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Study on ova structure of different gonadal stages of some Small Indigenous Fishes

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Abstract

The present study was conducted to examine the ova structure and fecundity patterns across different gonadal maturity stages in selected Small Indigenous Fish Species (SIFS). The investigation was carried out over a five-month period from August to December 2023 at the Laboratory of the Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal. Specimens were collected from multiple fish markets across Bhopal, including Kasturba, Vijay, Piplani, and Govindpura markets. Six species were selected for the study: *Rasbora daniconius, Amblypharyngodon mola, Gudusia chapra, Puntius ticto, Heteropneustes fossilis, and Clarias batrachus*. Total length, weight, fecundity, and ova diameter were recorded monthly. Ova diameter was measured using an ocular micrometer under a compound microscope, while fecundity was estimated using gravimetric methods by analyzing subsamples from different ovarian regions. Histological slides were prepared through alcohol dehydration, xylene clearing, eosin staining, and DPX mounting for structural observation of ova at various maturity stages. The study revealed significant interspecies and seasonal variations in fecundity and ova diameter. For instance, the maximum and minimum Fecundity for Rasbora daniconius are 13,912.29 in August and 3081 in September. For *Gudusia chapra*, the maximum and minimum Fecundity are 10,900 in September and 7013.83 in August. For *Puntius ticto*, the maximum and minimum Fecundity were found to be 23510.09 in August and 931 in November. For *Amblypharyngodon mola*, the maximum and minimum Fecundity were found to be 12147.07 in August and 1415.80 in September. For *Heteropneustes fossilis*, the maximum and minimum Fecundity were found to be

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19208.48 in September and 15174.77 in August, and for *Clarias batrachus*, the maximum and minimum Fecundity were found to be 18320 in August and 10564.2 in September. These findings contribute to a better understanding of reproductive biology in indigenous fish species, which is essential for their conservation, captive breeding programs, and sustainable aquaculture development.

Keywords: Ova structure, Gonadal stage, Fecundity, Small Indigenous Fishes.

1 Introduction

Small Indigenous Fish (SIF) species are those that typically reach a maximum length of about 25 cm at maturity. These species are rich in essential nutrients, including proteins, fatty acids, vitamins, and minerals. Notably, they are often consumed whole, including the head, bones, and eyes, which allows for the full utilization of available nutrients, particularly micronutrients.

In India, approximately 450 out of 765 documented native freshwater fish species are categorized as SIFs. The highest diversity of these species is found in the Northeast region, followed by the Western Ghats and Central India. SIFs play a crucial role in preventing malnutrition, especially due to protein and micronutrient deficiencies, thereby supporting both nutritional and livelihood security for rural populations. Species such as *Amblypharyngodon mola, Osteobrama cotio cotio, Esomus danricus,* and *Corica soborna* are known for their high content of vitamin A and other micronutrients. For instance, in Bangladesh, small fish like mola are the primary source of vitamin A and calcium for rural households.

Minnows, belonging to the family Cyprinidae, are a diverse group of small freshwater fish species. They typically have a laterally compressed body, a terminal mouth, and relatively large, shiny scales. Species such as *Amblypharyngodon mola, Gudusia chapra, Puntius ticto*, and *Rasbora daniconius* are examples of minnows. Catfish species like *Heteropneustes fossilis* and *Clarias batrachus* are characterized by cylindrical bodies and flattened ventral surfaces, which facilitate benthic feeding. They possess whisker-like barbels and are scaleless, distinguishing them from most other teleost fish.

Reproductive studies in fish often focus on gonadal development and maturation stages, which are critical for understanding spawning patterns and fecundity. Histological examination of gonads provides detailed insights into oocyte development, aiding in the determination of spawning periods and the estimation of annual fecundity. This information is essential for effective broodstock management and enhancing fish production.

Oocyte size distribution is a key parameter in assessing gonadal maturity. Ovaries are classified into three types based on oocyte development: synchronous, group synchronous, and asynchronous. Asynchronous ovaries, where oocytes at all stages of development are present, are often associated with species that have protracted spawning seasons and multiple spawning events.

Understanding the reproductive biology and fecundity of fish species is vital for sustainable aquaculture practices. This knowledge helps in optimizing breeding programs, improving fish production, and ensuring the conservation of aquatic biodiversity.

2 Methodology

1. Study Area

The study site was carried out at the Laboratory of the Department of Zoology and Applied Aquaculture of Barkatullah University, Bhopal, Madhya Pradesh.

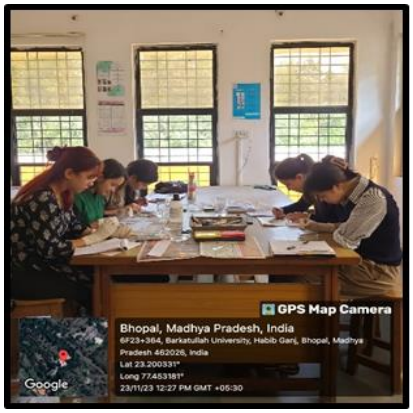


Fig 1: Laboratory at Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, Madhya Pradesh.

2. Collection of specimens and sampling

The different varieties of species are collected from various markets in Bhopal, Madhya Pradesh, viz. Kasturba market, Vijay market, Piplani market, and Govindpura market for a period of 5 months starting from August to December 2023.

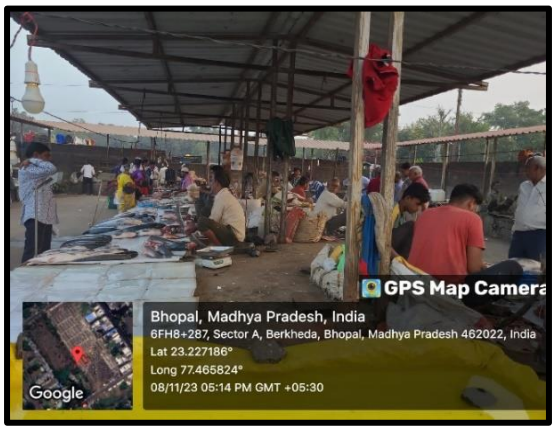


Fig 2: Vijay Market

Fig 3: Kasturba Market

Fig 4: Piplani Market

Fig 5: Govindpura Market

SPECIES NAME	**CLASSIFICATION**	
1. *Rasbora daniconius*	Phylum: Chordata Class: Actinopterygii Order: Cypriniformes Family: Cyprinidae Subfamily: Rasborinae Genus: Rasbora Species: R. daniconius	
2. *Puntius ticto*	Phylum- Chordata Class- Oesteichthyes Order- Cypriniformes Family- Cyprinidae Genus- *Puntius* Species- ticto	

3.*Gudusia chapra*	Phylum: Chordata Class: Actinopterygii Order: Clupeiformes Suborder: Clupeoidei Family: Clupeidae Subfamily: Alosinae Genus: *Gudusia* Species: *G. chapra*	
4.*Amblypharyngodon mola*	Phylum- Chordata Class-Oesteichthyes Order-Cypriniformes Family- Cyprinidae Genus- *Amblypharyngodon* Species-mola	
5. *Heteropneustes fossilis*	Phylum: Chordata Class: Actinopterygii Order: Siluriformes Superfamily: Siluroidea Family: Heteropneustidae Genus: *Heteropneustes* Species: *H. fossilis*	
6. *Clarias batrachus*	Phylum: Chordata Class: Actinopterygii Order: Siluriformes Superfamily: Siluroidea Family: Clariidae Genus: *Clarias* Species: *C. batrachus*	

3. Measurements of Length and Weight

The total length of the fish was measured by a measuring scale and the total body weight was measured by an electronic weighing machine.

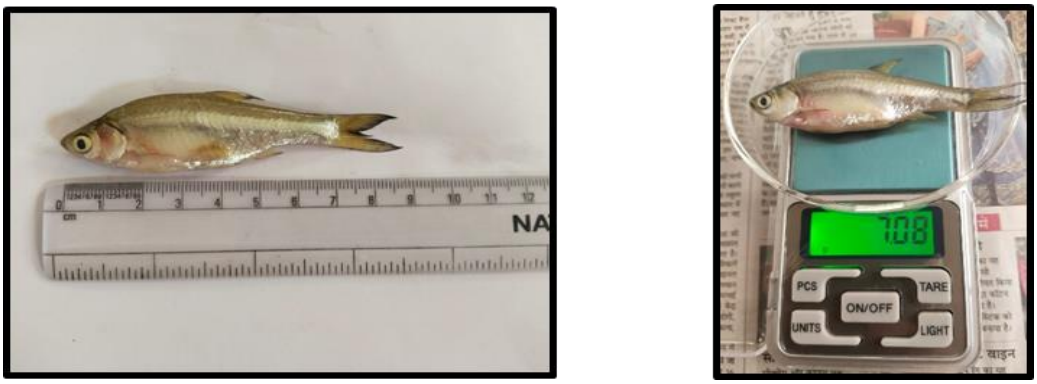


Fig 6: Length and Weight Measurement

4. Macroscopic determination of gonad maturity stages

The maturity stages were identified based on observable macroscopic features of the gonads. Specific characteristics such as color, overall condition, and morphometric measurements of the gonads were assessed. Additionally, the nature of the ova was carefully examined. The stage of gonadal development was determined using these external traits, including the size and appearance of the ovaries, following the I.C.E.S. scale described by Wood (1930).

5. Ova Diameter

To determine the diameter, a sample of ova from each maturity stages is taken in a Slide and is separated with the help of formalin solution and is recorded using an electronic compound microscope with the help of an Ocular micrometer

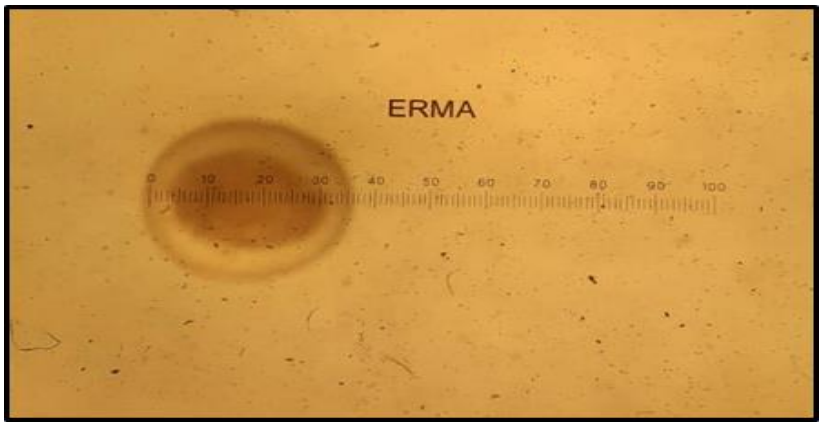


Fig 7: Figure showing the measurement of ova diameter

6. Fecundity

Fecundity was estimated by counting the number of mature eggs present in a known weight of fully mature or ripe ovaries. Subsamples were taken from the anterior, middle, and posterior sections of both

ovaries. These subsamples were evenly spread on a counting slide using a few drops of water, and the mature eggs were counted. The average number of eggs from the three sections was then used to calculate fecundity using the following formula:

$F = n \times G / g$

where F is fecundity, n is the number of eggs in the subsample, G is the total weight of the ovary, and g is the weight of the subsample.

Here,

F = Fecundity, n = Number of eggs in sub-sample, G = Weight of the ovary (gm),

g = Weight of the sub-sample (g)

7. Slides Preparation

For the structure of ova, ovaries of each maturity stage are preserved in permanent glass slides in alcohol for 24 hours.

- The ovaries are dehydrated with the help of a concentration of alcohol (30%, 50%, 70%, 90%) for 1 minute each.
- Xylene was applied to the slides as a clearing agent for 2 seconds.
- Staining was also done by using eosin as the required stain solution for 30 seconds.
- Again, it is dehydrated by using reverse osmosis concentrations of alcohol- 90%, 70%, 50%, 30% for 30 seconds each.
- Xylene is applied again for 2 seconds.
- Mounting is done with DPX, and placed on the slide is placed under the cover slip.
- Then the slides containing the ova are subjected for microscope observation.

Fig 8: Preservation of ova

Fig 9: Chemicals used for preparing slide preparation

3 Results and Discussion

Table 1: Monthly Variations of Length and Weight during gonadal development

Fish Species		August	September	October	November	December
Rasbora daniconius	TL	8.3116667	8.05	8.47	8.6	8.3579167
	TW	5.550556	4.9475	6.212	6.825	5.883764
Amblypharyngodon mola	TL	9.0333333	-	-	9.6285714	9.3309524
	TW	7.7266667	-	-	9.5314286	8.6290476
Gudusia chapra	TL	12.225	16.043333	12.766667	11.905714	13.235179
	TW	20.8	20.8	24.106667	16.75	20.614167
Puntius ticto	TL	11.6667	11.025	-	-	11.34585
	TW	25.2467	20.775	-	-	23.01085
Heteropneustes fossilis	TL	20.45	21.7	-	-	-
	TW	54.999	70.642	-	-	-

Clarias batrachus	TL	-	23.04	23.975	-	-
	TW	-	196.82	173.0725	-	-

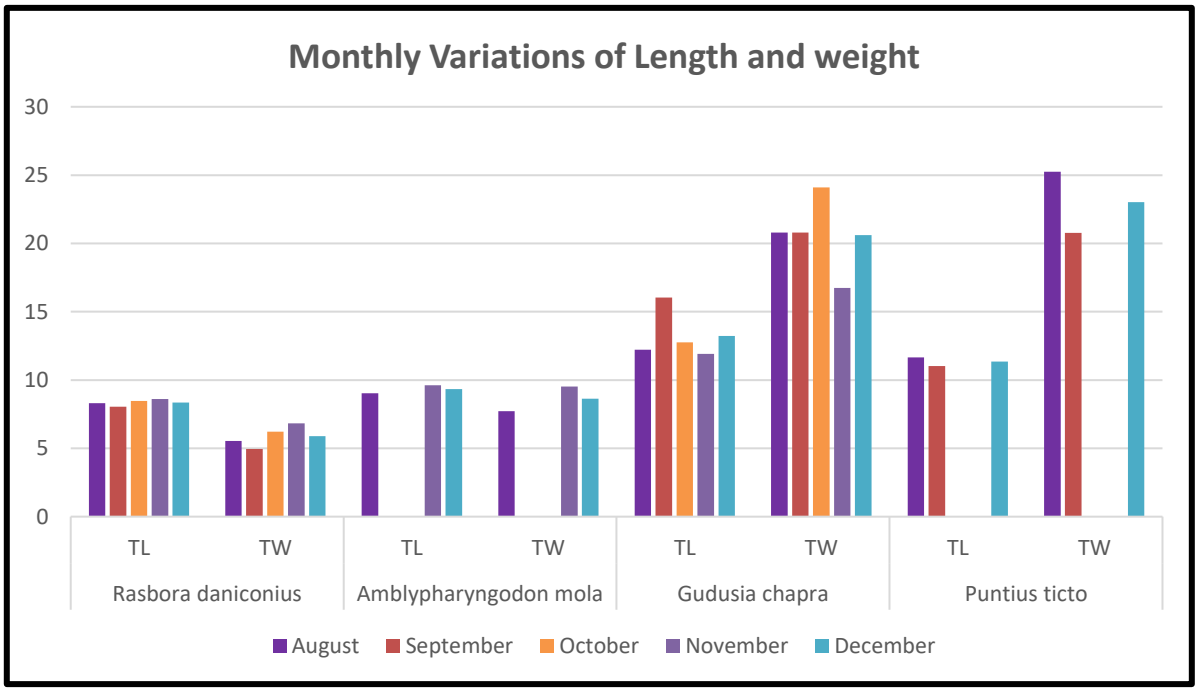


Fig 10: Monthly variations of average Length and Weight

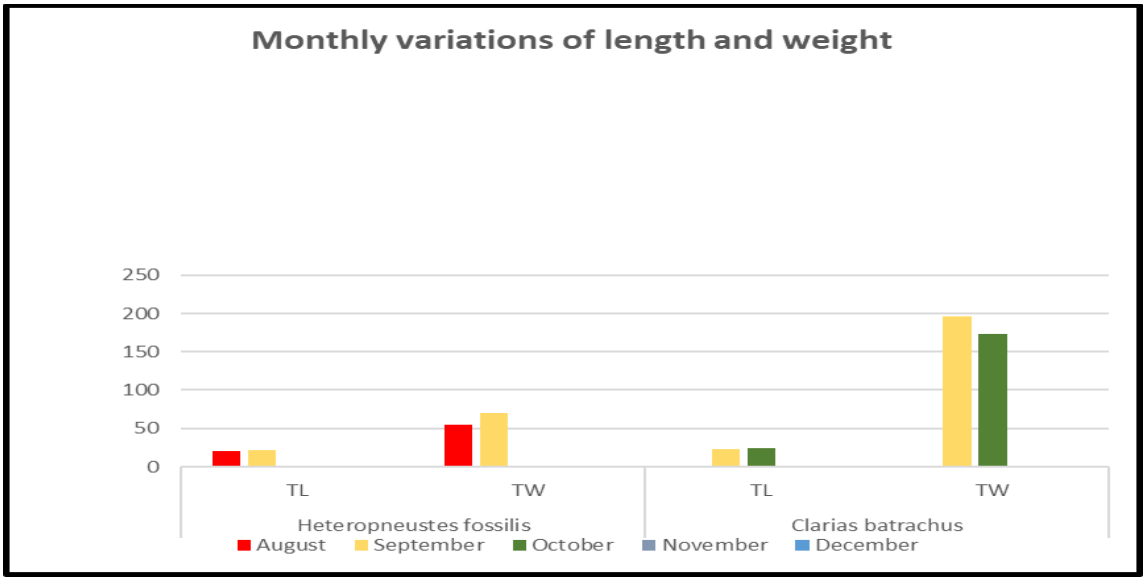


Fig 11: Monthly Variations of average Length and Weight

In Table 1, monthly variations of fish samples for length and weight were observed for a period of 5 months, i.e., from August to December. The results showed that the maximum and minimum total length for *Rasbora daniconius* is 8.6 cm in November and 8.0 cm in September and the maximum and minimum total weight is 6.82 g in November and 4.9 g in September.

The results showed that the maximum and minimum total length for *Amblypharyngodon mola* is 9.62 cm in November and 9 cm in August, and the maximum and minimum total weight is 9.53 g in November and 7.7 g in August. And the maximum and minimum total length for Gudusia chapra is 16.04 cm in September and 11.9 cm in August, and the maximum and minimum total weight is 24.1 g in October and 16.75 g in November.

The maximum and minimum total length for *Puntius ticto* is 11.6 cm in August and 11.0 cm in September, and the maximum and minimum total weight is 25.24 gm in August and 20.7 gm in September respectively and the maximum and minimum total length for *Heteropneustes fossilis* is 21.7 cm in September and 20.45 cm in August, and the maximum and minimum total weight is 70.6 gm in September and 54.99 gm in August.

The maximum and minimum total length for *Clarias batrachus* is 23.97 cm in October and 23.04 cm in September, and the maximum and minimum total weight is 196.82 g in September and 173.07 g in October.

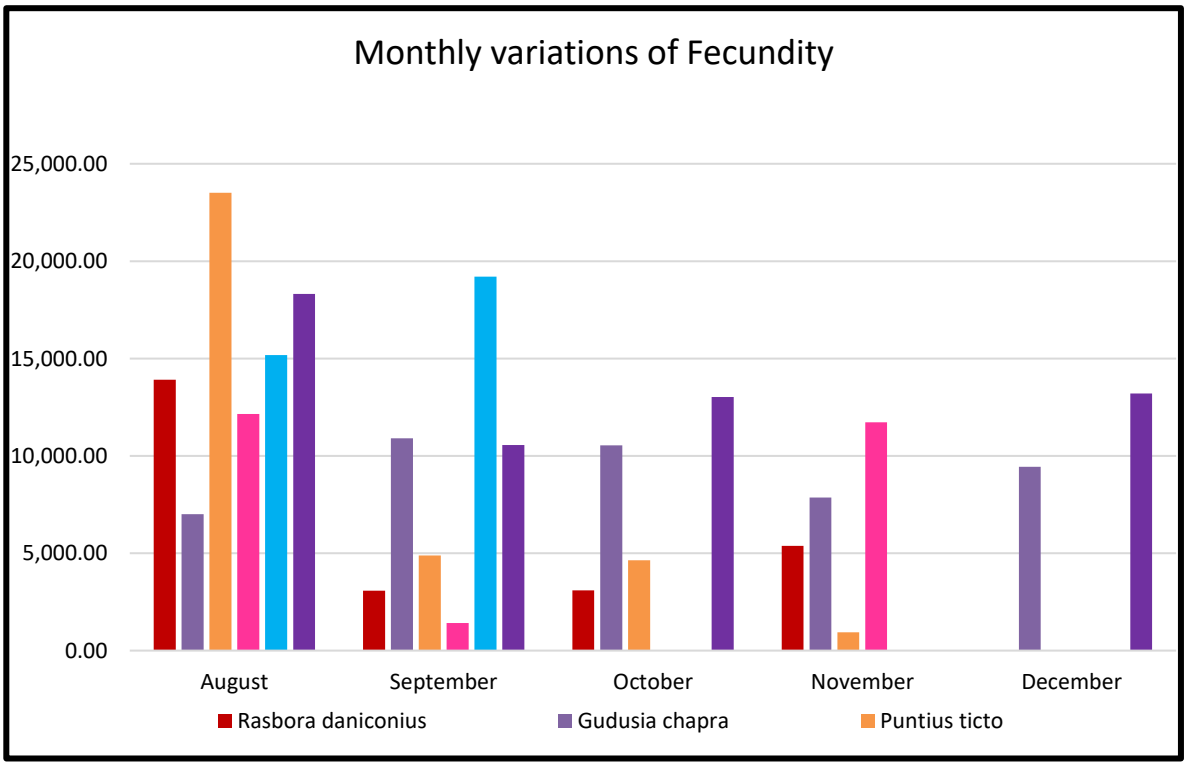


Fig 12: Monthly variations of average Fecundity

The Average Total Length and Weight of each species were arranged for each individual month and plotted against each other to understand the monthly variations (Fig 10 and 11).

Months	***Rasbora daniconius***	***Gudusia chapra***	***Puntius ticto***	***Amblypharyngodon mola***	***Heteropneustes fossilis***	***Clarias batrachus***
August	13,912.29	7,013.83	**23510.09**	12147.07	15174.77	**18320**
September	3081.431	**10900.07**	4,892.68	**1,415.80**	19208.48	10564.2
October	3092.556	**10,542.41**	**4640.65**		-	**13029.02**
November	5371	7858.667	**932**	11716.48	-	
December	-	**9433**	-	-	-	**13200**

Table 2: Monthly variations of Fecundity during gonadal development

From the above readings, Table 2 and Fig. 12, the maximum and minimum Fecundity for *Rasbora daniconius* are 13,912.29 in August and 3081 in September. For *Gudusia chapra*, the maximum and minimum Fecundity are 10,900 in September and 7013.83 in August. For *Puntius ticto*, the maximum and minimum Fecundity were found to be 23510.09 in August and 931 in November. For *Amblypharyngodon mola*, the maximum and minimum Fecundity were found to be 12147.07 in August and 1415.80 in September. For *Heteropneustes fossilis*, the maximum and minimum Fecundity were found to be 19208.48 in September and 15174.77 in August, and for *Clarias batrachus*, the maximum and minimum Fecundity were found to be 18320 in August and 10564.2 in September. The average fecundity values were plotted against each month to understand the monthly variations (Fig. 12).

Table 3: Monthly variations of Ova Diameter

Months	***Rasbora daniconius***	***Gudusia chapra***	***Puntius ticto***	***Amblypharyngodon mola***	***Heteropneustes fossilis***	***Clarias batrachus***
August	0.894	0.51	**0.99**	0.56	1.1625	**0.645**

September	0.945	**0.47**	1.075	**0.63125**	1.2	1.14
October	1.008	**0.39**	**0.97**	**0.75**	-	**1.2975**
November	0.953	0.47	**1**	0.754	-	**1.22**
December	-	**0.35**	-	-	-	-

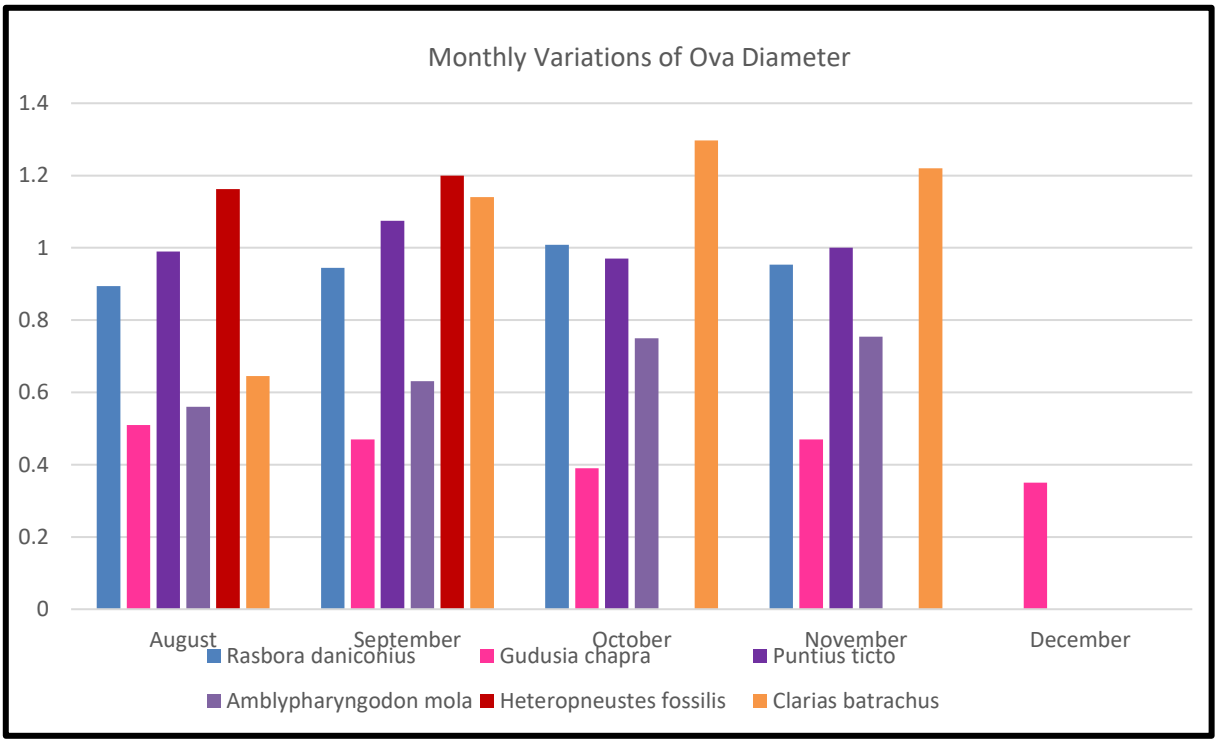


Fig 13: Monthly variations of Ova diameter

For *Rasbora daniconius*, the maximum and minimum ova diameter is 1.008 in October and 0.894 in August. For *Gudusia chapra*, the maximum and minimum ova diameter are 0.51 in August and 0.35 in December. For *Puntius ticto*, the maximum and minimum ova diameters are 1.075 in September and 0.97 in October. For *Amblypharyngodon mola*, the maximum and minimum ova diameters are 0.754 in November and 0.56 in August. For *Heteropneustes fossilis*, the maximum and minimum ova diameters

are 1.2 in September and 1.16 in August. For *Clarias batrachus*, the maximum and minimum ova diameter is 1.2975 in October and 0.645 in August.

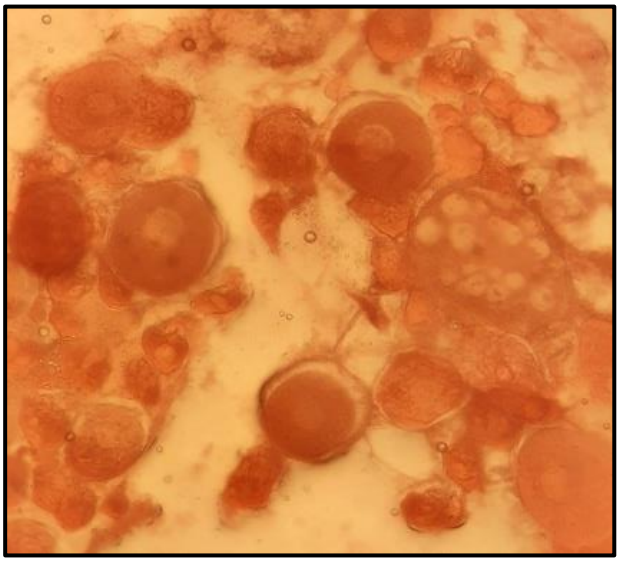


Fig 15*: Puntius ticto*

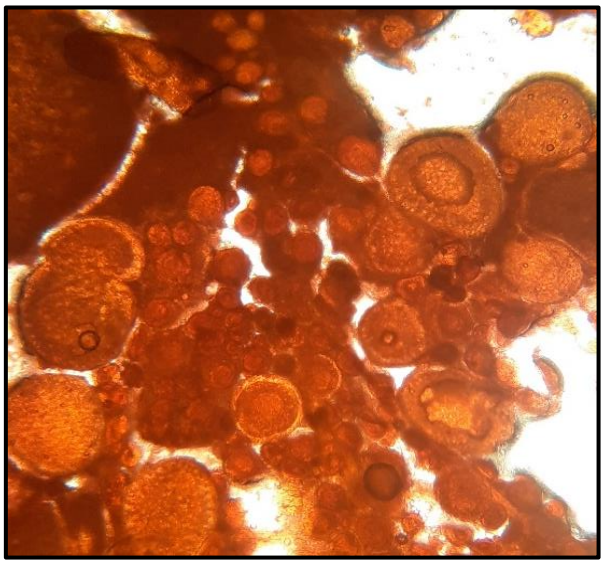


Fig 14*: Rasbora daniconius*

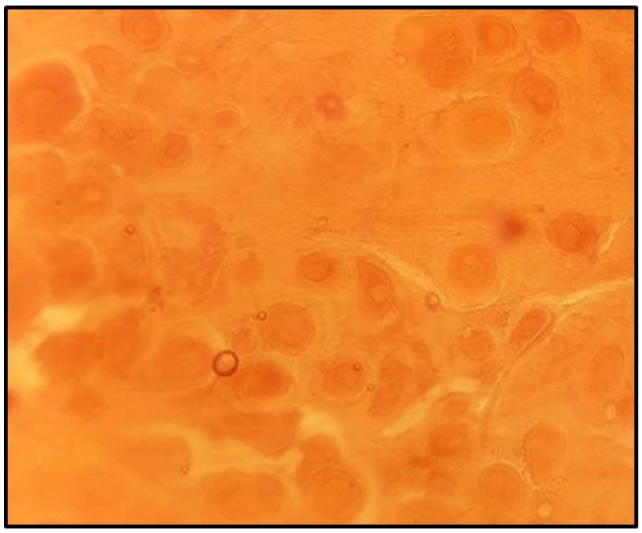


Fig 17: *Gudusia chapra*

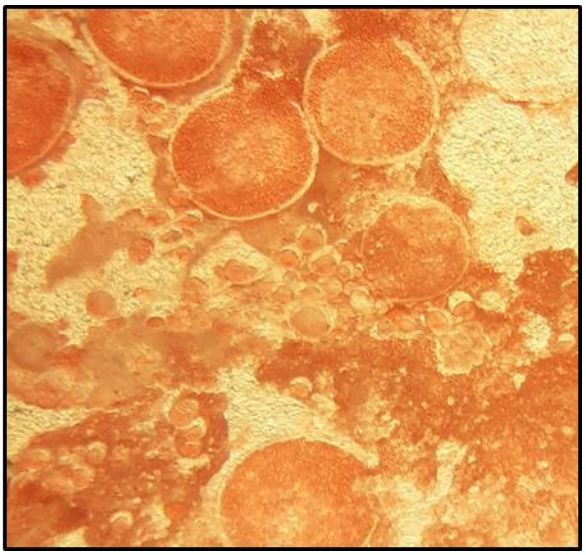
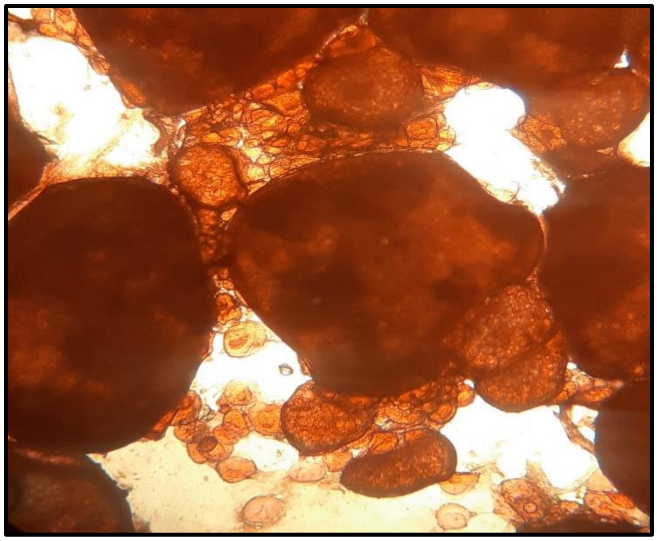


Fig 16*: Amblypharyngodon mola*



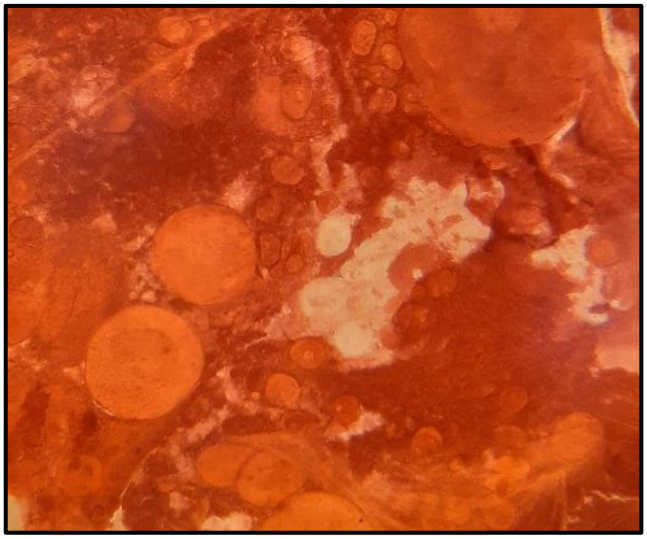


Fig 19*: Heteropneustes fossilis*

Fig 18*: Clarias batrachus*

Fig: Ovarian structure of mature stage of Different species

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