

Kurdistan Region Government- Iraq

Ministry of High Education

University of Salahaddin

College of Agricultural Engineering Sciences

Fish Resources and Aquatic Animals Department

Forth Stage

(Fish Breeding and Biotechnology)

**Ibrahim R. Ibrahim
2022- 2023**

Course title in English: **Fish Genetics, Breeding and Biotechnology**

Mode of completion and number of credits: **Exam**
(3 credits)

Mode of delivery/Timetabled classes: full-time, 2 (hours of lectures per week (theory) /3 hours of practic per week)

Language of instruction: English

Level of course: Bachelor

Semester: 2019 – 2020

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(Fish Breeding and Biotechnology)

Objectives:

- To understand some techniques in biotechnology.
- Using modern biotechnology in fish, animal and plant science.
- To understand the basic principles of genetics and breeding and their application to fisheries management and aquaculture.
- Based on our knowledge of genetics and molecular genetics to acquaint students with traditional and modern breeding methods and procedures point to the potential use of genetic varieties praxi. Second part of the course focuses on using the latest molecular techniques and procedures in practical fish breeding.

The aim of this course is to teach students; fish breeding techniques, innovations in fish breeding, monitoring the reflection of studies related to the fish breeding to our country's fishery, biotechnological developments in fish.

Fish Biotechnology Lecture Titles:

-Polymerase Chain Reaction “PCR”

Types of PCR:-

Gel electrophoresis:-

RESTRICTION ENZYMES

Biotechnology and its application to aquaculture:

Genetic modification, polyploidy and surrogate broodstock technology

SDS-PAGE

Western Blotting Analysis

Suggested Readings

1. Principles of Genetics:2nd Ed. Snustad & Simmons;
2. Genetics for Fish Hatchery Managers: 2nd Ed. Douglas Tave;
3. Genetic bases of fish selection: V.S.Kirpichnikov;
4. Fish genetics and Biotechnology: W.S.Lakra;
5. Genetics and Fish Breeding: C.E.Purdom;
6. Practical Genetics for Aquaculture: C. Greglutz
7. Theory and Problems of Genetics: William D. Stansfield
8. Genetics and Analysis of Quantitative Traits: Lynch M, Walsh B.
9. Gene transfer to animal cell by R.M. Twyman, 2005
10. Animal Biotechnology M.M. Ranga, Agrobios Publishers, India, 2003
11. Animal cloning by Joseph Pano, 2005

Journals

1. Journal of Animal Breed and Genetics
2. Journal of Genetics
3. Journal of Heredity

Learning Outcomes

1	To be able to evaluate the sterile fish production and its commercial benefit
2	To be able to define the genetic structure of natural population
3	To be able to develop ideas about increasing product quality in aquaculture by planning fish breeding applications.
4	To be able to provide reduction of production cost by using genetics features.
5	To be able to practice different techniques, that can be used for some advantages on production, by the features related with heredity and kinship.
6	To be able to use the advantages of molecular techniques which are gained to reach the desired goal
7	To be able to select suitable biotechnological methods while producing the breeding programs.
8	To be able to design related with fish breeding
9	To be able to correlate with the sex conversion and economical advantages on some commercial species
10	To be able to select the suitable breeding technique
11	To be able to practice the control of the fish physiology
12	To be able to provide selection ability on some techniques about the genetics sex control.

Course Contents

The importance of fish breeding in aquaculture, population genetics and variation, basic statistical concepts in fish breeding, selection and the methods which are used in fish farming, inbreeding aquaculture, heritability, hybridization, designing fish breeding programs, biotechnological applications.

Week	Theoretical	Practice	Laboratory
1	The importance of the fish breeding in aquaculture		Fish breeding practice samples
2	Basic genetic -Population genetics and genetic variation. Environmental variance, genetic and environmental interactions		Practices about Mendel heredity -Problem solving about the calculation of allele frequencies, Examples of the biometric calculation about qualitative and quantitative characteristics
3	Fish marking and tagging		Pit tag and VI Alpha application
5	Aquaculture methods by selection		Selection index practices
6	Heritability: quantitative inheritance		Calculation of heritability
7	Inbreeding aquaculture, hybridization, determining heterosis degrees		-Calculation of inbreeding aquaculture coefficient -Presentation artificial insemination and cryopreservation practices
8	Maturation and the external stimulation methods in spawning		Presentation related with the various methods which are used in practice
9	Modern bio-technology and aquaculture -Designing of breeding programs		Recognition of DNA markers and laboratory investigations -Problem solving about the estimation of breeding value
10	Sex control in fish farming		Dissection practices with the fish
11	Control of the genetic sex		sterilization of the inland water fish
12	Production of sterile fish		Examination of histological sections
13	Chromosomal genetic and chromosome manipulations		Examples of applications practiced on various fish species
14	Exam		

01. AGRICULTURAL BIOTECHNOLOGY

Unit 1: Cell Structure and Function

Prokaryotic and eukaryotic cell architecture, Cell wall, plasma membrane, Structure

and function of cell organelles: vacuoles, mitochondria, plastids, golgi apparatus, ER,

peroxisomes, glyoxisomes. Cell division, regulation of cell cycle, Protein secretion and

targeting, Cell division, growth and differentiation.

Unit 2: Biomolecules and Metabolism

Structure and function of carbohydrates, lipids, proteins and nucleic acids,

Synthesis of carbohydrate, glycolysis, HMP, Citric acid cycle and metabolic

regulation, Oxidative phosphorylation and substrate level phosphorylation, Vitamins,

plant and animal hormones. Functional molecules, antioxidants, nutrient precursor,

HSPs, anti-viral compounds.

Unit 3: Enzymology

Enzymes, structure conformation, classification, assay, isolation, purification and

characterization, catalysis specificity, mechanism of action, active site, regulation of

enzyme activity, multienzyme complexes, immobilized enzymes and protein

engineering, immobilized enzymes and their application.

Unit 4: Molecular Genetics

Concept of gene, Prokaryotes as genetic system, Prokaryotic and eukaryotic

chromosomes, methods of gene isolation and identification, Split genes, overlapping

genes and pseudo genes, Organization of prokaryotic and eukaryotic genes and

genomes including operon, exon, intron, enhancer promoter sequences and other

regulatory elements. Mutation – spontaneous, induced and site-directed,

recombination in bacteria, fungi and viruses, transformation, transduction, conjugation, transposable elements and transposition.

Unit 5: Gene Expression

Expression of genetic information, operon concept, Transcription – mechanism of transcription in prokaryotes and eukaryotes, transcription unit, regulatory sequences and enhancers, activators, repressors, co-activators, Co-repressors in prokaryotes and eukaryotes, inducible genes and promoters, Transcription factors post transcriptional modification and protein transport, DNA-protein interaction, Genetic code. Mechanism of translation and its control, post translational modifications.

Unit 6: Molecular Biology Techniques

Isolation and purification of nucleic acids. Nucleic acids hybridization: Southern, northern and western blotting hybridization. Immune response monoclonal and polyclonal antibodies and ELISA, DNA sequencing. Construction and screening of genomic and C-DNA libraries. Gel electrophoretic techniques. Polymerase chain reactor spectroscopy, rtPCR ultracentrifugation, chromatography, FISH, RIA etc.

Unit 7: Gene Cloning

Restriction enzymes and their uses. Salient features and uses of most commonly used vectors i.e. plasmids, bacteriophages, phagmids, cosmids, BACs, PACs and YACs, binary vectors, expression vectors. Gene cloning and sub-cloning strategies, chromosome walking, genetic transformation, Basis of animal cloning. Biology. Risk assessment and IPR.

Unit 8: Molecular Biology

Ribosome structure and function. Protein biosynthesis in prokaryotes and eukaryotes.

Post-translational modification. Gene regulation, RNA processing and Post transcriptional modifications. Bioprospecting, biofortification, gene pyrimiding and gene fusion, nbozyme technology.

Unit 9: Plant Molecular Biology

Photoregulation and phytochrome regulation of nuclear and chloroplastic gene expression. Molecular mechanism of nitrogen fixation. Molecular

biology of various stresses, viz. abiotic stresses like drought, salt, heavy metals and temperature; and biotic stresses like bacterial, fungal and viral diseases. Signal transduction and its molecular basis, molecular mechanism of plant hormone action mitochondrial control of fertility, structure, organization and regulation of nuclear gene concerning storage proteins and starch synthesis.

Unit 10: Tissue Culture

Basic techniques in cell culture and somatic cell genetics. Regulation of cell cycle and cell division.. Clonal propagation. Concept of cellular totipotency. Anther culture, somaclonal and gametoclonal variations. Hybrid embryo culture and embryo rescue, somatic hybridization and cybridization. Application of tissue culture in crop improvement. Secondary metabolite production. In vitro, mutagenesis, cryopreservation and plant tissue culture repository.

Unit 11: Plant Genetic Engineering

Isolation of genes of economic importance. Gene constructs for tissue-specific expression. Different methods of gene transfer to plants, viz. direct and vectormediated.

Molecular analysis of transformants. Potential applications of plant genetic engineering for crop improvement, i.e. insect-pest resistance (insect, viral, fungal and bacterial disease resistance), abiotic stress resistance, herbicide resistance, storage protein quality, increasing shelf-life, oil quality, Current status of transgenics, biosafety norms and controlled field trials and release of transgenics (GMOs).

Unit 12: Molecular Markers and Genomics

DNA molecular markers: Principles, type and applications; restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA sequences (RAPD), Simple sequence repeats (SSR), Single nucleotide polymorphism (SNP), Structural and functional genomics, gene mapping, genome mapping, gene tagging and comparative genomics and application of genomics.

16. ANIMAL BIOTECHNOLOGY

Unit 1: Cell Biology

Prokaryotic and eukaryotic cell architecture. Molecular organization and functions of cell membrane. Organisation and functions of the cytoplasm, cell organelles, endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, lysosomes, nucleolus and subnuclear structures. Protein secretion and targeting. Intracellular digestion. Oxidative phosphorylation. Cell division. Cell growth and differentiation. Control of proliferation and self regulation. Cell motility. Cell trafficking and signaling. Apoptosis.

Unit 2: Molecular Biology

DNA replication in prokaryotic and eukaryotic cells. Structure and functions of DNA polymerases. Molecular mechanisms of DNA repair. Synthesis and processing of different types of RNA. RNA polymerases. Protein biosynthesis. Genetics of mitochondria and plasmids. Transposons and intervening sequences. Minisatellites and macrosatellites. Molecular mechanism of spontaneous and induced mutations. Site directed mutagenesis. Recombination in bacteria, fungi and viruses. Molecular mechanism of genetic recombination, transduction, transformation and conjugation.

Unit 3: Gene Structure and Expression

Organisation of prokaryotic and eukaryotic genome. Repeated and non-repeated DNA sequences. The structure and chemical nature of the gene. Expression of genetic information, transcription – mechanism of transcription in prokaryotes and eukaryotes, transcription unit, regulatory sequences and enhancers, transcription factors, post-transcriptional modifications. DNA-protein interactions. Genetic code. Mechanism of translation and its control, post-translational modifications. Control of gene expression in prokaryotes and eukaryotes.

Unit 4: Genetic Engineering and Recombinant DNA Technology

Isolation and purification of DNA / RNA from prokaryotes / eukaryotes. Reverse transcription. Restriction endonucleases. Generation of DNA fragments, Cloning and expression vectors, plasmids, cosmids, phages, viruses (vaccinia, herpes, retrovirus and adenovirus), shuttle vectors. Cloning and expression in prokaryotic and eukaryotic hosts. DNA libraries, screening and characterization of DNA clones, transformation of bacterial and animal cells. Oligonucleotide synthesis. In situ mutagenesis. DNA amplification. Production of diagnostics and vaccines using r-DNA technology. Genetically modified foods / products. Genetic manipulation of rumen microbes. Safety aspects of genetic engineering.

Ethical issues related to use of biotechnology products. Patenting and Intellectual Property Rights.

Unit 5: Animal Tissue culture and Hybridoma Technology

Development of cell (tissue) and organ culture techniques. Nutrient requirements of mammalian cells. Media for culturing cells. Growth supplements. Primary cultures. Established cell lines. Stationary, Roller and Suspension culture techniques. Largescale production of cells using bioreactors, microcarriers and perfusion techniques.

Characterisation and maintenance of cells, karyotyping, cryopreservation and revival.

Detection of contaminants in cell cultures. Isolation and culture of lymphocytes.

Application of cell and organ cultures.

Micromanipulation of cells. Cell cloning. Cell fusion and Somatic cell hybrids.

Principles and methods of hybridoma technology. Production and characterization of

monoclonal antibodies and their application in animal health and production.

Unit 6: Embryo Transfer and Related Techniques

Induction of superovulation. Embryo collection and evaluation. Embryo splitting.

Embryo sexing. Embryo transfer. Advantages of embryo transfer in farm animals.

In vitro fertilization. Embryo cloning. Nuclear transplantation. Production of

transgenic animals and gene farming. Identification and transfer of gene influencing

production and disease resistance.

Unit 7: Molecular Biology Techniques

Quantitation of nucleic acids. Gel electrophoretic techniques. Isolation of plasmids. Production of radioisotopic and non-radioisotopic probes. Nucleic acid hybridization. In situ hybridization radioisotopic methods of biochemical analysis. Autoradiography. Blotting techniques. Nucleic acid sequencing methods. Methods of peptide synthesis. Protein purification methods. Restriction Fragment Length Polymorphism (RFLP). DNA fingerprinting. Polymerase Chain Reaction (PCR). Computer applications in molecular biology. Animal cloning and transgenic technology.

17. ANIMAL GENETICS AND BREEDING

Unit 1: Overview of Genetics

History and development of genetics. Classic researches and pioneer scientists in genetics. Mendalism and its deviations. Chromosomes and heredity. Sex in relation to chromosomes and genes. Linkage and crossing over. Artificial transmutation of genes. Penetrance and expressivity. Multiple factor inheritance. Gene modifiers. Non-chromosomal genes and their inheritance, Chromosomal aberrations. Mosaicism and chimerism.

Unit 2 : Advanced Genetics

Fine structure of chromosomes and chromosomal banding. Gene and mechanism of gene action. DNA replication. Central dogma. Protein synthesis. Genetic code and DNA cloning. Recombinant DNA technology. PCR. Gene banks. Split gene. Genetic control of hormone coordination, metabolism and metabolic diseases. Use of biotechnological tools in improving animal productivity. Application of immunogenetics. Biochemical polymorphism. Chromosomal studies in livestock improvement programmes. Development of clones in relation to animal productivity and maintaining biodiversity. Production of transgenic animals. Gene mixing for useful functions.

Unit 3: Overview of Breeding

Brief history of domestication of livestock. Important breeds of livestock & poultry with special reference to economic characters. Evolution of genetic systems. Isolating mechanisms and origin of species / sub-species, their adaptation. Mating systems for different livestock and poultry. Genetic and phenotypic consequences and applications of inbreeding and out-breeding. Genetic basis of heterosis and its use.

Diallele and polyallele crossing. Reciprocal and reciprocal-recurrent selection. Combining ability. Developments in population and production of livestock and poultry in India. Status of Animal Genetic Resources in India.

Unit 4: Genetic Properties of Population

Population Vs individual. Inheritance and continuity of population. Effective population size. Biodiversity. Description of animal population. Value and means; Average effect of gene and gene substitution. Components of total phenotypic variance of a population. Resemblances between relatives. Concept of heritability, repeatability; & phenotypic, genetic and environmental correlations. Methods of estimation, uses, possible biases and precision of estimates.

Unit 5: Population Genetics

Gene and genotypic frequencies and factors affecting them. Hardy Weinberg Law and consequences of it. Prediction of selection response by different methods. Selection for threshold characters. Indirect selection and correlated response. Theoretical basis of change of population mean and variance on inbreeding and cross breeding. Genotype – environment interaction. Metric characters under natural selection. Quantitative trait loci and their applications. Marker-assisted selection.

Unit 6: Genetic Strategies

Purpose-wise breeding strategies for livestock and poultry under different agroclimatic zones of India. Evaluation of past genetic improvement programmes for livestock and poultry in India. Bottlenecks in implementation of livestock breeding

programmes in India. Evaluation and characterization of various indigenous breeds of livestock and poultry. Ex-situ and In-situ conservation of animal and poultry genetic resources. Development of new breeds / strains for better productivity in animals. Open nucleus breeding system in livestock improvement in India. Biotechnology and its role in improving animals and poultry production. Role of artificial insemination / frozen semen / embryo transfer / ONBS / MOET technology in animal breeding. Formulation of breeding programmes : Purpose-wise, breed-wise, regionwise for different species of livestock and poultry. Programmes for genetic improvement of non-descript livestock population of different species. Evaluation and current

recommendations of cross breeding programmes of cattle, sheep and goat in India.

Unit 7: Selection & Selection Experiments

Basis and methods of selection. Construction of selection indices. Different methods of sire evaluation. Selection differential and intensity of selection. Prediction of response. Improvement of response. Effect of selection on variance. Realised heritability. Long-term and short-term objectives of selections. Selection experiments in livestock and poultry. Role of control population in selection experiments. Selection for disease resistance and development of general and specific

disease resistant strains / breeds. Purpose based selection and breeding of domestic animals and poultry. Genetic-slippage. Estimation of genetic divergence and its implications in livestock improvement programmes. Selection for better feed conversion efficiency in meat animals and poultry.

Unit 8: Genetic Laboratory Techniques

Culturing Drosophila stock. Study of Drosophila with markers. Gene sequencing. Blood group typing. Karyotyping and chromosomal mapping. Concept of recombinant DNA techniques cloning and gene mapping. Nucleic acid hybridization.

Development of breed descriptors at molecular level for different livestock and poultry breeds. Biochemical polymorphism analyses – blood groups, transferrins, milk proteins. Collection and storage of samples for DNA fingerprinting; isolation and quantification of DNA from blood and semen; Restricted enzyme digestion of genome

DNA, Analysis and transfer of DNA from agarose electrophoresis; Nucleic acid hybridization; Analysis of DNA fingerprinting, PCR-RFLP assay. Cryogenic preservation of animal germplasm.

Unit 9 : Research Techniques for Quantitative Animal Genetics

Use of computers in handling animal breeding data. Estimation of variances and

covariances. Development of statistical models for analyses of breed data and to

quantify environmental variance. Estimation of inbreeding and relationship.

Estimation of inbreeding rate in a closed herd / flock. Estimation and interpretation

of genetic and phenotypic parameters. Development of efficient selection programmes

and procedures. Estimation of genetic gains. Designing and evaluation of breeding

strategies like reciprocal recurrent selection, diallele and polyallele crossing.

Designing field based progeny testing programmes. Development of efficient methods

and traits for genetic evaluation of males under indigenous conditions. Data bank

concept.

Unit 10 : Laboratory Animal Breeding

Laboratory animal species viz mice, rat, guinea pig, rabbit, dog and monkey – Their

chromosome numbers – genome size – major genes. Physiological, nutritional,

reproduction parameters, maintenance protocol – pedigree recording, planned

mating. Selection and Mating methods /systems- monogamous, polygamous and

others. Genetic control and monitoring-Record keeping-Ethics and legislation for

management and use of laboratory animals. Nomenclature for different strains, inbred lines (SPF line, Knockout mice, etc.) – Animal model for human disease.

Specific utility of different laboratory species for different requirements.

39. FISH GENETICS & BREEDING

Unit 1. Principles of Fish Genetics and Breeding

Mendel an inheritance, genetic variation, chromosome theory, genetic basis for

sex determination and sex linked genes in fish, Gametogenesis, linkage and

crossing over, DNA as a genetic material, gene code and protein synthesis,

DNA replication, DNA and plasmid isolation, chromosome manipulation, ploidy

induction, sex reversal, gynogenesis, natural and induced.

Unit 2. Population and Quantitative Genetics

Genetics of population, 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, 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 3: Genetic Tools for Aquaculture Application

DNA markers in stock identification: Alzymes, RFLP, RAPD, AFLP,

Microsatellites, ESTs, SNPs, Type I and II markers, mtDNA and nuclear DNA

markers, Real-time PCR and EST markers, Lab assays for markers. Next generation sequencing, Applications for species identification, hybridization, stock identification, genetic diversity and conservation, Parentage, Linkage and QLT mapping, microarray genes, karyotyping and chromosome banding. Transgenics, GMO and biosafety regulations, designer ornamental fish, transgenic containment.

Unit 4: Breeding of Fish and Shellfish

Genetic basis for Selection of fish for breeding, QLT and marker assisted selection, sire and dam evaluation, selection for threshold characters, inbreeding effects, cross breeding and hybridization, selection and mating designs for select traits, selection for disease resistance, monosex, cryopreservation of gametes. Endocrine control of reproduction in fish and shellfish, synchronization of spawning , broodstock development and management, use of happa, care of fertilized eggs, assessing stripping, induced normality and mortality. Live feed development for larvae, larval feeding and maintenance, packaging and transport of shrimp post larvae, fish and fingerlings, eyed larvae or spat of molluscs. Nursery systems and their operation.

Unit 5: Conservation, bioinformatics

Breeding strategies for threatened species for restocking and live gene bank

(LGB), in situ and ex situ conservation, pluripotent embryonic stem (ES) cells

and germ cells, primary and established cell line cultures, cell cloning, cell

hybridization, hybridoma technology, data mining tools, submission of DNA

sequences, GenBank sequence database, Genome diagnostics, genome and

transcriptome analysis, protein information resources, EST database,

phylogenetic analysis, microarray informatics.