Dietary and Hormonal Biotechnology in Advancing Gonadal Maturation for Quality Seed Production of Indian Major Carps and Catfish

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Introduction

- Aquaculture expansion
- Freshwater aquaculture
  - Indian major carps - 3 species
  - Exotic carps – 3 species
- Brackishwater aquaculture
  - Seabass culture
  - Milkfish culture
- Mariculture
  - Cobia
  - Grouper

Freshwater aquaculture (aquaplosion)

- India ranks 2nd in freshwater aquaculture after China.
- Two technologies
  - Induced breeding (Hypophysation)
  - Composite fish culture (Polyculture)

Induced breeding (Hypophysation)

- Pituitary gland extract (PGE)
- HCG
- Gonadotrophin hormone (GtH)
- Gonadotrophin-releasing hormone (GnRH)

Fish Reproduction

- **Indian major carps (*Catla catla, Labeo roghita* and *Cirrhinus mrigala*) and freshwater catfishes, *Clarias batrachus* (Magur) and Heteropneustes *fossilis* (Singhi) are cultivable species of higher consumer preference.**
- **The air-breathing catfishes do have higher haemoglobin content (11.58%) and live out of water for several hours if skin kept moist.**
- **May be cultivated in swampy and derelict water bodies unsuitable for carp culture.**
- **Attain maturity in the second year and breed once during the monsoon season.**

- **Reproductive cycle is divided into four periods:**
  - **(i) Preparatory period (February-April),**
  - **(ii) Pre-spawning period (May-June),**
  - **(iii) Spawning period (July-August) and**
  - **(iv) Post-spawning period (September-January)**

- **It has been possible to advance maturation of the fish as early as April and breed four times by photo-thermal manipulations under the laboratory conditions.**

Changes in gonadosomatic index (GSI) and oocyte diameter of *H. fossilis* during various stages of ovarian maturation

Maturity stage	Number of Individuals	Body length (cm)	Body weight (gm)	GSI	Average ova dia. (µm)
Resting phase	10	21.2	62± 3	1.22±0.46	102±8.6
Early maturing phase	10	22.3	65±4	2.14±0.57	116± 6.9
Advanced maturing phase	12	24.4	78±4	4.84±0.62	152 ±8.2
Mature phase	12	24.2	84±6	7.24±0.88	228±11.2
Spawning phase	12	24.3	82± 4	9.46±0.78	248±10.8
Spent phase	10	23.2	73± 2	4.12±0.46	109 ± 9.8

Dietary manipulations on gonadal growth of catfish

- 2+ years *H. fossilis* brood fish (50-95 g) were maintained in the earthen ponds of 0.05-0.10 ha @ 20,000-30,000 fish/ha.
- Before stocking, ponds were freed from predators and weed fishes. Bleaching powder (250-300 kg/ha/year), lime 200 kg/ha/year (adjust pH of the water), cow dung 10,000 kg/ha/year (first dose being 2,000 kg/ha).
- After stocking, ponds were fertilized with raw cow dung 10,000 kg/ha, urea 25 kg/ha and single-super-phosphate 30 kg/ha.

- **Control diet (i): groundnut oilcake: rice bran (1:1) (crude protein 26%; gross energy 3,275 kcal/kg).**
- **Semi-balanced diet (ii): fish meal 30%, groundnut oilcake 25%, soybean oilcake 20%, wheat flour 10%, rice bran 14.8%, trace mineral mixture 0.1% and vitamin mixture 0.1% (crude protein content 32.50%; gross energy 4,280 kcal/kg).**
- **Semi-balanced diet (iii): fish meal 15%, groundnut oilcake 30%, soybean oilcake 30%, rice bran 13.5%, wheat flour 10%, Supplivit 0.5%, lysine 0.5% (Lysomix) and methionine 0.5% (Methiomix) (crude protein 32.50%; gross energy 4,280 kcal/kg).**

- **Fed @ 6-8% of body weight in two split doses morning/evening (pellet 2 mm diameter).**
- **With the semi-balanced diet (ii) maturity in the catfish was observed during the second week of May while in the fish on control diet (i), maturity was recorded by end of June/early July.**
- **With the semi-balanced diet (iii), maturity could be advanced by the second week of April and the fish were bred successfully by ovaprim as well as ovatide (0.5 ml/kg body weight male; 0.8 ml/kg body weight female) administration.**

- **Fertilization in the catfish maintained on semi-balanced diet (ii) and (iii) were 65-68% and 80-85% during May and 70-75% and 90-96% in June. Hatching success on both the diets were 50-52% and 70-72% in May and 55-60% 80-85% in June.**

Dietary manipulations on gonadal growth of carps

- Indian major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) fed on semi-balanced diet supplemented with lysine and methionine (0.5%) (crude protein 28%) attained maturity by end of April and were bred successfully with ovaprim (sGnRH+Domperdone) (male 0.2 ml/kg body weight and female 0.4 ml/kg body weight) with 96% fertilization and 85% hatching success.

Hormonal manipulations in catfish

Catfish (*H. fossilis)* both the sexes administered with 25 or 50 IU HCG showed enhanced GSI from 2.09±0.26 to 12.70±0.32 in females and 0.164±0.02 to 0.841±0.04 in case of males after 4 week of the hormone treatment.

Gonadal maturity in both the sexes were observed by end of April and fishes were bred with ovaprim administration.

Quality of eggs were better in the hormone treated fish. Eggs of HCG treated fish were well formed and separated while eggs of control fish were came out in lump after more pressure on belly indicating poor egg quality.

Fertilization ranged between 80-95% and hatch-ing success 60-80% in the experimental group as compared to the control (fertilization 20-25%).

Larvae of the HCG treated fish hatched out after 18-20 hours, yolk absorption completed by 72 hours, feeding commenced on day 4 whereas larval development of the control fish was arrested after a few hours leading to mortality in majority of cases.

Even oral drops of HCG given in two doses - 25 IU and 50 IU delivered below the pharyngeal region using micropipette/syringe gave almost similar breeding responses in the catfish.

Low doses of WOVA-FH 0.1-0.2 ml/kg body weight advances maturity in *H. fossilis*.

Effects of HCG and WOVA-FH administration on gonadosomatic index and breeding responses of *Heteropneustes fossilis.*

Treatment success	Sets taken	Gonadosomatic Index (GSI)	Fertilization (%)	Hatching (%)
Control	6	4.24±0.26	20-25	10-15
HCG 25 IU	6	6.88±0.48	78-85	68-73
HCG 50IU	6	8.72±0.32	82-88	75-82
WOVA-FH	6	6.54±0.18	72-76	65-69

Hormonal manipulation in the Indian major carps

- Even low doses (0.1-0.2 ml/kg body weight) of ovaprim/ovatide given before 15 days of induced spawning improves the quality and quantity of gametes in the Indian major carps, silver carp (*Hypophthalamix molitrx)* and grass carp (*Ctenopharyngodon idella*).
- Maternal thyroxine hormone administration improves the quality of egg production in Indian major carps and catfish.
- Thyroxine and cortisol (bath treatment) enhances metamorphosis and survival in the larvae of carps and catfish.

Fig. 1: Ovary of *H. fossilis* showing immature oocytes in ovigerous lamellae. H&E. x 200.

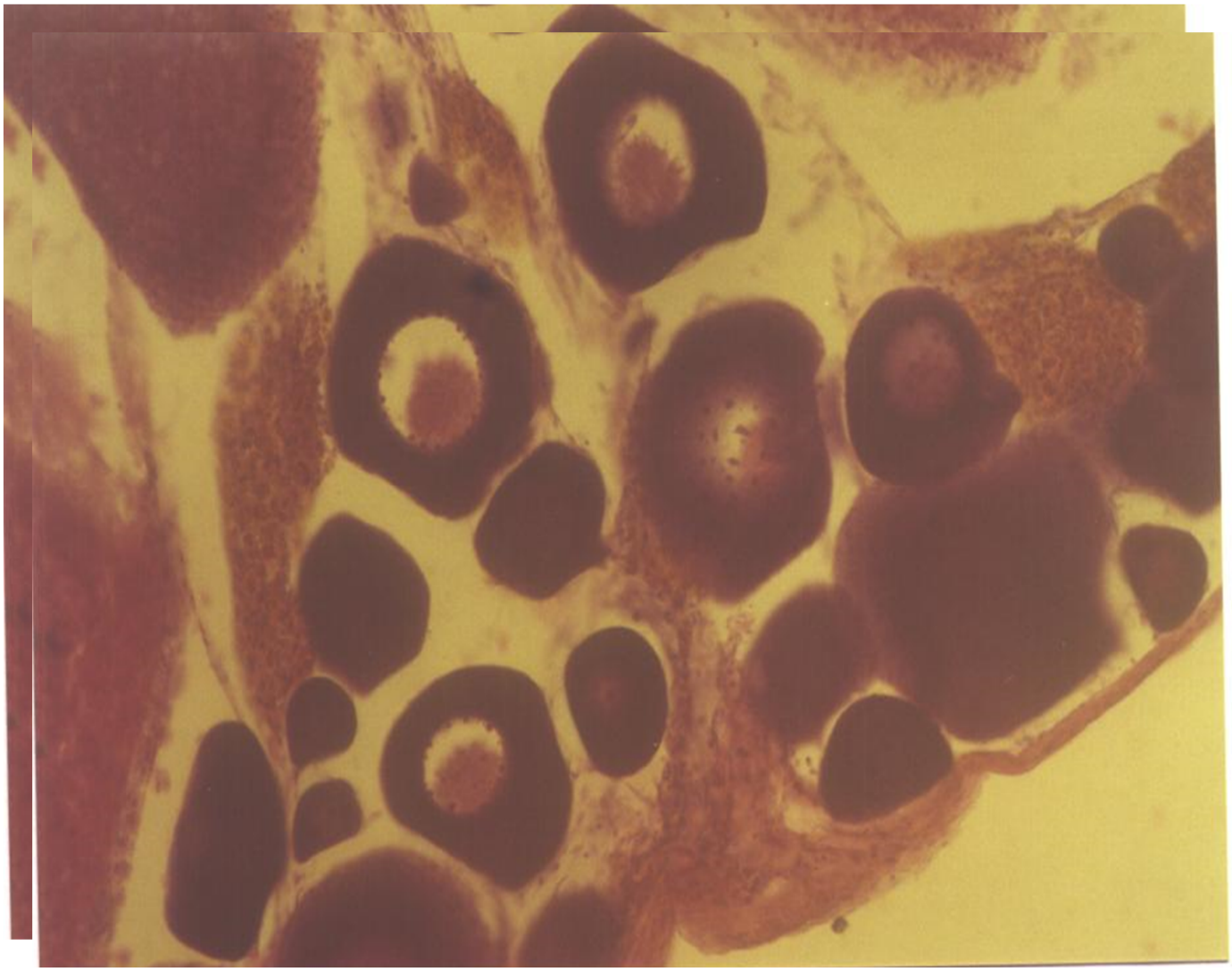


Fig. 2: Ovary of *H. fossilis* depicting immature and maturing and oocytes. Bromphenol blue. x 200

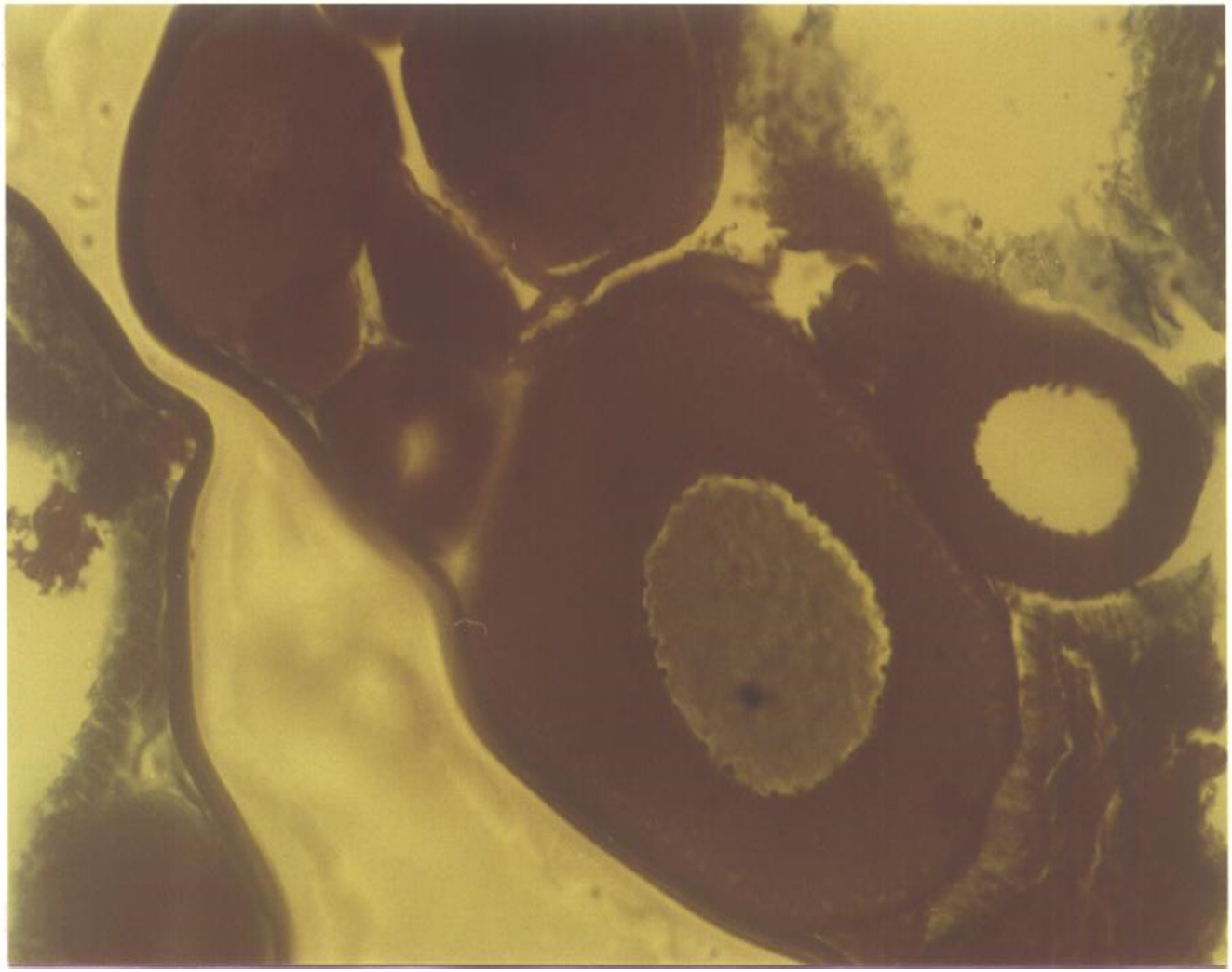


Fig. 3: Ovary of *H. fossilis* exhibiting immature and maturing oocytes. Toluidine Blue. x 200.

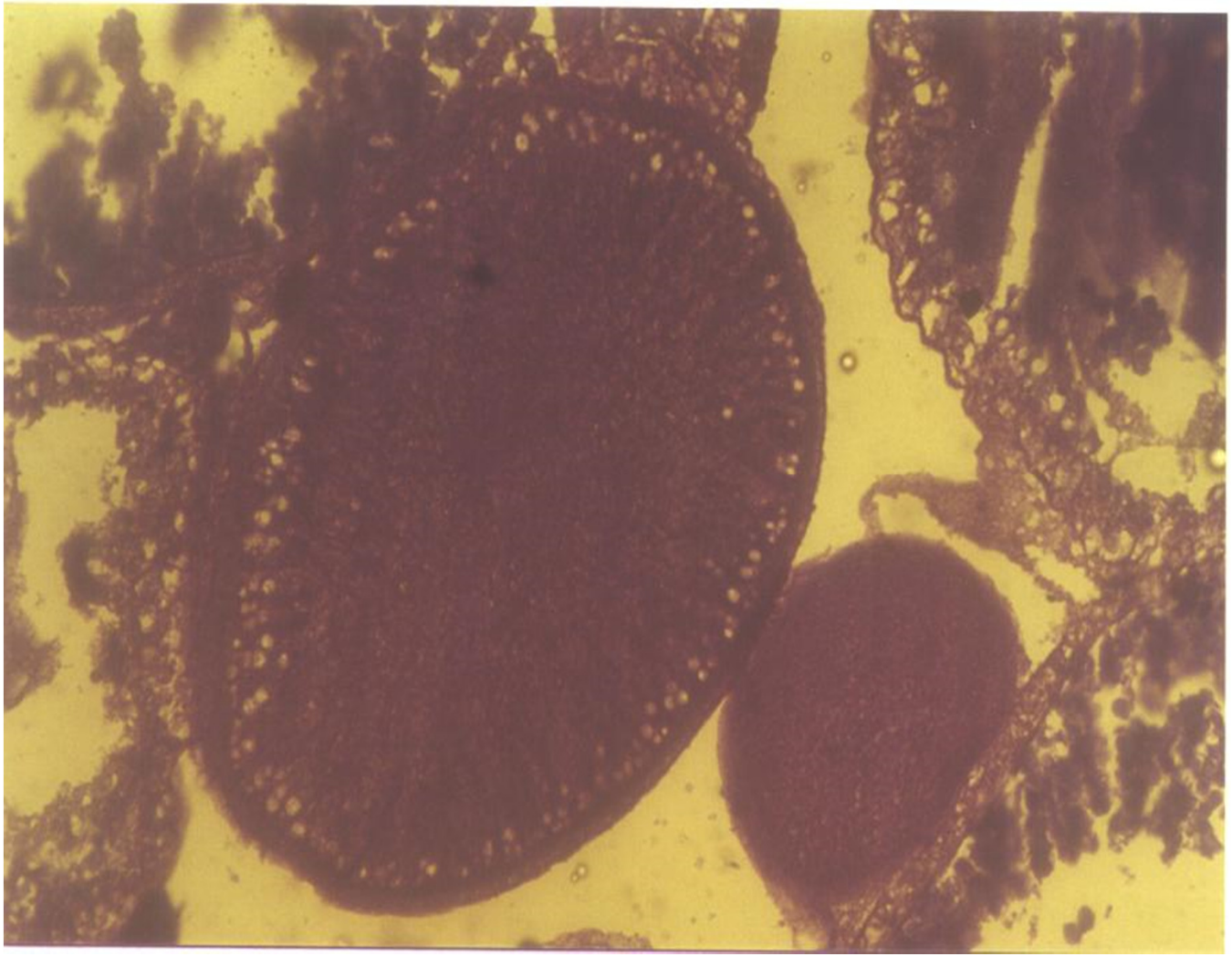


Fig. 4: Ovary of *H. fossilis* showing maturing oocytes. Also mark presence of immature oocytes in the ovigerous lamellae (arrow). H&E. x 200.

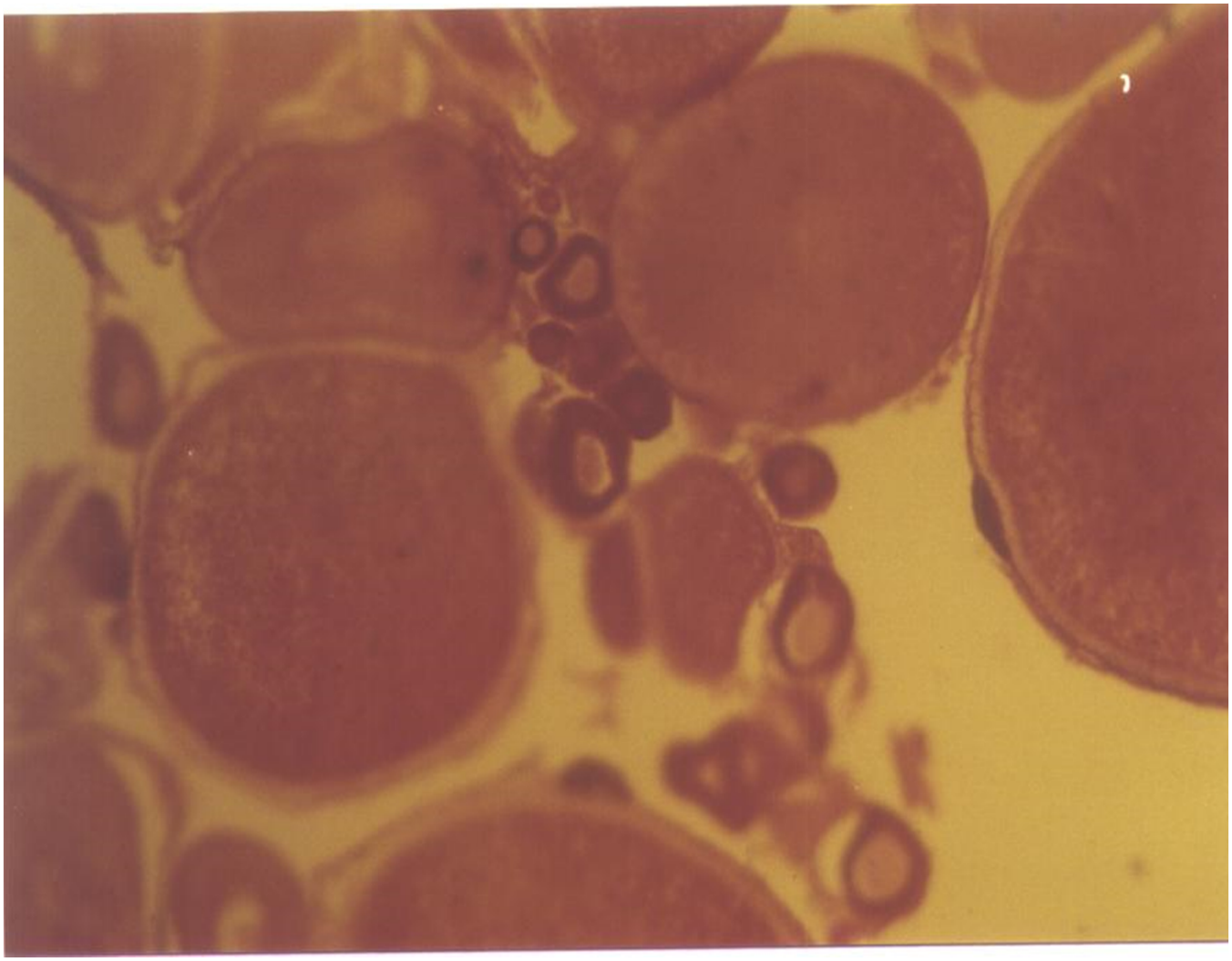


Fig. 5: Ovary of *H. fossilis* showing mature as well as maturing oocytes with oil globules and central nucleus (arrow). H&E. x 200

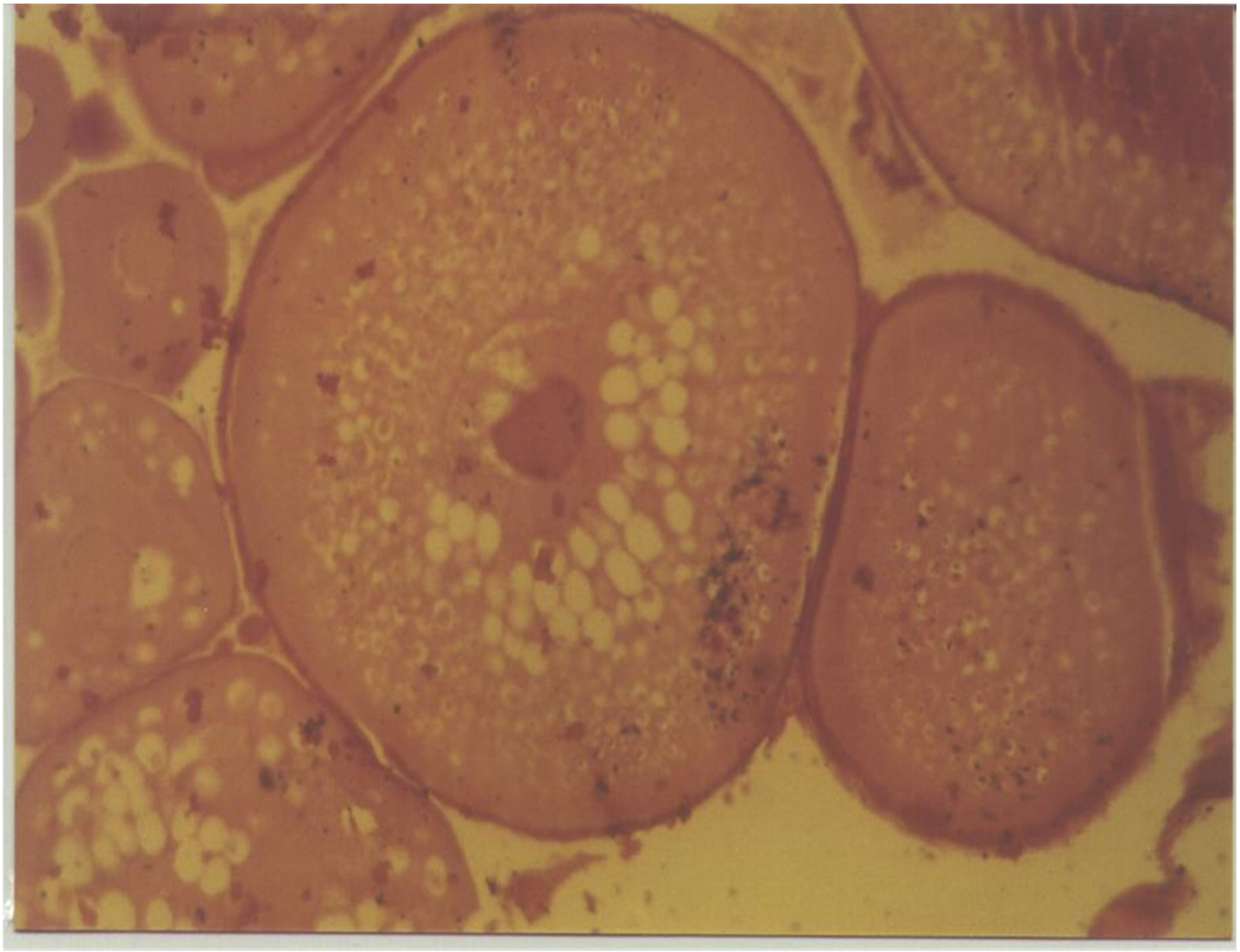


Fig. 6: Ovary of *H. fossilis* exhibiting mature oocytes with heavy deposition of yolk. An immature oocyte is also seen (arrow). H&E. x 400.

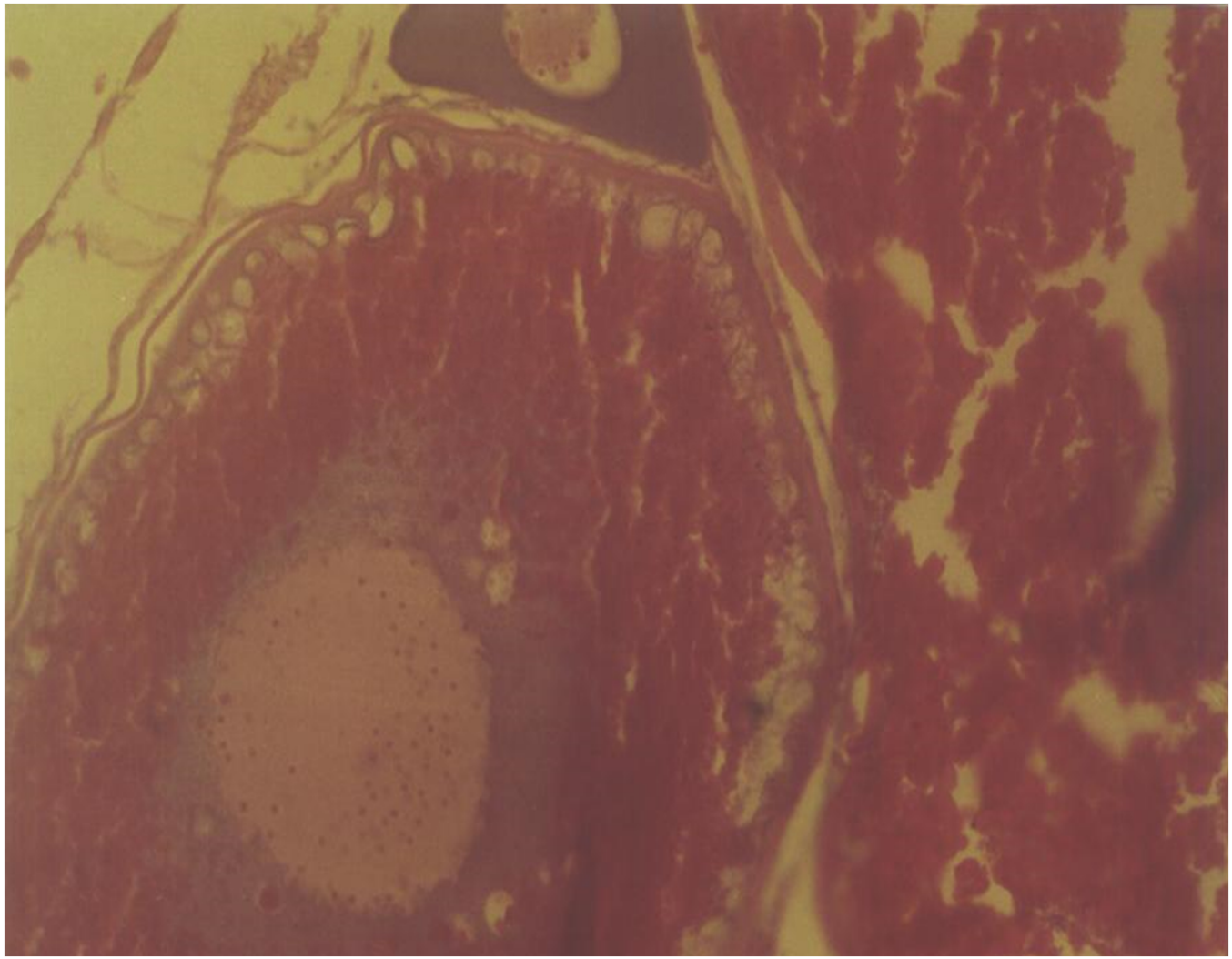


Fig. 7: PPD of maturing *H. fossilis* in May showing accumulation of secretory granules in the gonadotrophs. Mallory's triple. x 1,000.

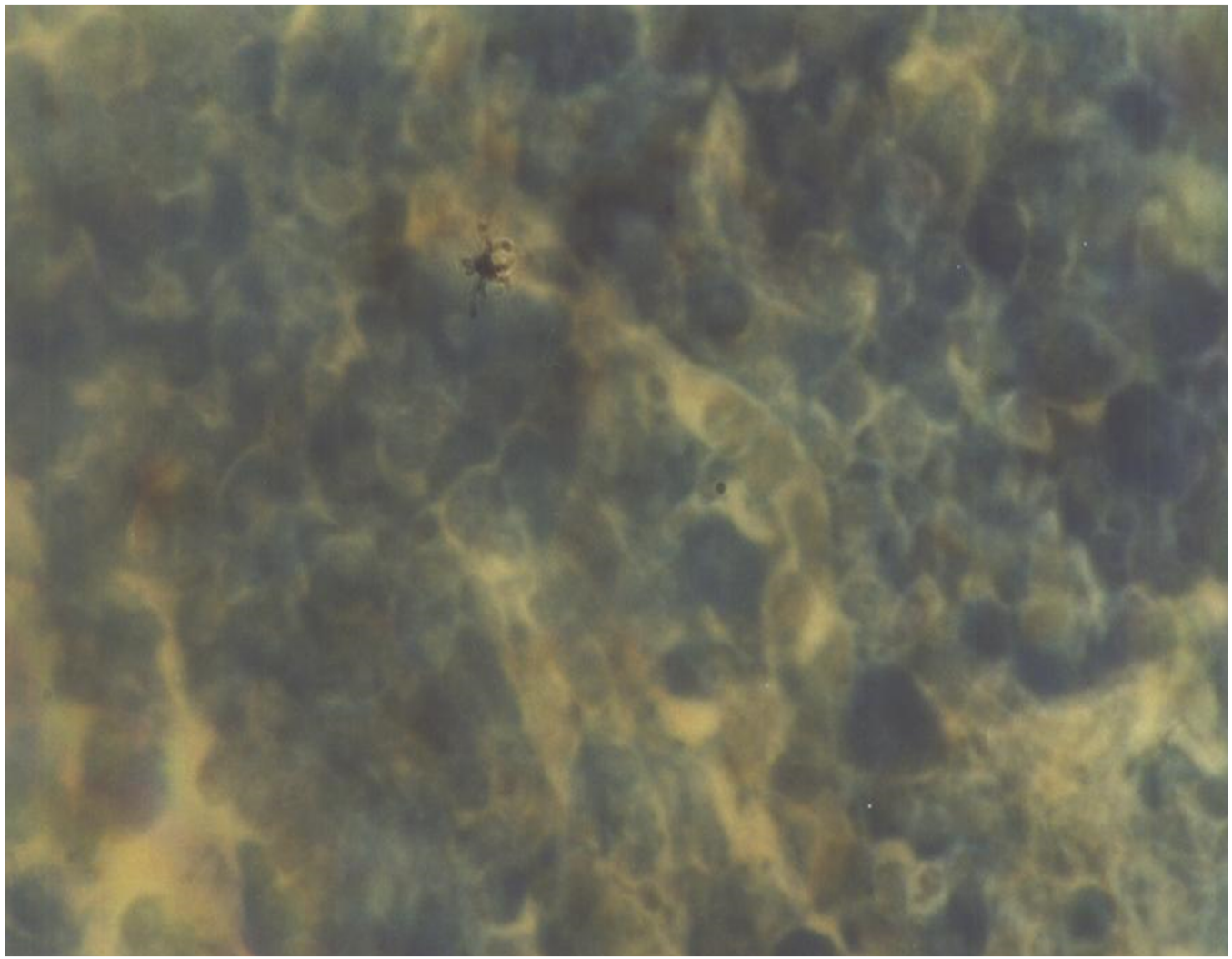


Fig. 8: PPD of female *H. fossilis* in June showing heavy accumulation of secretory granules. Mallory's triple. x 1,000

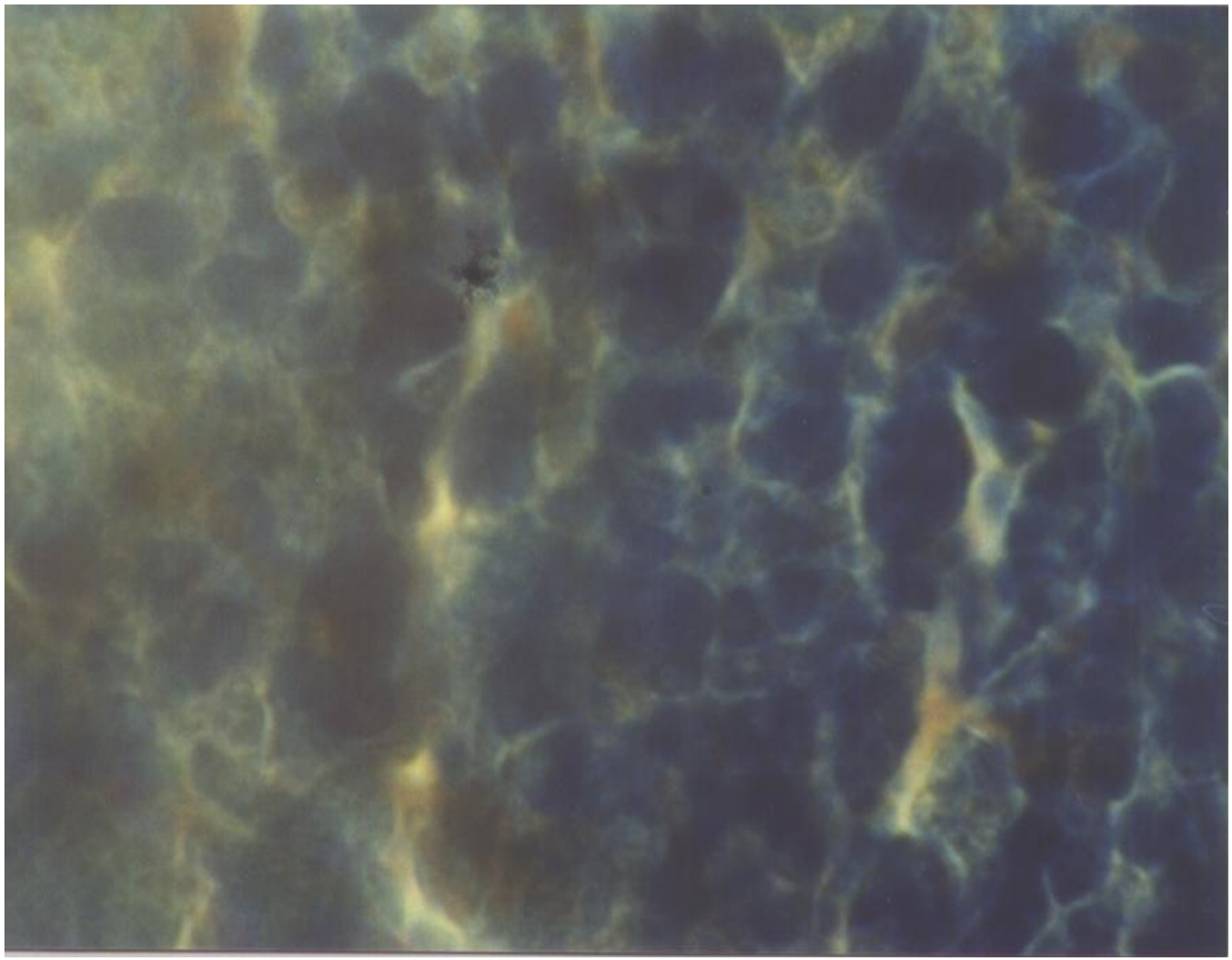


Fig. 9: Nucleus preopticus (NPO) of female *H. fossilis* in July showing vacuolization in PMC and PPC cells. Mallory's triple. x 1,000

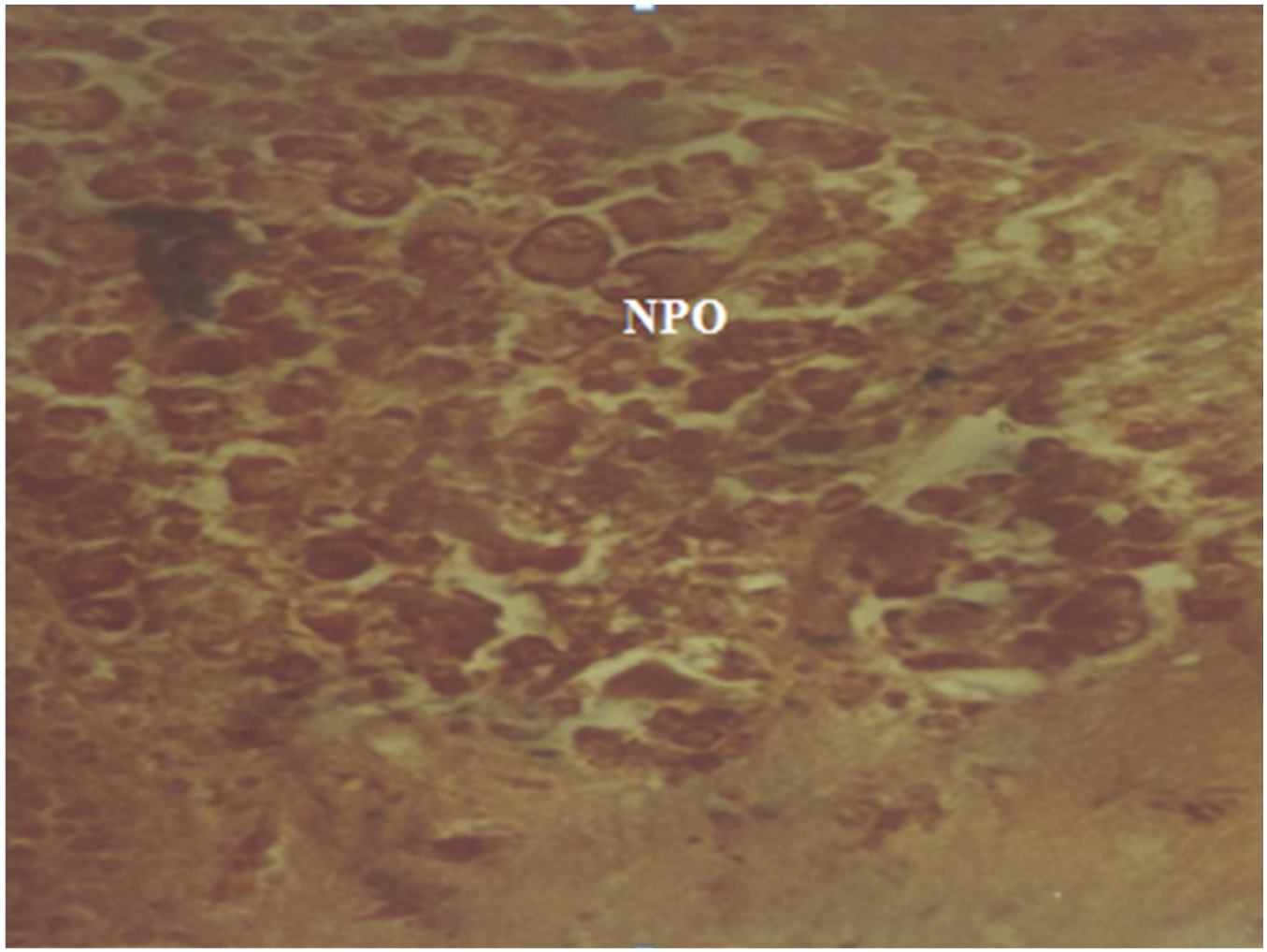


Fig. 10: Nucleus lateralis tuberis (NLT) of matured female *H. fossilis* in June depicting active neurosecretory cells. Mallory's triple. x 1,000.

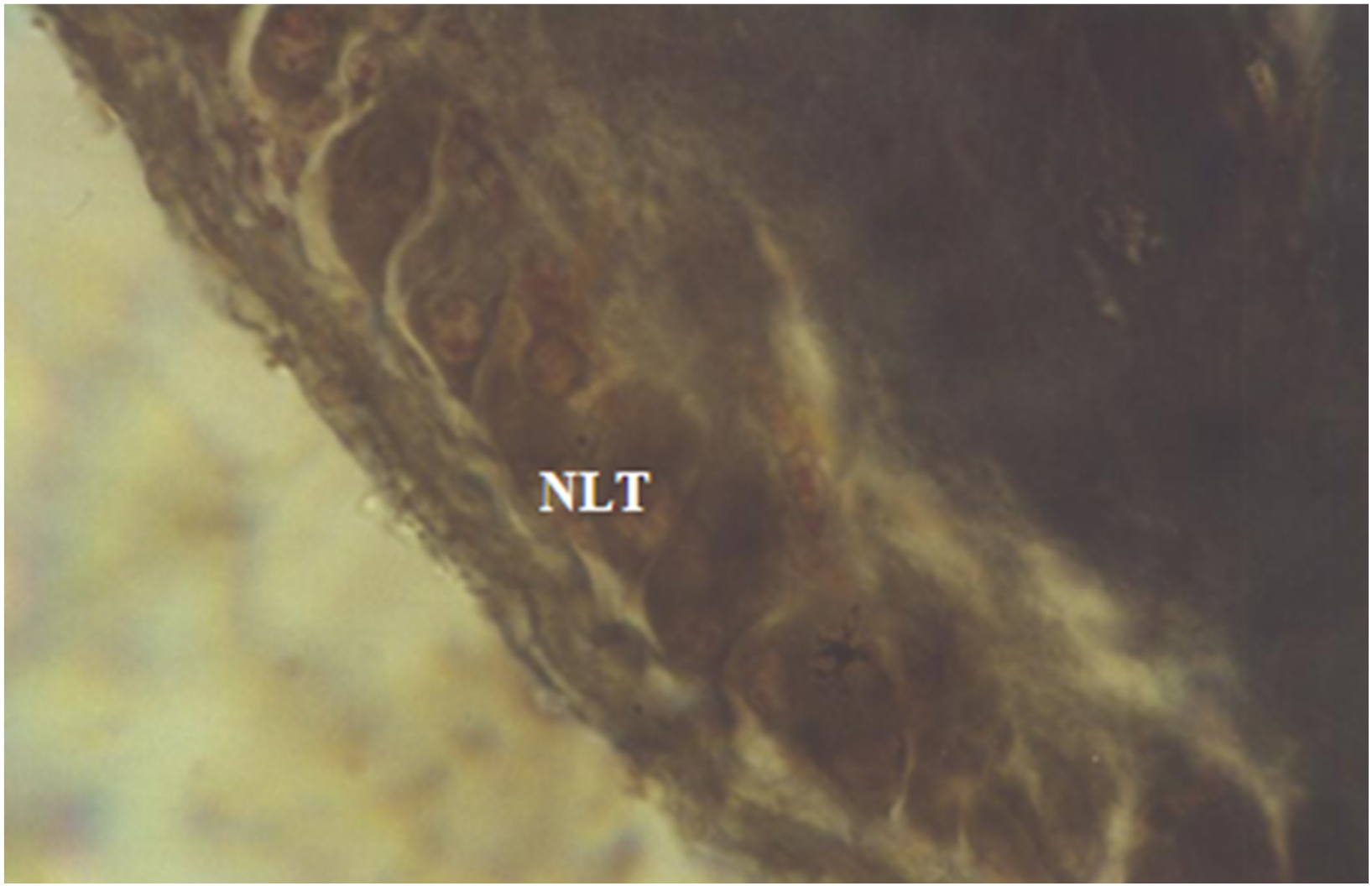


Fig. 11: Broodstock of *H. fossilis*.



Fig. 12: Broodstock of female *H. fossilis*.

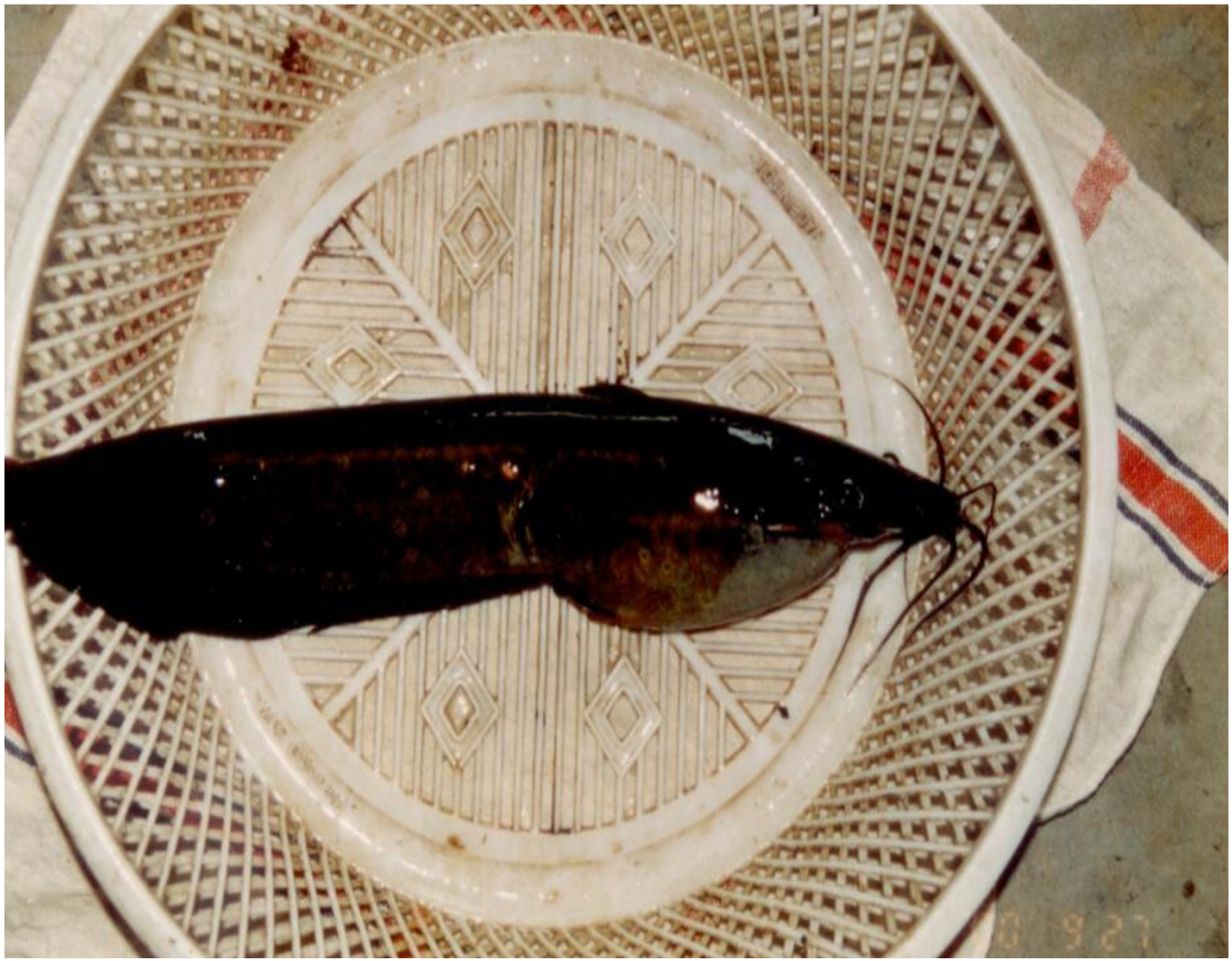


Fig. 13: Broodstock of female *H. fossilis*.



Fig. 14: Broodstock of female *H. fossilis* showing ovarian maturity.

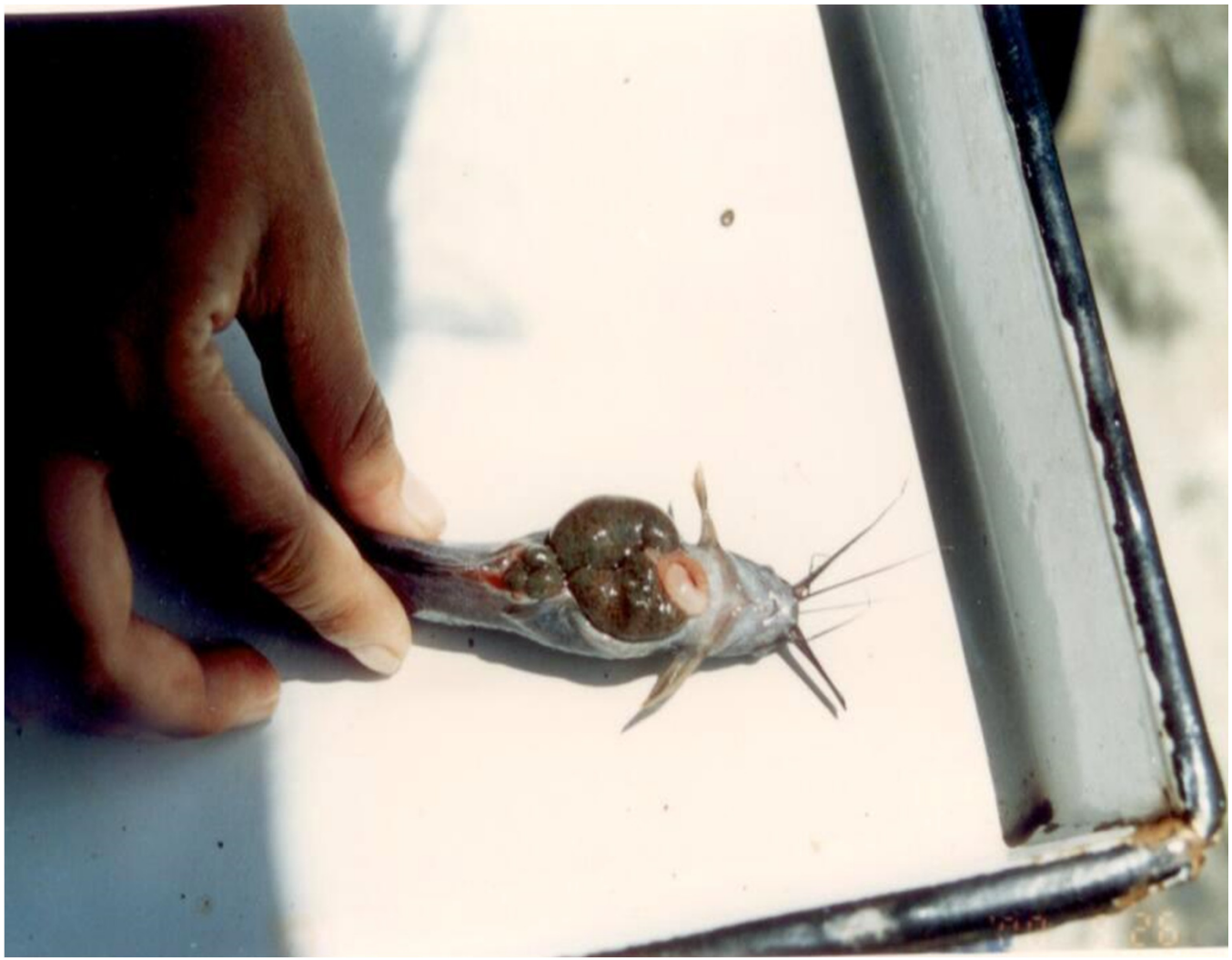


Fig. 15: Broodstock of female *H. fossilis* showing ovarian maturity.



Fig. 16. Eggs released from *H. fossilis* after stripping.



Fig. 17: Fertilized eggs of *H. fossilis*.

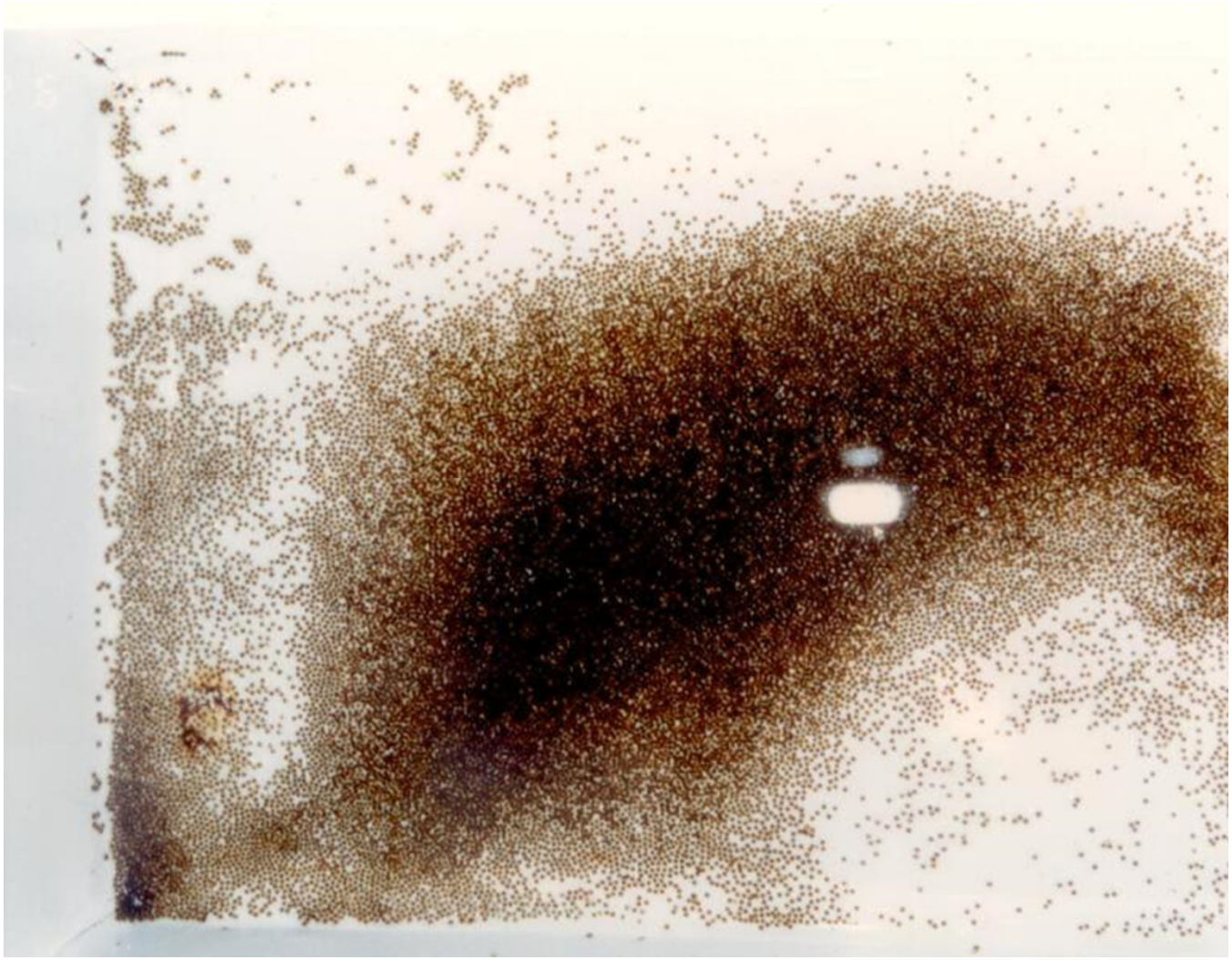


Fig. 18. Fertilized eggs of *H. fossilis.*



Fig. 19: Juveniles of *H. fossilis* after 6 months of rearing under hatchery conditions.



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Thanks