B. Sc. III, ZOOLOGY
Practical – I, II, III & IV
Syllabus Distribution & Practical Schedule

Note: All Online Practicals Platform – Google Meet

Sr. No.	Date	Time	Name of the faculty	Name of Experiment
1.	**3/7/2021**	2 to 4 pm	Dr. P. P. Yadav	**Practical – I** 12. Whole Mount of chick embryo – 18, 24, 33, 48 & 72 hours.
	3/7/2021	4 to 5.30 pm	Dr. Vijay S. Jadhav	**Practical – II** 1. Apiculture
2.	**4/7/2021**	2 to 4 pm	Dr. (Mrs.) S. M. Pawar	**Practical – IV** 12. Histology of following mammalian organs
	4/7/2021	4 to 5.30pm	Dr. P. B. Teli	**Practical – I** IV. Study of Histological structures of placenta V. Examination of Gametes
3.	**5/7/2021**	2 to 4 pm	Prof. M. H. Karrennnavar	**Practical – III** 1. Preparation of permanent histological slides by HE technique
	5/7/2021	4 to 5.30	Dr. Reshma Sanadi	**Practical – IV** b. Study of mosquito born diseases c. Study of sandfly born diseases
4.	**6/7/2021**	2 to 4 pm	Mr. Nasir Shaikh	**Practical – II** B) Immunology
	6/7/2021	4 to 5.30pm	Mrs. Punam Patil	**Practical – IV** 10. Endocrine glands (Anatomy & Histology)
5	**7/7/2021**	2 to 3.30pm	Prof. S. A. Khabade	**Practical – IV** Study of different kinds of mouth parts of insects
	7/7/2021	3.30-5.30 pm	Mrs. Asha Potalwad	**Practical** - I 1. Comparative Study of Skin, Digestive system & Respiratory system

6.	**8/7/2021**	2 to 3pm	Dr. (Miss.) P. S. Bhandare	**Practical – II** 10. Goat farming
	8/7/2021	3 to 4.30 pm	Miss. Punam Nalawade	**Practical – III** 2. Histochemical technique a. AB PH – 1 technique b. AB PH 2.5 technique
	8/7/2021	4.30 to 5.30	Dr. (Miss.) N. H. Shaikh	**Practical - I** 1.Comparative Study of Heart & Brain of Vertebrates
7.	**9/7/2021**	2 to 4 pm	Dr. Sunil Kamble	**Practical – IV** 2. Identify the zooplanktons 5. Determination of free CO_2 7. Estimation of total hardness of water
	9/7/2021	4 to 5.30 pm	Dr. M. B. Sajjan	**Practical – I** 13. T. S. of Chick Embryo-18. 24, 33, 48 & 72 hours VI. Preparation of Whole Mount Chick Embryo
8.	**10/7/2021**	2 to 3.30	Dr. (Mrs.) P. K. Waghmare	**Practical – III** c. PAS Technnique
	10/7/2021	3.30 to 5.30	Dr. Shilpa Khairmode	**Practical-III** 2. DNA Isolation 3. Demonstration of DNA by Feulgan technique
9.	**11/7/2021**	2 to 3pm	Mrs. Punam Patil	**Practical – IV** 6. Determination of alkalinity
	11/7/2021	3 to 4 pm	Dr. (Mrs.) S. M. Pawar	**Practical - IV** 4.Determination of dissolved oxygen
	11/7/2021	4 to 5.30 pm	Dr. Reshma sanadi	**Practical – II** 9. Freshwater Prawn Culture

Clubbing College Name

1. P. D. V. P. Mahavidyalaya, Tasgaon
2. Shikshanmaharshi Dr. Bapuji Salunkhe Mahavidyalaya, Miraj
3. Raje Ramrao Mahavidyalaya, Jat
4. Dattajirao Kadam Arts, Commerce & Science College, Ichalkaranji
5. Lal Bahadur Shastri College of Arts, Science & Commerce, Satara

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Jul 9, 2021

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Thank You

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2.Determination of ABO Blood group
3.Preparation
of stained blood smears to study various types of
blood cells.

Thanking you and regards

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7/12/21

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Practical – III
Molecular biology, Animal biotechnology, Biostatistics & Biotechniques

LOCALISATION OF MUCOPOLYSACCHARIDES BY PAS TECHNIQUE

AIM: Demonstration of neutral mucin in the given tissue by PAS technique

PRINCIPLE:

The periodic acid of Schiff's reaction with carbohydrates is an oxidative process where some polysaccharides react with the periodic acid producing an oxidized compound, an aldehyde. Aldehydes are revealed by the red or pink or magenta coloration due to the fixation of the colorless Schiff's fuchsin. PAS is linked to its association with diastase enzymes which are responsible for the convention of starch to maltose and sequentially to glucose. During glucose inversion, the stain appears pink which defines the intra or extracellular mucins persistence. Hematoxylin or methyl green is used to stain the nuclei.

or

Tissue or cell containing 1,2-diglycol group are converted into dialdehyde with the help an oxidizing agent which then react with Schiff's reagent to give bright magenta colour.

MATERIAL:

Fixation:

CAF: Calcium acetate formaldehyde

10% formalin.

Section: paraffin spread ribbon of Transverse sections of intestine of Rat at 5 µm.

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SOLUTIONS AND REAGENTS:

0.5% Periodic Acid Solution

0.5% Sodium metabisulphite

Schiff's reagent

Mayer's hematoxylin

Alcohol grades (30%-100%)

Xylene

DPX

PROCEDURE:

1. **Deparaffinize** the sections in **xylene for 10 min.**

2. **Hydration:** Hydrate the sections passing through **100% to water** through different grades **(100% - 30%).**

3. **Oxidation:** Put the section **0.5% of the Periodic acid** solution for oxidation for **5-10 minutes.** Rinse in 3 changes of distilled water.

4. Transfer the sections in **Schiff's reagent for 15 minutes** (Sections become light pink colour during this step).

5. **Wash in** tap water for 5 minutes (Sections immediately turn dark pink).

6.**To remove** the excess stain put the sections **in 0.5% Sodium metabisulphite** for **5 to 6 min.**

7. Counterstain **in Mayer's haematoxylin** for **1 minute**.

8.**Wash** in tap water for **5 minutes** then rinse in distilled water.

9. **Dehydrate** the sections through different alcohol grades **(30%-100%), clear and clean the slide in Xylene** mount using based mounting media **D.P.X** Cover the section with coverslip.

10.**Observe** under compound microscope.

MECHANISM :

The first reaction -periodic acid acting as an oxidising agent to oxidise the carbon-to-carbon bonds between two hydroxyl groups. This produces Schiff reactive aldehyde groups.

Second reaction- This comprises a mixture of basic fuchsin, hydrochloric acid, and sodium metabisulphite. The basic fuchsin in the mixture reacts with newly formed aldehyde groups in the tissue to produce a bright magenta colour.

Finally, when the section is rinsed in water, bound fuchsin molecules in the tissue then produce a bright magenta colour.

Haematoxylin is then typically used as a counter stain to visualise other tissue elements. When PAS is used to demonstrate fungal organisms, however, a light green counter stain is preferred.

Glycol —(Periodic acid)→ **Aldehyde**

Aldehyde —(Schiff's reagent)→ **Magenta colour.**

INTERPRETATION:

- **PAS positive material – Magenta or Purplish red colour**
- **Background – blue**

The goblet cell contains rich amount of neutral mucin in their cytoplasm. These mucins are due to periodic acid treatment get oxidised into aldehydes which react with Schiff's reagent to develop a pink magenta colour. The

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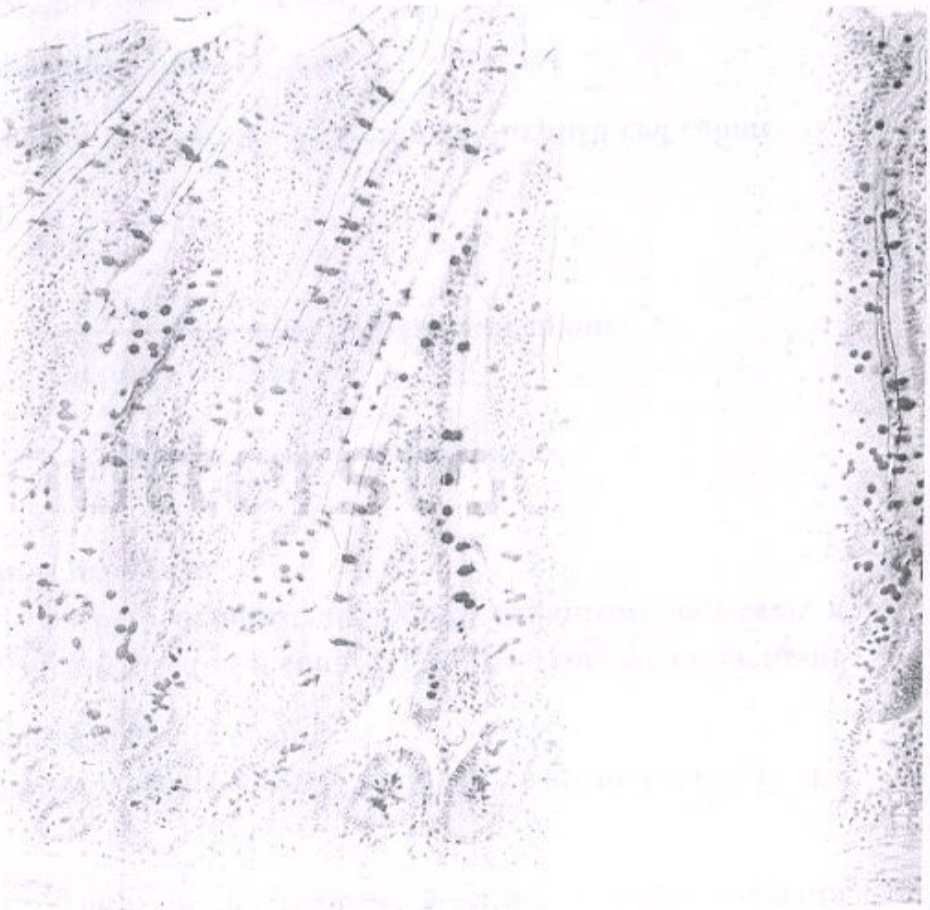
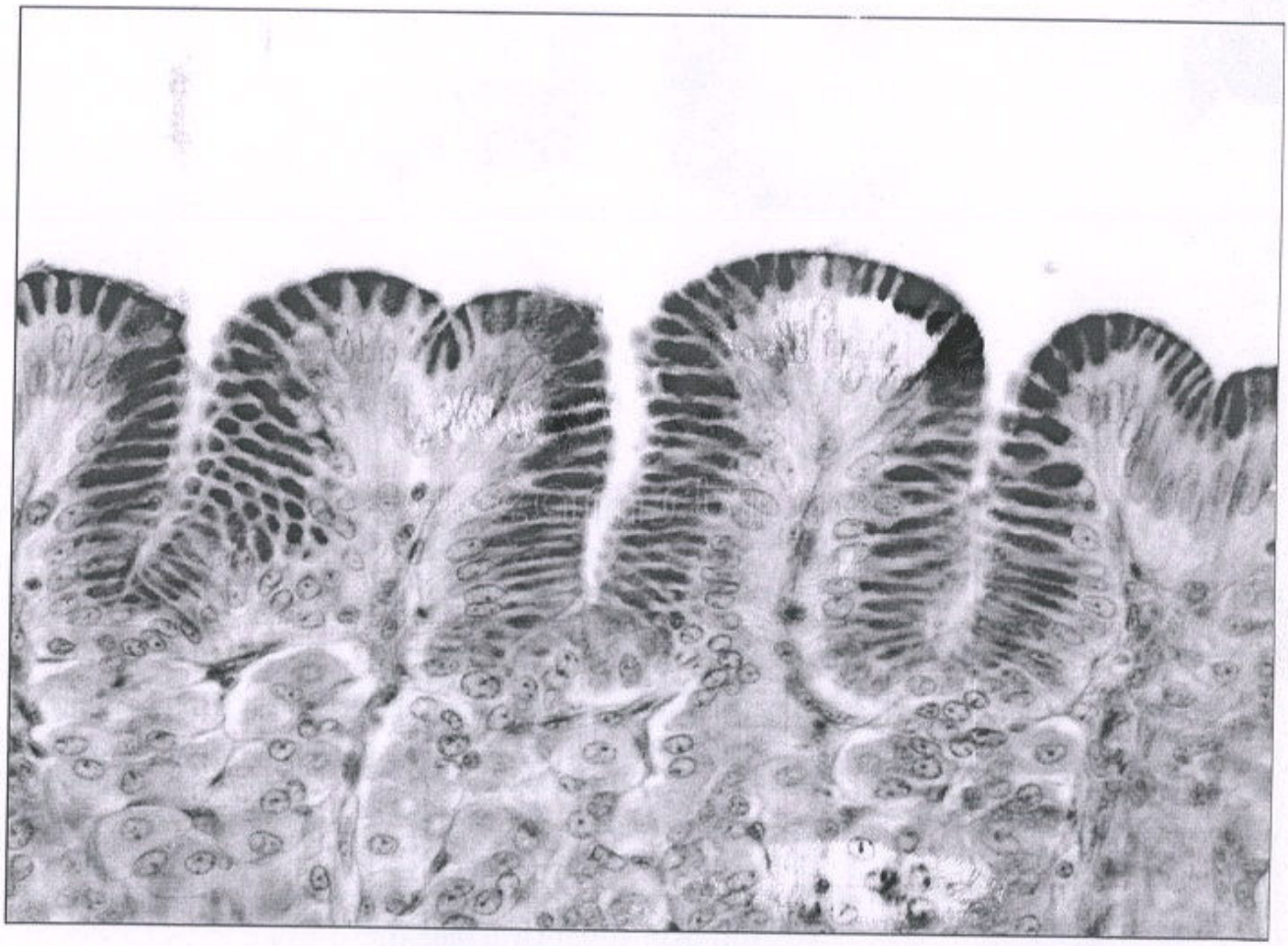


muscular coat contains glycogen which is also positive + ve to the reagent and hence the colouration.

RESULTS :

Neutral mucin(mucopolysaccharides) and Glycogen, and some basement membranes are stained in magenta colour (red/purple).

Fungi ---------- red/purple
Background ---------- blue



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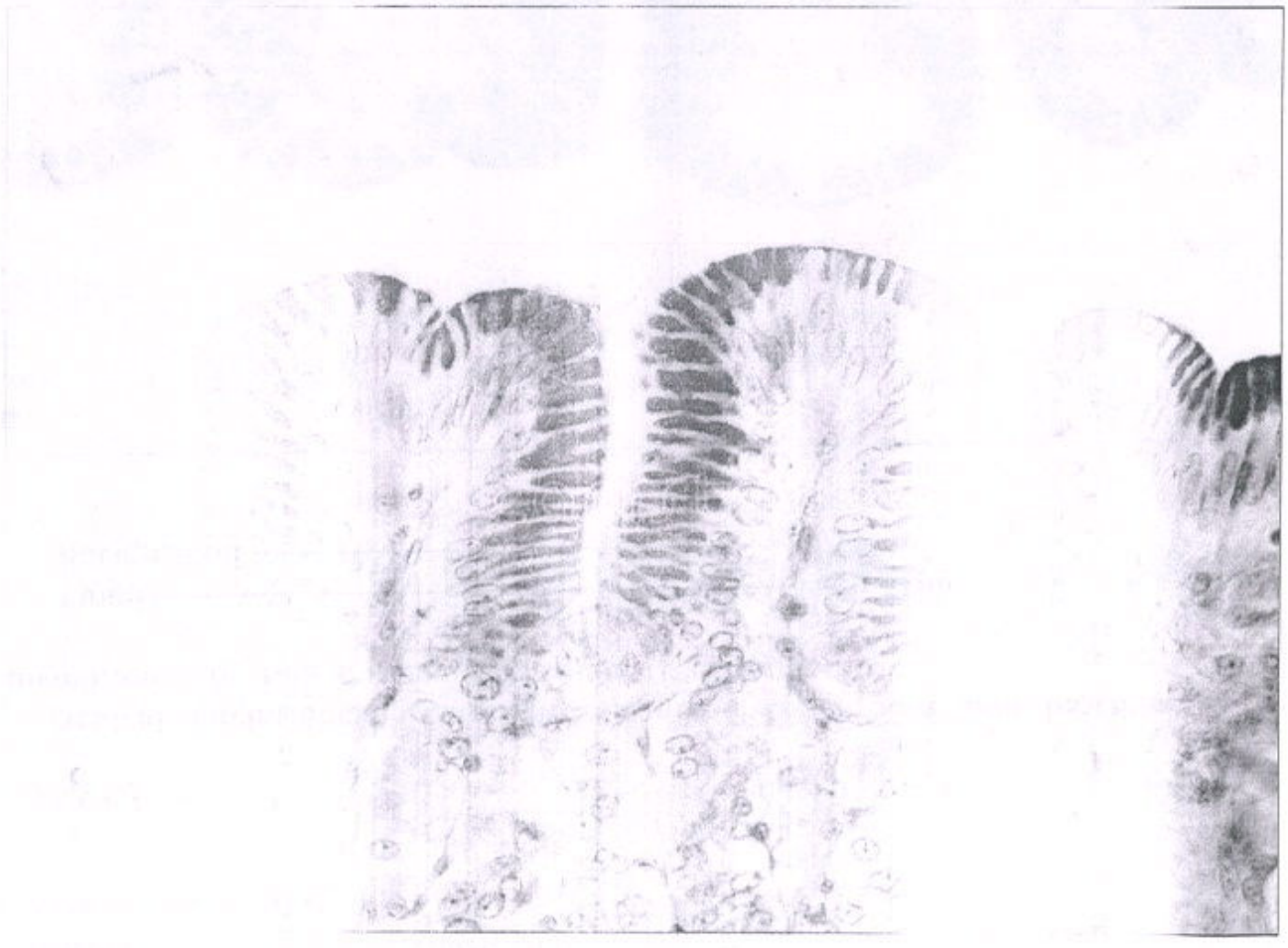

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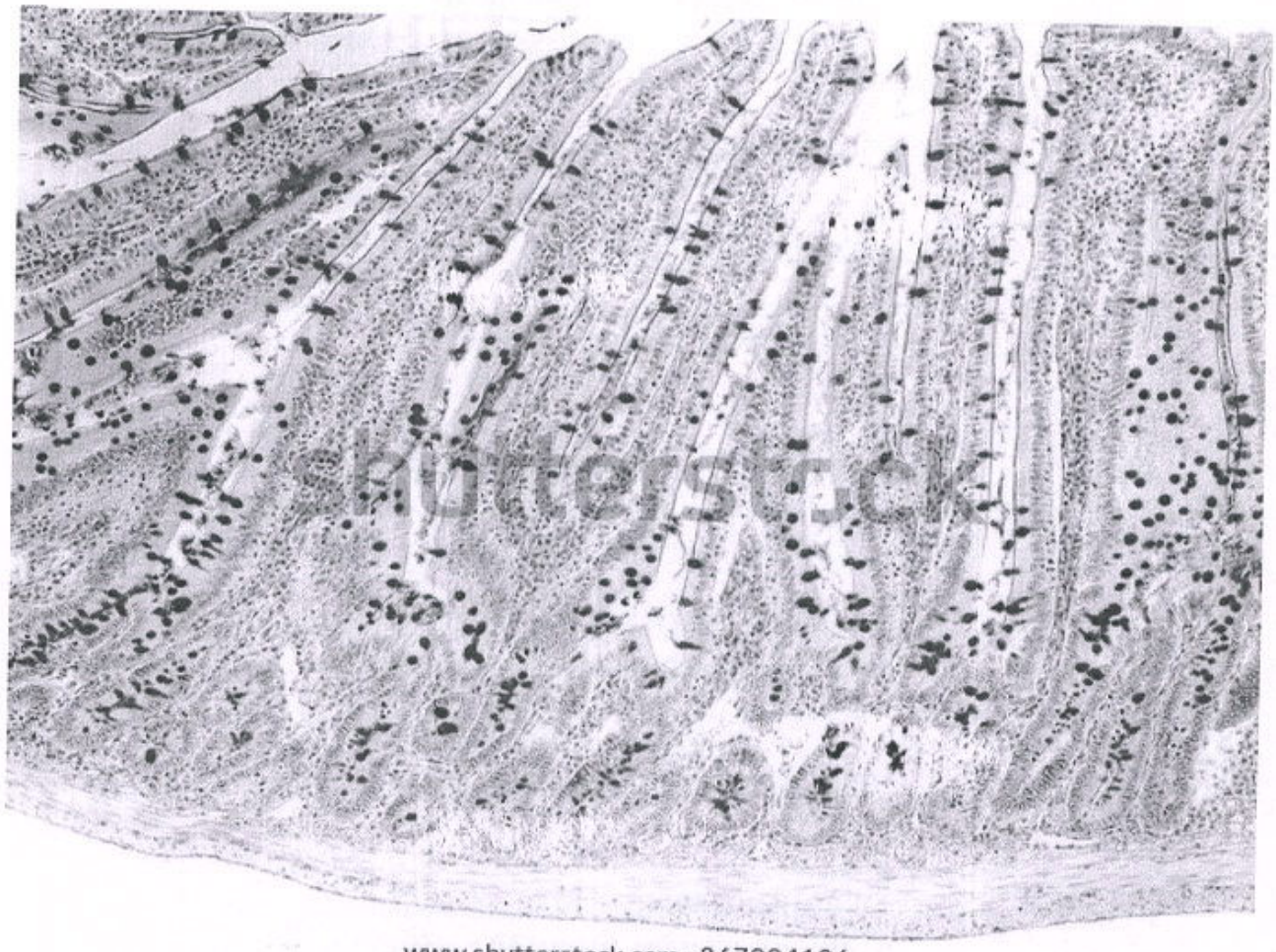

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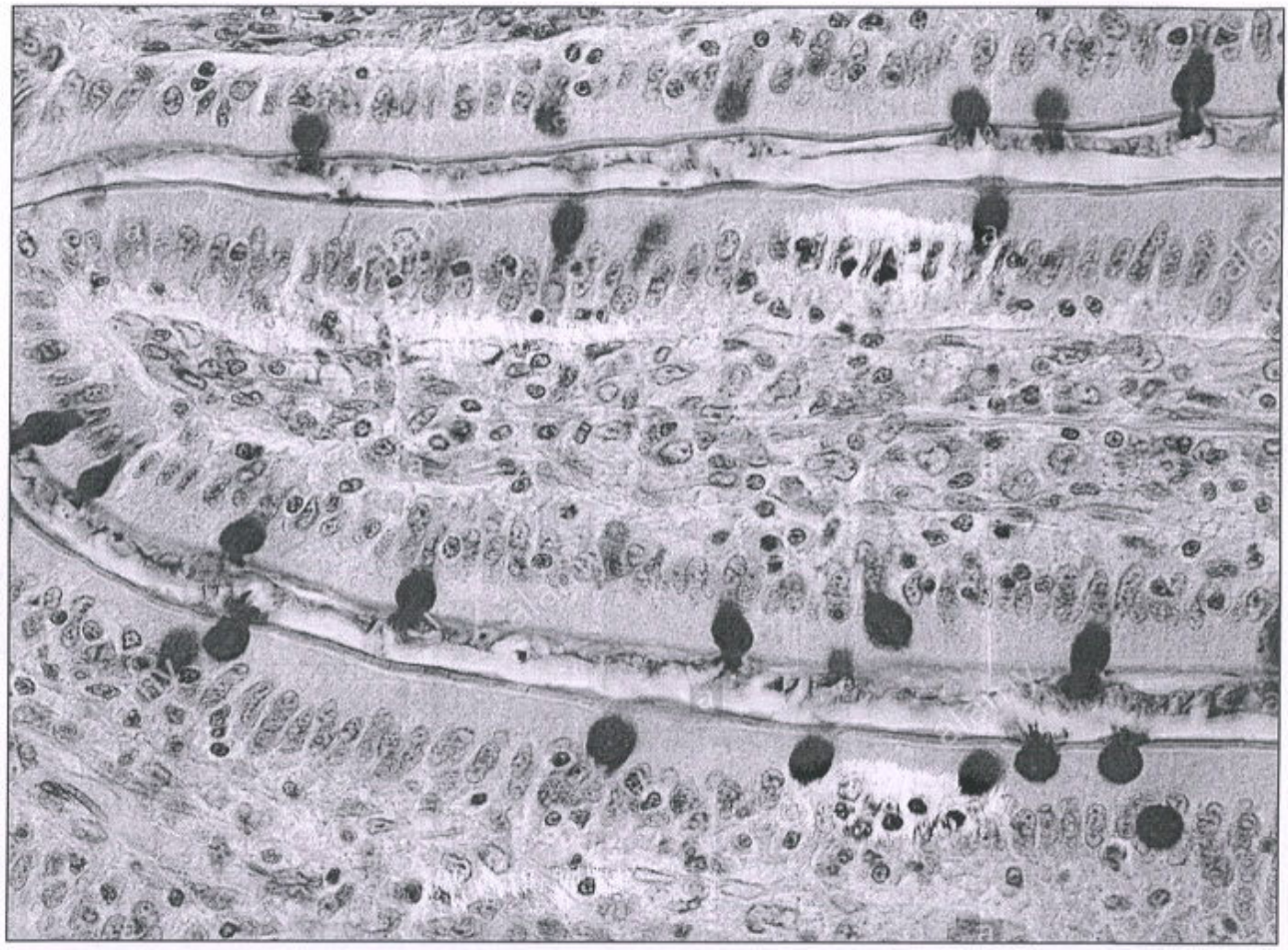


Intestinal villi stained with the Periodic Acid Schiff (PAS) staining technique. This method highlights the goblet cells as well as the brush border

FRESHWATER PRAWN CULTURE

Content

a. **Species of Prawn**
b. **Site selection**
c. **Farm Construction**
d. **Production system**
e. **Harvesting**

Introduction :

1. Freshwater prawn farming has emerged since early 1960 in world, in India1990.
2. Marine shrimp are grown in earthen ponds located in coastal areas of countries with tropical and subtropical climates.
3. The fresh water prawn attains a good size in relative less time.
4. Fresh water prawn commonly inhabits the Indian lakes, reservoirs, ponds and low salinity areas.
5. Prawn farming is a quickly expanding area.

Fresh Water Species

- Fresh water prawns of the genus Macrobrachiumare very suitable for intensiveculture.
- The main species of Macrobrachiumareas-:

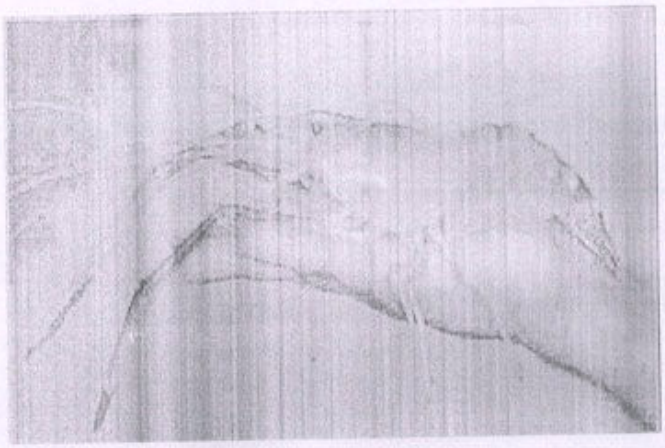
- Rivers
  - *Macrobrachium rosenbergii*
  - *Macrobrachium malcomsonii*
- Ponds
  - *Macrobrachium birmanicum*
  - *Macrobrachium rude*
  - *Macrobrachium idea*

Classification of fresh water prawn - *Macrobrachium rosenbergii*

- **Kingdom : Animalia**
- **Phylum: Arthropoda**
- **Subphylum: Crustacea**
- **Class: Malacostraca**
- Order: **Palaemonoidea**
- Family: **Palaemonoidae**
- **Genus : *Macrobrachium***
- **Species *: rosenbergii* (giant riverprawn)**

Identity and Morphology:

- The adult *Macrobrachium rosenbergii* can easily be identified from other species in the genus by the following characteristics:
- Adult male has a pair of very long legs (chelipeds).
- The rostrum is long and bent in the middle with 11–13 dorsal teeth and 8–10 ventral teeth.
- The movable finger of the leg of the adult male is covered by a dense mat of spongy fur.
- Distinct black bands on the dorsal side at the junctions of the abdominal segments.



Life cycle:

In the prawn life cycle four distinct phases comes under:

1) Egg
2) Larva (zoea)
3) Post larva (PL)
4) Adult

Adults spawn live in river, but the eggs lay in the inshore estuaries where the juveniles grow.

Embryonic development

- The egg development begins with the successful mating between ripe females and mature males.
- Incubation of the fertilized eggs takes 18–21 days, depending on the temperature (28°-30°C).
- The number of eggs carried by a female depends on her size, and varies from 3000 to 80,000.

Larval development:

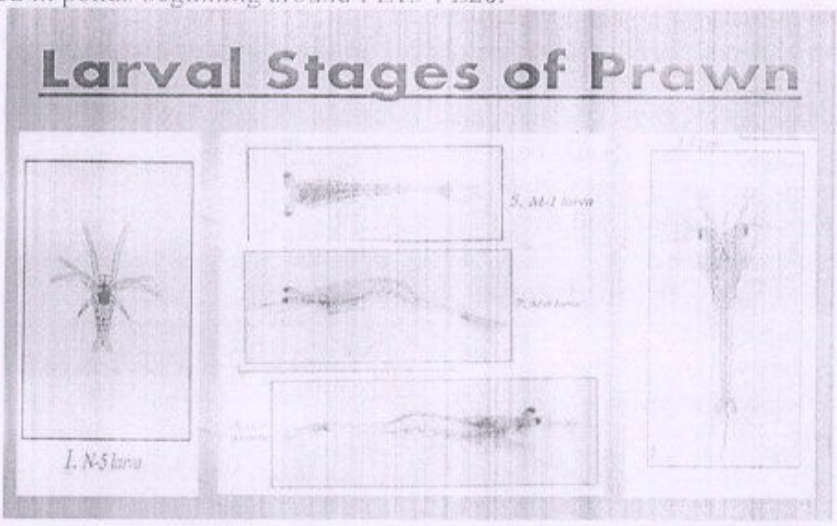
- The newly hatched larvae require brackish water within 1–2 days, or they will die.
- As the larvae moult, they not only increase in size but also increase in complexity, with new body features appearing at each stage.
- There are 11 distinct larval stages and it takes about 22–35 days for a larva to complete these 11 stages, to become a post larva (PL).
- The change from the larva form to the PL form is called metamorphosis.

Key for identification of larval stages of *Macrobrachium rosenbergii*

Larval stage	Age (days)	Characteristic feature
I	1	Sessile eyes
II	2	Stalked eyes
III	3-4	Uropods present
IV	4-6	2 dorsal teeth
V	5-8	Telson narrows and elongated
VI	7-10	Pleopod buds present
VII	11-17	Pleopods biramous
VIII	13-20	Pleopods with setae
IX	15-22	Endopods of pleopods with appendices internae
X	17-23	3-4 dorsal teeth on rostrum
XI	23-35	Teeth on half of upper dorsal margin
PL	23-35	Adult behaviour

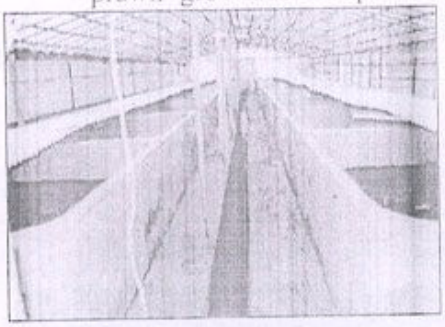
Post larva to adult

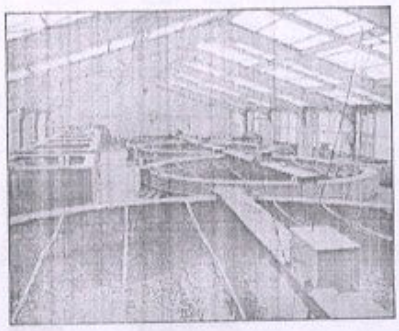
- Post larvae (PL) –
  - PL1: one day PL (0.0008g/PL1)
  - PL 20: 20 day PL (0.02g/PL20)
- Swimming seta present on pleopods.
- Reared in tanks or race ways.
- Stocked in ponds beginning around PL15-PL20.



Prawn Hatchery

- The hatchery building is usually associated with the nursery tanks and grow-out ponds in terms of water supply and other requirements.
- A freshwater prawn hatchery produces PL for growing out in ponds and for sale to other prawn grow-out enterprises.





Farming Concepts:

- Fresh Water Prawn Culture is grown in earthen ponds located in coastal areas of countries with tropical and subtropical climates.
- Ponds are filled with freshwater, Prawn is reproduced and raised in captivity are stocked into the ponds and are ready for harvest in 90 to 120 days.

Farming of Fresh Water Prawn

- Fresh Water Culture are depend upon following things:

1. Location of farm
2. Farm Permits
3. Farming Strategies
4. Pond Construction
5. Crop Species &Feeding
6. Stocking
7. Management
8. Harvesting

Farm Location

- A study of the potential market for the product and careful selection of suitable sites for prawn culture, whether it be for the larval (hatchery) or grow-out phases, is an essential prerequisite for successful farming .
- Farm constructed near of fresh water body.
- Road access, power supply, communication facilities and emergency generator are all essential components to run the equipment and operating systems in the hatchery.

Water quality

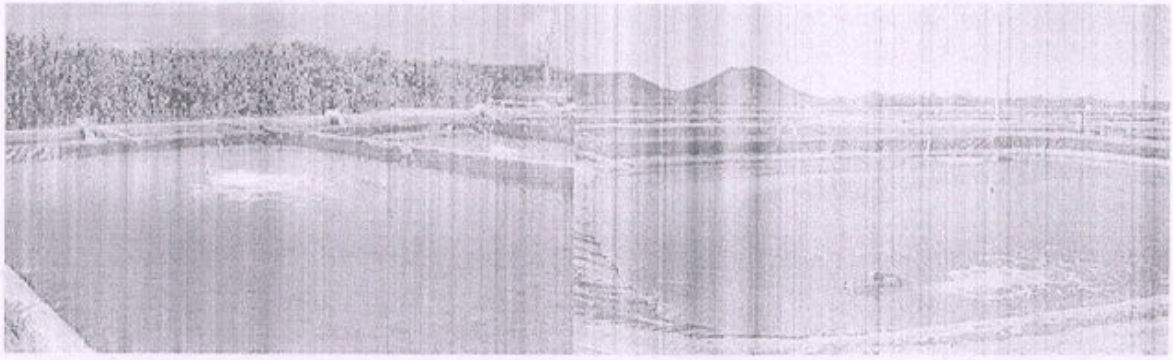
- Freshwater from a river, stream or lake, rainwater, or groundwater can be used.
- Hardness (as $CaCO_3$) should be in the range 50–100ppm.
- Seawater is needed to mix with the freshwater to produce brackish water for the larvae.
- The seawater is disinfected with 10 ppm of calcium hypochlorite and stored for at least a week before use.

Water Quality for Grow out Ponds

Temperature	≥	68F
Salinity	-	0.5-35ppm
Dissolved oxygen	≥	5ppm
pH	-	7.0-8.3
Unionized ammonia	≤	0.01ppm
Nitrite	≤	1.0ppm
Nitrate	≤	60 ppm

Pond Construction

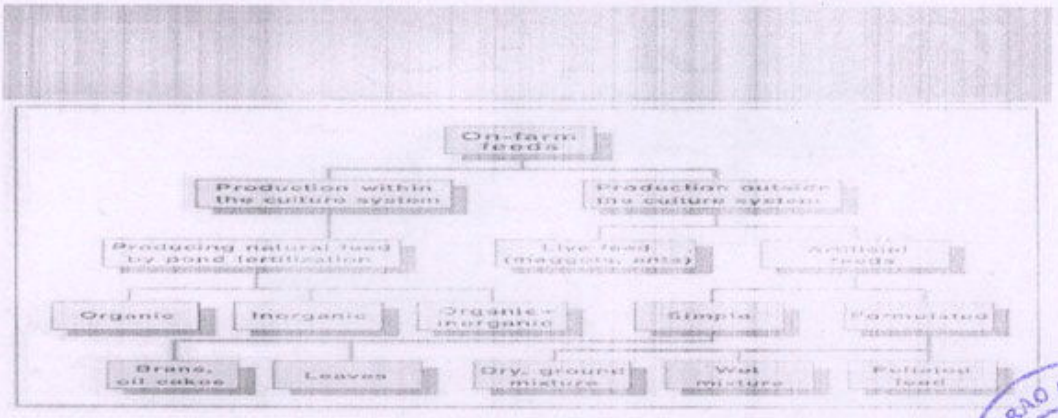
- Ponds range in size from 1-10acres
- 4-7 feet deep
- Gentle bottom slope
- Well maintained level



Levels of Pond Culture

Levels of Pond Culture

Supplements	Extensive	Semi-Intensive	Intensive	Super-Intensive
Density	0.2 – 5 /m²	5 – 20 /m²	15 – 50 /m²	50 – 200 /m²
Nutrition	Nat. Prod.	Supp. + Nat. Prod.	Feed	Feed
Aeration	None	Sometimes	Continuous	Continuous
Water Exchange Rate/day	Tidal	1-20% Evap. Loss	5-30%	50-200%



Hatchery components

Some of the basic hatchery components and equipment are:

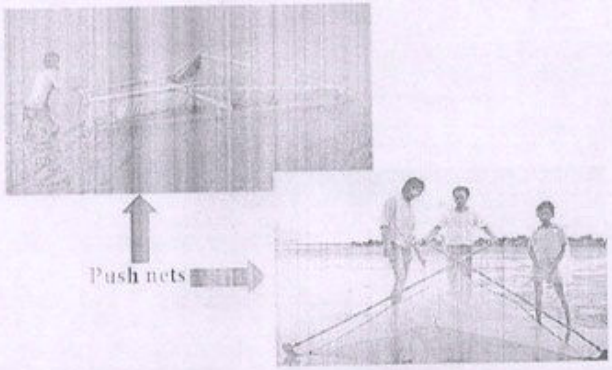
- Building to house the larval rearing space
- Hatch tanks 1000L
- Larval rearing tanks (LRTs)
- Holding tanks 1000 L for PL, also used for brood stockholding
- Nursery tank 5000 L for PL (optional)
- Freshwater storage tank
- Saltwater storage tank
- Mixed water storage tank
- Water pump management
- Plastic buckets, basins, containers
- Equipment for packing and transport of PL
- Feed and chemicals
- Ponds (200–400 m2) for rearing and maintaining adult prawns for breeding

Harvest

- Harvest in October month
- Drain and seine pond net
- Direct market or sell to processor
- Store after dry

Prawn Harvesting in River &Pond

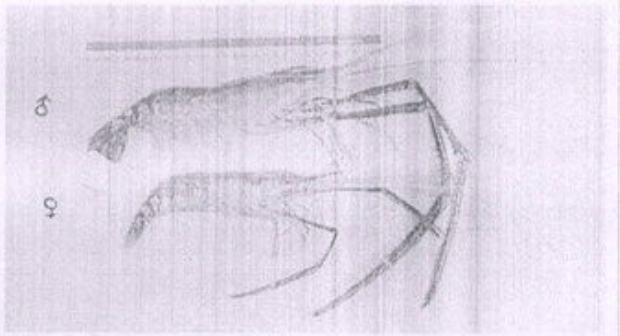
Prawn Harvesting in River & Pond



Brood stock

- The adult male and female prawns chosen for breeding are called brood stock.
- On average, 500–1000 prawns (male and female) need to be kept as brood stock.
- Berried females 10–12 cm long usually carry about 10,000–30,000 eggs each and 40 g females, 35 g males.

Fresh Water Prawn Brooder



Male and female *Macrobrachium rosenbergii* (scampi)

Fresh Water Prawn Brooder

- Growth to maturity
- The PL grow to maturity within 4–7 months in freshwater ponds.
- The PL grow to maturity is depending on temperature, food and environmental conditions.
- The maturity stages of females and male can be determined by external examination of the ovary, and testis.

Feeding brood stock

An example of a pellet feed formulated for brood stock feeding should roughly consist of:

1.	Protein	:	40%
2.	Fat	:	10%
3.	Carbohydrates	:	33%
4.	Ash	:	09%

Hatch tank management:

- Berried females ready for spawning should not be disturbed and should be kept secluded in the hatch tanks.
- Start with 500 Liter freshwater in a 1000 Liter hatch tank, and stock a maximum of 3–4 berried females.
- Keep the temperature at 25°–30°C and pH 7.0–7.3 until the eggs hatch.
- Tank water should be kept clean and free of dirt and debris through regular water changes and bottom-siphoning

Yields are:

- Extensive –500kg/ha/crop
- Semi-intensive –1,000 to 1,500 kg/ha/crop
- Intensive –10,000 to 20,000kg/ha/crop

The end.