



PRACTICAL MANUAL OF M. Sc. ZOOLOGY

— (PGZY 105 & PGZY 110) —

A PRACTICAL APPROACH TO UNDERSTANDING ANIMAL LIFE



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(PGZY- 105 and PGZY-110)

Introduction

Welcome to the Post-Graduate Practical Program in Zoology. This manual is designed as a comprehensive roadmap for your journey through the diverse landscapes of animal life, from the microscopic intricacies of molecular pathways to the complex dynamics of evolutionary change. At the M.Sc. level, the transition from theoretical understanding to empirical investigation is vital. These practical sessions are curated to transform abstract concepts into tangible experiences, fostering the analytical mindset required of a modern zoologist.

The curriculum spans several core pillars of the biological sciences:

- **Disease Biology & Parasitology:** Understanding the delicate (and often deadly) dance between host and pathogen is crucial in an era of global health challenges. This module provides hands-on experience in identifying parasites and understanding the pathology of infectious agents.
- **Physiology & Biochemistry:** Here, the focus shifts to the "how" of life. You will explore the chemical engines driving cellular metabolism and the physiological systems that maintain homeostasis, providing a mechanistic view of survival.
- **Animal Behaviour (Ethology):** By observing and quantifying animal actions, you will learn to interpret the evolutionary strategies and neurological triggers that dictate how organisms interact with their environment.
- **Chordata and Developmental Biology:** These modules bridge the gap between form and function. By examining the structural blueprint of vertebrates and the miraculous progression from a single cell to a complex organism, you will gain a profound appreciation for biological architecture.

- **Genetics and Evolution:** These sections delve into the code of life and the history of change. Through genomic analysis and evolutionary modelling, you will trace the lineage of traits and the forces that shape biodiversity.

For students pursuing their Master's through the Open and Distance Learning mode, these practical sessions represent a critical "bridge" between independent study and professional scientific practice.

1. **Validating Theoretical Knowledge:** In a distance learning setup, most learning happens through text and digital media. The laboratory serves as a physical touchpoint where you can verify the theories you have studied, ensuring that your knowledge is grounded in reality.
2. **Skill Acquisition:** Proficiency in handling sophisticated equipment—such as microscopes, spectrophotometers, and centrifuges—is essential for any career in research, diagnostics, or academia. These sessions provide the intensive, hands-on training necessary to build technical competence.
3. **Peer Interaction and Networking:** The practical laboratory sessions offer a unique opportunity to interact with faculty and fellow learners. This collaborative environment fosters the exchange of ideas, peer-to-peer learning, and the building of a professional community that is often missing in solitary study.
4. **Developing Scientific Temperament:** Distance learners must be exceptionally disciplined. Engaging in experimental design, data collection, and result analysis refines your critical thinking and problem-solving skills, preparing you for independent research projects and dissertation work.

As you embark on these experiments, remember that the laboratory is a place of discovery. Approach each task with curiosity, precision, and a commitment to scientific integrity.

PGZY-105 (Semester- I):

(Practicals)

PGZY 101 (Disease biology and parasitology):

1. Qualitative and Quantitative Analysis of Helminth Eggs in Fecal Samples

This practical focuses on the diagnostic stage of helminths, using specific gravity to separate parasite eggs from organic debris.

- **Objective:** To detect and identify helminth eggs (trematodes, cestodes, or nematodes) and estimate the worm burden (Eggs Per Gram - EPG).
- **Protocol (Salt Flotation Method):**
 1. **Sample Collection:** Collect 3–5 grams of fresh fecal matter.
 2. **Dilution:** Mix the sample with 30–40 ml of saturated salt solution (specific gravity ~1.2) in a beaker until it becomes a "soupy" consistency.
 3. **Filtration:** Strain the mixture through a fine sieve or cheese cloth into a flat-bottomed vial.
 4. **Flotation:** Fill the vial until a positive meniscus (slight bulge) forms at the top. Place a clean glass coverslip on the meniscus and let it sit undisturbed for 15–20 minutes.
 5. **Observation:** Carefully lift the coverslip and place it on a glass slide. Examine under 10x and 40x objectives to identify egg morphology.
- **Alternative (Sedimentation):** Use normal saline or tap water for heavy trematode eggs that do not float easily.

2. Differential Staining of Blood Smears for Hemoprotozoans

This experiment teaches students to identify intracellular parasites and microfilariae in host blood, a critical skill for clinical parasitology.

- **Objective:** To prepare and stain thin and thick blood films to identify *Plasmodium* (Malaria), *Trypanosoma*, or Microfilariae.
- **Protocol (Giemsa Staining):**

1. **Smear Preparation:**

- **Thin Smear:** Place a small drop of blood on one end of a slide and use a spreader at a 45° angle to create a tongue-shaped film.
 - **Thick Smear:** Place 2–3 drops and spread them in a circle (~1 cm diameter) using the corner of a slide.
2. **Fixation:** Air-dry both smears. Fix the **thin smear only** in absolute methanol for 1–5 minutes. Do not fix the thick smear (it must be de-hemoglobinized).
 3. **Staining:** Immerse the slide in a 1:20 dilution of Giemsa stain for 30 minutes.
 4. **Washing & Drying:** Gently wash with tap water and air-dry.
 5. **Microscopy:** Examine under oil immersion (100x). Identify ring stages (for *Plasmodium*) or spindle shapes with undulating membranes (for *Trypanosoma*).

3. Preparation of Whole Mounts of Helminth Parasites

This practical provides an in-depth look at the internal anatomy and taxonomic features of flatworms or roundworms.

- **Objective:** To permanently preserve and stain helminth parasites for detailed morphological study.
 - **Protocol (Borax-Carmine Staining):**
1. **Killing and Fixation:** Place live parasites in 70% alcohol or AFA (Alcohol-Formalin-Acetic acid) fixative to prevent contraction.

2. **Staining:** Immerse the specimen in Borax-Carmine stain for several hours (depending on size) until it is deeply colored.
3. **De-staining:** Transfer the parasite to acid-alcohol (70% alcohol + 1% HCl) to remove excess stain until the internal organs are clearly visible against a lighter background.
4. **Dehydration:** Pass the specimen through a series of increasing alcohol concentrations (70%, 90%, and absolute alcohol) for 15–30 minutes each.
5. **Clearing:** Place in Xylene or Clove oil until the specimen becomes transparent.
6. **Mounting:** Place on a slide with a drop of Canada Balsam or DPX and apply a coverslip.
7. **Observation:** Observe this slide under the 10X and 40X and study details.

PGZY-102 (Physiology and Biochemistry):

4. Estimation of Serum Glucose using the GOD-POD Method

This is a staple of clinical biochemistry that teaches students about enzymatic specificity and spectrophotometry.

- **Objective:** To quantitatively determine the glucose concentration in a biological sample.
- **Principle:** Glucose oxidase (GOD) oxidises glucose to gluconic acid and hydrogen peroxide (H_2O_2). Peroxidase enzyme (POD) then reacts H_2O_2 with a chromogen to form a pink-coloured complex. The intensity is proportional to the glucose concentration.
- **Protocol:**
 1. **Preparation:** Label three tubes: **Blank**, **Standard**, and **Test**.
 2. **Pipetting:**
 - **Blank:** 1000 μL Reagent.

- **Standard:** 10 μ L Glucose Standard + 1000 μ L Reagent.
 - **Test:** 10 μ L Serum/Sample + 1000 μ L Reagent.
3. **Incubation:** Mix well and incubate for 10 minutes at 37°C (or 20 mins at room temperature).
 4. **Measurement:** Set the spectrophotometer to 505 nm (green filter). Zero the machine with the Blank.
 5. **Calculation:** $\text{Glucose (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}.$

5. Effect of pH or Temperature on Salivary Amylase Activity

This experiment demonstrates how physiological conditions directly dictate enzyme kinetics.

- **Objective:** To determine the optimum pH or temperature at which salivary amylase breaks down starch.
- **Protocol (Temperature Variant):**
 1. **Enzyme Collection:** Collect saliva, dilute 1:10 with distilled water, and filter.
 2. **Setup:** Prepare 5 test tubes, each containing 2 ml of 1% starch solution and 1 ml of phosphate buffer (pH 6.8).
 3. **Incubation:** Place tubes in water baths set at different temperatures: 0°C (ice), 20°C, 37°C, 60°C, and 100°C. Let them equilibrate for 5 minutes.
 4. **Reaction:** Add 1 ml of the diluted saliva to each tube.
 5. **Sampling (Achromic Point):** Every 2 minutes, take a drop from each tube and mix with a drop of Iodine on a porcelain tile.

6. **Observation:** Record the time taken for the blue-black colour to stop appearing (the "achromic point"). The tube that reaches this point fastest indicates the optimal temperature.

6. Quantitative Estimation of Total Soluble Proteins (Lowry's Method)

This is a fundamental biochemistry technique used to quantify protein content in tissues (liver, muscle, etc.).

- **Objective:** To estimate the protein concentration in a tissue homogenate.
- **Principle:** Copper ions react with peptide bonds under alkaline conditions (Biuret reaction), followed by the reduction of the Folin-Ciocalteu reagent by tyrosine and tryptophan residues, yielding a blue colour.
- **Protocol:**
 1. **Sample Prep:** Homogenise 100mg of tissue in 5ml of buffer. Centrifuge and use the supernatant.
 2. **Reagents:** Prepare Alkaline Copper Reagent (Reagent L) and 1N Folin-Ciocalteu Reagent.
 3. **Assay:**
 - Add 0.2 ml of sample to a test tube.
 - Add 2 ml of Reagent L; mix and let stand for 10 minutes.
 - Add 0.2 ml of Folin-Ciocalteu reagent rapidly with immediate mixing.
 4. **Incubation:** Incubate in the dark for 30 minutes at room temperature.
 5. **Measurement:** Read absorbance at 660 nm.
 6. **Standard Curve:** Compare results against a standard curve prepared using Bovine Serum Albumin (BSA).

PGZY-103 (Animal behaviour & Wild life biology):

7. Assessment of Phototaxis in Earthworms or Fruit Flies (*Drosophila*)

This experiment quantifies an organism's directional movement in response to light.

- **Objective:** To determine if the specimen exhibits positive or negative phototaxis.
- **Protocol:**
 1. **Apparatus:** Use a T-maze or a long glass tube. Cover one half with black paper (Dark) and leave the other half transparent (Light).
 2. **Acclimatization:** Place 10 individuals in the center of the tube and let them settle for 2 minutes in the dark.
 3. **The Run:** Introduce a light source at one end. After 10 minutes, count how many individuals moved to the light zone vs. the dark zone.
 4. **Repetition:** Rotate the tube 180° (to control for any internal bias) and repeat the experiment.
 5. **Analysis:** Use a **Chi-square test** to determine if the preference for light or dark is statistically significant.

8. Study of Social Hierarchy and Dominance in a Cichlid Fish Group

This practical explores the "peck order" and how social status affects resource access.

- **Objective:** To identify dominant and submissive individuals based on aggressive and submissive displays.
- **Protocol:**

1. **Setup:** Place 4–5 Cichlid fish (aquarium fish) of similar size in an aquarium with one "hiding spot" (e.g., a PVC pipe or ceramic pot).
2. **Observation:** Observe the group for 30 minutes daily for 3 days.
3. **Recording:** Log "Agonistic Interactions": Chasing, nipping, or lateral displays (dominant) vs. fleeing or hiding (submissive).
4. **The Matrix:** Create a **Sociometric Matrix** where you plot "Who attacked whom."
5. **Identification:** The individual with the highest "win" ratio is the Alpha. Note if the Alpha spends the most time in the "hiding spot" (resource guarding).

9. Food Preference and Foraging Behaviour in Ants (Choice Test)

This experiment demonstrates how insects communicate and prioritize high-energy resources.

- **Objective:** To determine the "Optimal Foraging" strategy based on nutrient type (Sugar vs. Protein).
- **Protocol:**
 1. **Baiting:** Place three identical filter paper discs near an ant trail: one soaked in sugar syrup, one with crushed protein (dried shrimp/egg), and one with plain water (Control).
 2. **Scouting:** Record the time taken for the first "scout" ant to find each bait.
 3. **Recruitment:** Every 2 minutes, count the number of ants present at each disc for 20 minutes.
 4. **Trail Analysis:** Observe if the ants follow a specific "scent trail" and how they communicate the find to others (Antennation).
 5. **Graphing:** Plot a recruitment curve (Number of ants vs. Time) for all three baits.

10. Shelter-Seeking Behaviour (Thigmotaxis) in Cockroaches

This experiment demonstrates an animal's preference for physical contact/confinement, usually as a defense mechanism.

- **Objective:** To measure the strength of the "wall-following" or "corner-seeking" instinct.
- **Protocol:**
 1. **Arena:** Use a large circular or square open-field arena.
 2. **Zoning:** Mentally divide the arena into "Edge Zone" (near walls) and "Center Zone."
 3. **Procedure:** Release a cockroach in the exact center of the arena.
 4. **Timing:** Start a timer and record:
 - **Latency:** Time taken to reach the edge.
 - **Duration:** Total time spent in the Center vs. the Edge over 5 minutes.
 5. **Modification:** Add a small cardboard shelter in the center. Observe if the "shelter instinct" overrides the "wall-following" instinct.

PGZY-110 (Semester- II): (Practicals)

PGZY- 106 (Chordata):

11. Comparative Study of the Brain in Vertebrates (Fish, Frog, Reptile, Bird, Rat)

This practical allows students to visualize the evolution of the telencephalon (cerebrum) and cerebellum across chordate classes.

- **Objective:** To identify and compare the lobes of the brain and their relative development.
- **Protocol:**
 1. **Selection:** Use preserved specimens or Skeleton (Head) of Labeo (Fish), Rana (Amphibian), Calotes (Reptile), Pigeon (Bird), and Rat (Mammal).
 2. **Dissection:** Carefully remove the cranium using fine forceps and bone cutters to expose the brain *in situ*.
 3. **Observation:** Identify the Olfactory lobes, Cerebral hemispheres, Optic lobes, Cerebellum, and Medulla oblongata.
 4. **Drawing:** Draw the dorsal and ventral views of each.
 5. **Comparative Analysis:** Note the transition from a prominent Optic lobe (Fish/Bird) to a dominant Cerebral cortex (Mammal) and the complexity of the Cerebellum in flying/active animals.

12. Microscopic Study of Integumentary Derivatives (Scales, Feathers, Hair)

Chordates are defined by their skin specializations. This study focuses on the histological and morphological differences of these structures.

- **Objective:** To prepare temporary mounts and identify types of scales and feather structures.

- **Protocol:**

1. **Scales:** Collect Placoid (Shark), Cycloid (Labeo), and Ctenoid (Anabas) scales. Clean in 1% KOH, wash in water, stain with Safranin, mount in glycerine, and observe under the microscope.
2. **Feathers:** Identify the Parts (Quill, Shaft, Vane) of a Contour feather and a Down feather. Examine the barbs and barbules under a microscope to see the interlocking hook mechanism.
3. **Hair:** Examine a permanent slide of a mammalian hair transverse section (T.S.) to identify the Medulla, Cortex, and Cuticle.
4. **Identification:** Draw and label the specific growth rings on cycloid scales and the dentine spines of placoid scales.

13. Skeletal Adaptations: Study of the Synsacrum and Pygostyle in Birds

This study focuses on how the chordate axial skeleton modifies for specialized locomotion (flight).

- **Objective:** To identify the fusion of vertebrae in the avian skeleton.

- **Protocol:**

1. **Specimen:** Use a cleaned, articulated skeleton of a fowl or pigeon.
2. **The Synsacrum:** Identify the fusion of the last thoracic, all lumbar, all sacral, and the first few caudal vertebrae with the pelvic girdle. Note how this provides a rigid "fuselage" for flight.
3. **The Pygostyle:** Observe the fused terminal caudal vertebrae which support the tail feathers (rectrices).

4. **Comparison:** Compare this with the flexible vertebral column of a mammal (Rat/Rabbit) to understand the loss of flexibility in favour of aerodynamic stability.

14. Study of Accessory Respiratory Organs in Teleosts

Many chordates show unique adaptations to low-oxygen environments. This practical examines "extra" lungs/breathing structures.

- **Objective:** To dissect and observe the dendritic organs or labyrinthine organs in air-breathing fishes.
- **Protocol:**
 1. **Specimen:** Select *Clarias* (Magur), *Anabas* (Climbing Perch), or *Channa* (Snakehead).
 2. **Dissection:** Carefully lift the operculum (gill cover) and remove the bony supra branchial chamber wall.
 3. **Observation:**
 - In *Clarias*: Look for the branched, tree-like Dendritic organs above the gills.
 - In *Anabas*: Observe the highly vascularized, leaf-like Labyrinthine organ.
 4. **Functional Link:** Note how these organs allow the fish to survive out of water by utilizing atmospheric oxygen.

PGZY- 107 (Development Biology):

15. Study of Different Stages of Chick Embryogenesis

This is the gold standard for vertebrate development, allowing students to see the primitive streak, somites, and heart formation.

- **Objective:** To identify developmental landmarks in 24, 48, 72, and 96-hour chick embryos.

- **Protocol:**

1. **Incubation:** Place fertilized eggs in an incubator at 37.5°C with 60% humidity.
2. **Windowing:** Gently tap the blunt end of the egg to create a small hole. Use fine forceps to remove a circular piece of the shell.
3. **Extraction:** Carefully pour the contents into a petri dish containing warm **Tyrode's solution** or Normal Saline (0.9% NaCl).
4. **Transfer:** Using a wide-bore pipette or a paper ring, lift the blastoderm and transfer it to a watch glass.
5. **Observation:** Use a stereomicroscope to count somites (to determine the exact age) and observe heart pulsations in the 48h-72h stages.

16. Vital Staining of Amphibian Eggs (Vogt's Method)

This experiment demonstrates "fate mapping" by marking specific cells in a living embryo to track their movement during gastrulation.

- **Objective:** To observe morphogenetic movements using non-toxic vital dyes.

- **Protocol:**

1. **Preparation:** Prepare small agar blocks soaked in vital dyes like **Neutral Red** or **Janus Green B**.
2. **Staining:** Gently press the dyed agar block against a specific area (e.g., the animal pole or dorsal lip) of a frog blastula for 5–10 minutes.
3. **Rinsing:** Remove the block and rinse the embryo in Spring water or Holtfreter's solution (NaCl: 3.46 g, KCl: 0.05 g, CaCl₂: 0.1 g, NaHCO₃: 0.2 g in 1000 ml water).

4. **Tracking:** Observe the embryo over 24 hours under a microscope. Note how the colored "spot" moves inward during involution or spreads over the surface during epiboly.

17. Preparation of Whole Mounts of *Drosophila* Larvae (Imaginal Discs)

This practical links larval structures to adult anatomy, focusing on the precursors of wings, legs, and eyes.

- **Objective:** To dissect and identify imaginal discs from third-instar larvae of *Drosophila* fly.
- **Protocol:**
 1. **Selection:** Choose large, crawling 3rd instar *Drosophila* larvae.
 2. **Dissection:** Place the larva in a drop of PBS (Phosphate Buffered Saline). Hold the posterior end with one pair of forceps and pull the head forward with another.
 3. **Isolation:** The internal organs will spill out. Look for small, translucent, flattened sacs (imaginal discs) attached near the brain or trachea.
 4. **Staining:** Stain with 2% **Acetocarmine** stain for 5 minutes to highlight the folded epithelial layers.
 5. **Mounting:** Clear in 70% alcohol and mount in glycerine to observe the distinct shapes of wing vs. leg discs.

18. Skeletal Preparation (Alizarin Red Staining)

This technique is used to study the progress of ossification in developing vertebrate fetuses (usually rat or chick).

- **Objective:** To differentiate between bone (red) and cartilage (blue) in a developing foetus.
- **Protocol:**
 1. **Fixation:** Fix the specimen (e.g., 15-day-old rat foetus) in 95% Ethanol for 3 days.

2. **Maceration:** Place the specimen in 1% KOH (Potassium Hydroxide) until the soft tissues become transparent and the skeleton is visible.
3. **Staining:** Submerge in a solution of Alizarin Red S (stains calcium/bone) and Alcian Blue (stains cartilage) for 24 hours.
4. **Clearing:** Transfer through a series of Glycerine and KOH mixtures (25%, 50%, 75%) until the tissues are crystal clear.
5. **Storage:** Store the final specimen in pure Glycerine with a pinch of Thymol to prevent fungal growth.

PGZY- 108 (Genetics and Evolution):

19. Karyotyping and Ideogram Preparation (Human)

This practical introduces cytogenetics and the systematic classification of chromosomes.

- **Objective:** To arrange chromosomes based on size, centromere position, and G-banding patterns.
- **Protocol:**
 1. **Slide Preparation:** Use pre-prepared slides of human lymphocyte metaphase spreads treated with Colchicine (to arrest cells in metaphase stage).
 2. **Photography/Drawing:** Capture a clear image of the metaphase spread or draw it using a Camera Lucida.
 3. **Measurement:** Measure the length of the long arm (q) and short arm (p) of each chromosome.
 4. **Classification:** Calculate the **Centromeric Index** and categorize chromosomes as Metacentric, Sub-metacentric, Acrocentric, or Telocentric.

5. **Construction:** Cut out individual chromosomes and paste them in homologous pairs on a sheet, starting from the largest to the smallest, to create a formal **Ideogram/ Karyotype**.

20. Genetic Mapping in *Drosophila* (Three-Point Cross)

This is the fundamental method for determining the linear order and distance between genes on a chromosome.

- **Objective:** To calculate recombination frequencies and map distance (centimorgans).
- **Protocol:**
 1. **Crossing:** Cross a homozygous recessive "triple mutant" male (e.g., *yellow body*, *white eyes*, *singed bristles* OR *curly wing*, *ebony body*, *sepia eye*) with a wild-type female.
 2. **F1 Generation:** Observe the F1 phenotype (all wild-type).
 3. **Test Cross:** Cross an F1 female with a triple mutant male.
 4. **F2 Scoring:** Categorize at least 500 F₂ offspring into 8 phenotypic classes (Parental, Single Crossovers, and Double Crossovers).
 5. **Calculation:** Use the formula: $\text{Map Distance} = \frac{\text{Total Crossovers} + \text{Double Crossovers}}{\text{Total Progeny}} \times 100$.

21. Demonstration of the Hardy-Weinberg Equilibrium using Bead Models

This simulation helps students visualize how allele frequencies remain stable unless acted upon by evolutionary forces.

- **Objective:** To calculate genotypic frequencies over generations in a hypothetical population.
- **Protocol:**
 1. **Setup:** Use a container with 100 beads of two colours (e.g., 60 Red, 40 Blue). Here, $p = 0.6$ and $q = 0.4$.

2. **Random Mating:** Without looking, draw two beads at a time (representing an individual's genotype). Record if it is AA, Aa, or aa.
3. **Replacement:** Return the beads to the container, mix, and repeat for 50 "offspring."
4. **Comparison:** Compare the observed genotype counts with the expected values ($p^2 + 2pq + q^2$) using a **Chi-square test**.
5. **Evolutionary Stress:** Remove all aa (lethal) individuals and repeat for 5 generations to observe how the frequency of q decreases (Natural Selection).

22. Isolation of Genomic DNA from Animal Tissue (Liver/Blood)

This provides the "raw material" for all modern genetic and evolutionary studies (like PCR and DNA Sequencing).

- **Objective:** To extract high-purity DNA using the Phenol-Chloroform method.
- **Protocol:**
 1. **Homogenization:** Grind 100mg of tissue in **Lysis Buffer** (containing SDS to break cell membranes).
 2. **Digestion:** Add **Proteinase K** and incubate at 55°C to degrade cellular proteins and histones.
 3. **Purification:** Add an equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1). Centrifuge at 10,000 RPM.
 4. **Extraction:** Carefully pipette the top aqueous layer (containing DNA) into a new tube.
 5. **Precipitation:** Add chilled Isopropanol. A "white thread" of DNA will appear. Centrifuge, wash with 70% Ethanol, air-dry, and resuspend in TE buffer.

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