# **DEPARTMENT OF MARINE LIVING RESOURCES**

# **Revised Syllabus**

# With effect from 2022-2023



# ANDHRA UNIVERSITY Visakhapatnam

## DEPARTMENT OF MARINE LIVING RESOURCES, ANDHRA UNIVERSITY Scheme of Examination (With effect from 2022-2023admitted batches) M.Sc. Marine Biotechnology-I Semester

Paper No	Paper Title	Ma	aximum Marks	5		Credits	
NO		Theory (End	Practical	Total	Theory	Draatical	Toto1
		evam ±	(Semester end)	Total	Theory	Flactical	Total
		Mid+Asonmt	()	marks			
11	Oceanography and Marine	70+20+10	50	150	4	2	6
1.1	Biology	70120110	50	150		2	0
1.2	Biochemistry	70+20+10	50	150	4	2	6
1.3	Reproductive Physiology and	70+20+10	50	150	4	2	6
	Endocrinology						
1.4	Molecular Biology	70+20+10	50	150	4	2	6
	Total marks	400	200	600	16	8	24
	M.Sc.	<b>Marine Biote</b>	chnology -II S	emester			
		Theory (End	Practical	Total	Theory	Practical	Total
		exam +	(Semester end)	marks			
	~	Mid+Asgnmt)		1.50			
2.1	Coastal Aquaculture	70+20+10	50	150	4	2	6
2.2	Fish Genetics	70+20+10	50	150	4	2	6
2.3	Microbiol Technology and	/0+20+10	50	150	4	2	6
2.4	Coll & Tissue Culture	70 + 20 + 10	50	150	1	2	6
2.4	Total marks	70+20+10 400	200	600	4	2	24
	Total marks	400 Marina Biata	200 chnology III (	Somostor	10	0	24
	IVI.SC	Theory (End	Practical	Total	Theory	Drastical	Tota1
		exam ±	(Semester end)	notai	Theory	Flactical	Total
		Mid+Asonmt)	()	marks			
3.1	Health Management in	70+20+10	50	150	4	2	6
0.11	Aquaculture	/0120110	00	100		_	Ũ
3.2	Immunology	70+20+10	50	150	4	2	6
3.3	Enzymology and Enzyme	70+20+10	50	150	4	2	6
	Technology						
3.4	Marine Pollution and Bio-	70+20+10	50	150	4	2	6
	deterioration				-		
3.5	MOOC-I						2
	IPR (Value added course)	400	200	(00	1(		26
	1 otal marks	400	200	600	16	8	26
M.Sc. Marine Biotechnology–IVSemester				T . ( . 1			
		Theory (End	Practical	1 otal	Theory	Practical	Total
		Mid+Asanmt)	(Semester end)	marks			
4.1	Applications of Biotechnology	70+20+10	50	150	4	2	6
	in Aquaculture	70120110	20	100			
4.2	Genetic Engineering	70+20+10	50	150	4	2	6
4.3	Bioactive Marine Natural	70+20+10	50	150	4	2	6
	Products						
4.4	MOOC-II						2
4.5	Project	100		100	4	-	4
	Viva-voce		50	50		2	2
	Research Methodology						
	Total marks	400	200	600	16	8	26

# **M.Sc. Marine Biotechnology**

**Course Objective**: To supply trained manpower in the field of Marine Biotechnology. After completion of the course, the post graduate in Marine Biotechnology should be able to

Master the concepts in Oceanography, Biochemistry, Molecular biology, Reproductive physiology and Endocrinology of Marine cultivable organisms.

Master the culture of fish/shellfish/seaweeds in coastal waters, concepts of genetics, Microbiology and tissue culture in the contemporary world of Marine Biotechnology.

Practice the techniques of health management in Aquaculture, Immunology, Enzymology and also the control measures related to Marine pollution.

Apply Biotechnological tools including Genetic Engineering techniques in Fish Transgenic besides acquiring knowledge in Bioactive Marine Natural products.

### M.Sc. Marine Biotechnology

## First Semester Paper 1.1: Oceanography and Marine Biology

#### **Learning Objectives:**

To impart knowledge on the various aspects such as temperature, light, salinity, waves, tides as physical parameters and as ecological parameters; heat distribution between continent and oceans, micronutrient distribution, regeneration of nutrients, dissolved oxygen, carbon dioxide and other important gases, their biological importance in the productivity of oceans.

To give knowledge to the students about the various national and international marine research institutions.

#### **Outcome:**

The student will get sound knowledge on the above aspects which helps the student understand importance of the physical and chemical properties seawater as ecological parameters and physical parameters to apply the same in the management of culture candidate species when they undertake culture activity.

The student will get knowledge on the role played by the various properties of seawater on the sustainability of organisms and on the overall productivity of oceans.

The student will be aware of the various marine research institutions in India and abroad to prepare himself to seek a position in the institute or to pursue higher studies.

#### Unit - I

Dimensionsofoceans; Physical parameters of sea: Tides, waves, light, temperature, cur rents, density, pressure. Heatbudget of the oceans.

Soundanditspropagationinthesea.

#### Unit - II

Chemicalparametersofthesea:salinity,dissolvedoxygen,carbondioxide,pH,nutri entsandtraceelements.Compositionof seawaterandbrackish water.

#### Unit -III

Classification of marine habit at sand ecological divisions of the

ocean.Plankton, nekton, benthos and theiradaptations,

methods of collection

Ecologyofcoralreefsandmangrovehabitats; their spec

 $ial features. \\ Sea-ranching of economically important$ 

marineorganisms.

#### Unit - IV

Lawpertainingtotheseas:HistoricalperspectivesinInternationalnegotiationsandsett lementsoveropen seas. Modern law of the sea.

Remote sensing applications in ocean ography and marine biology.

Elements of Geographic Information Systems (GIS) and its role in ocean ography.

#### Unit - V

NationalandInternationalInstitutes ofmarine research: NIO, CMFRI, CIFE, CIFT, CIBA, MPEDA, INCOIS, NRSA their affiliation, thrust areas of research, administrative hierarchy, scientist recruitment. Scripp's Institute of oceanography, Woods Hole Institute of Oceanography, Rosential School of Marine Sciences, Hawaiian Institute of Marine Biology, National Oceanography and atmospheric administration, Plymouth Laboratories.

References:			
Svedrup et al	The Oceans	Prentice Hall	
Tait RV	Elements of marine ecology	Butterworths	
Riley & Skirrow	Chemical Oceanography	Academic Press	
Newell RC	Biology of intertidal animals Logos Press		
Kinne O (Ed)	Marine ecology	John Wiley & Sons	
Mann KH	Ecology of coastal waters		
King CAH	Introd. Phys. & Biol. Oceang.	ELBS	

- 1. Determination of Salinity of the sea water sample adopting Harvey's Method
- 2. Determination of the Dissolved Oxygen concentration in the sea water adopting Winkler's Method
- 3. Determination of Alkalinity of the sea water sample following Titrimetric Method
- 4. Determination of pH of sea water sample using a digital pH meter.
- 5.Identification of Phytoplankton, Zooplankton, Nekton, Intertidal, sub-tidal organisms, corals and mangroves.

## Paper 1.2: Biochemistry

### Learning Objective:

To provide knowledge on biochemical functions of different biomolecules and their metabolism.

### **Outcome:**

To gain knowledge on basic concepts of biochemistry, chemical bonds, structure and properties of biomolecules and their metabolism. To learn about metabolic regulation, bioenergetics, biological membranes, vitamins, hormones and antibiotics.

### Unit-I

Basic concepts of biochemistry: An overview, types of chemical bonds. Structure & properties of biomolecules - Carbohydrates; Metabolism of carbohydrates - Glycolysis, Gluconeogenesis, Kreb's cycle, Oxidative phosphorylation.

### Unit-II

Structure & properties of biomolecules – proteins, lipids ; Protein metabolism, lipid metabolism-Beta fatty acid oxidation. Principles of Metabolic regulation- regulatory steps

## Unit-III

Proximate compositions of fish and shellfish.

Bioenergetics: Basic principles, Equillibria and concept of free energy; Coupled processes

### Unit-IV

Structure and properties of Vitamins and hormones; Prostaglandins, leucotrienes, thromboxanes, interferons, interleukins . Types of antibiotics

#### Unit-V

Biological membranes: organization-sideness and function, transport of biomolecules and cytoskeletal organization. signals and second messengers Biochemical pathway of photosynthesis and factors affecting.

Stryer H	Biochemistry	Freeman
Lehninger AL	Principles of biochemistry	CBS
Voet & Voet	Biochemistry	
Plummer	An introduction to practical	biochemistry.

- 1. Estimation of moisture in fish/shrimp muscle
- 2. Estimation of protein in fish/shrimp muscle by Lowry's method
- 3. Estimation of carbohydrate in fish/shrimp muscle by Anthrone method
- 4. Estimation of lipid in fish/shrimp muscle by Sulpho- Phospho vanillin method
- 5. Separation of proteins by Acrylamide GEL Electrophoresis
- 6. Estimation of glycine by formal titration
- 7. Analytical method to detect carbohydrates
- 8. Analytical method to detect amino acids-
- 9. Analytical method to detect proteins
- 10. Analytical method to detect lipids
- 11. Equipments in Biochemistry

# Paper 1.3: Reproductive Physiology and Endocrinology

# Learning Objectives:

To study the anatomy and histological changes in gonads of fish and shellfish.

To study sexual dimorphism and sex differentiation.

To study the factors responsible for reproduction and sexual dimorphism.

To study endocrine system of finfish and shell fish and its role in reproduction.

To study the applications of biotechnology in accelerating reproduction and gonadal development.

# **Outcome:**

To acquire knowledge on reproductive system of finfish and shell fish.

To study the reproductive cycles, spawning season, gonadal development and fecundity studies of finfish and shellfish.

Importance and role of endocrine system.

Applications of biotechnology in aquaculture.

# Unit - I

Anatomy and histology of gonads in fin fish and shellfish.

Development of gonad: oogenesis; spermatogenesis, metabolic changes during oogenesis and spermatogenesis and gonadal steroidogenesis.

# Unit – II

Sex determination and differentiation, factors affecting sex differentiation.

Sexual dimorphism, primary and secondary sex characters, bisexual reproduction, inter-sexes, hermaphroditism

Sex reversal in fish and shellfish, factors affecting sex reversal.

# Unit - III

Breedingbiology: Annual reproductive cycles and breeding patterns in fin fish and shell

Fish, artificial insemination

Role of environment - photoperiod, temperature, rainfall, nutrition

Pheromones and reproductive behaviour.

# Unit - IV

Role of hypothalamo-hypophyseal system and pineal gland Neuro-endocrine systems in fish and shell fish and its role in the regulation of reproduction.

# Unit - V

Cryopreservation of gametes Application of biotechnology for accelerating gonadal growth In vitro fertilization.

Adiyodi KG & Adiyodi RG. 1971. Endocrine Control of Reproduction in Decapod Crustacea. Biology Reviews.

Agarwal NK. 2008. Fish Reproduction. APH Publ.

Bell TA & Lightner TA. 1988. A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society.

Ghosh R. 2007. Fish Genetics and Endocrinology. Swastik Publ. & Distr.

Hoar WS, Randall DJ & Donaldson EM. 1983. Fish Physiology. Vol. IX. Academic Press.

Maria RJ, Augustine A & Kapoor BG. 2008. Fish Reproduction. Science Publ.

Matty AJ. 1985. Fish Endocrinology. Croom Helm.

Mente E. 2003. Nutrition, Physiology and Metabolism in Crustaceans. Science Publ.

Nikolsky GV. 2008. The Ecology of Fishes. Academic Press.

**Thomas PC, Rath SC & Mohapatra KD. 2003.** Breeding and Seed Production of Finfish and Shellfish. Daya Publ. House.

- 1. Dissection of reproductive system fish, shrimp, Celina
- 2. Estimation of spawning season
- 3. Estimation of fecundity
- 4. Gonadal maturity stages in fish, shrimp, mollusca
- 5. Dissection and display of pituitary gland from fish
- 6. Preparation of pituitary gland extract
- 7. Estimation of GSI
- 8. Dissection and display of various endocrine organs of fish/shrimp

# Paper 1.4: Molecular Biology

## **Learning Objectives:**

To understand cell structure and functions in prokaryotes and Eukaryotes including Cell cycle and signal transduction.

To understand structure of Nucleic Acids, their replication, protein synthesis,

To understand molecular models of DNA recombination, mutations, Mutagens, DNA damage and repair.

To understand regulation of gene expression in prokaryotes and eukaryotes.

### **Outcome:**

After study of the subject the candidate should be able to understand and apply principles molecular biology in aquaculture. Molecular biology forms the basis for development of kits for disease diagnosis

## Unit-I

Cell structure and function in prokaryotes and eukaryotes with reference to nucleus, mitochondria, chromosomes and ribosomes. Cell cycle and its regulation.

## Unit-II

Nucleic Acids: Structures of DNA and RNA; DNA replication, damage and repair in prokaryotes and eukaryotes; DNA recombination: homologous, non-homologous and site-specific recombination

#### **Unit-III**

Transcription in Prokaryotes and eukaryotes; Regulatory elements, processing of tRNA and rRNA; Genetic code, Translation and Translational machinery: Ribosomes, composition and assembly; Transport of proteins and molecular chaperones, Protein stability, Protein turnover and degradation; Post-translational modifications

## **Unit-IV**

Regulation of gene expression in prokaryotes and eukaryotes - Operon concept – Lac, Tryp, Ara, Gal and His; DNA methylation, regulatory sequences and transacting factors

## Unit-V

Environmental regulation of gene expression

Mutations: Types, Physical, chemical and biological mutagens, Role of transposons in mutations. Oncogenes and tumor suppressor genes.

Lewin B	Genes IX	John Wiley
Watson et al	Molecular biology of gene	Benj. Cumm.
Frifielder D	Microbial genetics	
Lodish et al	Molecular cell biology	Freeman

- 1. Qualitative analysis of Nucleic acids.
- 2. Quantitative analysis of nucleic acids.
- 3. Plasmid DNA and Genomic DNA isolation & quantitation: Plasmid mini preparations
- 4. Isolation of RNA from yeast cells/ fish
- 5. Purification of DNA from an agarose gel
- 6. Restriction digestion of DNA
- 7. Bacterial transformation

#### M.Sc. Marine Biotechnology

Second Semester

#### 2.1: Coastal Aquaculture

## Learning Objectives:

To study the importance and present status of aquaculture in Inia and abroad

To study the various aspects of site selection and construction of fish/ shrimp ponds and hatcheries

To study the various culture systems and practices for culture of various finfish/ shellfish and sea weeds.

## Outcome:

The student can acquire sound knowledge on culture of various types fishes, shrimps, molluscans and sea weeds, those have high demand in the domestic as well as foreign market.

### Unit-I

Definition and importance of aquaculture. Overview and status of aquaculture in India. Global aquaculture scenario, production, consumption and emerging trends.

## Unit-II

Engineering aspects of aquaculture: Design and construction of pond and hatchery. Types of culture systems: Ponds, cages, pens, race ways. Types of culture practices: Traditional, extensive, semi-intensive, intensive, super intensive, ultra-intensive, mono- and poly culture, integrated and organic farming.

## Unit-III

Biology and life cylces of *Penaeus monodon, Penaeus vannamei, Macrobrachium rosenbergii, Panulirushomarus, Scylla serrata, Crassostrea madrasensis Pinctada fucata, Loligo sps., Chanos chanos*and *Lates calcarifer*.

## Unit-IV

Criteria for selection of species for coastal aquaculture.

Finfish culture: Milk fish, mullets, Asian sea bass.

Shell fish culture: Shrimps, prawns, crabs, lobsters, oysters, mussels and cephalopods.

## Unit-V

Life cycles of seaweeds: *Ulva fasciata, Gracilaria corticata* and *Sargassum tenerrimum.* Culture practices of seaweeds in India and Abroad. Farming of Agar, Algin and Carrageenan yielding seaweeds. Breed improvement in Sea weeds.

Bardach JE et al. Aquaculture Wiley interscience
Pillay TVR Aquaculture: principles and practices FNB
Bardach, John.E. 1997 Sustainable Aquaculture. John Wiley and Sons.
Chapman, V.J., 1980. Seaweeds and theirs uses Chapman and Hall London.
Wheaton, F.W. 1977. Aquaculture Engineering. John Wiley and Sons, New York.
Stickney, 1995. Principles of Aquaculture, John Wiley & Sons.
Santhanam R et al Coastal aquaculture CBS

- 1. Identification of fish based on morphometric and meristic data.
- 2. Identification of shrimp based on morphological characters.
- 3. Dissect and display the digestive system of fish, shrimp, molluscs
- 4. Identification of cultivable organisms: Seaweeds, Crustaceans, Molluscs and Finfish
- 5. Identification of larval forms of cultivable organisms: Crustaceans, Molluscs and Finfish.
- 6. Aquaculture equipment.
- 7. Design and describe shrimp hatchery and fish farm.

## Paper 2.2: Fish Genetics

## Learning objectives:

To understand basic principles of fish genetics, Mendelian and non-Mendelian inheritances, sex determination in fish, Chromosomal polymorphism and Population genetics. To understand various DNA markers, and assays to detect DNA damage, Transgenics and GMOs.

## **Outcome:**

After studying the subject the candidate should be able to understand and apply principles of Genetics for the production of hybrids, development of specific pathogen resistant species and transgenic fish.

## Unit-I

Scope and Inportance of fish genetics.Mendelian inheritance, Non- chromosomal & Mitochondrial inheritance, genetic variation, chromosome theory, genetic basis for sex determination and sex linked genes in fish, linkage and crossing over.

## Unit-II

Chromosomal polymorphism, Gynogenesis, androgenesis, production of super males. Genetic improvement in fish.Population genetics: Changes in allelic and genotypic frequency, effective population size, inbreeding and coefficients, Fitness, qualitative and

quantitative traits, Components of variance- additive and non-additive variance.

## **Unit-III**

Genetic variability and differentiation, Genetic similarity and Nei's genetic distance, Genetic bottle neck and mutation drift equilibrium null alleles, population genomics, outlier loci and adaptive variation in trait-related genes.

#### **Unit-IV**

DNA markers in stock identification: Allozymes, RFLP, RAPD, AFLP, Microsatellites, ESTs, SNps, Type I and Type II markers, mt DNA and nuclear DNA markers, Laboratory assay of markers, Hybridization, genetic diversity and conservation, Parentage, Linkage, QLT mapping and microarray genes.

#### Unit-V

Karyotyping and chromosome banding- C-banding, G-banding, NOR banding.

Fluorescence In-situ Hybridization (FISH), Transgenics, GMO and biosafety regulations, designer ornamental fish. Comet assay, Sister chromatid exchange, MNT(Max's Next Tango).

Pandian TJ, Strussmann CA & Marian MP Biotechnology Lakra/Singh Mirza Akbar Khan Fish Genetics & AquacultureSciencePub

Fish genetic resources Genetic Embryology and fishes

- 1. Exercises on Mendelian laws
- 2. Estimation of gene and genotypic frequencies
- 3. Estimation of effective population size
- 4. Building of pedigree size
- 5. Protein, mt DNA and nuclear DNA extraction.
- 6. ANOVA in genetic variance
- 7. Estimation of heritability by half-sib, full-sib and mid-parent analysis.
- 8. Procedures for estimating breeding values/coefficient of inbreeding.
- 9. Estimation of genetic diversity and relatedness using molecular information.
- 10. Preparation of chromosome spreads; karyotyping, banding techniques, commet assay.

# Paper 2.3: Marine Microbiology and Microbial Technology

### Learning objectives:

To understand the general principles of microscopy, microbiology including the morphology, taxonomy and culture methods of Virus, Bacteria, Virus, Microalgae and Protozoans.

To understand techniques of sterilization, enumeration and preservation of Bacteria.

To understand the general principles and design of bioreactors used for fermentation .

To understand the importance of pathogenic micro organisms in fish preservation and concepts of quality management in fish processing.

To understand the principal aspects of planning and implementation of HACCPs.

## **Outcome:**

After study of the subject the candidate should be able to understand and apply the concepts of microscopy, microbiology, fish processing, microbial culture and quality management in Aquaculture processing industry besides design and utilization of bioreactors.

# Unit-I

Microscopy: Principlesand Working mechanisms of Light, Phase contrast, Transmission and Scanning electron microscopes. Virus, bacteria, fungi, protozoans and their classification Microbes in extreme environments and their significance- thermophiles, psychrophiles, halophiles and barophiles.

## Unit-II

Physical and chemical methods of Sterilization. Viruses:morphology,isolation and culturemethods. Bacteria: Morphology, Enumeration, culture, classification and preservation. Culture of Fungiandprotozoans.

## Unit-III

Bioreactors: Design and types. Fermentation and bioconversion by microorganisms. Water borne pathogens of public health importance: Protozoan, Bacteria, Entero viruses, microbial toxins. Microbial standards for different water uses.

## **Unit-IV**

Role of bacteria and moulds in fish preservation. Microorganisms in frozen, canned and dried products and their control. Fish quality, evaluation and different indices of quality, total quality management in sea food processing.

# Unit-V

HACCP: Practical aspects of planning and implementing HACCP systems.

Hazards in sea foods, risk assessment. National and international standards – ISO 9000, ISO 22000. Role of Bureau of Indian Standards, Export Inspection Agency, EIC and FSSAI. Traceability issues in international trade.

Pelczar et al	Microbiology	McGraw Hill	
Cappuccino & Sherman	Microbiology- Lab manual		
Stainer RT et al	General Microbiology MacMillan		
Luria et al	General Virology	Wiley	
Rehm & Reed	Biotechnology		
Reed G et al	Industrial microbiology	CBS	

- 1. Sterilization Techniques
- 2. Preparation of Different Bacterial, Fungal and Protozoal culture media,
- 3. Isolation, identification of Bacteria.
- 4. Estimation of total heterophilic Bacterial counts in water and soil sediments,
- 5. Estimation of total Vibrio counts in water and soil sediments,
- 6. Isolation, Culture and identification of fungi
- 7. Antibiotic sensitivity tests.
- 8. MPN of coliforms and confirmation
- 7. Detection of food borne pathogens Salmonella, Listeria and schizella
- 8. Detection of antibiotic residues in Fish/Shrimp.
- 9. Measurement of bacterial growth.

## Paper 2.4: Cell and Tissue culture

#### Learning Objectives:

To impart knowledge to the student about the cell culture and tissue culture; importance of tissue culture, types of culture media, growth of cells in the cell culture flask; cell lines, viability, toxicity, cells harvesting, cloning, culture of shrimp/ fish cell lines, cloning of cell lines; cancer cell and stem cell culture, applications of stem cell culture, apoptosis, 3D cultures and tissue engineering. The student will be provided the knowledge of

Culture of marine microalgae used as live feed in aqua industry, mangrove plants for restoration of mangrove forests, mantle of molluscs to be used in the pearl oyster culture Lymphoid organ and hematopoietic tissue culture of shrimp which are used in the study of shrimp immune system.

#### Outcome

The student will obtain knowledge in

Cell culture, tissue culture, culture of cell lines, culture of stem cells, organ culture, microalgal culture, differentiating cancer cells from healthy cells, preparation of culture media for tissue culture and microalgal culture, determining viability of cells, cytotoxicity, apoptosis, industrial production of cells and tissues, harvesting of cultured cells and tissues.

The student with the above knowledge can serve the industry, aquaculture sector, cancer research laboratories and institutes.

#### Unit- I

An overview of tissue culture, equipments and materials for tissue culture. Culture media: Types and preparation.

#### Unit- II

Cell cultures: Primary, secondary cultures and their maintenance.

Cell lines: Primary and secondary cell lines establishment. Measurement of viability and cyotoxicity assay, measuring parameters of growth.

#### Unit- III

Pure culture: isolation, separation, characterization, identification, maintenance and preservation, Cloning of cell lines.

Development of cell lines of shrimp and fish.

#### Unit- IV

Organ cultures, large scale cultures.

Stem cells: Stem cell differentiation, Blood cell formation, Fibroblasts and their differentiation,

Differentiation of cancerous cells and role of proto-oncogenes.

#### Unit- V

Stem cell cultures, embryonic stem cells and their applications; Measurement of cell death & apoptosis, Three dimensional culture and tissue engineering.

Cell culture techniques of marine macroalgae, mangrove plants and molluscan mantle.

Industrial applications of tissue culture. **References: Gupta PK** Biotechnology **Barnes D & Mathur PJ** Animal cell culture methods Academic **Basega R** Cell growth & division: a a IRL Practical approach **Clynes** Animal cell culture techniques Springer **Freshney I** Culture of animal cells: a manual Wiley-Liss Of basic techniques **Harrison et al** General techniques of cell culture Cambrid. U. **Lan FR** Culture of animal cells Wiley-Liss **Masters RW** Animal cell culture: practical approach Oxford **Hoar & Randall** Fish Physiology Vol V

- 1. Preparation of tissue culture media.
- 2. Preparation of Balanced Salt Solutions: DPBSA and Eagle's BSS.
- 3. Preparation of F2 and Convey's medium for microalgal culture.
- 4. Isolation of protoplast from seaweeds.
- 5. Tissue degradation by trypsinization- Warm/cold.
- 6. Cell count using Haemocytometer.
- 7. Spore culture of seaweeds.
- 8. Detection of dead and live cells using calcofluor white stain

## M.Sc. Marine Biotechnology

# Third Semester Paper 3.1: Health management in Aquaculture

### Learning objectives:

To understand the general principles of pathology, Epizootiology and diagnosis by microbiological, Histopathological, immunological, molecular diagnostic methods. To understand various aetiological agents and their pathogenicity, prophylaxis and treatment methods.

To understand the action of probiotics, immunostimulants, antibiotics and vaccines in the disease management of fish.

### **Outcome:**

After study of the subject the candidate should be able to understand and apply the principles of disease diagnosis, prevention and treatment of fish diseases in culture systems.

## Unit-I

Disease: Definition, Host-pathogen-environment relationship, environmental stress, Inflammation response to diseases.

## Unit-II

Infectious bacterial, viral and mycotic diseases in fish and shrimp: Epizootiology, Diagnosis, Life Cycle, Prevention and treatment.

Non-infectious Diseases: Nutritionally induced diseases, water, soil, environmental parameters and their effecton fish health.

#### **Unit-III**

Diagnostic techniques in aquaculture: Microbiological, haematological, histopathological, immunological andmolecular techniques, Disease surveillance and reporting.

#### **Unit-IV**

Disease control and management: Environment management, chemotherapeutic agents, host management, prophylaxis-vaccines, adjuvents, immunostimulants.

## Unit-V

Probiotics.Use and abuse of antibiotics and chemicals in health management. Fish health and quarantine systems. Seed certification, SPF and SPR stocks- development and applications.

Bardach JE et al., Aquaculture Wiley- Interscience

Pillay TVR Aquaculture: Principles & practices FNB

Santhanam R et al., Coastal aquaculture CBS

Inglis V, Roberts RJ and Bromage NR. Bacterial diseases of fish . Blackwell

**Iwami G & Nakanishi T (Eds.).** The fish immune system- organism, pathogen and environment. Academic Press.

**Roberts RJ.** Fish Pathology 3<sup>rd</sup> Ed. WB Saunders

Schaperclaus W. Fish diseases. Vols. I, II. Oxonian Press.

Shankar KM and Mohan CV. 2002. Fish and Shellfish Health Management. UNESCO Publ.

**Sindermann CJ.** Principal diseases of marine fish and shellfish. Vols. I, II. 2<sup>nd</sup> Ed. Academic Press.

Stickney RR. Principles of aquaculture Wiley & Sons

- 1. Procedure of disease diagnosis.
- 2. Tissue fixation, Microtomy and histology of various fish/shrimp tissues.
- 3. Isolation of pathogenic bacteria/fungi and their identification.
- 4. Disease diagnositic methods: Necroscopy, Agglutination tests.
- 5. Identification of various parasites
- 6. Diseases of fin fish and shell fishes.
- 7. PCR for disease diagnosis, serological techniques in disease diagnosis.
- 8. Diseased fish/shrimp specimens.
- 9. Fish/Shrimp haemotological parameters- TEC, DLC, TLC, Hb, Total protein, lipid profile, creatine Urea and enzymes in blood.

# Paper 3.2: Immunology

## Learning objectives:

To understand innate and adaptive immune systems including the structure and diversity of antibodies.

To understand the disorders of immune system and immunotherapy methods.

To understand types of vaccines and their mode of action.

# **Outcome:**

After study of the subject the candidate should be able to understand and apply the concepts of immunology in Biotechnological industry .

# Unit-I

Immunity and typesofimmunity.Fundamental concepts and anatomy of the immune system, components of innate and acquiredimmunity, Phagocytosis, Complement and inflammatory responses, Organs and Cells of the immune system-primary and secondary lymphoid organs, Lymphatic system, Lymphocyte circulation, Lymphocyte homing, Mucosal and Cutaneous associated Lymphoid tissue (MALT & CALT); Mucosal immunity.

## Unit-II

Antigens: Types and properties. Major Histocompatability Complex(MHC) - MHC genes, Immune responsiveness and disease susceptibility and HLA typing.

# Unit-III

Immunoglobulins: Structure, types and functions. Immunogenetics:Genetic basis of antibody diversity.Molecular biology of B and T cells. Complement proteins and cytokines.

## Unit-IV

Disorders of immune system: autoimmunity- types of autoimmune diseases.

Immunodeficiency- Primary immuno deficiencies, Acquired or secondary immuno deficiencies, hypersensitivity.

Immunotherapy, Immunostimulants-beta glucagons; Active and passive immunization; vaccines- Live, killed, attenuated, sub unit vaccines and antibodies. Non-specific immunity of shellfish.

# Unit-V

Hybridoma technology- mono and polyclonal antibodies. ELISA, RIA and Immuno electrophoresis applications. Transplantation- Immunologiocal basis of graft rejection. Tumor immunology- Tumor antigens; immune response to tumors and tumors evasion

of the immune system, Cancer immunotherapy.

Eli Benjamini	Immunology – a short course	
I. Riott	Essentials of immunology	Blackwell
I Riott et al	Immunology	Molsby
Aruna B	Manual of practical immunology	Palani-
		paramount

- 1. Preparation of antigens, Immunization and methods of bleeding, serum separation and storage.
- 2. Antibody titre by ELISA method.
- 3. Isolation and purification of Ig G from serum or Ig Y from chicken egg.
- 4. Blood smear identification of leucocytes by Giemsa stain.
- 5. Separation of leucocytes by dextram method.
- 6. Demonstration of phagocytosis.
- 7. Immuno electrophoresis, Isolation of antibody from serum, Nonspecific immune response (NBT and Prophenol oxidase tests).

## Paper 3.3: Enzymology and Enzyme Technology

### Learning Objectives:

Concepts of nomenclature and classification of enzymes. Enzyme kinetics and regulation. Knowledge on extraction, isolation and purification of enzymes. Basic concepts of Immobilized enzymes. Impart knowledge on importance of enzymes in disease diagnosis and industries.

## **Outcome:**

Student able to acquire knowledge on enzymes, enzyme kinetics, regulation and their importance.

### UNIT -I

Nomenclature & classification of Enzymes. Enzyme structure and properties, co-enzymes, co-factors. Enzyme specificity; factors affecting enzyme action.

### UNIT -II

Mechanism of enzyme action: activation energy; characterization of active site; activators and inhibitors, Enzyme kinetics: Michaelis- Menten Kinetics, steady state kinetics; single and multi-substrate interactions, Competitive, non-competitive and uncompetitive enzyme substrate kinetics.

#### UNIT -III

Multi-enzyme complex; single and multi-substrate systems. Regulatory enzymes: Allosterism, covalent modification and feedback mechanisms; ATPase, glutamine synthetase.Haemoglobin and myoglobin.

## UNIT -IV

Membrane-bound enzymes: Extraction, purification, assay and enzyme storage Immobilization of enzymes: methods of enzyme immobilization; applications; Merits and demerits of immobilized enzymes.

## UNIT -V

Biosensors and modifications.

Synthetic enzymes, isozymes and their importance.

Enzymes of industrial and diagnostic importance.

Boyer P D	The Enzymes	Freeman
Fersht A R	Enzyme structure & mechanism	Freeman
Palmer T	Enzymes	Horwood
Siseman	A Handbook of Enzyme technology	
Trevan M D	Immobilized enzymes	Wiley & Sons
Plowman K M	Enzyme kinetics	MacGraw Hill
Rehm & Reed	Biotechnology Vol II: Bioreactors	

- 1) Preparation of maltose standard curve using 3, 5- Dinitrosalicylate reagent.
- 2) Assay of amylase in saliva.
- 3) Preparation of ammonia standard curve using Nessler's Reagent
- 4) Assay of urease activity.
- 5) Time course of enzyme activity.
- 6) Effect of temperature on enzyme activity.
- 7) Effect of pH on enzyme activity.
- 8) Common instruments used in enzymology.

## Paper 3.4: Marine Pollution and Bio-deterioration

#### Learning Objectives:

To impart thorough knowledge to the students in marine pollution, sources of pollutants to coastal oceans, transport paths and agents, domestic, industrial and agricultural discharges, composition, fate in the marine environment. Waste water treatment methods. To study the fouling and boring activities of marine organisms on marine structures, controlling of boring and fouling activities of marine organisms. To study the strategies of global environmental methods.

#### **Outcome:**

The students will get knowledge about the various types of pollutants, their sources, transport paths, transport agents, their fate in the environment; controlling, monitoring and management methods of all those pollutants. The students will get commendable knowledge on the recent topics such as application of biotechnology to mitigate the pollutants generated by industrial activities, to treat the pollutants; EIA methods and enzymatic removal of hazardous substances. The knowledge acquired by the students on marine pollution may encourage them to develop, conduct and participate in the programmes against release of pollutants into the coastal environment.

#### Unit - I

Sourcesofmarinepollution:dynamics,transportpathsandagents.

Composition of domestic, industrial and agricultural discharges; their fate in the marine environment. Toxicity and treatment methods.

#### Unit - II

Oilpollution: Sources, composition and its fate in marine habitats. Toxicity and treatment methods.

Thermalandradioactivepollution:sources,disposalsystemsofheatedeffluents,effect sandtreatment.

Soliddumping, mining and dredging operations: their effects on marine ecosystem, management of solid waste.

#### Unit - III

Biofouling and bio-deterioration: Biofilm formation-primary, secondary,

tertiary colonizers. Effects of biofouling and control measures:

manual, mechanical, and chemical

Borers: Effects and control measures.

Corrosion-

definition, reactions, classification. Factors and preventive measure

s.

## Unit - IV

Globalenvironmental monitoringmethods: status, objectivesand limitations.

Monitoring strategies of marine pollution:critical pathway approach and mass balancing.EnvironmentImpactAssessment:

Assessmentofdamageandproblemsofmeasuringtheimpact

Unit - V

Roleofbiotechnologyinmarine pollution control. Bio-deterioration: Biofilm formation- primary, secondary and tertiary colonization of organisms on marine structures. Enzymatic removal of hazardous substances. Wastesfromfishprocessingunitsandtheirtreatmentremovalofnitrogenandphosphorus. Aquatic macrophytes in treatment of waste water.

#### **References:**

NielsenSE Tropical pollution
Kinne O Marine Ecology Vol. V John Wiley
Johnson R (Ed) Marine pollution Academic Press
Patin SA Pollution and Boil. Resources of oceans. Butterworths
Goldberg, E. D. 1974. The Health of the oceans, UNESCO Press. Paris.
Park, P .K, Kester D.R., J.W. Deudall and B.H Ketchum, 1983. Wastes in the Ocean. Vols. 1 to 3. Wiley Interscience Publishers, New York.
Eckenfelder WW. 2000. Industrial Water Pollution Control. McGraw Hill.
Gray NF. 2004. Biology of Wastewater Treatment. Oxford University Press.
Trivedy RK. 1998. Advances in Wastewater Treatment Technologies. Global Science.

### **Practicals:**

1. Determination of BOD in the polluted sea water sample.

2. Determination of nutrients in the polluted sea water sample: nitrites, nitrates, silicates and phosphates

- 3. Determination of toxic elements in the polluted sea water sample: ammonia, sulphide
- 4. Estimation of particulate organic matter in the polluted sea water sample
- 5. Spotters: Foulers- primary, secondary and tertiary colonizers; Borers

# M.Sc. Marine Biotechnology Fourth Semester Paper4.1: Applications of Biotechnology in Aquaculture

### Learning Objectives:

To learn the plant live feeds and animal live feeds. Collection, isolation and culture of live feeds. To impart knowledge on applications of genetics in aquaculture and development of high quality strains.

**Outcome:**Student can understand about the various important applications of biotechnology in aquaculture.

## UNIT-I

Role of biotechnology in aquaculture. Natural and artificial feeds, probiotics, nutraceuticals, Growth promoters, Energetics of food conversion.

#### UNIT- II

Plant live feeds (*Chaetoceros, Isochrysis, Nannochloropsis*&*Tetraselmis*)- collection, isolation, culture and enumeration.Animal live feeds (Artemia, Rotifers, Copepods): Collection, isolation and culture. supplementary feeds: feed formulations, methods & strategies.Importanceanduseofanti-oxidantsandantibioticsinfeeds.

#### UNIT- III

Applicationofgeneticsinaquaculture: genetic selection, inbreeding, and cross-breeding, sex control, polyploidy and transgenesis, methods of gene transfer in fishes. Development of high quality strains.

#### UNIT- IV

Synthetic hormones for induced breeding - molecular endocrinology with emphasis on use of analogues for breeding like GnRH, pheromones, growth hormone signal peptide for secretion, biotechnological approaches for peptide synthesis, Antimicrobial peptides and their applications.

#### UNIT- V

Microalgae-biotechnological approaches for the identification and production of commercially important compounds, single cell protein from Spirulina, Microalgae for nutrition and cosmetics. Applications of nanotechnology in aquaculture.

Colwell RR Biotechnology in Marine Science

Felix S. 2007. Molecular Diagnistic Biotechnology in Aquaculture. Daya Publ. House.

Fingerman M, Nagabhushanam R & Thompson MF. 1997. Recent Advances in Marine Biotechnology, Vols. I-III. Oxford & IBH.

Pandian TJ, Strusmann CA & Marian MP. 2005. Fish Genetics and Aquaculture Biotechnology, Science Publ.

Reddy PVGK, Ayyappan S, Thampy DM & Gopalakrishna. 2005. Text book of fish Genetics and Biotechnology, ICAR

Nair PR. 2008. Biotechnology and Genetics in Aquatic Biotechnology. Science Publ.

#### **Practicals:**

- 1. Hatching and production of Artemia nauplii for shrimp feed.
- 2. Dissection and display of various endocrine glands of fin fishes & shell fishes.
- 4. Isolation of pituitary gland from fish, and preparation of extract,

5. Types of various artificial fish and shrimp feed.

- 5. PCR for diagnosis of viral diseases (WSV, EHP, MBV)
- 6. Synthetic GnRH for induced breeding, in fish-preparation and dose determination.
- 7. Induced breeding in shrimp- Eyestalk ablation, cauterization, squeezing.
- 7. Induced breeding in bivalves and sea urchin.
- 8. Live feed organisms: animal & plant live feed.
- 9. Quantification of protein in Spirulina dry biomass.
- 10. Extraction of lipid from micro algae dry biomass.
- 11. Quantification and characterization of lipid by GCMS.

## Paper 4.2: Genetic Engineering

**Learning Objectives:** To acquaint the students about enzymes, vectors, cloning methodologies, techniques involved in genetic engineering. To provide knowledge on PCR, types and applications. To understand the basic principles of DNA and RNA sequencing, next generation sequencing technology. To provide knowledge on gene silencing and gene therapy.

To introduce the students to bioinformatics, DNA and protein databases, sequence retrieval and phylogenetic analysis and their applications.

## **Subject Objective:**

Student acquire the basic concepts in Genetic engineering and Bioinformatics in-depth on the techniques available for genetic engineering.

### UNIT -I

Basic concepts: Restriction enzymes, DNA ligases, Klenow fragment,T4DNApolymerase,autoradiography, Radio-active and non-radioactive probes, hybridization techniques (Southern,Northern,Westernandcolonyhybridization).

## UNIT -II

DNA finger printing and DNA foot printing, Methyl interference assay, transposons. Cloningvectors:Plasmids,bacteriophages- Lambda,M13,cosmids, Yeast vectors, shuttlevectors, cohesive&bluntendsligation,Cloning methodologies: Insertion of foreign DNA into the host cells, Transfectiontechniques.

## UNIT -III

ConstructionofGenomicandC-DNAlibraries, Jumping and hopping libraries. Southwestern andfarwestern cloning. Analysis of cloned genes.PCR and its applications: Basic principles of PCR; types of PCR- Multiple, Nested, reverse transcriptase, real time, touchdown, hot start, colony. Loop Mediated Isothermal Amplification (LAMP) Site specific mutagenesis, PCR based mutagenesis.DNA-micro arrays.Transgenic fish.

## UNIT -IV

Sequencing and Gene-therapy methods: DNA sequencing- chemical, Enzymatic, automated. RNA sequencing. Next Generation Sequencing, CRISPER technology.

Gene silencingtechniques- Si RNA, Si RNA technology, Micro RNA, Construction of RNA vectors. GeneKnock-outsandgenetherapy- suicidegenetherapy,genereplacement and genetargeting.

## UNIT -V

Bioinformatics: History, definition, scope and applications, Data base: mining tools; database searching, similarity search, FASTA, BLAST.

Information networks: Gene bank sequence database, EBI-net; NCBI, Genome net, Protein database.

Phylogenetic analysis; Comparative genome analysis; Microarray analysis.

**RW Old & SB** Primrose Principles of Gene manipulation Blackwell **H Lodish et al** Molecular cell biology Scientfic American **RF Weaver & PW** Hedrick Genetics WCB **S. Mitra** Genetic Engineering

J Sambroo & DW Russel Molecular cloning- Lab manual Vol. 1-3

- 1. Quantitative isolation of DNA
- 2. PCR and Agarose Gel Electrophoresis
- 3. Restriction digestion, Ligation
- 4. Primer Designing
- 5. Generating Fasta Sequence from an Autoradiogram
- 6. Usage of various bioinformatic tools, software packages, web portals
- 7. Pair-wise Alignment (FASTA/BLAST)
- 8. Multiple Sequence Alignement (Clustal W)
- 9. Phylogenetic Tree Construction and Phylogenetic Analysis
- 10. Protein Structure Prediction
- 11. Amplification of viral/bacterial DNA

## Paper 4.3: Bioactive Marine Natural products

## Learning objectives:

To understand the general principles and techniques of extraction, separation, characterization of Bioactive compounds of marine origin.

To understand the sources and action of various antibiotic, anti tumour, anti inflammatory, anti viral, and anti fouling compounds of marine origin.

To understand basic principles of pharmacology including the action of drugs.

### **Outcome:**

After study of the subject the candidate should be able to extract, separate, characterize the bioactive compounds of marine origin besides conducting bioautographic and pharmacological studies .

## UNIT-I

Introduction: Significance of marine natural products.Principle and applications of<br/>colorimeter, Flame photometer; Atomicabsorptionspectrophotometer,<br/>rophotometer,<br/>Inductively Coupled plasma Spectrophotometer (ICP) in quantification of compounds.

### UNIT-II

Isolation techniques: Liquid - liquid extraction, membrane separation methods, chromatography techniques- Paper, Thin layer, Gas and liquid chromatography, HPLC, Ion-Exchange chromatography.Characterization techniques: IR, UV, NMR, Mass Spectroscopy.

#### UNIT-III

Typesofimportantproducts:Antibiotic,anti-tumour,tumour-promotor, anti-inflammatory, analgesic, cytotoxic, anti-viral, anti-fouling compounds of marineorigin.

#### UNIT-IV

Marinetoxins:Saxitoxin,brevitoxinandciguatoxin.Marinepeptides&alkaloids:pyridoacridine,pyrrolocridineindole,pyrrole,isoquinoline,alkaloids.

#### **UNIT-V**

Basic principles of pharmacology: Classification and action of antibiotics and other antimicrobials.

Green fluorescent protein from jelly fish and its applications. Pharmaceutical values and drug action.

David HA et al	Marine Biotechnology	Plenum
Scheur PJ	Marine Natural Products	Academic
DS Bhakuni DS Rawat	Bioactive marine natural produc	cts Springer& Anamaya

# Practicals:

1. Separation of amino acids by paper chromatography

2. Identification of amino acids by paper chromatography

3. Preparation of crude extract by methanol: hexane solvent from molluscs, sponges, mangrove plants

4. Separation of crude extract fractions by column chromatography

5. Separation of fractions/compounds by TLC

6. Testing of Anti-bacterial activity by crude extract

7. Bioautography testing