrete data and continuous data or numeric data.

Ratios, proportions-factorial values-fatality proportion- prevalence –proportion of affected—rates dynamic measurement of an event or prediction of an event.

Incidence rate –difference between prevalence and incidence

Investigation of disease outbreaks—morbidity and mortality curves. Pattern of disease occurrence curve.

Propagative source curves-epedemiform curve and sigmoidal curve---conclusion drawn on the movement of etiological factor.

Point source curve---high virulence of causative agent or single attack

Broad source curves---long period of exposure and slow incubation or transmission.

Investigative approach— disciplines essential--microbiology, pathology, physiology, toxicology, immunology, limnology, ecology and Epidemiology---environment, host- and agent related factors.

Retrospective studies-confirm the nature of the problem—characterize the events—identify the population at risk---establish proportions—identify the suspected associated causal factors—formulate test hypotheses—presentation of recommendations—experimental testing.

Prospective Epidemiology and its usefulness.

Lecture 10. **K.C.George**

Epidemiology.

Concept and cause---multifactorial nature—component cause---sufficient cause---necessary cause.

Risk factors—increase in risk---protective factors—casual and non-casual risk factors—quantification of risk factors—formulation of intervention strategies.

Outcomes and associations---separation of population into groups for comparison---identification of variables in association with outcomes

Disease as outcome—examples of EUS and WSS—case definition

Quantitative epidemiological studies.

Observational studies—natural cases in different populations without interventions—descriptive—distribution, frequency---animal and place----hypothesis---case-control studies—units with disease and without disease.

Intervention and theoretical studies—experiment imposed on population level—mathematical modeling

Steps in designing and execution of an aquatic epidemiological study---clear questions and objectives---target populations and units—study design—physical and financial resources—questionnaires, measurements, sampling and laboratory tests—random sampling—95%

Lecture 11. **K.C.George**

Viral disease.

General virology—nature and structure of virus. Architecture of virus and cellular architecture-organelles and metabolic machinery utilization of host cell. Size of virions. Genome of virus. Capsid and capsomeres- lipoprotein envelopes- glycoproteins

Nucleic acids found in virus--- double or single stranded--+ve or-ve strand. +ve RNA messenger RNA. –ve DNA synthesis + ve DNA then transcription. –ve RNA transcripts. +ve strand

Protein coat—capsid—symmetry—icoshedral, helical and complex.

Icoshedron-polyhedron with 20 equilateral triangular faces. capsomeres arrangement in equilateral triangular facets

Helical symmetry—long rod shaped. Nucleic acid in spiral form—capsomeres around nucleic acid. Vertebrate virus—helical enveloped—DNA viruses. Envelope origin host cell—incorporation of viral glycoprotein—spikes—attachment to receptors—haemmagglutination—neuraminidase—fusion of cells.

Complex structure---poxviruses.

Viral enzymes—neuraminidase, reverse transcriptase.

Viral antigens—4 to 100—group specific, sero type, genus—strains—study—electrophoretic mobility. Antigenic variation—gene mutation—substitution, deletion.

Virus classification –204 families—vertebrate viruses—nature of genome---structure of virion. Family—genera, species strain—serology. Fish viruses—DNA virus—Herpes viruses, Irido viruses and Adeno viruses. RNA virus—Calci viruses, Rhbdo viruses, Birna viruses, Reo viruses, Orthomyxo viruses, paramyxo viruses, Retro viruses and ungrouped viruses

Virus replication—need for live cells—eclipse phase, intracellular appearance, logarithmic phase, release and decline—graphic depiction.

DNA virus attachment—internalization—un-coating—transcription of early viral genes—is shutting down host cell nucleic acid and protein synthesis. Enzymes required for replication viral nucleic acid.Late viral gene transcription—structural proteins Cleavage and glycosylation.

Attachment –receptors-specificity—tissue tropism— virulence—trypsinisation.

Lecture 12. **K.C.George**

Viral disease.

Penetration—receptor mediated endocytosis—fusion with the plasma membrane-enveloped virus through glycoproteins.

Translocation—non enveloped icoshedral virus—direct passing through cell membrane.

Un-coating—process differ in DNA and RNA viruses—virus fusing with cell membrane and lysosomes are believed to be un-coated.

Transcription--- general—eukaryotic cells ds DNA transcription in nucleus—DNA dependent RNA polymerase—one messenger RNA-one polypeptide—Methyl caps at the 5' end and poly A tail at 3' end. Prokaryotic cell the m RNA is ploycistronic—more than one peptide

Six genome type in virus

1. Ds DNA—replication in nucleus of host—host DNA dependent RNA plolymerase. Herpes virus, adenovirus and papovirus

DNA dependent replication in cytoplasm. A DNA dependent RNA polymerase of viral origin. Poxvirus and iridiovirus.

2. (+) SS DNA replicate in nucleus of host—host enzymes—dsDNA—DNA dependent RNA polymerase. Parvovirus.

3. ds RNA—RNA is segmented. Viral RNA dependent RNA polymerase transcribe each segment---separate m RNA. Reovirus.

4. (+) SS RNA viral RNA act as m RNA—genome size polypeptide cleaved to several structural protein. Picornavirus, togavirus, coronavirus and calcivirus.

5. (-) SS RNA—structural SS RNA dependent RNA polymerase. RNA segmented. Myxovirus and paramyxovirus.

6. (+) SS RNA reverse transcriptase—(-) SS RNA—ds DNA—mRNA. Retrovirus.

Translation—early proteins—enzymes and proteins. Regulation of transcription and translation. Next structural proteins--- may do double function. Migration of components to site of assembly. Glycoprotein---cell machinery-RER—ribosmes—enzymes—Golgi apparatus—plasma membrane.

Lecture 13. **K.C.George**

Viral disease.

Replication of nucleic acids---six classes of NA. Primers and promoters. Enzymes polymerase, replicase and ligase. Complementary strand—5'to 3' terminal direction. Start at 3' end of parental strand.

1. ds DNA a) continuous—terminal loop in one strand—synthesis 5'to 3' direction—parallel strand displaced—template for another strand. b) Semi continuous—both strands simultaneously synthesize. Synthesis in lower strand in short sequence—joined by ligase. Circular DNA—early viral protein unwind. Papovavirus, iridiovirus and poxvirus.
2. (+) ss DNA—terminal loop method—ds DNA—loop is broken—ds DNA—template for new viral DNA.
3. Ds RNA—viral m RNA—transcription—(-) ss RNA---combines with m RNA—ds RNA
4. (+) ss RNA—(-) complement—(+) ss RNA.
5. (-) ss RNA—complementary positive strand—negative strand.
6. (+) ss RNA—ss DNA—ds DNA—integration to host genome—reverse transcriptase.

Assembly of virus—structural proteins-capsomeres aggregate spontaneously around a nidus—viral nucleic acid. Enveloped virus—synthesis of viral glycoproteins via RER Golgi—incorporation in host cell membrane—nucleocapsid alignment underneath membrane—budding.

Release of virus

Non enveloped—lysis. Enveloped—budding.

Defective replication—genome defective—helper virus—interference.Latency—DNA integration—herpes virus. Transformation—integration—neoplasia—oncogenic

Effects of virus infection in cells—reversible changes—cytopathic effects—function loss—transformation-neoplasia—integration in host genome—altered staining property—inclusion body—giant cells. Effect of virus infection in fish—clinical disease—silent infection—Carrier State—persistent infections. Diagnosis of viral diseases—tissue culture techniques—CPE—confirmation—serological .

Lecture 14. **K.C.George**

Viral disease.

Microscopy—light and electron microscope—inclusion body and virion. Detection of specific virus components—virus proteins—antibodies labeled antibodies—FAT, ELISA, and immuno-dots—viral nucleic acids—southern blotting.

Inactivation of virus—temperature—radiation—X-rays, gamma rays, UV and sunlight. Chemical methods—pH—2% sodium hydroxide, quick lime—oxidizing chemicals—halogens—hypochlorites, iodine—tincture iodine—providone iodine—idophore. Ozone. Alkylating agents—formaldehyde, glutaraldehyde. Detergents—enveloped virus—act on lipids

Prevention and control of virus disease in fish—avoidance—stock, water-attenuated live vaccine, killed vaccine—cloning viral antigens. Chemotherapy—theoretical. Genetic selection.

Reservoirs of infection and viral transmission.

Common viral diseases of fish—DNA virus—herpes virus—group characters—channel catfish virus—morphology—nucleocapsid-DNA-100nm—envelope 175-200nm diameter. 162 capsomeres—viral survival. Culture, detection and diagnosis—BB and CCO cell lines. Immunosuppression and detection. Southern blot.

Clinical signs and gross pathology—spiral swimming and vertical hanging. haemorrhage in abdomen and fins, abdominal distension, pale haemorrhagic gills, exophthalmos. Enlarged kidneys, haenorrhages in musculature, liver, kidneys and spleen. Stomach--mucous secretions. Yellow exudate in peritoneum.

Histopathology—haemorrhages in vital organs. Kidney—increase-lymphocytes and necrosis of PCT—viral particles—EM. Brain herpes encephalitis.

All cat fish—transmission—persistent infection by genome integration.

Herpes salmonis—rainbow trout. *Oncorhynchus masou virus*—oncogenic nature—perioral tumors Iridoviruses—group characteristics—icoshedral symmetery. 130-300nm diameter. Ds DNA. Five fish iridovirus—lymphocystis disease virus—nodular lesions in fresh water, brackish water and marine fishes. Virus morphology 130-300nm. Genome structure and biochemistry. Virus survival. Culture, detection and diagnosis BF-2, BF-W and MCT cell lines. Clinical signs—cream coloured nodules.

HP—fibroblast like cells enlarge—basophilic. Cytoplasmic inclusions

Erythrocytic necrosis virus—cytoplasmic inclusions in erythrocytes—alterations in c haematology—wider distribution

Adenoviruses –ds DNA non enveloped—isometric—fish adenovirus identified by EM. Cod adenovirus, sturgeon adenovirus and dab adenovirus.

RNA virus—calciviruses—isometric—icoshedral 35—40nm. SsRNA—non enveloped—san miguel sealion virus

Rhabdoviruses—bullet shaped 130-380x60-95 nm. Envelope-5-10 nm spikes—(-) ss RNA—helical—RNA dependent RNA polymerase—pike fry rhabdovirus—epizootic red disease/ hydrocephalus—virus survival---culture FHM cells—CPE 40 hours. Clinical signs—high mortality in fry and fingerlings—loss of equilibrium, exophthalamos and hydrocephalus. Haemorrhages—skin, gills, muscles, spinal cord, spleen, pancreas, kidney and haematopoietic tissue.

Spring viraemia of carp (SVC)—two rhabdovirus—SVC&SBI(swim bladder inflammation)—*Rhabdovirus carpio*. Both are two parts of disease complex—infectious dropsy/ dropsical syndrome—carp erythrodermatis—bacterial etiology. Morphology and biochemistry—120x60-90nm—ss RNA—fhm cell line—CPE—granulation and rounding. Serology –similar. Clinical signs and pathology—reduced respiratory rate, loss of balance, abdominal distension exophthalamos. Darkening. SBI—loss of weight, darkening abdominal distension and exophthalmos.

Peritonitis, ascites haemorrhagic enteritis. Transmission-water, blood sucking parasites. Control.

Viral haemorrhagic septicaemia—egtved disease –180x60-90nm enveloped rhabdo virus—ss RNA--two serotypes. RTG2 and FHM CPE—rounding—disease of farmed trout—rainbow trout—winter disease--15°C—4 weks to adult—persistent infection. Clinical signs and patholgy—acute phase—chronic phase—latent stage. Transmission and control.

Infectious haemopoietic necrosis—160x95nm-enveloped—cell cultures—CPE—animals affected—infection and disease—clinical signs and pathology—pathognomic lesions.

Lecture 16. **K.C.George**

Viral disease.

Symptoms and pathology

Disease free population triphasic—acutephase—high mortality-dark colour—haemorrhage external and internal. Third stage—no virus isolation—no mortality—nervous signs—looping swimming. Darkness, exophthalmos, kidney swelling and discoloration

Birna virus—non-enveloped isometric ds RNA—two segments IPN virus—molecular size, replication of virus. Virus survival. Sensitivity to heat, acid etc. Serological relations with different strains—intrastrain antigenic variations. Defective-interfering virus—culture and detection—CHSE-214, BF-2, FHM—CPE—immunoperoxide, FAT clinical signs—pancreas lesions—host—salmonids pathogenesis— largest fry affected first feeding to 20 weeks dark colour spiral swimming exophthalmos distension of abdomen pancreatic lesions—petichae, pale liver and spleen. No food in GI focal and generalized necrosis pancreas, kidney and liver necrosis intestine Macknight cells chronic cases cachexia—islets of Lagerhans. Spread of virus—virulence and genetics, persistence and immunity

Other viral diseases.

Toxicological and other non infectious diseases

Toxicological diseases—dietary mineral toxicity—selenium-nephrocalcinosis

Calcium—metabolism—metastatic calcification—visceral granuloma—nephrocalcinosis

Mycotoxins—aflatoxins—hepatotoxicity—hepatocarcinoma—general carcinogenicity—immunosuppression etc. Toxic algae—algal blooms—red tide

Plant toxin—rag wort—cotton seed—gossypol—cyclopropenoid fatty acid

Ipil-ipil—*Leucaena* –mimosin. Pesticides—organo-phosphates, organochlorines, pyrethrins etc.—liver and kidney lesions Photosensitizing chemicals—electron emission by UV-local tissue damage—phenothiazine

Lecture 17.

K.C.George

Toxicological diseases

Silk worm toxins—carp—islets of Langerhans—diabetes—retinopathy and nephropathy—Sekoke disease.

Visceral cataracts—animal offal feeding—role of pirons?

Single cell protein toxicity—biliary carcinoma.

Drug toxicity—erythromycin, sulphonamides.

Miscellaneous non-infectious diseases—gas bubble disease—super saturation—how water gets super saturated. Clinical features, histopathological features etc

Low temperature diseases—myxobacterial diseases—two stages of disease.

Water borne irritants—pH ranges—cement, dust, slits and ammonia---gill pathology—proliferative—nodular gill disease

Blue sac, white- spot and yolk sac deformity disease of larvae—metal ions, ammonia etc.

Colouration anomalies in cultured marine fishes—pseudo-albino, partially pigmented and reversely pigmented---lighting used. Sunburn disease—necessity of shade and depth—UV radiation—UV-A and UV-B. UV-A melanin stimulation. UV-B sunburn UV-B penetration 1m. Fish at depth of 0.5m—keratinized layer. Histology of lesion—swelling of cells, karyorrhesis, oedema, ulceration etc photosensitization—UV-A—UDN—natural prophyrins—marine fish. Traumatic injuries---secondary infections—*saprolegnia.* Tagging lesions. Conditions presumed to be genetic origin—malignant melanoma, Siamese twins in salmonids, over shot and under shot jaws, anomalous fin and foreshortened opercular growth

Ulcerative dermal necrosis (UDN)—incidence and prevalence—lack of diagnostic test—pattern of lesions secondary infections—histopathology—necrosis—*Aeromonas hydrphila*—etiology—viral isolation attempt. Central nervous system and perpheral ganglion lesions. Symmetry of lesions—photosensitization.

Acute anaphylaxis—sensitization stage—circulating antibody—antigen-antibody complexing—release of mediators from reagin sensitive cells---generalized vascular reaction—relevance in fish—tilapia carp—high protein—rigor-dark colour-rigid body—pre-medication with antihistamine drugs.

Lecture No. 18 **N.K. Sanil**

Parasitology – general aspects, definitions and common terms

Parasitology – Study of parasites - different types of animal associations - symbiosis.

Phoresis – definition - degree of interactions involved – examples;

Commensalism – definition – degree of interaction between partners – dependency – example.

Mutualism – definition – degree of interactions – metabolic/biochemical dependency – example

Parasitism – definition – degree of interaction involved – biochemical/physiological dependency – nature of host-parasite adaptation and pathogenicity - antigen sharing, antigenic disparity – host–parasite equilibrium – overlapping with other forms of associations.

Classification of parasites – Obligatory parasites – definition - examples Facultative/opportunistic parasites – definition – examples.

Definition with examples of the following terms - Ectoparasites , endoparasites, histozoic parasites, coelozoic parasites, intercellular parasites, intercellular parasites.

Monogenetic parasite – digenetic parasite – monoxenous parasite – oligoxenous parasite – polyxenous parasite

Accidental parasite – hyper parasite – carrier – vector

Classification of hosts –Definitive host – intermediate host – paratenic host – transport host – reservoir host – infection and infestation – intermediate hosts and vectors - definition with examples

Protozoan parasites – definition – salient features; metazoan parasites – definition – salient features

Lecture No. 19

N.K. Sanil

Life cycle patterns.

Protozoan life cycles – Reproduction – binary fission – multiple fission – infective stages/cysts

Direct life cycle: passive transmission – oral uptake – active transmission – infective stages

Life cycle patterns and developmental stages in Dinoflagellates - Hexamita and spironucleus – Cryptobia – Ichthyobodo - Microsporeans – Coccidians – Ichthyophthirius - Ectoparasitic ciliates

Indirect life cycle- role of intermediate hosts/vectors

Life cycle patterns and developmental stages in Trypanosomes –Trypanoplasma - Myxosporeans – Development in fishes - role of tubifex worms as intermediate hosts – development in tubifex worms

Metazoan life cycles - Monogenetic trematodes: life cycle – larval stages - oncomiracidium – infection

Digenetic trematodes: Different life-cycle patterns –miracidium – development in the first intermediate host- sporocyst - redia – free swimming stage – cercaria – different types of cercaria - development in second intermediate host – metacercaria – paratenic host.

Cestode: coracidium – development in the first intermediate host - procercoid - development in second intermediate host – plerocercoid – paratenic hosts.

Acanthocephalans: development in first intermediate host - Acanthor larvae – acanthella – cystacanth – paratenic hosts.

Nematodes: egg - larval stages – first / second intermediate hosts – paratenic hosts

Crustacean parasites: life cycle patterns in crustaceans

Lecture No. 20

N.K. Sanil

Parasitic adaptations

Parasitic adaptations - morphological – physiological / biochemical – aiding transmission. Morphological – specialized structures depending upon the mode of life – for attachment / feeding – Ectoparasites – hooks, spines, suckers, adhesive glands / secretions etc. – specialized mouth parts – Endoparasites – loss of appendages and sense organs – suckers for attachment – flattened body shapes – villi on surface – resistant cuticle.

Physiological/ biochemical – Nutrition through body surface – presence of villi on the surface – enzymes – modified cuticle. Cysts with thick / resistant walls - excystment Cystogenous glands – penetrating glands – mucous threads– histocytic and other specialized enzymes – Anaerobic respiration – altered metabolic pathways – Immune suppression – immunomodulation – immune evasion – antigenic coating – antigenic variation – variation in amino acid sequence – relapse strains.

Adaptations for parasitic transmission – very high reproductive potential – Protozoa – multiplies by fission – spore/ cyst/ egg with resistant wall – Metazoans – highly developed reproductive system – polyembryony / internal budding – very large number of eggs – Larva – parthenogenetic development in intermediate host – complex life cycle with intermediate host and vectors – hermaphroditism – hormonal synchronisation circadian rhythm correlated with hosts activity – hypobiosis – altered host behaviour.

Lecture No. 21 **N.K. Sanil**

Factors affecting pathogenicity in parasitic infections

Nature and degree of adaptation between fish and parasite – pathogenicity – introduction of species – Host –parasite balance.

Site of infection – abnormal host - abnormal site – pathogenicity – host reaction.

Number of parasite / intensity of infection – effect on the host – Effect of parasites on weak /old/very young fish.

Protozoa – proliferate independent of initial intensity of infection. Metazoa – intensity depend on initial infection.

Duration of contact with parasite – ectoparasite – pathology - duration - size of parasite - intensity of damage to host. Condition of fish – Effect of health/ nutrition on resistance / immunity – Age of host and susceptibility to diseases– very young/ old – susceptible – Effect of other diseases – modifies pathology. Mobility of parasite – enhancing pathogenicity in ectoparasites. Living habits / behaviour – migration – parasite – enhance / control infection – shoaling – influence on the rate of parasite transmission and pattern of infection – Environmental condition – over crowding – stress – increase in rate of transmission – Unbalanced nutrition – reduced oxygen – altered pH - altered temperature – enhancing proliferation of parasite -

Pollution – organic / inorganic – toxins – very high organic loads – defective sanitation – frequent disturbance / handling etc. – Stress – Effect on fish health and occurrence of diseases

Pathology of Ectoparasitic infestations – Effects of Attachment/feeding – enzymes/ toxins / metabolic wastes – effects of irritation & inflammation – excess mucus secretion and its effects – interference with gaseous exchange – effect on respiratory / excretory efficiency of gills.

Inflammation and swelling of tissues – edema – repair - fusion of gill filaments – effect of altered tissue architecture – hyperplasia of epithelium - reduced respiratory / excretory efficiency - anoxia –death

Necrosis – role of secondary invaders –role of peeling/sloughening of cells in osmoregulatory breakdown – haemodilution - circulatory failure and death.

Lecture No. 22

N.K. Sanil

Host-parasite interactions

Host specificity – factors involved - Innate immunity – factors – role of epithelial cells and mucus – resistance – alternate pathway of complement activation. Aquired immunity – cell mediated immunity and humoral immunity – immunity to homologous infection - complement fixing and neutralizing antibodies. Temperature and immune response/ course of diseases - Humoral immunodepression in *Cryptobia salmositica* infections - Balanced Host-parasite relations – absence of immune response against myxosporean spores - Cell & tissue reactions to parasites – myxosporeans – host cell envelope-inflammation – granuloma formation –

Pathology of histozoic forms – pressure atrophy - organ alterations – hyperplasia, hypertrophy – tissue destruction; coelozoic forms – inflammation necrosis- muscle lesions.

Tissue reactions in atypical hosts & sites – Extend of damage – factors – species – lifecycle stage s- intensity of infection – host reaction

Microsporidia – cell specificity – infected cells – xenoma - hyperbiotic development - hypertrophy & fragmentation of nucleus – non xenoma causing species.

Isolation and elimination of parasite – various stages of tissue response

Inflammation & connective tissue capsule formation – coccidians

Monogeneans – species specific- 'skin immunity' – tissue response – hyperplasia – deformation – respiratory failure - immunity to reinfection

Digenea – host specific & non specific cases – immune responses - migration – inflammation – fibrous capsule formation – melanophores – humoral antibody production – response to blood flukes

Cestodes – migratory stages - cellular response with inflammation – antibody production

Nematodes – hemorrhage – inflammation – adhesion – oedema – necrosis- encapsulation – granuloma formation

Copepods – natural resistance – acquired resistance.

Lecture No. 23 **N.K. Sanil**

Economically important parasites

Parasites of fishes – Protozoan parasites – amoebae, flagellates, sporozoans and ciliates – Flagellate infections - dinoflagellates – majority photosynthetic – *Amyloodinium oellatum* – velvet disease – specificity – course of disease – symptoms - pathology-epizootics. Infections with *Piscinoodinium* sp., *Crepidoodinium* sp.

Hexamitosis & spironucleosis – disease – symptoms - pathology.

Ichthyobodosis – (Coastiasis) *I. Necator* – disease – symptoms - pathology – Secondary infections. Ichthyobodosis in marine fishes.

Cryptobiosis – ecto and endo parasitic species - *C. (C) branchialis, C. (C) iubilans, C. (T) salmositica & C. (T) borreli* - disease – symptoms - pathology

Trypanosomosis- in fresh water and marine fishes - disease – symptoms - pathology –

Amoebic infections – opportunistic invasion – amoebic gill infections.

Systemic amoebiosis - *Acanthamoeba* spp.

Myxosporean infections – pathogenicity – host specificity

Sphaerospora infection in carps – disease – symptoms - pathology

Myxobolus koi infection in cyprinids – disease – symptoms - pathology

Myxobolus cerebralis infection in salmon – disease – symptoms - pathology

Ceratomyxa shasta infection in salmonids – disease – symptoms - pathology

Muscle liquefaction in marine fishes – disease – symptoms - pathology

Proliferative kidney disease - disease – symptoms - pathology

Microsporedian infections – Pleistophora infections – disease – symptoms - pathology

Glugea infection – Xenoma formation – disease – symptoms - pathology

Lecture No. 24 **N.K. Sanil**

Economically important parasites (continued)

Coccidia – Eimeria infections - disease – symptoms - pathology.

Enterococcidiosis – disease – symptoms - pathology

Nodular coccidiosis and other Goussia infections-- disease – symptoms - pathology

Ciliate infections –*Ichthyophthirius multifilis* infection – specificity - disease – symptoms - pathology – epizootic potential

Cryptocaryon irritans (*I. Marinus*) infection – disease – symptoms - pathology

Ectoparasitic ciliates – Chilodonella infection - disease – symptoms - pathology

Brooklynella infection disease – symptoms - pathology

Trichodina infections – ectoparasitic and endoparasitic forms -disease – symptoms - pathology

Peritrich ciliate infections – fouling – solitary and colonial forms – host specificity – disease – symptoms - pathology -

Sessiline peritrichs – Epistylis – epizootics – role of other pathogens.

Metazoan parasites – Monogenetic and digenetic trematodes - Cestodes – Acanthocephalans – Nematodes – Crustaceans.

Monogenian infections –

Neobenedenia infections - disease – symptoms - pathology

Dactylogyrosis – host specificity – disease – symptoms - pathology

Gyrodactylosis – host specificity – disease – symptoms - pathology

Diplozoon infections – disease – symptoms

Trematode infections – Ectoparasites and endo parasites – stages infecting fishes

Lecture No 25 **N.K. Sanil**

Economically important parasites (continued)

Diseases caused by adult trematodes and its pathology. Trematode infections in farmed fishes .

Blood flukes – Sanguinicola and Aporocotyles - disease – symptoms - pathology

Diseases caused by larval trematodes – metacercaria – encystment in tissues and viscera/vital organs - Black spot disease, yellow grub disease - ,metacercarial eye infections.

Cestode infection – stages infecting fishes – pathology in gut – fish tape worms of economic importance. Larval cestode infections – pathology.

Nematode infection – role of fish as definitive/ intermediate host – pathology caused by adult nematodes in fishes - Larval nematodes in fishes – visceral migration – pathology.– Philometra larva in carps – Anisakis larva in marine fishes.

Acanthocephalan infection – pathogenicity – Echinorhynchus infection in cyprinids and Salmonids - Acanthocephalus infection in cat fishes

Crustacean parasites – ecto parasites on gills/ skin – damages caused by feeding on blood/ tissues/ tissue fluids – attachment/ penetration damages – secondary infections

Copepods – skin and gill parasites – Ergasilus infections – disease – pathology

Lernaeosis – anchor worm –host specificity – disease –pathology

Caligus infections – disease – pathology . Lernaeocera infections - disease – pathology

Sphyrion infections - disease – pathology

Branchiura – Argulus (carp lice) – specificity – disease – symptoms

Isopod parasites - Cymathoids – disease caused -pathology

Annelid worms – leeches – vector potential

Lecture No. 26 **N.K. Sanil**

Parasitic diseases of Shellfishes

Parasitic diseases in Oysters/mussels/clams:

Perkinsosis – etiological agent – symptoms of the disease – pathological changes . Coccidial infection in kidney - etiology – symptoms – pathological changes.

Gregarine infections - developmental stages in molluscs – inflammation - local pathology. Microsporidiosis – infection of eggs – infections in oysters – cyst formation in connective tissues. Delaware Bay Disease – *Haplosporidium coastale* – development in connective disease - environmental factors – pathogenicity/mortality

Haplosporidiosis - *Haplosporidium nelsoni* – organs infected- pathological changes – mortality – environmental factors. Martelia infection – development in tissues – pathology/ mortality. Bonamiasis – symptoms – pathology – mortality.

Hexamitasis - pathogen – symptoms – pathology. Trichodina infections - pathology

Trematode infections – metacercarial infections – pathology – effect on growth

Red worm disease – pathology; Parasitic copepods -Pea crabs in mantle cavity

Parasitic diseases of crustaceans: Paramoebiasis - disease, occurrence – pathology. Ciliate diseases. Microsporidiosis – milk/cotton shrimp diseae – pathogen – symptoms – pathological changes – mortality. Paranophrys infection - systemic infection – mortality

Rhizocephalan disease – symptoms – effect on growth – castration. Peritrich ciliates – effect on host – role of high organic loads. Gregarine parasites – effect on hosts

Parasitic disease of Crabs: Microsporidiosis – symptoms - pathology – mortality.

Paramoebiasis – symptoms – pathology – mortality- epizootic potential. Haematodinium disease – pathology – mortality. Bitter crab disease. Rhizocephalan infections – Sacculina infection – parasitic castration – growth disturbances.

Trematode infections

Fish parasites causing problem for human health

Parasitic Zoonosis – human diseases transmited through fishes – fishes as intermediate hosts – role of food habits.

Protozoans – Cryptosporidium – host range and pathology

Cestodes – *Diphyllobothrium latum* geographic distribution – *D. lanceolatus & D dendriticus* in Japan - life cycle pattern. Symptoms of disease – megaloblastic anaemia – diagnosis

Trematodes – metacercaria in fishes – *Clonorchis sinensis* – natural hosts – site of infection - symptoms– metacercariae in cyprinid fishes

Opisthorchis tenuicollis, O. viverreni, O felineus - natural hosts – fish intermediate hosts - symptoms – prevalence.

Heterophyes heterophyes , *Metagonimus yokogawai* - Natural hosts – intermediate hosts - site of infection - symptoms – prevalence.

Chlonorchis sinensis – Natural hosts – intermediate hosts - site of infection - symptoms – prevalence

Pargonimus westermanii – *Paragonimus kellicotti* – lung flukes – role of crustacea, crab, cray fish in transmission – symptoms

Nanophyetus salmincola – metacercaria in salmonids – vector potential for *Neoricketsia helminthoeca*

Nematodes – Anisakiasis – *Anisakis simplex* and *Pseudoteranova* (*Phocanema*) *decipens & Contracaecum* – parasites of marine mammals – natural hosts - acute illness – symptoms – prevention

Eustrongyloides sp. – symptoms. Spirometra – symptoms and disease – transmission.

Capillariasis – Host range & natural hosts – crustacean intermediate hosts - fish intermediate hosts – transmission – symptoms

Parasites as vectors – Role of parasites in the transmission of bacterial and viral diseases

Lecture-28 **K.S.Sobhana**

Defence mechanisms in finfish-1

Introduction to teleost immune system – Innate/non-specific immunity and adaptive/specific immunity – co-operation between innate and acquired immunity *in vivo*

Non-specific defence mechanisms- surface barriers- skin , mucus and alimentary tract enzymes

Non-specific humoral factors- transferrins, interferons, enzyme inhibitors, complement (complement activation- classical and alternate pathway), Lysozyme, agglutinins, precipitins, lectins and C-reactive protein

Non-specific cellular factors- granular and semi-granular cells – macrophages, monocytes and neutrophils- Reticulo endothelial system (RES)' Non-specific cytotoxic cells (NCC), eosinophils, basophils and mast cells, eosinophilic granular cells (EGC'S)

Phagocytosis- cells involved in phagocytosis, phagocytic activation- factors like chemotactic agents- Lymphokines, complement factors, opsonins etc.-recognition and attachment of particles to the ingesting leucocyte- engulfment with subsequent formation of a phagocytic vacuole- killing/bactericidal activity- respiratory burst- production of oxygen free radicals

Inflammatory response- protective response to tissue injury- non-specific humoral and cellular factors involved- functions of inflammatory response.

Lecture-29 **K.S.Sobhana**

Defence mechanisms in finfish-2

Specific defense mechanisms – humoral immunity and cell mediated immunity. Immunological memory - cells involved- B and T lymphocytes
Antigens- nature, antigenic determinant sites (epitopes), haptens and carriers

Humoral antibody response- role of B lymphocytes- origin and differentiation of B cells- haemopoeitic stem cells, plasma cells, effector cell and memory cells- clonal selection theory- primary and secondary response.

Immunoglibulins – structure of antibody- heavy chains and light chains- -constant and variable regions of antibody- Fab and Fc fragment- kappa and lambda variability of light chains- antibody binding sites- immunoglobulin subclasses- IgG, IgM, IgD, IgA and IgE.

Functions of antibodies- agglutination, precipitation, opsonisation, neutralization, complement activation etc.

Lymphoid organs in fish – primary and secondary lymphoid organs
Thymus- morphology, ontogeny- thymus as a primary lymphoid organ
Kidney- renal haemopoietic tissue- reticulo endothelial system and lymphoid system in kidney, melano macrophage centers, antigen trapping in kidney, circulation of lymphocytes
Spleen- Ellipsoid system and associated melano macrophage centers- uptake of foreign material in spleen
Mucosa associated lymphoid tissue- local immune responses-associated with gills, skin and gut (gut associated lymphoid tissue- GALT)

Lecture-30 **K.S.Sobhana**

Defence mechanisms in finfish-3

Cell mediated immunity (CMI)- role of T-lymphocytes- T cell antigenic recognition and T cell receptors

T lymphocyte sub populations- T helper cells, T killer/cytotoxic T cells, T lymphokine producing cells, T suppressor cells.

Antigen processing and presentation – T dependent and T independnt antigens- antigen trapping and uptake- role of major histocompatabality complex (MHC) in antigen processing and presentation- endogenous/ cytosolic pathway and class I MHC – role of cytotoxic T cells with CD8 receptors

Exogenous/ endocytic pathway and class II MHC molecules- macrophages/monocytes/B lymphocytes as antigen presenting cells – role of T cells with CD4 receptors

Principles of fish vaccination- vaccines stimulate specific immune system by producing antibody, group of memory cells and T cells.

Types and methods of vaccination- oral vaccination, injection vaccination and immersion vaccination Effectiveness of a vaccine- function of safety and immunogenecity . methods of assessing vaccine effectiveness- relative percentage survival(RPS) and increase in lethal dose 50 percent

Facors affecting immune responses- extrinsic factors- temperature, antigen dose, nature of antigen, route of administration, adjuvants, seasonal effects, environmental effects- Intrinsic factors- immune complexes, helper activity, suppressor activity and ontogenic maturation.

Lecture-31 **K.S.Sobhana**

Defence mechanisms in crustaceans

Introduction to invertebrate defence mechanisms
Physical barriers- cuticular exoskeleton, cuticular lining of foregut and midgut

Internal cellular defences- include wound repair and coagulation, phagocytosis, nodule formation and encapsulation, cytotoxicity

Invertebrate blood cells- haemocytes- hyaline cells, semigranular cells and granular cells – classification based on presence of granules and staining reactions

Haemopoeisis in crustacea – role of haemopoietic tissue and progenitor/ stem cells
Role of haemocytes in phagocytosis, nodule formation, encapsulation and cytotoxicity.
The clotting reaction – role of haemocytes in clotting

Humoral factors- Lectins- carbohydrate bindig proteins, agglutinins and lysins, other antimicrobial factors, other inducible humoral factors, coplement like activity

The prophenol oxidase activating system- control, release and activation of pro- Po system- antimicrobial compounds- terminal compounds of the pro-Po system
Role of immunostimulants in pro-Po activation- participation of pro-Po components in cell to cell communication.

Lecture-32 **K.S.Sobhana**

Economicaly important bacterial diseases of coldwater and warm water finfish-1

Introduction to fish bacteriology- taxonomy- structure of bacterial cell wall- gram reaction

Pathogenecity mechanisms of gram negative and gram positive bacteria- processes of bacterial disease development- mechanisms of cell and tissue damage- persistence of infection – factors responsible for predisposition

Types of bacterial diseases- Acute systemic type, surface ulcerative type and chronic granulomatous type of infections

Important bacterial fish pathogens:

Vibrionaceae- there are several representatives of fish pathogens under this group.
Vibriosis - serious disease in marine and migratory fish- 'red pest' in eels - aetiological agent- *Vibrio anguillarum*- a number of biotypes present.
Other vibrios involved- *V.damsela, V.vulnificus, V. alginolyticus, V. carchariae*
Habitat- only found in marine and estuarine environment, Isolation- culture- morphology

Clinical pathology-disease takes 3 forms depending on culture practices, virulence of pathogen etc.- per acute, acute and chronic forms, Pathogenecity- Histopathology.

Control- husbandry practices play an important role in control- use of antibiotics and other antimicrobial agents- successful vaccines have been also developed.

Lecture-33 **K.S.Sobhana**

Economicaly important bacterial diseases of coldwater and warm water finfish-2

Aeromonas hydrophila: aetiological agent of motile aeromonad septicaemia- a facultative pathogen- Habitat- isolation –culture-morphology- epizootiology

Clinical pathology- depends on state of the disease- Histopathology

Other motile aeromonads pathogenic to fish- *Aeromonas caviae* and *Aeromonas sorbia*

Aeromonas salmonicida: Aetiological agent of furunculosis in salmonids- obligate pathogen- disease occur in both fresh water and marine fishes- sub species- *A.s.salmonicida, A.s.achromogenes, A.s.masouda*

Habitat- isolation – morphology-epizootiology- Clinical pathology- purely depends on form of disease- peracute, acute and chronic- presence of large number of furuncles (raised lesions) on the body surface in chronic infections

Histopathology- very little host response- absence of inflammatory response

Virulence factors- cell surface antigens and extra cellular proteins(ECP'S)-

Development of vaccines.

Myxobacteria: gram negative bacteria capable of gliding motility

Cytophagaceae- gram negative single rods or filaments motile by gliding movements- pigmented bacteria forming yellow orange of red colonies

Flexibacter columnaris: aetiological agent of columnaris disease- Habitat- associated with mucus of both normal and diseased fish- mostly freshwater fish- Isolation- culture- morphology- epizootiology- clinical pathology- lesions confined to the skin of head and back of gills-Histopathology

Diseases caused by other cytophagales- Bacterial gill disease – common in cultured salmonids and ornamental fish- cotton wool disease – skin ulcers

Flexibacter psychrophila: cold water disease (peduncle disease) in cultured salmonids

Lecture-34 **K.S.Sobhana**

Economicaly important bacterial diseases of coldwater and warm water finfish-3

Enterobacteriaceae: *Edwardsiella ictaluri:* aetiological agent of enteric septicemia in channel cat fish- host specific- a primary pathogen -Clinical pathology- histopathology

Edwardsiella tarda: pathogen of freshwater eels -Habitat- Isolation-morphology-culture –epizootiology- Clinical pathology- histopathology

Yersinia ruckeri: aetiological agent of enteric red mouth disease (ERM) in salmonids- Habitat-morphology-epizootiology-clinical pathology-histopathology

Pasturellaceae: *Paturella piscicida* : a pathogen of marine fish –causes pseudo-tuberculosis and severe epizootics in cultured yellow tail and black sea bream.

Pseumonadaceae: *Pseudomonas flourescens:* causative agent of red spot disease in Japanese eels

Flavobacterium: gram negative non motile rods with rounded ends- yellow or orange pigmented colonies

Bacillaceae: gram positive bacilli which form endospores. *Clostridium botulinum:* Habitat- isolation- epizootiology

Coryneform group: nonsporing gram positive rods. *Renibacterium salmoninarum:* aetiological agent of bacterial kidney disease (BKD) in salmonids- Habitat-isolation-culture-epizootiology

Streptococcaceae: gram positive, nonmotile, spherical cells in single or chains

Streptococcus spp.: habitat- isolation – epizootiology

Mycobacteriaceae: *Mycobacterium marinum*- pathogen of marine fish- Isoaltion –culture-epizootiology-other Mycobacteria- *M. fortuitum, M.chelonei*

Nocardiaceae: causative agnets of nocardiosis in fresh water fish- *Nocardia asteroids, N. kampachi*

Control of bacterial diseases- use of antimicrobial agents and vaccines

Lecture-35 **K.S.Sobhana**

Economicaly important fungal diseases of coldwater and warm water finfish and shellfish- 1

Fungi- introduction- classification- most of ichthyoparasitic fungi are members of Eumycota (true fungi)

Subdivision- Mastigomycotina- characterized by spores with motile sexual stage and either a motile or non motile asexual stage

Class- Oomycetes- most important group of fish fungal pathogens- produce motile, biflagellate spores- hyphae generally without cross walls

Order: Saprolegniales- Fly. Saprolegniaceae- Eg. *Saprolegnia, Achlya, Aphanomyces* (*Aphanomyces astaci*- cray fish plague fungus and *Aphnomyces invadens*- EUS fungus)

Fly: Leptolegniaceae- eg. *Leptolegnia marina*

Fly: Haliphthoraceae: eg. *Haliphthoros milfordensis*

Order: Lagenidiales- Fly-Laginidiaceae- eg. *Lagenidium* spp.

Fly: Sirolpidiaceae: eg. *Sirolpidium*

Orser: Leptomitales- Fly. Leptomitaceae- eg. *Leptomitus* spp.

Order: Peronosporales- Fly. Pythiaceae- eg. *Pythium* spp.

Class: Chytridiomycetes- Order- Chytridiales- *Dermocystidium* spp.

Subdivision:Zygomycotina-produce non motile sporangiospores(aplanospores)

Order: Entomophthorales- eg. *Basidiobolus spp.*

Sub division : Ascomycotina- possess septate mycelium and no flagellated structures- produce ascospores- eg. *Trichomaris invadens*

Sub division: Deuteromycotina (Fungi imperfecti): lack sexual stage in life cycle- possess septate mycelium- reproduce by asexual method by producing conidia. Eg. *Phoma herbarum, Fusarium solani, Ochroconis sp., Exophiala, Phialophora, Aspergillus*

Other asceptate fungi: *Branchiomyces* spp.: produce aplanospores. Eg. *B. sanguinis* and *B. demigrans.*- infects gill tissue causing brancyomycosis

Ichthyophonus hoferi: causes systemic granulomatosis (Ichthyophoniasis) in freshwater and marine fish.

Lecture-36 **K.S.Sobhana**

Economicaly important fungal diseases of coldwater and warm water finfish and shellfish- 2

Epizootic ulcerative syndrome: Introduction – Mycotic granulomatosis (MG); Red spot disease (RSD) and Epizootic ulcerative syndrome (EUS)

Species affected- Aetiology- disease occur in wide geographical area and diverse range of habitats

Bacteriology of EUS – virulent *Aeromonas hydrophila* strains isolated from EUS affected fish caused EUS like lesions in Tilapia- in natural outbreaks tilapine species are refractory to EUS.- possible reasons for frequent isolation of *Aeromonas* and *Pseudomonas* spp. from EUS lesions

Virology- no consistent isolation of a single strain/genus and all EUS associated rhabdoviruses do not belong to one taxonomic group - failure to show any consistent lesion in pathogenecity studies

Mycology - fungal presence and mycotic granulomatous conditions have been consistently seen in EUS affected fishes from different parts of the world.

-involvement of a highly invasive fungus, *Aphanomyces invadens*- necessary cause for EUS- Fungi can penetrate spinal cord and intramuscular bones, cause pathology in internal organs and can cause ulcers in the contra lateral side

Types of EUS lesions – Type I lesion (Erythamatous dermatitis) – type II lesion (Necrotising dermatitis) – type III lesion (Dermal ulcers) – type IV lesions (Healing ulcers).

Environmental factors affecting EUS- Temperature – rainfall and related water quality variables – flooding.

Lecture-37 **K.S.Sobhana**

Shrimp viral diseases

Introduction to viral diseases- RNA and DNA viruses

Important viral diseases affecting shrimps: taxonomic affiliation- host species – geographic distribution- impact on the host – diagnostic techniques

White spot virus (WSV) : wide host range- causes mass mortality in cultured shrimp – clinical signs – histopathology – basophilic intranuclear inclusion bodies in the hypertrophied nuclei of ectodermal and mesodermal origin tissues.

Baculovirus penaei (BP) : wide host and geographic range – cause mass mortalities especially in larval stages – target organs – epithelial cells of hepatopancreas and anterior midgut – single or multiple eosinophilic intranuclear polyhedral inclusion bodies in affected cells.

Monodon baculovirus (MBV) : Cause serious mortalities in cultured penaeids – target organs – hepatopancreatic tubule and duct epithelium and mid gut epithelium of PL, juveniles and adults – MBV occlusion bodies appear as prominent eosinophilic, single to multiple round bodies within the hypertrophied nuclei of affected cells.

Infectious hypodermal and haematopoietic necrosis virus (IHHNV) : affect many species of penaeids- conspicuous eosinophilic intranuclear inclusion bodies in ectodermally and mesodermally derived tissues in acute infections

Hepatopancreatic parvo like virus (HPV): cause mortalities in juvenile shrimps – single prominent basophilic intranuclear inclusion bodies in the affected epithelial cells of hepatopancreatic tubules and epigastric caecal epithelial cells

Other shrimp viruses : Reo like virus (REO); Rhabdo virus of penaeid shrimp (RPS); Yellow head virus (YHV); Lymphoid organ vacoulisation virus (LOVV); Baculoviral midgut gland necrosis (BMN); Taura syndrome virus (TSV).

Lecture-38 **K.S.Sobhana**

Nutritional diseases

Introduction- interaction between nutrition and fish health

Reasons for nutritional disease – insufficient food – imbalanced diet- poorly formulated diets – improper storage of feed – toxic factors in diet

Absolute nutritional deficientcy – signs of starvation – causes and effects

Deficiencies and imbalances of major dietary components:

Dietary macronutrients : Protein : nutritional value of protein – role of essential aminoacids – signs of amino acid deficiency/imbalance and inadequate protein in the diet

Lipids : role of essential fatty acids – effect of feeding excess fat , diets deficient in essential fatty acids and diets containing oxidized fat – fatty infiltration of liver – lipoid liver disease

Carbohydrates : effect of low energy diets deficient in carbohydrates – effect of excess carbohydrate levels – hepatocyte degeneration – glycogen deposition

Dietary micronutrients :Vitamins: water soluble vitamins – deficiency diseases due to vitamin losses during preparation and storage of feed and leaching in water – deficiency signs of vitamin C and B complex vitamins.

Fat soluble vitamins – chances for hypervitaminosis – Deficiency signs of Vitanins A, D, E and K.

Minerals : deficiency results mainly from reduction in bioavailbility – zinc deficiendy cataracts – goiters due to iodine deficiency – iron deficient anaemias

Dietary toxicity – Dietary mineral toxicity – Cadmium toxicity – nephrocalcinosis -Toxic organic compounds in the diet – poisonous plant alkaloids and fungal metabolites – mycotoxins – algal toxins – plant toxins – cotton seed toxin – ipil-ipil toxin-contamination with pesticides – drug toxicity

Nutritional diseases in farmed shrimps – Ascorbic acid deficiency syndrome (black death syndrome) – cramped muscle syndrome – chronic soft-shell syndrome

M.F.Sc - Mariculture

LECTURE OUTLINE

Course No. Mc-509

(MARICULTURE GENETICS)

Faculty

Dr.P.C.Thomas
Dr. P.Jayasankar
Dr. A.Gopalakrishnan

(Credits : 1+ 1)

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

Topic : Structure and function of nucleic acids

Nucleic acids :- DNA - RNA ; Primary functions:- hereditary material - direction of proteins synthesis by DNA - protein structure code; Structure:- Polynucleotides;

Constituents and primary structure :- (1) pentose sugar (ribose – RNA ; 2' deoxyribose – DNA). (2) purine base – (adenine/guanine) , pyrimidine base - (cytosine/thymine: DNA and cytosin/uracil : RNA); Nucleoside – Sugar + base ; (3) phosphate group – 5' sugar carbon – ester bond ; Nucleotide = nucleoside phosphate = nucleoside + phosphate; Different nucleotides :– adenosin - guanosine – cytosine – thymidine nucleotides; Polynucleotide formation;– nucleotide linking – 5' to 3' phosphodiester bond; Polarity of DNA – free 5' phosphate , 3' hydroxyl group;

DNA differences : – nucleotide sequence differences ; Notation :- A,T,G,C – 5' to 3' ; Secondary structure: – double stranded DNA – sugar phosphate backbone – inner projecting bases – facing each other.; Complimarity : – A-T, G-C ; Orientation of DNA strands: – anti parallel; Holding force – hydrogenbonds. Double helix :– opposite sugar direction – off the centre sugars base attachment – coiling/twisting of double strand; Direction & degree of base pair twist: – clockwise, 36° per base pair – Number of bases & length of complete turn (360°) = 10 base pairs - length 34 A°; Common & variant DNAs :- **A** -DNA, **B**- DNA, **C**- DNA, **D** –DNA and **X** – DNA. Distance of one complete turn - (length of 10 base pairs) : 34 A°; Double helix diameter :– 20 A° / 2 nanometers ; Designation of DNA strands :– plus (+) and minus (-) strands. Plus strand – transcribed strand.

Denaturation of double stranded DNA:- meaning – cause – role of temperature- start 70°C – complete 90° C; Melting temperature (Tm) :- 50% denaturation temperature. Hyperchromic effect: – absorbance of more light of 260 nm by single stranded DNA ; Renaturation :– re-doubling denatured single strands - slow cooling ; Rapid cooling – random coiling;

Types of RNA:- mRNA – rRNA – t RNA; Base pairing of complimentary nucleotides of RNA single strand – clover leaf tRNA; DNA , RNA pairing / hybridization;

Lecture No: 2 Prepared by: Dr. P. Jayasankar

Topic: **Molecular basis of heredity**

DNA replication: Mechanism of synthesis of DNA – Watson & Crick double helix model - Types of DNA replication – Semiconservative method – Universally accepted method – proposed by Watson & Crick and supported by Meselson & Stahl experimentally - conservative method – proposed by Cavalieri & Rosenberg – no separation of individual strands of DNA - dispersive method – parent molecule breaks down - mechanism of DNA replication – Origin of replication on the chromosome - replication forks - cell division stage – metaphase - strand separation – helices – topoisomerases – nicking - semiconservative replication – priming – RNA primer - requirement of reaction conditions – Synthesis of new DNA strands – DNA polymerases, 5'-3' nucleotide synthesis or leading strand sysnthesis – dNTPs - Okazaki fragments – synthesis in 3'-5' direction - DNA ligase

Lecture No: 3

Prepared by: Dr. P. Jayasankar

Topic: **Molecular basis of heredity**

Transcription and translation of DNA: Definition of transcription – the central dogma of molecular biology – DNA – mRNA – proteins - mechanism of transcription – template strand – promoter – initiation site – RNA polymerase – RNA strand elongation in 5'-3' direction - termination site or pause site – poly A tail – protein factors – primary transcript or nascent RNA - processing of mRNA, tRNA & rRNA – primary transcripts of mRNA - heterogenous nuclear RNA - tRNA – clover leaf structure - rRNA - definition of translation – mechanism of translation – activation of amino acid – aminoacyl adenylate - attachment of activated amino acid with tRNA – aminoacylation of tRNA – initiation of polypepetide chain – formylated and nonformylated methionins – initiation complex – elongation of polypeptide chain – dipeptide bond – termination of polypeptide chain – termination codon -

Lecture No: 4 Prepared by: Dr. P. Jayasankar

Topic: **Chromosome individuality**

Structure of chromosome: Self-reproducing thread-like structures in the nucleus – Hofmeister - Structural details of typical somatic chromosome – pellicle – thin and nongenetic – matrix – bulk of chromosomes - chromonemata – somatic or standard coil – euchromatin – heterochromatin - heteropycnosis - chromomeres – inter chromomeres – centromere – highly staining region – primary constriction – 5 zones of centromere – kinosome – 3 major functions of centromere - secondary constriction – subterminal position – nucleolar organizers - satellite bodies – SAT chromosomes - telomeres – polarity of chromosomes - fine structure of chromosome – DNA helices - chromatids

Lecture No: 5

Prepared by: Dr. P. Jayasankar

Topic: **Chromosome individuality**

Sex determination in fish: Sexual differentiation and differences – sex chromosomes – exceptions for X-Y sex determination – W-Z chromosomes – undifferentiated gonads - gonochorism - hermaphrodites – secondary sex characters – sexual dimorphism – fin length – body colour – genital papillae – brood pouches – courtships – aggressive display – sex-related size differences – Synchronous hermaphroditism – occurrence of ripe ovary and testis – Consecutive hermaphroditism - protandrous hermaphroditism – protogynous hermaphroditism - primary & secondary sexes – Endocrine control of sex reversal and hermaphroditism – production of all female population – controlled breeding - aquaculture management - sex determination in fishes – practical importance of sex determination – hereditary pattern of sex determination - sex linkage – sex related differences in chromosomes – chromosome engineering - sex-linked inheritance of traits — colour patterns – natural occurrence of hermaphrodites - genetic mechanism of sex determination - polygenic determination of sex – environmental influence of sex determination – hormone mediated sex reversal – hormonal transdetermination of sexes – heterogamety

Lecture No. 6 **Prepared by A. Gopalakrishnan**

Topic: Gynogenesis

Definition of Gynogenesis; Types of gynogenesis; fundamental aspects of fish oogenesis (arrest and resumption of I meiosis; stage of oocytes at the time of ovulation; sperm penetration and trigger for the release of II polar body; parthenogentic development of fish oocytes upto 6 hours after water hardening); meiotic and mitotic gynogenesis; UV-irradiation of sperms; details of experimental set up; effect of distance between UV-lamp and sperm suspension; Optimum thickness of sperm suspension in the container; sperm-egg fertilization; time of release of II polar body; type of shock (pressure and thermal - (cold & heat shock)); time and duration of shock to arrest the release of II polar body in the case of meiotic gynogenesis in various species; time and duration of shock to arrest the I parthenogentic cleavage in the case of mitotic gynogenesis; Differences between meiotic and mitotic gynogenesis; methods to ascertain gynogenesis, level of homozygosity in meiotic and mitotic gynogenesis; application of gynogenesis in fisheries/aquaculture; species in which experimental gynogenesis are produced; Gynogenesis attempts in Indian species and abroad.

Lecture No.7 **Prepared by A. Gopalakrishnan**

Topic: Androgenesis

Definition of androgenesis; fundamental aspects of fish oogenesis in brief; parthenogenetic development of fish oocytes; destruction of nuclear material in ripe egg using UV; γ and X rays and related problems; sperm penetration into eggs through micropyle; parthenogenetic cleavage of haploid male nucleus inside enucleated fertilized oocyte; time and duration to arrest I parthenogenetic cleave of sperm nucleus; diploidisation; application of androgenesis in fisheries; recovery of endangered/extinct species using androgenesis and milt cryopreservation. Bottlenecks in androgenetic techniques; androgenetic experiments in abroad and in India; species in which androgenetic experiments have been carried out; supermale (YY males) production and androgenesis.

Lecture No.8 **Prepared by Dr.P.C.Thomas**

Topic : Polyploidy

Chromosome number – specified for each species; Ploidy – chromosome number variations ; Cause :- irregularities during meiosis – mitosis – fertilization;

Ploidy classification: – Euploidy and Aneuploidy ;

Euploidy : Definition:- duplication / loss – complete chromosome set ;

Euploids: – organisms – contain complete chromosome sets – any number.

Euploidy types: – (1) Monoploidy – one set of chromosomes in the somatic cells (n); (haploidy – monoploidy in gametes); Monoploids – generally sterile.

(2) Diploidy – two chromosome sets – (2n) in somatic cells; Diploidy – related with fertility - balanced growth – vigour – adaptability - survivability etc.

(3) Polyploidy – more than 2 sets of chromosomes.; Polyploids :- triploid (3n); tetraploid (4n); pentaploid (5n); hexaplod (6n) etc.; Polyploiy levels higher than tetraploids – not commonly in nature.

Kinds of polyploidy:

(1) Autopolyploidy:- chromosome sets homologous – all from same species.
Eg: Autotriploid (AAA) – autotetraploid (AAAA) ;

(2) Allopolyploidy:- All chromosomes sets in cell not homologous – belong to two different species – Allotetraploid : AABB ;
Allopolyploids – behave like new species ;

Significance of polyploidy:

Genetic – taxonomic – evolutionary – economic significance.
The genetic significance:- understanding dosage effect ; The taxonomical and evolutionary significance: - forms new species.

Autopolyploidy – additio of no new alleles to the genome – less significant - than allopolyploidy in forming new specie.

Allopolyploidy – produce new adaptive gene combinations – provides better adaptability to species – wider variety of habitats - increased chance of being successful in natural selection; Polyploid plants – gigantism - accumulation of more vitamins etc.,

Triploid animals – infertile – wastage of no energy – reproduction – better growth rate ; Triploid plants – seedless fruits – gigantic fruits.

Lecture No.9 Prepared by Dr.P.C.Thomas

Topic : Aneuploidy

Aneuploidy : – duplication - loss of part of – chromosome complement ;

An aneuploid – an individual whose chromosome numbers differs from the wild type- (diploid number) by - part of a chromosome set ;

Aneuploid chromosome set – differs from wild type by – one or small number of chromosomes; Cause of aneuploidy :- Non-disjunction during mitosis - meiosis - cause most aneuploidies ; Type of aneuploidy :-

Monosomy :- diploid organisms – lack only one chromosome – from a single homologous pair – monosomic – (General formula = 2n-1)

Types of gametes from monosomic individual – two types – i.e. (n) and (n-1)

Turner syndrome – human – monosomic condition – 46-1 – absent chromosome – an X chromosome;

Nullisomy:- diploid organisms – lose a pair of homologous chromosome – General formula = 2n-2 ; Effect of nullisomy: - lethal mostly – effect in survivers - vigour reduction – fertility reduction – survivability reduction ;

Trisomy :- Diploid organism – extra chromosome –. 2n+1 ; Types of gametes produce – two – (n) and (n+1) ;

Klinefelter syndrome – trisomy in males – X chromosome duplication – 44Autosomes + XXY - extra X chromosome in male; Down syndrome:- trisomy of chromosome 21.

Tetrasomy:- duplication – one homologous pair – 2n+2 ; Type of gametes – one– (n+1) ; Double trisomy : – diploid organism – chromosomal addition – two different chromosomes have an extra – 2n+1+1 ;

Lecture No.10 Prepared by **Dr.P.C.Thomas**

Topic: Characteristics of Qualitative and quantitative traits

Qualitative traits - Mendelian traits :- Genetic control - one of few pairs of genes. Types of gene action involved :- Intra-allelic interaction – dominance - incomplete dominance - co-dominance;- Inter-allelic interaction (More than one locus) : epistatic interaction;- All or none traits / Either or traits - can be classified into distinct phenotypes. - discontinuous variation

Qualitative gene expression - not affected by environmental factors.
Study of qualitative traits - counting progenies number with different phenotypes - finding their ratios.

Quantitative traits:- Polygenic inheritance-Multiple gene inheritance-many genes.

Gene actions: different types may be involved in expression of quantitative traits.

Additive gene action - predominant type involved in the quantitative traits - contribution of each allele (of the same locus or different locus) - additive or cumulative;- Individual gene's contribution – vary - cannot be noticed;

Continuous variation - no sharp distinction between phenotypes of different genotypes - many gradations of phenotypes available - continuous variation.
No distinct phenotypic classes - overlapping.;
Genotype of individuals - indistinguishable by the Mendelian ratios.

Economically important characters - generally quantitative.(length- weight, etc.).
Measurable traits - metric units used for measurement - metric traits.
Study of quantitative traits - made as populations of individuals.

Genetic analysis – description of genetic architecture of the population - statistical procedures -statistical genetics / population genetics.

Expression of quantitative traits - modified by environment -very much.
Genotype sets - maximum limit to which the individual can express the character.

Additive gene action :
The predominant gene action - in quantitative / economic traits.

Effect of alleles - (of the same locus or different locus) - cumulative in effect.

Example: Skin colour in trouts and human ; four alleles –two each at two locus.

Black color: due to four alleles AABB. White : due to alleles aabb.

Economically important traits - many genes - magnitude of contribution by the alleles – vary - but will be additive.

No sharp distinction - between phenotypes resulting from different genotypes - many gradations of phenotypes - show continuous variation of phenotypes.

Individuals - cannot be segregated into distinct phenotypic classes - overlapping.- Individual genotype - not distinguished ; Effect of individual genes - unrecognizable by the Mendelian ratios.

Expression of additive genes are affected/modified by environment.

Genotypic and phenotypic distribution

Normal distribution :- Individuals normally distributed - Bell shaped curve;

Quantification : Additive phenotype quantification - measures of central tendency and dispersion - mean value, range, standard deviation, variance,coefficient of variance; Mostly used parameters - Mean and standard deviation (SD).
SD = average of squared deviation of individual values from the mean - accurate measure of variation in a population - describe population effectively together with means ; Coefficient of variation = SD x100 - unitless, standard measure of variation - compare populations.;
Phenotypic and genotypic values: First division of phenotypic value = genotype (heredity) and environment ; Genotype confer certain value to individual - environment cause deviation - one direction or other direction.; Phenotype = G enotype+ Environmental deviation(P = G + E).
The environment:- all non genetic factors.; Similar genotype in different environments - may differ in (quantitative) phenotype.; The value conferred by the genotype to individual - genotypic value.
Sum total of all environmental deviations in a population = zero ; mean phenotypic value = mean genotypic value(when a large population is considered).

Components of genotypic value: Quantitative characters - different gene action - each contribute its share to genotypic value; Genotype value = sum of the different types of gene actions contributing ;

Breeding value or Additive genetic value:-Main component of genotype value = additive genotypic value - that part of that part of genotypic value due to additive gene's contribution; Breeding value -BV:- superiority of individual's genetic value - over population value on account of additive genes - (instead of absolute values);

Transmission of additive genetic value / the BV - additive genetic value - can be and will be transmitted to the progeny - predictable transmission.; Transmission of non additive and epistatic values - unpredictable.

Estimation of additive genetic value or BV - from the mean value of its progenies.

Calculation of BV:- Individual mated to a number of random females; Breeding value = twice the mean deviation of the progeny from the population mean- (deviation doubled as the one parent in question provides only half the genes in the progeny, the other half coming at random from the population.)
Genotypic value of the progeny = half the BV of the one parent in question.
B.V - measurable. BV is indicated as 'A'.

Norm of Reaction

Definition - table or graph showing the range of phenotypes expressed by a given genotype in different environments .

Basis - genotype environment interaction-certain genotypes perform better than others in one environment compared to other environment .

Topic : Variance ; Genetic variance of quantitative traits

Heridity - (genotype or genetic make up) - fixed at the time of conception - except for mutation - remains the same throughout life.

Dissimilar genetic make up for all individuals - except identical twins.

Offspring produced by the same pair of parents - dissimilar genetic make (Since parents are not homozygous for all the genes they posses).

Parents - homozygous for more genes pairs - produce progenies more similar genetically - than those produced by parents having less of homozygous pairs.

Amount of genetic variation - within species – unlimited. (Two parents heterozygous for the same 20 pairs of genes located on 20 different homologous chromosomes - can produce 3^{20} different genotype (3.5 million genotypes).

Greater variability for quantitative traits - because many genes and different gene actions involved

Same quantitative genotype - differ in phenotype of – in different environments.

The population exhibit - lot of phenotypic variation.

The genetic study of metric traits - centers around the study of variation - study of variation of individual values from the population average; Quantitative genetic questions are explained - in terms of variations.

The variation in the population - estimated - expressed as variance.

Phenotypic variance(V_P):- variance of the phenotypic values - measured from individuals of the population.

Partitioning of total variance -(phenotypic variance) into component parts - genetic study of quantitative traits - involve partition of total variance - (phenotypic variance) into its component parts.

The phenotypic variance (V_P) = sum total of the variance caused by genotype(V_G)-environment(V_E) - interaction between the two(V_{GE}).

The phenotypic variance can be partitioned into - genetic variance -variance due to the environment - genotype environment interaction variance --- $V_P = V_G + V_E + V_{GE}$

V_G - of greatest importance - breeding programme to improve the stock – exploit the genetic variance in the stock.

The genotypic variance(V_G) - further divided into additive genetic variance V_A, dominance variance V_D and interaction variance V_I

The interaction variance $V_I = V_{AA} + V_{AAA}, V_{AD} + V_{DD}$

Topic : Variance

Additive genetic variance:

Variance due to additive genotype - (variance of breeding value) :- component most important - chief cause of resemblance between relatives - chief determinant of observable genetic properties of the population;

Additive genetic variance - (V_A) - proportionately and predictably inherited by the progeny from the parent - V_D and V_I are not ;

Additive genetic variance - only component readily estimated from population performance data - (since additive genetic effects are proportionately transmitted to the progenies).

Common practice - partition total variance into additive variance against all the rest, the rest being non additive genetic variance.

Genotypic and environmental components of variance (V_G and V_E) – cannot be estimated from single population.

Estimation of V_G and V_E in experimental populations :- if one component can be completely estimated then phenotypic variance would provide an estimate of other component.

Environmental component :- includes all non-genetic variances - beyond experimental control - cannot be removed completely.

Environmental genotypic variance - can be achieved experimentally.

Individuals of identical genotype – for estimation of V_E - highly inbred lines , clones propagated from single individuals, identical twins ; Phenotypic variance of identical twins, clones (grown in the normal range of environment or circumstances) = estimate of environmental variance V_E ;

Genotypic variance (V_G) = Substraction of V_E from the phenotypic variance(V_P) of a genetically mixed population ;

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

Topic : Heritability

Heritability - estimate of proportion of genetic variance - out of phenotypic variance in a population.

Importanance of heritability :- genetic variance portion of V_P only transmitted to the progeny - choice of selection and breeding programme for genetic improvement depend on magnitude of proportion of the genetic variance out of the phenotypic variance (heritability)

Heritability in broad sense - (H^2): - fraction of the total phenotypic variance(V_P) which due to all genetic variance (V_G) ; $H^2 = V_G / V_P$

Heritability in the narrow sense - (h^2) = estimate of the fraction of V_P due to V_A = V_A/V_P

Range of heritability : 0 to 1.

Application of h^2 :- narrow sense heritability (h^2) : metric traits – (1) indicat[on of predominant gene action involved in genetic variance (2) prediction of breeding value of individual from phenotypic value (3) prediction of selection response .

Reason - only V_A proportionately and predictably inherited by progeny from the parents - unlike V_D and V_I.

Genetic gain from selection :- prediction formula : response to selection (R) or genetic gain (A_G) = selection differential (SD) x heritability.

When heritability is 1, R = SD x 1 = SD itself.
When heritability is 0, R = SD x 0 = 0

Prediction use : not mandatory - enable breeder to anticipate whether gain = worth the effort needed.

Selection and breeding strategy determination: heritability (h^2) magnitude - indicate predominant gene action in genetic variance - help planning selection and breeding strategies for maximum genetic gain.
High narrow sense heritability - predominant additive gene action - simple mass selection or individual selection useful -for trait improvement.
High broad sense but low narrow sense - predominance of non additive gene action -reduced efficiency of mass selection – need special selective breeding programmes to exploit non additive genetic variance for genetic improvement.

Heritability (h^2) above 0.25 - mass selection; heritability below 0.15 - mass selection inefficient.

Low heritability traits - fitness related traits - crucial for the survival of the species in the environment . Reproductive traits - low heritability. Meristic traits - larger heritability.

Lecture No.15 **Prepared by Dr.P.C.Thomas**

Topic : Gene frequency: Binomial distribution of genotypes

Gene frequency - allelic frequency - relative abundance or rarity of a gene/ allele in a population. Genotypic frequency - relative abundance or rarity of a particular genotype in a population. Example: Trouts with GG genotype normally pigmented ; G'G' genotype golden coloured and GG' palomino.

Population of 1000 trouts : 360 GG, 160 G'G' and 480 GG' . Allele frequencies :

$$G = \frac{360+360+480}{2000} = \frac{1200}{2000} = 0.6 \; ; \quad G' = \frac{160+160+480}{2000} = \frac{800}{2000} = 0.4$$

Binomial distributionof genotypes :normally distributed: bell shaped cure on plotting
Alleles = G and a ; Genotypes = AA, Aa, aa
Genotype ratio = 1 AA + 2Aa =1 aa
Population of 1000 trouts : GG=360, Gg'=480, G' G' =160.

$$\text{Frequency of GG} = \frac{360}{1000} = 0.36 \; ; \quad \text{Frequency of GG'} = \frac{480}{1000} = 0.48$$

$$\text{Frequency of Rr} = \frac{160}{1000} = 0.16 \; ; \quad \text{Frequency of G} = \frac{360+360+480}{2000} = 0.6$$

$$\text{Frequency of G'} = \frac{480+160+160}{2000} = 0.4$$

Total of frequency of alleles at a locus = 1 ; Frequency of allele **A** is 1 (all individuals in that population - homozygous for that allele) - then frequency of allele **a** is zero ; Range - bwtween 0 and 1 ;

Estimation of allele frequency of dominant /recessivegene action:- Dominant and heterozygous genotypes - indistinguishable phenotypically - estimation frequency - basing Hardy-Weinberg law.

Gene frequency:Hardy-Weinburg law (1908):

Definition - gene frequency remains constant from generation to generation in random mating populations in the absence of selection.

Hardy-Weinburg ratio – allele frequency p and q - then p+q equals one - frequencies of the three offspring genotypes will be = p^2, 2pq, and q^2 .

Homozygous dominant individual = p^2 · Hterozygous individual = 2pq
Homozygous recessive individuals = q^2

Conditions for Hardy-Weinberg law : random mating large population without – selection, mutation, migration, mixing of populations.

The estimation of gene frequencies using Hardy-Weinburg ratio:
(1) Frequency of recessive gene = root of homozygous recessives frequency.
(2) Frequency of dominant gene =1 minus the frequency of recessive allele
(2) Frequency of homozugous dominant I = square of frequency dominant gene
(4 Frequency of heterozygous individuals = 2 times the sum of the frequency of dominant allele and frequency of recessive allele.

Factors affecting gene frequency:- selection, mutation, migration and genetic drift .

Lecture No.16 Prepared by Dr.P.C.Thomas

Topic : Selection for genetic improvement

Selective breeding – time tested – conventional method – in plants and live stocks genetic improvement of fish – applicable ;

Definition : Selection – Process of preferring certain individuals in a population over others – production of next generation;

Classification:

Natural Selection:- Survival of the fittest – main force responsible for nature's selection.; Artificial Selection:- selection practiced by man – to increase frequency of desirable genes in the stock – selecting only good individuals for breeding ;

Classification on the basis of selection criteria:

Individual Selection :- Selection of breeders –criteria of selection – individual's own phenotypic performance – use phenotype – indicator of genotype –Mass selection.

Effectiveness:

Quantitative traits:- Most effective for additive and highly heritable traits; Reliability - enhancement –evaluation of when all individuals - under uniform environmental conditions.

Qualitative traits:- Most effective for- codominant trait ; less reliable if dominant - over dominant -epistatic;

Genetic effect of selection:- creation of no new genes - increase desirable gene's frequency- in the population; Increased proportion of homozygous individuals.

Genetic gain in quantitative trait from one generation of selection :-

$$\Delta_G = h^2 \times Sd \; ; \quad \text{where } Sd = (P_s - P)$$

Yearly generic gain: $\Delta_{Gt} = \frac{h^2 \times Sd}{I_g}$

Lecture No. 17 **Prepared by Dr.P.C.Thomas**

Topic : Pedigree Selection

Definition :- ancestor's performance - performance pedigree - selection basis.

Pedigree :- record of individual's ancestors - related through its parents.

Pedigree selection for qualitative traits:-

Advantage:- detect[on of the possibility of recessive genes in the individual.

Disadvantage:- culling good individuals – on possibility of recessive gene carrier;

Pedigree selection for quantitative traits:- Advantages / applicability :- (1) increased accuracy of probable breeding value – estimate of individual based on its own performance ; (2) lowly heritable traits - records of ancestors enhance accuracy of individual selection.

Method:- Ancestors ranking – in comparison with contemporaries – using trait ratios ; Trait ratios:- form average performance record of the contemporaries – subtraction from performance record of ancestor – expressed as percentage of the average performance of its contemporaries.

Family selection : Selection basis - family mean ; Family : full-sibs / half sibs family ; unit of selection - full-sibs / half sibs family ;

Applicability: 1) when h^2 is low - individual selection is inefficient. (2) when environmental variance uncontrollable - example: Non-synchronous spawning, hatching etc. - (negation of V_E by family means - genetic differences are better revealed) ; 3) slaughter traits - sex limited traits.

Expensive than mass selection - require more facilities - more record keeping.

Between family selection: Ranking of mean values of each family – whole family selected – culled; save a random and equal number of individuals – from each selected families; estimation of separate male and female means – separate selection made if sex dimorphism exists ; Disadvantage- culling of individuals with good phenotype - since entire family rejection.

Within family selection: family considered temporary sub-population –retaining of best individuals from each family . **Combined-between and within family selection :** First step – selection of best families - second step- selection of best individuals of selected families for breeding.

Lecture No. 18 Prepared by Dr.P.C.Thomas

Topic : Selection on the basis of progeny testing

Selection base - average merit of offsprings – comparison of average merit of progeny - from contemporary breeders ; Frequent use – selecting sires in terrestrial animals; Advantage in fish – selection of dams.

Uses : Qualitative traits:

Identification of homozygous - heterozygous breeders of dominant gene action traits ; Applicability in fish : - multiparous species - efficient.

Producion of off springs for progeny testing – mating with testers.

Testers:- homozygous recessives – known carriers of the recessive genes.

Other methods : mating with own daughters – unselected half sibs – full sibs.

Quantitative traits:

Reliability :- more accurate – compared to mass selection – at low heritabilities.

Diallel crossing for progeny testing – reduce importance of maternal influence in – progeny test results – (Diallel cross : determining BV of two or more males by – breeding to the same two or more groups of females – comparing sire wise progeny averages).

Accuracy: - progeny test more accuratly predict – BV than using own performance-better progress per generation.

Progeny test increases generation interval - lower progress per year - for additive traits; Many to be progeny tested - few to be - based on the ranking; labour intensive -very expensive-.

Lecture No.19 **Prepared by Dr.P.C.Thomas**

Topic : Methods of selection for more than one trait

The value of animal – depend on several traits – selection of multiple traits.
Selection methods :- Tandem method - Independent culling - Selection Index.

(1) Tandem method:- one trait at a time – realize satisfactory improvement – relax selection efforts – direct towards efforts another trait..
Efficiency:- Least efficient method – depend on genetic association (genetic correlation) between traits.; Positive V_G desirable – Negative V_G undesirable;
Completion of selection in all traits – long duration required – breeder may change goals – leads to poor results.

(2) Independent culling method :- Simultaneous selection – two or more traits;
Minimum standard set for each trait – above the standard saved for breeding;
Disadvantage :- fail to meet standard in one trait – animals with excellent genes for other traits culled .
Advantage :- all round – simultaneous improvement.

(3) Selection Index method:
Value of each trait – separate determination – addition of values
Index :- total score of all traits, animal wise – compare index – high index selected. Appropriate weightage – to value of each trai t- weightage depend upon relative economic importance – h^2 – genetic correlation among the traits.
Efficiency:- efficient than independent culling method – allows selection of individuals superior in some traits – but deficient in other traits.

Lecture No.20

Prepared by Dr.P.C.Thomas

Topic : Inbreeding

Definition:- system of mating – closely related mates – than average members of the population being inter mated; Practical view – mating of individuals having common ancestors – preceeding 4 - 6 generations;

Mild inbreeding:- parents related as second cousin; Intense inbreeding:- Full sib mating - parent offspring mating etc ; Most intense form inbreeding – self fertilisation by plant .

Inbeeding effects:- increased homozygosity ; Selfing - homozygosity after 'n' generatons of selfing = $1-(1/2)^n x100$;
Increased homozygosity from full sib mating = less than half the rate of selfing.

Coefficient of inbreeding (F): represent the probable increase in homozygosity – resulting from mating of related individuals;- Range 0 to 1 – 0 to 100% ; Viewed from standpoint of individual locus – F represent probability of a random locus , originally heterozygous, has become homozygous due to inbreeding ;

Formula for estimation of inbreeding coefficient :- $Fx = \Sigma (1/2)^{n+1} (1+F_A)$;

Genetic effects : Frequency change in genotypes:- homozygotes – increased frequency ; heterozygotes – decreased frequency ; In small populations – alleles lost, as inbreeding progress – gene freqency may fluctuate extremely – some genes be lost – others fixed (homozygosis) ;
Large populations – gene frequency fluctuations – less extreme – only few alleles may be lost.

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

Topic : Out breeding

Definition : Mating system – mating individuals – less closely related than average of group which to they belong ; Practical point of view – mating animals -no common ancestors in the preceding 4 - 6 generations.

Types of out breeding :

Out crossing: – unrelated animals, within same breed; Cross breeding – mating between breeds ; Crossing inbred lines – within same breed ; Species hybridization – between different species; Grading up – Mating males of established breed with nondescript females and their progenies – generations after generations ;

Genetic effects :- Exactly opposite of inbreeding –increased heterozygosity.

Outbred animals – less likely to breed true than inbreds less likely to transmit same type of genes to their offspring ;

Example: homozygous animal (AA BB CC SS) produce – ABCD gametes , heterozygous animal (Aa Bb Cc Dd) produce -16 different types gametes.

Hybrid vigor / Heterosis:

Definition: - increased vigor – offspring –over parents – when unrelated individuals mated – (Technically : positive or negative heterosis);

Crop, terrestrial animal examples:- Poultry – breed cross – superior growth and egg production ; Heterosis from species hybridisation – mule - cross of male ass and female horse ; Hybrid vigor by crossing inbred lines – high yielding corn - hybrid vigor from are used in the ; Heterosis not mandatory for all traits ;

$$\text{Percent heterosis} = \frac{\text{Mean of offsprings} - \text{mean of parents}}{\text{Mean of parents}} \times 100$$

Genetic factors of heterosis :- Heterozygosity of no-additive genes – Masking deleterious recessive genes by dominant alleles – Heterozygosity at overdominant locus – epistatic gene action -No heterosis from additive gene action.

M.F.SC. MARICULTURE

LECTURE OUTLINE

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

Lecture No 1 **Prepared by Shri Wilson T Mathew**

TOPIC : INTRODUCTION TO COMPUTERS

What is computer- definition. Why we need computer? Computer- detailed definition.
Important landmarks in the evolution of computer- the abacus, the first mechanical calculating machine developed by Pascal- the difference engine made by Charls Babbage- the development of punched card machine by Hollerith- the ENIAC, first general purpose computer.

Computer Generations:
First generation computers, Second generation computers, third generation computers, fourth generation computers, fifth generation computers.

Types of computers-digital and analog
The characteristics of a computer-speed –accuracy and reliability-storage capacity or memory –
Structure of a computer-the input device-the central processing unit, the output device
(With detailed diagram)
Types of memory-ROM and RAM
ROM-definition. Programmable ROM-definition-EPROM-definition
Random Access Memmory: Definition of RAM- difference between ROM and RAM.
Hard Disk- definition, definition of bit, byte, kilobyte, Mega Byte, Giga byte and Terra Byte.
Computer programming languages: What computer programming means. Different types of languages: -machine language, Assembly language, High level language (detailed definitions with examples of each).
Hardware:Definition of hardware
Software: definition of software- operating system software and utility software with examples of each.

Binary number system: What is binary number system and its importance in the computer field.

Course Title: Bio-Statistics and Computer Applications

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

Lecture No 2 **Prepared by Shri Wilson T Mathew**

TOPIC : FUNDEMENTALS OF DOS

What is DOS? Embedded DOS in windows operating system.- Working in DOS- how does DOS works? - Versions of DOS Activating DOS from Windows operating system.- The DOS prompt- The meaning of the prompt C:/>

Commands in DOS-Path- illustrated definition

Naming of Files and Directories:- extension name- rules for naming files and directories

Dos commands- DIR command with its several options.

COPY command with its options- MOVE command with options

The wild card and question mark.

DEL command – deleting single and a group of files. Delecting files from floppy while you are in the C drive.

The create directory command MKDIR/MD

The root directory and sub directories and sub-sub directories. With tree chart illustrated

The CD command (Chang Directory) -Returning to root directory from any directory .

Returning step-by-step from any directory to the root directory.

The FORMAT command and its use- The CLS command -The REN command to rename a file.

How to copy the contents one floppy disk to another floppy disk using DOS.

The DOS Editor- how to activate and close -The TYPE command and its use

The MORE command and its - How to get help about a command in DOS.

Course Title: Bio-Statistics and Computer Applications

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

Lecture No 3

Prepared by Shri Wilson T Mathew

TOPIC : **Windows Operating System**

What is meant by an Operating System-Definitions of keywords- Drive, Folder, File extension and icon. Descriptions of desktop-Task bar-System Tray-My Computer-Recycle Bin-My Documents-Internet Explorer-Shortcut icons-Shutting down Windows 98 and restarting it. What is stand by mode?

Parts of a Window-Control box, Border, Title Bar, Minimize button, Maximize button, Restore button, Close Button, Menu bar, Tool Bars, Work Area, Status Bar- how to switch between windows- ho to move window around on desk top-cascading window-tiling your windows-scroll bars and their working.

Opening a program-How to remove an item from the Program menu- how to rename an item on the program menu-how to delete a file from the Program menu -Finding files on your computer (with several options).

Using Windows Explorer Program-creating a folder-Deleting a file or folder- moving/ coping files to different folders- copying a file/folder to floppy disk-copying file/folder from floppy disk to other drive-Renaming a file/folder-how to change the date and time?
Desktop shortcut—how to create a desktop short cut- how to delete a desktop shotcut- how to change the icon associated with an object- wallpaper -Screen saver-screen resolution.

Icon-Menu-Short Cut Key-selection-cut, copy and paste -Description of Short cut keys . Saving a file-Familiarizing Accessories: Wordpad-Notepad- Calculator
System Tools: Scan Disk

Course Title: Bio-Statistics and Computer Applications

Course No. MC. 510 Course Credit 2+1

Lecture No 4 **Prepared by Shri Wilson T Mathew**

TOPIC : Introduction to Microsoft EXCEL -I

Excel –its features, getting started in Excel- 3 ways:1) click on the start button, choose programs and then click on the Microsoft Excel menu item (2)From Double click on any any excel file.(3)Double-click the Execl shortcut icon on your Windows desktop.

Exiting Excel : Three ways (1)Click on the X button in the upper-right corner of the Excel workspace(2) choose File, Exit (3)Click on the Excel icon in the upper-left corner of the Excel Window to display the control Menu, then choose Close (4) Press ALT+F4.

Getting familiar with Microsoft Excel- illustrative explanation of Title bar

Illustrative explanation of the Menu bar and its components. viz.File, Edit, View, Insert, Format, Tools, data, Window, help,

Illustrative explanation of the Standard Tool bar-New, Save,Print preview, print, open, cut, copy, paste, format painter, undo, redo,insert hyperlink, Autosum, paste function, sort,Zoom and Help.

Illustrative explanation of the Formatting Tool Bar

Illustrative definition of Work sheet(s)- the formula bar- the status bar- scroll bars-Sheet tabs and scrolling buttons.

Definition of cell and Active cell or Selected cell.- how to select a new active cell using the keyboard's arrow keys or using the mouse. –Functions of [PageDown] and [pageUp] and [Home] keys- how to select one cell- how to select entire row- how to select entire column- how to select entire worksheet and how to select cluster of cells.

Definition of Block. What are Constant values. What are formulas?. Entering data and text- how excel studies the entered data to determine its type?

Course Title: Bio-Statistics and Computer Applications

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

Lecture No 5 **Prepared by** Shri Wilson T Mathew

TOPIC : Introduction to Microsoft EXCEL- II

Entering numbers- what is the meaning if #### symbol occurs when you, make a numeric entry?

Entering formulas-operators that can be used to form a formula- significance of prefixing every formula with (=) sign.What if you type the formula with out the (=) sign.

Excel functions –how to enter a formula that contains a function?-The function wizard.

Saving workbooks, Saving multiple files, Saving files in other formats

Difference between work book and work sheet-Moving to a new worksheet-Editing a worksheet data-editing a Cell by using the Formula bar-Moving and copying cells-pasting Cut and Copying Cells- Clearing Cells-Drag and Drop

Undo and redo, Finding and replacing worksheet data-spell checking the worksheet-spell checking the data-options available in the spelling dialog box

Using auto correct- selecting auto correct option from the Tools menu-how auto correct works.?

Formatting worksheets-Formatting tool bar-if tool bar is not visible how to make it visible. Format cells dialog box-number tab, alignment tab, font tab, border and pattern tabs. Resizing Rows and Columns, styles –style dialog box, format painter, Auto format, Aligning data-Orienting text

Controlling text within a cell- wrap text-shrink to fit-merge cells

Changing fonts, applying text format, applying boarders, applying patterns, working with graphic objects- adding clip art- add an image from a file.

Conditional formatting- Cell referencing- relative and absolute.

Page setup-page, margin, and header/ footer

Printing worksheets-printing an area-Print preview-printing worksheets

Getting help

Key board shortcuts- for document actions-cursor movement- selecting Cells- Text style-Formatting- formulas.

Course Title: Bio-Statistics and Computer Applications

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

Lecture No 6 **Prepared by Shri Wilson T Mathew**

Topic : Measures of central tendency- mean, median and mode -I

What is the necessity of central tendency values (averages) –Definition of central tendency- Objects of measures of central tendency.

Different methods of measuring central tendency-Arithmetic Mean, Median, Mode, Geometric mean, Harmonic mean

Definition of Arithmetic Mean, denoting arithmetic mean for a population and sample-How to calculate Arithmetic Mean for a discrete series of data and for continuos series of data.- demonstration of calculating Arithmetic Mean with examples. Nature and significance of Arithmetic Mean- The advantages and disadvantages of A.M.

Definition of median-how to calculate median in case of discrete and continuos series-how to denote median-What is the relevance of median with regard to the O- give curves. Use of median in statistical analysis- calculation of median for a discrete series with example. Calculation of median in a continuos distribution with example.

Course Title: Bio-Statistics and Computer Applications

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

Lecture No 7 **Prepared by Shri Wilson T Mathew**

TOPIC : Measures of central tendency- mean, median and mode -II

Definition of mode. Is there any effect on mode by the occurrence of one or a few extremely high or low values- discussion with example. Calculation of mode in discrete series and in a continues distribution with examples. Discussion about multimodal distribution. Nature and significance of mode. Discussion of these three central tendency values with the help of a problem.

Explanation of symmetrical distribution and skewed distribution. Relationship between mean, median and mode in (a) symmetrical distribution (b) Positively skewed distribution (c) in a negatively skewed distribution.

Definition of geometric mean and Harmonic mean.-Problem solving using Geometric mean and Harmonic mean.

Course Title: Bio-Statistics and Computer Applications

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

Lecture No 8 **Prepared by Shri Wilson T Mathew**

TOPIC : Measures of variation- Standard Deviation, range, coefficient of variation -I

Definition of dispersion-How dispersion is related to the measurers of central tendencies-Importance of Measures of Dispersion . characteristics of a good measure of dispersion.
Difference between absolute and relative measures of dispersion – necessity of relative measures of Dispersion

Various absolute measures of dispersion.

Range-its definition- merits and demerits of range (b)Mean deviation-its definition- merits and demerits of mean deviation (c)Semi-Interquartile range or Qurtile deviation-its definition, merits and demerits of quartile deviation. (d) The mean deviation –definition, merits and demerits of mean deviation (e) the standard deviation –definition, merits and demerits. Uses of standard deviation and variance- empirical relation between mean, quartile and standard deviation.

Course Title: Bio-Statistics and Computer Applications

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

Lecture No 9 **Prepared by Shri Wilson T Mathew**

TOPIC : Measures of variation- Standard Deviation, range, coefficient of variation-II

Choice of a measure of dispersion- depends on (a) the properties of different measures (2) the nature of the data and (3) the purpose for which the measure is to be used. Illustration with exercises on how to calculate the above measures of dispersion.

Relative measures of dispersion- the necessity of finding out relative measures of dispersion. Difference between absolute and relative measures of dispersion. Definition of coefficient of variation How to compare the consistency of two series of data with the help of coefficient of variation.- problem solving to find out relative measures of dispersion .Interpretation of consistency with regard to coefficient of variation.

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

Course credit: 2 + 1

Lecture No.: 10 **Prepared by: Dr. T.V. Sathianandan**

Topic: Elements of probability – Definition & addition theorem

Origin of probability theory – uncertain events – hidden pattern in uncertain events – the concept of experiments and outcomes – uncertainty in outcomes of an experiment – equally likely outcomes.

Sample Space – definition of sample space – notation used – classification of sample space – finite sample space – infinite sample space – discrete sample space and continuous sample space. Event – definition of event in relation to outcome of experiment – event as a subset – elementary event/simple event – compound event – use of set theory to represent relationships among events – universal set – union – intersection – subset – superset – empty set – complement set – mutually exclusive events.

Different definitions of probability – relative Frequency – probability of an event in terms of relative frequency – definition of probability based on the classical concept – definition based on the frequency concept and axiomatic approach for definition of probability. Probability as a personal degree of belief (subjective probability) – probability defined as a long-run relative frequency and definition in terms of equally likely outcomes.

Probability of an event – probability measure – the functional set up – tossing of a coin and associated events and probabilities – experiments with dice. Addition Rule for probability of events – general formula for addition of events – formula for mutually exclusive events and independent events.

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

Course credit: 2 + 1

Lecture No.: 11 **Prepared by: Dr. T.V. Sathianandan**

Topic: Elements of probability - conditional probability and multiplication theorem

Conditional probability – Reduction of sample space due to conditioning – Conditional probability of an event knowing the fate of another event of the same sample space – notation used to denote conditional probability – Illustrative example of the family with two children – Definition of Independent events in relation to conditional probability.

Multiplication Rule to determine probability of events – general rule, rule for independent events.

Law of Total Probability – Probability of an event defined in terms of its association with other events.

Bayes' Theorem – the simplest form using multiplication rule and law of total probability. Baye's general formula on conditional probability of events. Applications of Bayes' theorem. Example to demonstrate the use of Bayes' theorem.

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

Course credit: 2 + 1

Lecture No.: 12 **Prepared by: Dr. T.V. Sathianandan**

Topic: Elements of probability - random variables, discrete and continuous

Definition of random variable as a function mapping events in a sample space to real numbers – Domain and range for random variables – Discrete and continuous random variables – probability function of a discrete random variable – domain and range for the probability function – Probability density function – conditions to be satisfied by a probability density function – calculation of probabilities using probability density function for continuous random variables – Example of a continuous random variable.

Probability distribution of a discrete random variable – properties of probability function – cumulative distribution function – properties of cumulative distribution function – method of calculation of cumulative distribution function of discrete and continuous random variables.

Independent random variables – condition for independence of two random variables in terms of their cumulative distribution function.

Mathematical expectation – the concept of mathematical expectation of random variable – properties of mathematical expectation – expressions for mean and variance of a random variable in terms of mathematical expectation.

Probability-Probability (P-P) Plot, its construction and use.

Quantile-Quantile (QQ) Plot to determine the distribution followed by a data set – construction of quantile-quantile plot.

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

Lecture No.: 13

Prepared by: Dr. T.V. Sathianandan

Topic: **Elements of probability - Binomial distribution**

Binomial distribution – The experimental setup for Binomial distribution – Bernoulli trials – requirements to be met by the Bernoulli trials – Bernoulli random variables – mean and variance of a Bernoulli random variable – Definition of Binomial random variable in terms of Bernoulli trials – Domain and range for the Binomial random variable – probability function of a Binomial random variable – probability distribution of Binomial random variable – the general binomial expansion – recursive formula for computing probabilities of binomial random variable.

Derivation of expression for the mean of a Binomial random variable – expression for the variance of Binomial random variable – expressions for skewness and kurtosis of the Binomial distribution.

Binomial frequency distribution – expression for the frequency distribution – approximating a frequency distribution by binomial distribution – estimation of parameters of the distribution from the data – computing the probabilities and expected frequencies – examining the fit.

Probability distribution of proportions – the expected value and variance for the random variable representing proportions.

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

Course credit: 2 +1

Lecture No.: 14 **Prepared by: Dr. T.V. Sathianandan**

Topic: Elements of probability - Poisson distribution

Poisson distribution – the law of rare events – definition for a Poisson random variable – range of values that a Poisson random variable can assume – necessary requirements for a random variable to have Poisson distribution – probability function of a Poisson random variable – Poisson distribution as a limiting case of Binomial distribution – conditions for approximation of Binomial distribution by Poisson.

Cumulative distribution function of Poisson random variable – recursive formula for calculation of probabilities of a Poisson random variable – derivation of expression for the mean of a Poisson random variable – expression for variance of Poisson random variable – relation between mean and variance – expressions for measures of skewness and kurtosis of the distribution.

Poisson frequency distribution – Fitting Poisson distribution for a given frequency table – estimation of parameter of the distribution from data – computing probabilities for the classes – finding the expected frequencies – examining the fitted distribution.

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

Course credit: 2 + 1

Lecture No.: 15 **Prepared by: Dr. T.V. Sathianandan**

Topic: Elements of probability - Normal Distributions

Normal Distribution – Continuous random variable – definition of continuous random variable – probability density function of a continuous random variable – properties of probability density function – cumulative distribution function of a continuous random variable.

Hitting the bulls eye example for the derivation of probability density function of normal random variable – Definition of normal random variable – probability density function of normal random variable – alternate name for normal distribution – range of values for the normal random variable – parameters of normal distribution.

Properties of normal distribution – shape of the normal probability curve – symmetry of the distribution – point of maximum probability – maximum value for the normal probability density function – points of inflection for the normal curve – approximation to large class of distributions – normal approximations to Binomial and Poisson distributions – expected value of a normal random variable – mean and variance of normal random variable – method of calculation of probability for normal distribution – cumulative distribution function of a normal random variable – distribution of error terms in linear models – angular transformation on proportions.

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

Course credit: 2 + 1

Lecture No.: 16 **Prepared by: Dr. T.V. Sathianandan**

Topic: Elements of probability - Standard normal distribution and its properties

Standard normal distribution – properties of standard normal distribution – total area under the normal curve – cumulative distribution of standard normal variate – transforming a normal random variable to standard normal form – calculation of probabilities using the cumulative distribution table of standard normal variate.

Approximating a frequency table by a suitable normal distribution – estimation of parameters of the distribution – transforming the class limits by standardizing – calculation of probabilities for the classes – computing expected frequencies – examining the goodness of fitted distribution.

Application of standard normal distribution in sampling distributions – Students t – Chi-square – Fitting normal distribution to length frequency data.

Course Title :	**Biostatistics and Computer Applications**
Courese No :	**MC – 510** Course Credit : **2 + 1**

Lecture No 17 ***Prepared by Mini, K.G.***

TOPIC : SAMPLING DISTRIBUTION

Introduction – Descriptive statistics – Inferential statistics – Random samples – Sample mean – Sample variance – Properties of sample mean – Properties of sample variance – Definition – Properties of the Sampling Distribution of Means – Central limit theorem – Definition – Formula – Illustration – Sampling From a Finite Population – Correction factor – Formula – Example – Sampling Distribution of Sample Proportion – Formula – Example.

CHI-SQUARE DISTRIBUTION

Introduction – Standard normal distribution – Chi-square variate – Definition – Probability density function – Range – Parameter – Probability distribution function – Mean – Median – Mode – Variance – Skewness – Kurtosis –Coefficient of variation

Generating functions of chi-square distribution – Moment generating function – Cumulant generating function – Limiting form of Chi-square distribution for large degrees of freedom – Characteristic function of Chi-square distribution – Additive property of Chi-square variate – Chi-square probability curve

Applications of Ch-square distribution – Chi-square test of goodness of fit – chi-square test for population variance – Test for independence of attributes – Chi-square test for homogeneity of correlation coefficient – Test for homogeneity of several independent estimates of the same population variance.

Course Title :	**Biostatistics and Computer Applications**	
Courese No :	**MC – 510**	Course Credit : **2 + 1**

Lecture No *18* ***Prepared by Mini, K.G.***

TOPIC : t DISTRIBUTION

Introduction – Student's t definition – William S Gosset – Constants of t distribution – Moments – Mean and Variance of t distribution – Measures of Skewness – Measures of Kurtosis – Moment generating function of t-distribution – Limiting form of t-distribution – Relationship to the normal curve

Characteristics of t distribution – Bell-shaped – Symmetric about t=0 – Area=1 – The degrees of freedom is n-1 (i.e. one less than the sample size) – t is Used for normal distribution when standard deviation is unknown and n <=30 – Graph of t-distribution – Critical values of t – Small sample confidence interval for the mean

Applications of t-distribution – t-test for single mean – Assumptions for t-test – test for difference of two means – paired t-test for difference of means – t-test for the significance of an observed sample correlation coefficient – test for the significance of an observed regression coefficient – testing the significance of an observed partial correlation coefficient.

F DISTRIBUTION

F-distribution – Introduction – F statistic – Definition – Ratio of two chi-square distribution – Probability density function – Range – Parameter – Probability distribution function – Mean – Variance – Mode – Skewness

Applications of F distribution – F test for equality of population variances – Critical values of F-distribution – F test for testing the significance of an observed multiple

correlation coefficient – F test for significance of an observed sample correlation ratio – F test for testing the linearity of regression – F test for equality of several means – Relation between t and F distributions – Relation between F and Chi-square distributions.

Course Title :	**Biostatistics and Computer Applications**	
Courese No :	**MC – 510**	Course Credit : **2 + 1**

Lecture No 19 *Prepared by Mini, K.G.*

TOPIC : Z DISTRIBUTION

Introduction – Fisher's z definition – Moment generating function of z-distribution – Fisher's z transformation

Applications of z distribution – Test whether observed value of correlation coefficient differs from population correlation coefficient – Test the difference between two independent sample correlation coefficient .

CONCEPT OF ESTIMATES

Introduction – Parameter space – Types of Estimators – Point estimation – Interval estimation – Properties of point estimator – usually based on sample from a population – usually is a statistic – notation of point estimator

Characteristics of estimators – Unbiasedness –Consistency – Sufficiency – Efficiency

Unbiasedness – Definition – Conditions for unbiasedness – Examples

Consistency – Definition – Sufficient conditions for consistency – Examples

Sufficiency – Definition – Conditions for sufficiency – Examples

Efficient Estimators – Definition – Conditions for efficiency – Examples

Method of moments – Maximum likelihood estimation – Minimum variance unbiased estimators – Definition – Interval estimation – Confidence interval – Confidence limits – Case where variances are unequal – Case where variances are known

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

Course credit: 2 + 1

Lecture No.: 20

Prepared by: Dr. T.V. Sathianandan

Topic: Test of significance – statistical hypothesis, Type-I and Type-II errors, critical region, levels of significance of tests, Null hypothesis and alternate hypothesis.

The concept of population and sample. Explanation about the terms – parameter – estimate – statistic – sampling distribution and sampling error. The need for testing hypothesis – Possible errors when decision about populations is made based on samples – four different situations when decision is based on sample – faulty rejection and wrong acceptance.

Statistical hypothesis – definition of hypothesis – types of hypothesis – simple hypothesis – composite hypothesis – null hypothesis – alternate hypothesis.

Definition of type-I and type-II error in terms of probability – Level of significance – alternate names for type-I and type-II error – Definition of critical region and power of a test – Notations used for representing the two types of errors – Relation between power and type-II error – association between the two types of errors.

Test Statistic and its use – choice of a test statistic – critical value for a hypothesis test – relation between critical value and the significance level – one sided and two sided tests – the choice between a one-sided and a two-sided test – partition of the sample space into critical region and acceptance region – Conclusion after a statistical test.

The probability value (p-value) of a statistical hypothesis test – decision based on p-value of a test.

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

Course credit: 2 + 1

Lecture No.: 21

Prepared by: Dr. T.V. Sathianandan

Topic: Test of significance - tests based on z and Students t distributions.

The z test statistic – distribution on which the test is based – probability distribution of z statistic – conditions to be satisfied for the application of z test – the quantities to be known – condition on the sample size – standardization of a random variable.

Testing the mean of a distribution using z test – Testing equality of means of two populations using z test – limitations of the test – advantages and disadvantages of the test. Small sample tests – the Student's t statistic – probability function of the Student's t test statistics – Situations under which Student's t test can be applied – testing the mean of a population using Student's t test – the hypothesis, calculation of the test statistic – degrees of freedom for the test statistic – level of significance and the critical value for the test – comparison with the critical value – conclusion based on the test.

Testing equality of means of two populations using students t test – basic requirements for application of the test – the null hypothesis and alternate hypothesis for the test – expression for the test statistic for testing equality of means – degrees of freedom for the test – level of significance and the critical value for testing equality of means using students t test – comparison with the critical value and the conclusion based on the test.

Paired t test – situation for paired t test – the random variable – expression for the t statistic for paired t test and its degree of freedom.

Testing significance of correlation coefficient using students t test – the null hypothesis and alternative hypothesis for the test – computation formula for the test – degrees of freedom – level of significance and the critical region.

Testing the significance of regression coefficient using Students t test – null hypothesis and alternate hypothesis – test statistic and its computation formula – degree of freedom and critical region.

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

Lecture No.: 22 **Prepared by:** Dr. T.V. Sathianandan

Topic: Test of significance - tests based on chi-square and F distributions

Testing the variance of a population for a given quantity using chi-square test – The underlying distribution of the population – test statistic and the expression for the test statistic – critical value for the test – situations when the population mean is known and when it is not known. Chi-square for testing the goodness of fit in frequency tables – frequency distribution, fitting probability distributions for frequency distributions – classes – observed frequencies – expected frequencies computed using the fitted distribution or according to some hypothesis about the distribution – the test statistic – computation formula for the test statistic – degrees of freedom for the test statistic – critical value for the test. Use of chi-square for testing the independence of attributes in contingency table – classification based on attributes to form contingency table – observed frequencies in contingency tables – the null hypothesis and alternate hypothesis for the test – test statistic – expression for the test statistic – degrees of freedom for the test – critical value and conclusion after performing the test – Testing homogeneity of frequencies in a contingency table using chi-square test. Bartlett's chi-square test for testing homogeneity of variances of several populations – alternate name for the test – conditions and assumptions necessary for the application of the test – the test statistic – computational formula and necessary steps for the calculations – null hypothesis and alternate hypothesis for the test – degrees of freedom of the test and the critical region. Testing equality of variances of two populations using F test – F test as the ratio of two independent sum of squares – computation formula for the test – degrees of freedom – critical value and level of significance for the test. Testing equality of means of several populations under homosedasticity using F test – expression for the test statistic – degrees of freedom for the test – level of significance and critical value for the test.

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

Lecture No.: 23 *Prepared by : Dr. Somy Kuriakose*

TOPIC: NON-PARAMETRIC STATISTICS

Non-parametric Methods – Introduction and Basic Ideas – Use of Statistical Tests in Research – Choosing an Appropriate Statistical Test

Non-parametric Tests – Definition – Assumptions regarding the form of the Population – Independence – Continuity of Variable – Probability Density Function – Existence of lower order moments– Advantages of Non-parametric Methods – Disadvantages of Non-parametric Methods – Comparison with parametric methods

Chi-square Test

Chi-square Test – Introduction – Conditions for the Validity of Chi-square Test– Chi-square Test for Independence of Attributes – Contingency tables – Definition – r x s contingency table – Expected frequency – Observed frequency – Null hypothesis – Alternate hypothesis– Test statistic – Degrees of freedom – Critical values – Examples .

Sign Test

Sign Test – Introduction – When it is used – Underlying assumptions – Deviations expressed in positive or negative signs – continuous distribution – independence – Null hypothesis – Alternate hypothesis – Procedure – Small samples – computation of test statistic – level of significance – critical values – Large samples – Test statistic – Approximation to normal distribution – Inferences – Advantages and disadvantages of sign test – comparison with paired t-test – Illustration.

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

Lecture No.: 24 ***Prepared by : Dr. Somy Kuriakose***

TOPIC: NON-PARAMETRIC STATISTICS

Median Test

Median Test – Introduction – Basic Concepts – testing how two independent ordered samples differ in their central tendencies – Method – ordered sample – determination of median value – null hypothesis – alternative hypothesis – distribution – test statistic in small samples – hyper geometric distribution – large samples – normal approximation – Alternative method in large samples – use of 2 × 2 contingency table – Test using chi-square statistic – Advantages and disadvantages of Median test – Location and shape of distribution – Comparison with t-test – Example.

Run test

Run test- Introduction – when to use run test – what is a run – definition – how to determine the number of runs – Null hypothesis – Alternate hypothesis – Procedure – Test for randomness – test statistic – critical values – drawing inferences – Examples.

Kolmogrov Smirnov two sample test

Kolmogrov Smirnov two sample test– Function and Rationale – Method – Null hypothesis – Alternate hypothesis – Determination of cumulative frequency – distribution – observed cumulative distribution function – Test statistics – small samples – large samples – one tailed test – two tailed test – critical values – Inferences – Illustrative examples – Power – efficiency – comparison with t-test, chi-square test

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

Lecture No.: ೩೯ *Prepared by : Dr. Somy Kuriakose*

TOPIC: ANALYSIS OF VARIANCE - ONE WAY CLASSIFICATION

Analysis of Variance - Introduction and Overview - Key Concepts - Why testing of equality of several means related to Variance - Difference between group means - Sample sizes in each group - Variance of the dependent variable

ANOVA - One way classification - General Statistical Model - Parameters of the model - Grand mean - Factors - Levels - Effect of factor - Random Error - Notations in ANOVA - factors- subscripts - means - Null hypothesis - Alternate hypothesis

Assumptions of ANOVA - Independence of Observations - Normal Population - Homogeneity of Variances – samples are from populations that are categorized in only one way – checking assumptions-transformations-alternatives

Partitioning of Variance - Total variability - Between groups variability - Within group variability - Treatment effects - Individual differences - Experimental Error

ANOVA Table - Sources of Variation - Degrees of Freedom - Sum of Squares - Mean sum of Squares - F-ratio

Computation of Sum of Squares - Grand Mean - Mean of different groups - Total sum of Squares - Between group sum of squares - within group (Error) sum of squares

Degrees of freedom - Total degrees of freedom - Between groups degrees freedom - Error degrees of freedom

Computation of Mean Sum of Squares - Between groups mean square - Error mean square

F-ratio - Ratio of Between Group Mean Square to Error Mean Square - Degrees of freedom for test statistics- Value of test statistic under null hypothesis - Critical Values

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

Lecture No.: 26 ***Prepared by : Dr. Somy Kuriakose***

TOPIC : ANOVA – TWO WAY CLASSIFICATION WITH SINGLE OBSERVATION PER CELL

Two way classification – Introduction – Layout – Factor – levels – interaction – Treatment groups – Main Effect – Interaction effect – general model Assumptions of ANOVA – Independence of samples – Normal populations – Homogeneity of variances – sample size of each group

Test of significance – Null hypothesis – Alternate hypothesis – Estimation of model parameters – Partitioning of Variance - Total variability - Between groups variability - Within group variability – Treatment effects – Interaction effects – Individual differences – Experimental Error

ANOVA Table – Sources of Variation – Degrees of Freedom – Total degrees of freedom – Between groups degrees freedom – Error degrees of freedom – Sum of Squares – Computation of Sum of Squares – Grand Mean – Mean of different groups – Total sum of Squares – Between group sum of squares – within group (Error) sum of squares – Mean Sum of squares – Computation of Mean Sum of Squares – Between groups mean square – Error mean square – F-ratio – Ratio of Between Group Mean Square to Error Mean Square – Degrees of freedom – Value of test statistic under null hypothesis – Critical Values – Inferences

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

Lecture No.: 27 *Prepared by : Dr. Somy Kuriakose*

TOPIC : ANOVA – TWO WAY CLASSIFICATION WITH MULTIPLE EQUAL OBSERVATION PER CELL

Two way classification – Introduction – Layout – Factor – levels – interaction – Treatment groups – Main Effect – Interaction effect – general model Assumptions of ANOVA – Independence of samples – Normal populations – Homogeneity of variances – sample size of each group

Test of significance – Null hypothesis – Alternate hypothesis – Estimation of model parameters – Partitioning of Variance – Total variability – Between groups variability – Within group variability – Treatment effects –Interaction effects – Individual differences – Experimental Error

ANOVA Table – Sources of Variation – Degrees of Freedom – Total degrees of freedom – Between groups degrees freedom – Error degrees of freedom – Sum of Squares – Computation of Sum of Squares – Grand Mean – Mean of different groups – Total sum of Squares – Between group sum of squares – within group (Error) sum of squares – Mean sum of Squares – Computation of Mean Sum of Squares – Between groups mean square – Error mean square – F–ratio – Ratio of Between Group Mean Square to Error Mean Square – Degrees of freedom – Value of test statistic under null hypothesis – Critical Values – Inferences

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

Course credit: 2 + 1

Lecture No.: 28 **Prepared by: Dr. T.V. Sathianandan**

Topic: Co-variance analysis for One-way classification

The experimental situation for covariance analysis – use of a covariate in the analysis – logic behind the analysis of covariance – advantage over analysis of variance.

Analysis of covariance for one-way classified data – the linear model – parameters of the model – assumption about the random error component in the model.

Decomposition of the total variance and covariance into sum of squares and sum of products due to different components – total sum of squares due to main variate – total sum of squares due to covariate – main variate sum of squares due to the factor – covariate sum of squares due to the factor – sum of products due to the factor – error sum of squares due to the main variate – error sum of squares due to the covariate – error sum of products.

Expression for estimates of regression coefficients and correlation coefficients in terms of error sum of squares and error sum of products – expressions for adjusted total sum of squares – adjusted error sum of squares and adjusted sum of squares due to the factor.

The analysis of covariance table – expression for the F statistic for testing homogeneity of levels of the factor – computation of critical difference for pair wise comparison of factor levels.

Course Title :	**Biostatistics and Computer Applications**
Courese No :	**MC – 510** Course Credit : **2 + 1**

Lecture No 29 *Prepared by Mini, K.G.*

TOPIC : CORRELATION

Simple linear correlation – Introduction to correlation – When are correlation methods used? – Univariate distribution – Bivariate distribution – Looking at data: scatter diagrams – Examining scatter plot – Positive association – Negative association – Karl Pearson's coefficient of correlation – Concepts of: Sum of cross products – Sums of squares of X & Y – Computational formula for the Pearson Product-Moment Correlation Coefficient – Notations for the linear correlation coefficient

Population correlation coefficient – Sample correlation coefficient – Characteristics of coefficient of correlation – How to Interpret the Values of Correlation coefficient – Positive linear correlation – Perfect positive correlation – Negative linear correlation – Perfect negative correlation – No linear correlation – What is correlation coefficent ? and what it is not ? Interpretation when r=0, may be due to – random scatter – curved relation – outliers – parallel lines – different linear trends

Properties of linear correlation coefficient – Correlation is not causation – Common errors involving correlation – Interpreting the correlation – Factors which could limit a product-moment correlation coefficient –Assumptions in order to use the Pearson product moment correlation – Linear relationship – X & Y are metric variables – Randomly drawn sample – X & Y are normally distributed in the population – Spurious Correlations

Course Title :	**Biostatistics and Computer Applications**	
Courese No :	**MC – 510**	Course Credit : **2 + 1**

Lecture No 30 ***Prepared by Mini, K.G.***

TOPIC : LINEAR REGRESSION

The concept of linear regression – Model building – Typesof models –Regression line – The use of a scatterplot in determining a bi-variate relationship – The relationship between x and y

Population Regression Model – Sample regression model – Estimation of model parameters – Method of least squares – Definition – Notations – Criteria for judging the fit of a straight-line – Raw sum of squares for x – Corrected sum of squares for x – Raw sum of squares for y – Corrected sum of squares for y – Raw sum of cross products – Corrected sum of cross products – Estimation of least squares line – Formula for the parameters

Inference about parameters of regression line – Interpretation of the slope of a line – Interpretation of the intercept of a line – How to plot a regression line – Two Regression Lines – Interpretation of same intercept and different slope – Interpretation of same slope and different intercepts – How to make predictions with a regression equation – Guidelines for using the regression equation

Residuals – Least square property – Marginal change – Outlier – Influential points — Total deviation – Explained deviation – Unexplained deviation – Partitioning of sums of squares – Total sum of squares – Regression sum of squares – Error or residual sum of squares Total variation – Interpretation of coefficient of determination – The features of the coefficient of determination – Cautions about regression – Assumptions underlying linear regression – Confidence interval for the mean value of Y for a given value of X – Prediction interval for an individual value of Y for a given value of X

Course Title : **Biostatistics and Computer Applications**

Courese No : **MC – 510** Course Credit : **2 + 1**

Lecture No 31 ***Prepared by Mini, K.G.***

TOPIC : LINEAR REGRESSION

Assumptions in Regression Analysis – Residuals are not correlated with any of the independent variables – The relationship between the dependent variable and each of the independent variables is linear – Mean of the residuals is zero – Homogeneity of Variance – The independent variables are measured without error

Model Specification – Normality – The residuals are normally distributed – Violations of Assumptions – Measurement Error – Specification errors.

Properties of regression coefficients – Relation between correlation coefficient and regression coefficients – Arithmetic mean of the regression coefficients – Limits of regression coefficients – Effect of change in scale and origin – Angle between two lines of regression – Illustrations of different angles of two regression lines – Standard error of estimate

CURVILINEAR REGRESSION

Objectives – Linear Vs Non-linear models – Curvilinear relationships – what if the data are non-linear – Different types of transformations to make the model into simple linear models – Polynomial equation – Estimation of parameters – Hyperbola – Transformation to make the model linear – Exponential equation – Logarithmic transformation to make it a linear equation – steps for good modelling – Plot the data – Look for outliers – Look for trends / patterns – look for homogeneity of variance – write down the possible relationships – use relationships seen in scatter diagrams – experience trial and error – select best relationships using practical considerations and statistical considerations.

Course Title: Biostatistics and Computer Applications

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

Lecture No 32 ***Prepared by Mini, K.G.***

TOPIC : TESTS OF SIGNIFICANCE OF CORRELATION AND REGRESSION COEFFICIENTS

Additvity of Correlation coefficients – Hypothesis Testing – How to Determine Whether Two Correlation Coefficients are Significant – t-test for determining the significance of r – Null hypothesis – Alternate hypothesis – Test statistic – Inference – Causation – Common errors – Predictor and Criterion Variables– Intercorrelation matrix – Caveats in interpreting an intercorrelation matrix.

The relationship between correlation and linear regression – Angle between the two regression lines – Confidence intervals and hypothesis testing

t distribution to test the significance of a regression coefficient – Null hypothesis – Alternate hypothesis – Test statistic – Acceptance and rejection regions for the test – Computation of the p-value for the simple regression t test

Interval estimation for linear regression – Standard errors of estimated parameters in simple linear regression – Confidence interval for the average value of Y given X – Confidence interval for the predicted value of Y given X – The range of X values – Predictions made from regression lines for individual observations

Course Title :	**Biostatistics and Computer Applications**
Courese No :	**MC – 510** Course Credit : **2 + 1**

Lecture No 33 ***Prepared by Mini, K.G.***

TOPIC : MULTIPLE REGRESSION

The concept of multiple regression – Introduction to multiple regression model, and some simple cases - categorical/qualitative variable – estimating regression coefficients Calculating fitted values and residuals – Interpretation of the intercept and regression coefficients in multiple regression – The Analysis of Variance summary, and the coefficient of multiple determination – Regression assumptions – Linear relationship – Residuals – Normally distributed Homoscedastic over the levels of the predictors – Noncollinear predictor variables – Regression plane – example with independent covariates

Regression with two independent variables – Equation of the model – Estimation of additional parameters – Interpreting coefficients in the model – Interpretation of R and R^2

Partitioning the sums of squares in regression – Total sum of squares – Regression sum of squares – Residual (error) sum of squares – The coefficient of determination and the regression sum of squares

Inference about parameter estimates, and mean response and new observations – Choosing variables: How important is each variable in modelling the response? – Choosing variables: How important is each variable in modelling the response? – Partial Regression Plots – Checking for multicollinearity, or when the spread in the independent variables is not adequate – All possible regression procedure, and forward stepwise – Checking for influential observations – Autocorrelation

Course Title :	**Biostatistics and Computer Applications**	
Courese No :	**MC – 510**	Course Credit : **2 + 1**

Lecture No 34 *Prepared by Mini, K.G.*

TOPIC : MULTIPLE REGRESSION

Inference concerning the coefficients of the multiple regression model – Usefulness of the model if the coefficients equal to zero – steps in the test of hypothesis – Define the hypothesis to be tested – select the appropriate statistical measure to rephrase the hypothesis – Determine whether the hypothesis should be one-sided or two-sided – specify alpha the level of the test – select the appropriate test statistic – F statistic – Interpretation of small value of F – Drawing conclusion

Confidence interval for each of the coefficient – Testing hypothesis on individual beta coefficients – Inference on the model's predictions – confidence interval for the average value of Y given X – how good is the estimate – confidence interval for the predicted value of Y given X – Assessing the fit – Adjusted R^2 – Predictions – Dummy variables – sets of dummy variables – how to calculate the effect on t values – F test – Illustration by an example

PARTIAL REGRESSION

Introduction – Definition – Yule's notation – Partial regression coefficients – Residual – Plane of regression – Generalisation – Properties of residuals – Variance of the residual – applications of partial regression

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

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

Lecture No.: 35 ***Prepared by : Dr. Somy Kuriakose***

TOPIC : DESIGN OF EXPERIMENTS

Design of Experiments – Introduction – Experimental and observational studies–What is the purpose of the experiment – What are the treatments – experimental units – observational unit – What measurements are to be recorded – Strategy of Experimentation – Experimental Error Origins of Experimental error – Natural Variation among Experimental Units – Variability in Measurement of the Response – Inability in Reproducing Treatment Conditions exactly from one unit to another – Interaction of Treatments and Experimental Units – Extraneous Influences

Statistical Design of Experiments – Basic Principles of Experimental Design – Replication – Introduction – Definition – Role in Increasing Accuracy of Experiments –Determination of optimum number of replications – Randomization – Introduction – Why do we randomize – justification for methods of statistical inference – validity of the estimate of experimental error – unbiased estimates – systematic bias – selection bias – accidental bias – How do we randomize? – systematic plan. – random permutation – Local Control or Blocking – Basic Ideas – Selection of Uniform Experimental Units – Criteria for Blocking – Proximity – Physical Characteristics

Guidelines for Designing Experiments – Recognition and Statement of the Problem – Choice of Factors and Levels – Selection of Response Variable – Choice of Experimental Design – Performing the Experiments – Statistical Analysis – Conclusions

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

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

Lecture No.: 36 *Prepared by : Dr. Somy Kuriakose*

TOPIC : SIMPLE DESIGNS AND THEIR ANALYSIS

Simple comparative Experiments – Basic Statistical Concepts

Completely Randomized Designs – Introduction – Objectives – General Model – Assumptions – Advantages and disadvantages of CRD – Randomization and Layout – Illustrative example – Data – Analysis of the Model – Null Hypothesis – Alternate Hypothesis

ANOVA – Sources of Variation –treatments - Decomposition of total Sum of Squares – Treatment sum of Squares – Error sum of Squares – Mean sum of Squares – Error mean Square – Degrees of Freedom – Test Statistic – Computation of F - Value – Analysis of Variance Table – Test of Significance – Interpretation of Results

Comparisons Among Treatment Means – Comparing pairs of Treatment Means –The Least Significant Difference (LSD) – Duncan's Multiple Range Test – Turkey's Test

The Least Significant Difference (LSD) – Procedure – Test Statistic – Critical Values - Inferences

Duncan's Multiple Range Test – Procedure – Test Statistic – Critical Values – Significance Level – Inferences

Turkey's Test – Procedure of the Test – Test Statistic – Critical Values – Significance Level – Inferences – Comparision with Duncan's Multiple Range Test

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

Lecture No.: 37 ***Prepared by : Dr. Somy Kuriakose***

TOPIC : RANDOMIZED BLOCK DESIGNS AND LATIN SQUARE DESIGNS

Block Designs - Introduction and Basic Concepts - why blocking is done - example

Randomized Block Design (RBD) - Introduction and Definition - General Statistical Model of RBD - Advantages of RBD over CRD - Assumptions - Estimation of Model Parameters - Null Hypothesis - Alternate Hypothesis

Analysis of Variance - Sources of Variation - Partitioning of total sum of Squares - Treatment Sum of Squares - Block sum of Squares - Error Sum of Squares - Mean Sum of Squares - Degrees of Freedom- F-value - Analysis of Variance Table - Test of Significance using F-distribution - Conclusions and Drawing Inferences

Comparisons among Treatment Means - Least Significant Difference – Duncons Multiple Range Test- Tukeys Test

Latin Square Design (LSD) - Latin Squares - Introduction to Latin Squares of Order p. - Examples - General model in LSD- Assumptions - Estimation of Model Parameters - Null Hypothesis - Alternate Hypothesis

Analysis of Variance - Sources of Variation - Partitioning of total sum of Squares - Treatment Sum of Squares - Block sum of Squares - Error Sum of Squares - Mean Sum of Squares - Degrees of Freedom - F-value - Analysis of Variance Table - Test of Significance using F-distribution - Conclusions and Drawing Inferences - Comparisons among Treatment Means in LSD.

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

Course credit: 2 + 1

Lecture No.: 38 **Prepared by: Dr. M. Srinath**

Topic: Sampling Vs Census - probability sampling

Introduction to sample survey and probability sampling - basic concepts - Fields of Application of Sampling Techniques and its limitations – Sampling concept – Selection of part of an aggregate to represent the whole.

Definitions and Preliminaries. Census – Complete enumeration. Sampling Vs Census: Advantages of sampling over census – reduced cost of survey - greater speed in getting results. - greater accuracy - greater scope - adaptability - Principles of sampling - Probability and Non probability sampling. Concepts – Sampling unit – Sampling frame - Sampling error – Non sampling error - Unbiased ness – Accuracy – Precision. Principal Steps in a Sample Survey - Planning and organization of Survey - Objectives - Definition of population – Definition of sampling frame and sampling unit – Selection of proper sampling design – – data to be collected – precision desired – sample selection – pilot survey – sample size - Organization of field work – Summary and analysis of data.

Sampling and non-sampling errors – relationship of sampling error with sample size – estimation of population total – bias in the estimate – mean square error – difference between accuracy and precision.

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

Course credit: 2 + 1

Lecture No.: 39 **Prepared by: Dr. M. Srinath**

Topic: Simple random sampling

Simple Random Sampling (SRS) – definition of simple random sampling - a method of drawing sample such that every one of possible samples of required size from a given population units has the same probability. Properties of Simple Random Sampling – inclusion probability – probability of drawing specified unit at different draws.

Simple Random Sampling with and without replacement – selection of sample using random number table by remainder method – notations used – population characteristics – mean – total – ratio of two means - expression for the estimate of population mean and population total – proportion of units that fall into a defined class – estimation of population parameters

Unbiased estimate of population mean, $\bar{y}_n = \sum_{i=1}^{n} y_i$

Expression for variance of population mean estimate = $\left(\frac{1}{n} - \frac{1}{N}\right) S^2$

Estimate of variance of estimated population mean = $\left(\frac{1}{n} - \frac{1}{N}\right) s^2$

where $s^2 = \sum_{i=1}^{n} (y_i - \bar{y}_n)^2$

Standard error of the estimate – expressions for the variance and standard error of the estimate of population total – estimation of variance of population total estimates – computing confidence limits of the estimate – estimation of population proportion.

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

Course credit: 2 + 1

Lecture No.: 40 **Prepared by: Dr. M. Srinath**

Topic: Stratified sampling and cluster sampling.

Stratified sampling – The population of N units is sub-divided into k sub-populations called strata – procedure of taking samples from each stratum is called stratified sampling – objective of stratified sampling is to give better cross section of the population – principles of stratification – How to make stratification? Advantages off stratified sampling – Estimation of populate mean and its variance

$\bar{Y} = \sum_{i=1}^{L} W_i \bar{Y}_i$ L = number of stratum, W_i = weight of i^{th} stratum, y = character under study.

Estimate of variance $= \sum_{i=1}^{L} w_i \left(\frac{1}{n_i} - \frac{1}{N_i} \right) S_i^2$

Allocation of samples into different strata. Principles of Stratification – Advantages of Stratification - Relative Precision of Stratified Random Sampling with Simple Random Sampling – Estimation of Gain in Precision Due to Stratification – Formation of Strata – Determination of Number of Strata.

Cluster sampling – A group of elements is a cluster – When the sampling unit is a cluster, it is called cluster sampling – Principles of clustering – Advantages of cluster sampling over Simple Random Sampling – cost and precision considerations – Estimate of mean $\bar{y}_{cc} = \frac{1}{n} \sum_{i=1}^{n} \bar{y}_i$ where n is the number of clusters, $\bar{y}_i$ is the cluster mean.

Estimate of variance $= \left(\frac{1}{n} - \frac{1}{N} \right) S_b^2$; S_b^2 = sampling variance between clusters.

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

Course credit: 2+1

Lecture No.: 41 **Prepared by: Dr. M. Srinath**

Topic: Systematic sampling - two stage sampling.

What is systematic sampling – A technique in which only the first unit is selected at random and other set selected automatically according to some pre-designed pattern – Advantages and disadvantages – Simplicity – operational convenience – even spread – efficient estimates – increase in bias if there is periodicity – variance of estimates are not estimable. Estimation of mean and variance of the population through systematic sampling – expressions for computing estimates of population total and its standard error. Comparison of Systematic with Random Sampling – Comparison of Systematic with Stratified Random Sampling

Two stage sampling – A case of sub-sampling - Sampling is done in two stages – Type of sampling which consists in first selecting the cluster and then selecting a sample of elements with in a selected cluster – More efficient than Simple Random Sampling from cost and operations point of view – Less efficient than Simple Random Sampling from the point of view of sampling variability – Main advantage at the first stage, a frame of first stage units only required to be known – More flexible and permits different selection proceedings in different stages.

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

Course credit: 2 + 1

Lecture No.: 42 **Prepared by: Dr. M. Srinath**

Topic: Sampling design for the estimation of marine fish production.

Objectives of the sample survey conducted by CMFRI Sampling design – area covered by the survey – maritime states – sampling design adopted – the stratified multi-stage random sampling – stratification criteria – space and time – fishing intensity and geographical considerations – sampling frame and its updation – fishing zones and their categories – single centre zones – landing centres – schedules used – landing centre days – selection of landing centres for observation – night landings – sampling of boats landed – information being recorded – work schedules for data collection – distribution of enumerators for data collection – estimation procedure followed – expression for the estimation of catch – the administrative setup for monitoring the sample survey – processing of collected information – scrutiny of collected information – coding of data – grouping of species – estimation of species wise, gear wise, zone wise, district wise and state wise catch and effort – sampling coverage and cost involved.

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

Course credit: 2 + 1

Lecture No.: 42 **Prepared by: Dr. M. Srinath**

Topic: Sampling design for the estimation of marine fish production.

Objectives of the sample survey conducted by CMFRI Sampling design – area covered by the survey – maritime states – sampling design adopted – the stratified multi-stage random sampling – stratification criteria – space and time – fishing intensity and geographical considerations – sampling frame and its updation – fishing zones and their categories – single centre zones – landing centres – schedules used – landing centre days – selection of landing centres for observation – night landings – sampling of boats landed – information being recorded – work schedules for data collection – distribution of enumerators for data collection – estimation procedure followed – expression for the estimation of catch – the administrative setup for monitoring the sample survey – processing of collected information – scrutiny of collected information – coding of data – grouping of species – estimation of species wise, gear wise, zone wise, district wise and state wise catch and effort – sampling coverage and cost involved.

Course Title : **Biostatistics and Computer Applications**

Courese No : **MC – 510** Course Credit : **2 + 1**

Lecture No : 43 ***Prepared by Mini, K.G.***

Topic : Statistical analysis using MS Excel

Statistical data analysis using Excel analysis toolpak – How to install the analysis toolpak – The Statistical functions available

Measures of central tendency – AVERAGE -The average (arithmetic mean) of the arguments – GEOMEAN - Returns the geometric mean of an array or range of positive data – HARMEAN - Returns the harmonic mean of a data set – MEDIAN - Returns the median of the given numbers – MODE - returns the value of mode

Measures of dispersion – QUARTILE - Returns the quartile of a data set – STDEV - Estimates standard deviation based on a sample – STDEVA - Estimates standard deviation based on a sample – STDEVP - Calculates standard deviation based on the entire population – VAR - Estimates variance based on a sample – VARA - Estimates variance based on a sample. In addition to numbers, text and logical values – VARP - Calculates variance based on the entire population – VARPA - Calculates variance based on the entire population – SKEW - Returns the skewness of a distribution – KURT - Returns the kurtosis of a data set

Distributions – BINOMDIST - Returns the individual term binomial distribution probability – POISSION - Returns the Poisson distribution – NORMDIST - Returns the normal cumulative distribution for the specified mean and standard deviation – NORMSDIST - Returns the standard normal cumulative distribution function – BETADIST - Returns the cumulative beta probability density function – CHIDIST - Returns the one-tailed probability of the chi-squared distribution – EXPONDIST - Returns the exponential distribution – FDIST - Returns the F probability distribution – GAMMADIST - Returns the gamma distribution – HYPEGEOMDIST - Returns the hypergeometric distribution – LOGNORMDIST - Returns the cumulative lognormal

distribution of x – NEGBINOMDIST - Returns the negative binomial distribution – TDIST - Returns the Percentage Points (probability) for the Student t-distribution – TINV - Returns the t-value of the Student's t-distribution – WEIBULL - Returns the Weibull distribution – GAMMAINV - Returns the inverse of the gamma cumulative distribution – FINV - Returns the inverse of the F probability distribution – CHIINV - Returns the inverse of the one-tailed probability of the chi-squared distribution – BETAINV - Returns the inverse of the cumulative beta probability density function – LOGINV - Returns the inverse of the lognormal cumulative distribution function of x – NORMINV - Returns the inverse of the normal cumulative distribution – NORMSINV - Returns the inverse of the standard normal cumulative distribution

Correlation and Regression – COVAR - Returns covariance – CORREL - Returns the correlation coefficient of the array1 and array2 cell ranges – PEARSON - Returns the Pearson product moment correlation coefficient – RSQ - Returns the square of the Pearson product moment correlation coefficient – SLOPE - Returns the slope of the linear regression line

Testing of hypothesis – CHITST - Returns the chi-square test for independence – FTEST - Returns the result of an F-test – TTEST - Returns the probability associated with a Student's t-Test – Z TEST - Returns the two-tailed P-value of a z-test

Other functions available – PROB - Returns the probability that values in a range are between two limits CONFIDENCE - Returns the confidence interval for a population mean – COUNT- Counts the number of cells that contain numbers and numbers within the list of arguments – COUNTA - Counts the number of cells that are not empty and the values within the list of arguments – CRITBINOM - Returns the smallest value for which the cumulative binomial distribution is greater than or equal to a criterion value – FISHER - Returns the Fisher transformation at x – FISHERINV - Returns the inverse of the Fisher transformation – FORECAST - Calculates, or predicts, a future value by using existing values – FREQUENCY - Calculates how often values occur within a range of values – GAMMALN - Returns the natural logarithm of the gamma function – GROWTH - Calculates predicted exponential growth by using existing data – INTERCEPT - Calculates

the point at which a line will intersect the y-axis by using existing x-values and y-values – LARGE - Returns the k-th largest value in a data set – LINEST - Calculates the statistics for a line by using the "least squares" method to calculate a straight line that best fits your data – LOGEST – Calculates an exponential curve that fits the data —– MAX- Returns the largest value in a set of values – MAXA - Returns the largest value in a list of arguments – – MINA - Returns the smallest value in the list of arguments – – PERCENTILE - Returns the k-th percentile of values in a range – PERMUT - Returns the number of permutations for a given number of objects that can be selected from number objects –RANK - Returns the rank of a number in a list of numbers –SMALL - Returns the k-th smallest value in a data set – STANDARDIZE - Returns a normalized value from a distribution characterized by mean and standard_dev – – STEYX - Returns the standard error of the predicted y-value for each x in the regression –TREND - Returns values along a linear trend – AVEDEV - The average of the absolute deviations of data points from their mean – AVERAGEA - Calculates the average (arithmetic mean) of the values in the list of arguments –DEVSQ - Returns the sum of squares of deviations of data points from their sample mean – MIN - Returns the smallest number in a set of values

Course Title : **Biostatistics and Computer Applications**

Courese No : **MC – 510** Course Credit : **2 + 1**

Lecture No : 44 ***Prepared by Mini, K.G.***

Topic : Statistical analysis using MS Excel

Covariance – Concept – Input range – Grouping of data – Output range – Correlation – Introduction – Input range – Grouping of data – Output range

Descriptive statistics – Input range – Grouping of data – Confidence level for mean – Finding our maximum –Minimum – Output range – Exponential smoothing – Rationale – Input range – Damping factor – Labels – Output range – Chart output – Standard error – 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

Regression – Rationale – Input range of independent and dependent variable – Labels – Value of constant is zero – Output range – Residuals – Standardized residuals – Residual plots – Line fit plots

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

ANOVA - single factor – Basic concept – Input range – Row or column grouping – Significance level – Output range – ANOVA - two factor with replication – Concept – Input range – Rows per sample – Significance level – Output range – ANOVA – two factor without replication – Input range – Labels – Alpha – Output range.