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STUDIES ON SEXUAL DIMORPHISM AND GONADAL DEVELOPMENT OF AN INDEGENEOUS ORNAMENTAL FISH *TRICHOGASTER LALIUS*

DEBKANTA SUTRADHAR, SAMARNEDRA BEHERA, RINKU GOGOI* AND SANJEEV KUMAR
Department of Fisheries Resource Management, Faculty of Fishery Sciences,
WBUAFS, 5-Buderhat Road, Panchasayar - 700 094, Kolkata, INDIA
e-mail: rinkuz1986@rediffmail.com

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***Corresponding author**

ABSTRACT

The reproductive biology of dwarf gourami *Trichogaster lalius* was studied during 2007-2008 in the laboratory of West Bengal University of Animal and Fishery Sciences. The study reveals that *T. laius* is very much sexually dimorphotic. Male dwarf gourami is attractive and more colourful than female. However, this colour is more prominent during breeding season from (March-August) than non breeding season (Sep-Feb). Apart from colour some other secondary sexual characteristics like belly structure, dorsal fin structure and genital opening are studied in this species.This external morphology was found useful for sex identification in this species particularly during breeding season. Hence breeding and non breeding seasons were also determined. Gonadal maturation was studied on the basis of GnSI, TI and OI. The highest Gonadosomatic index (GnSI) in male was found to be 0.583±0.043 while in female the highest value of GnSI was recorded during the month of June (6.218±0.956). The average pre-spawning fecundity of *T. lalius* was 1024 to 1351 in number for the fish average length and weight of 43.6 mm to 46.33 mm and 1.244 g to1.808 g respectively.

INTRODUCTION

Ornamental fishes are called as 'living jewels' due to their lucrative colouration, unique shape of the body and there adaptive behavior. Ornamental fishes can be defined as "an attractive colourful fishes of peaceful nature that are kept as pets in confined space of an aquarium or a garden pool with the purpose of enjoying their beauty for fun and fancy" (Mils, 1990 and Dey, 1996).

The export of ornamental fish from our country started during the year, 1969 with a few species of tropical fresh water fish with insignificant export earnings, which increased to 10 crore rupees in 1994. It has also been noticed that Indian ornamental fishes are in greater demand in international market (Ayyappan and Jena, 2006). According to Ziauddin *et. al.*, (2007) India has a great potential to increase the level of fish export to US $ 30 million every year.

As far as the export of ornamental fishes from India is concerned, 90% of the total exports are wild caught fishes of fresh water origin and it constitutes of *Colisa fasciata, Trichogaster lalius, Dontiadeor spp, Tetraodon spp, Chanda ranga, Esomus danricus* and some other from North Eastern region. The remaining 10% are either tank reared or breed and reared varieties of exotic species of fresh water or marine origin.

Trichogaster lalius is an indigenous ornamental fish of West Bengal. It belongs to the family- Belontiidae, Sub-family-Trichogasterinae, commonly known as Dwarf gourami. In West Bengal, this fish is locally called 'Khalisa or LalKholse'. It is traditionally liked by the people for its good taste and fetches a market value of Rs. 130 to 150/ Kg. Though it is generally categorized under weed fish, Male of lalia has high foreign demand as aquarium fish due to lucrative colouration. It is naturally collected and exported to the foreign countries e.g. USA, Singapore, Japan, Republic of Korea, Sri Lanka, Germany, UK, Hong Kong, Taiwan, Thailand, Netherlands, Bangladesh, Malaysia and China (Biswas and Lepcha, 2004).

Due to high exploitation of this fish in the natural resources their habitat is lost for natural propagation. So it is to be needed to think over artificial propagation to meet up such high demand.The knowledge on reproductive cycle of the species will help in formulating breeding and culture technology of the commercially important species for mass propagation as well as conservation (Gogoi *et al.,* 2013). Hence, a proper knowledge of reproductive biology of *Trichogaster lalius* is barely necessary before going for artificial breeding. So the study was under taken on sexual dimorphism, gonadal development at different seasons to find out their breeding season, mode of maturation and development of sexual character etc.

MATERIALS AND METHODS

Rearing of fish in captivity

The study was conducted during 2007-2008 in the Department of Fisheries Resource Management, West Bengal University of Animal and Fishery Science, Kolkata, West Bengal. Adults of *Trichogaster lalius* ranging from the length 43 mm to 47 mm and weight of 1.1711 g to 1.8474 g (w/w) were collected from the pond of Faculty of Fishery Sciences, Chakgaria Campus and from the market of Galef Street, ornamental fish market, Kolkata. Fishes were collected from pond and market and then transported to the laboratory by plastic buckets either with manual aeration or by plastic bag with oxygen packing. Fishes were maintained in the laboratory condition for 12 months from November'07 to October'08. During the entire rearing period a fresh artificial feed (protein content 40%) was given to the stocked fishes at the rate of 2 to 3 % of their body weight daily i.e. during morning and a live feed (Tubifax) at the evening hours.

Sampling

In every fortnight period, sampling was done randomly from the stocked specimen. In each sampling, 30 numbers (15 numbers of male and 15 numbers of female) of healthy and disease free fishes were selected for observation.

Morphological study of specimen

The weight (w/w) and length (total length) of fishes were measured by standard procedure and recorded in g and mm respectively. The external features of live fishes were observed thoroughly viz., colour pattern of body, characteristic of vent as well as the structure of the belly, tip of the dorsal and anal fins (plate 1 to 4). On the basis of the keen observation on the external morphology the sexes of the fishes were assumed and segregated sex wise.

Morphological study of gonad

The fishes were dissected to take out the gonads for confirmation of the sex and dissected (testes/ovary) were taken out and put into physiological saline solution. The gonads were washed properly to remove the blood, adhering tissues and fats. The colour, size, length and weight of the gonads were recorded for each sample (Table 1). By using the parameters mentioned above, the gonadosomatic index (GnSI), testicular index (TI) and ovarian index (OI) of the species were also determined as per standard procedure of Lagler, 1956.

$$\text{GnSI (\%)} = \frac{\text{Gonad mass (g)}}{\text{Body mass (g)}} \text{ X } 100$$

$$\text{TI (g / cm)} = \frac{\text{Total testis mass / 2 (g)}}{\text{Total testis length / 2 (cm)}}$$

$$\text{OI (g / cm)} = \frac{\text{Total ovary mass / 2 (g)}}{\text{Total ovary length / 2 (cm)}}$$

Study of pre-spawning fecundity

For the study of fecundity, 2-5% formalin and Gilson's fluid modified by Simpson (1951) were used as fixative and preservative. The sample of the ovary was preserved in modified Gilson's fluid about 2 days or 48 hr. for liberates by breaking down the ovarian tissue. After two days the ova may be completely released from the ovarian tissue by vigorous shaking. The ovary was washed with fresh water up to about 100% water exchange. Then it was dried and counted. The numbers of ova were counted manually to get the fecundity by following formula -

$$\text{Fecundity} = \frac{\text{Total weight of ovary X No. of ova counted in sub-samples}}{\text{Weight of the sub-samples}}$$

Statistical analysis

To interpret the data statistically least square method was used to find out the relationships between the different variables like fecundity and fish weight / fish length / ovary volume / ovary weight / ovary length in the following formula -

$Y = a + b.X$

and in which logarithmic from is-

$\text{Log } Y = \text{Log } a + b \log X$

Where,

Y = Fecundity, X = Different body or gonadal parameter and a (intercept) and b (slope) are consant.

RESULTS AND DISCUSSION

Determination of maturity and spawning period by observing GnSI is of pivotal significance in the life of fish. The maturity cycle and the peak breeding seasons were determined by studying the trend of GnSI during the study.The maximum mean gonad weight of *Trichogaster lalius* was recorded 0.01 ± 0.004 g in case of male and 0.097 ± 0.014 g in female respectively in the month of June (Table 1). The maximum GnSI value was also found to be 0.583 ± 0.043 in case of male and 6.218 ± 0.956 in female respectively in the same month (Table 2).

Sexual dimorphism on the basis of external morphology

Sexual dichromatism is often common in several species of fish. In general males are more brightly coloured than their female counterparts. *Trichogaster lalius*is very much sexually dimorphic. Male and female of *Trichogaster lalius* were identified by observing their colour pattern both during breeding (March-June) and non-breeding season (July to February). During breeding season the male dwarf gourami is attractive and more colourful than the female. It has an almost translucent blue colour with vertical diagonal strips of alternating red and dark orange that extended into the fins. The female dwarf gourami, never achieving the brilliant colour likes males; the female remain a silvery colour or a dull silvery blue to gray colour. During non-breeding season, the colour of the bands of male was bright red whereas in female, it was grayish bands over the light orange laterally compressed body (plate I to VI). Apart from colour some other characteristic differences in belly structure, fin structure and genital opening is also prominent in *T. lalius* (plate VII to VIII). During breeding season, bulgingness of belly was found more in female in comparison to male Similar observation was also seen in *Anabas testudineus* (Khan, 1972; Dehadrai *et al.*, 1973; Banerji and Prasad, 1974; Das, 2002 and Ziauddin, 2002) and in *Colisa fasciatus* (Khan, 2004). The bulgingness of belly in female is more due to accommodation of mature eggs in

Table 1: Monthly variation of the mean length, weight and colourof the testes and ovary of *Trichogaster lalius*

Months	Maturation Phase	Breeding season	Testis of *Trichogaster lalius* Length (mm) Mean	SD	Weight (g) Mean	SD	Colour	Ovary of *Trichogaster lalius* Length (mm) Mean	SD	Weight (g) Mean	SD	Colour
November	Late resting	Non-Breeding Season	6.83	±1.67	0.005	±0.001	White	8.00	±1.00	0.032	±0.007	White
December			6.93	±1.98	0.005	±0.002	White	8.00	±0.93	0.033	±0.007	White
January	Preparatory		7.00	±1.89	0.006	±0.002	Creamy white	8.80	±1.37	0.057	±0.019	Creamy white
February			7.20	±1.85	0.007	±0.003	Creamy white	9.37	±0.67	0.066	±0.018	Creamy white
March	Pre-spawning*	Breeding Season	7.90	±2.35	0.007	±0.002	Yellowish white	9.87	±1.30	0.076	±0.021	Yellowish white
April			8.03	±1.82	0.008	±0.002	Yellowish white	10.07	±1.03	0.085	±0.014	Yellowish white
May	Peak-spawning*		9.17	±1.26	0.01	±0.001	Yellow	10.40	±1.35	0.094	±0.018	Bright Yellow
June			9.30	±0.75	0.01	±0.004	Yellow	10.50	±1.32	0.097	±0.014	Bright Yellow
July	Post-spawning	Non-Breeding Season	8.60	±0.89	0.008	±0.001	Dull yellow	10.00	±1.41	0.079	±0.019	Pale Yellow
August			7.70	±1.36	0.006	±0.001	Dull Yellow	9.43	±1.02	0.063	±0.018	Pale Yellow
September	Resting		6.70	±1.82	0.005	±0.002	Dull white	9.20	±1.08	0.046	±0.015	Dull white
October			6.70	±2.34	0.005	±0.002	Dull white	8.70	±0.84	0.031	±0.008	Dull white

the ovary. In case of male, tip of the posterior region of dorsal fin (soft ray) and anal fins are pointed while in female, that region of dorsal and anal fins are rounded or curved (plate IX). During breeding seasons the genital opening of female is stouter, less pointed and tipped with red colour but in case of male, the opening is conical shape, pointed and tipped with reddish colour (plate VI to VIII).

Testicular morphology and morphometry

The testes are paired and attached to each other all along the length. The colour of testis is creamy white during non-breeding season and creamy white to yellowish or pinkish red during breeding season. The colour and length change due to lobulation of testes and storage of testicular product (sperm) in percentage. These changes are seen according to the advent of maturation of testes.

Length and weight of testes was the more during breeding season (March to August).In pre-spawning stage the gonad showed increase in length and weight attained maximum in peak spawning stage. During post-spawning season (July to August), it started decreasing trend up to the resting phase (September to October) and late resting phase (November to December). During January to February the gonads get prepare for maturation (Table 1). During peak breeding season (May-June), sperm could be oozing out by pressing on belly. The colour of the testes was changing according to the maturation of testes and with the rhythm of seasonal cycle. The testes of fish show variation during different months of breeding and non-breeding season. In the present study, the colour of the testes was changing according to the maturation of testes and with the rhythm of seasonal cycle. During non-breeding season the colour of the testes became creamy white. With the onset of breeding season, the colour of testes turns creamy white to yellowish or pinkish red.

Ovarian morphology and morphometry

The external morphology of the ovary of *T. lalius* is changed according to the rhythm of the ovarian cycle, which is influenced by the season. The ovaries are paired elongated sac-like structures lying in the abdominal cavity, ventral to the kidneys. They are attached to the body wall by means of the Mesovarium. The anterior ends of the two ovaries are free but their posterior ends are united together into one. During non-breeding (July to December) season, the ovaries were pale, dirty white in colour or opaque and light yellow in colour. As the breeding season advanced, the ovary became bright yellow. During post-spawning phase (July-August) the ovaries became sac like and reduced in volume with dull appearance. During preparatory period (January-February), the length, weight of ovary was comparatively less than the pre-spawning period (March-April). The length, weight and volume of ovary were observed Maximum in peak-spawning period (May-June). Ripe ovary occupied maximum space of the body cavity. Ripe ova were coming out with a gentle pressure on abdomen. The meristic character and colour of the ovary were found to be changing accordingly to the maturity stages.

Gonado somatic index (GnSI)

The maximum mean gonad weight of *T. lalius* was recorded 0.01 ± 0.004 g in case of male and 0.097 ± 0.014 g in female respectively in the month of June (Table 1). The maximum

Table 2: Monthly changes in the Mean Gonadosomatic Index (GnSI) vs Mean Testicular Index (TI) of Male *Trichogaster lalius* and Mean Ovarien Index (OI) of Female *Trichogaster lalius*

Month	GnSI (Testes) Mean	SD	SEM(SD/″n)	TI Mean	SD	SEM(SD/″n)	GnSI (Ovary) Mean	SD	SEM(SD/″n)	OI Mean	SD	SEM(SD/″n)
November	0.321	±0.071	0.018	0.0008	±0.0002	0.00005	2.206	±0.479	0.124	0.004	±0.0008	0.0002
December	0.341	±0.114	0.029	0.0008	±0.0002	0.00006	2.307	±0.493	0.127	0.004	±0.0006	0.0002
January	0.364	±0.108	0.028	0.0009	±0.0003	0.00008	3.732	±1.046	0.27	0.006	±0.002	0.0004
February	0.397	±0.165	0.043	0.0009	±0.0003	0.00008	4.304	±1.151	0.297	0.007	±0.002	0.0004
March	0.453	±0.118	0.03	0.001	±0.0004	0.0001	4.907	±1.113	0.287	0.008	±0.002	0.0004
April	0.507	±0.101	0.026	0.001	±0.0001	0.00004	5.409	±0.802	0.207	0.008	±0.0009	0.0002
May	0.571	±0.061	0.001	0.001	±0.0001	0.00003	5.972	±0.956	0.247	0.009	±0.001	0.0003
June	0.583	±0.043	0.011	0.0011	±0.0005	0.00005	6.218	±0.956	0.247	0.009	±0.001	0.0003
July	0.48	±0.052	0.013	0.0009	±0.0001	0.00003	5.088	±1.087	0.281	0.008	±0.001	0.0003
August	0.401	±0.080	0.021	0.0009	±0.0002	0.00005	4.214	±1.119	0.289	0.007	±0.001	0.0004
September	0.301	±0.127	0.033	0.0007	±0.0002	0.00006	3.062	±0.840	0.217	0.005	±0.001	0.0003
October	0.304	±0.116	0.03	0.0007	±0.0002	0.00005	2.104	±0.537	0.139	0.004	±0.0008	0.0002

Table 3: Relationship between Spawning Fecundity with various body and ovary parameters of *Trichogaster lalius*

No. of Fish examined	Total length of Fish (mm) Range	Avg.	Weight of Fish (g) Range	Avg.	Length of Ovary (mm) Range	Avg.	Weight of Ovary (g) Range	Avg.	Fecundity (No. of eggs) Range	Avg.
3	43-44	43.6	1.100-1.300	1.244	6.0-7.5	6.67	0.020-0.045	0.038	1000-1040	1024
3	44-45	44.83	1.300-1.500	1.45	7.5-9.0	8	0.045-0.070	0.061	1100-1194	1156
3	45-46	45.5	1.500-1.700	1.613	9.0-10.5	9.67	0.070-0.095	0.09	1235-1280	1260
3	46-47	46.33	1.700-1.900	1.808	10.5-12	11.5	0.095-0.120	0.108	1341-1360	1351

Table 4: Relationship between Fish Weight, Ovary Weight and Relative Fecundity of *Trichogaster lalius*

No.of Fish examined	Weight of Fish (g)		Weight of Ovary (g)		% of ovary weightin total weight of fish(avg.)	Fecundity (No. of eggs)		No. of eggs/g weight of ovary		No. of eggs/g weight of fish (Relative Fecundity)	
	Range	Avg.	Range	Avg.		Range	Avg.	Range	Avg.	Range	Avg.
3	1.100-1.300	1.244	0.020-0.045	0.038	3.06	1000-1040	1024	22857-34965	27623	798-853	823
3	1.300-1.500	1.45	0.045-0.070	0.061	4.22	1100-1194	1156	16769-22267	19170	794-801	796
3	1.500-1.700	1.613	0.070-0.095	0.09	5.63	1235-1280	1260	13417-14310	13889	752-808	781
3	1.700-1.900	1.808	0.095-0.120	0.108	5.99	1341-1360	1351	11305-13711	12526	736-763	747

GnSI value was also found to be 0.583±0.043 in case of male and 6.218±0.956 in female respectively in the same month. In this study, GnSI value for female *T. lalius* revealed increasing trend from March to June with a peak value during May-June. There after sudden drop was noticed from July to October. Subsequently an increasing trend was noticed from November onwards.The present observation, these sequential changes in GnSI value indicated that the spawning season of *T. lalius*is restricted between March to June with a peak during May-June. However, Khan (2004) has reported the breeding season of *Colisa fasciatus* from April to July.

Fecundity

The average pre-spawning fecundity of *T. lalius* was 1024 to 1351 in number for the fish average length and weight of 43.6 mm to 46.33 mm and 1.244 g to1.808 g respectively. The number of eggs increases with the increase in body weight, body length, gonad length and gonad weight of the fish. Similar observation was made by Dasgupta (1988), Musa and Bhuiyan (2007) and Chavan and Muley (2014).

In present observation the relationship between fecundity and the length of fish, weight of fish, gonad length and gonad weight of fish was found to be directly linear and the correlation co-efficient 'r' was significant. However, fecundity of the fish is closely related to the fish length and weight. A straight line relationship has been observed between the fecundity and the fish length, and the fecundity and fish weight in a number of species. The similar observation was made by *Puntius sp.* (Mustafa *et al.*, 1982), *Anabas testudineus* (Nargis and Hussain, 1988 and Das, 2002), *Heteropneustes fossilis* (Reddy and Rao, 1991 and Faruq *et al.*, 1998), *Colisa fasciata* (Bhuiyan *et al.*, 1995 and Khan, 2004), *Oreochromis niloticus* (Bhuiyan and Afrose, 1996), *Nandus nandus* (Hossain *et al.*, 1997), *Barbodes gonionotus* (Bhuiyan *et al.*, 2000), *Periophthalmus barbarous* (King and Udo, 2001), *Mystus montanus* (Jesu *et al.*, 2004), *Channa punctatus* (Marimuthu and Haniffa, 2006) and *Channa striatus* (Haniffa *et al.*, 2010). In addition to these, fecundity is directly proportional to body weight (Simpson, 1951), since the weight is more closely connected with the condition of the fish than its length (Bagenal, 1967). Yuen (1955) and Varghese, (1980) found the relationship between fecundity and weight to be curvilinear, where as several

Plte I:♂*T. lalius*in non-breeding season

Plate II:♀*T. lalius*in non-breeding season

Plate III:♂*T. lalius*in pre-spawning season

Plate IV:♀*T. lalius*in pre-spawning season

Plate V:♂*T. lalius*in breeding season

Plate VI:♀*T. lalius*in breeding season

workers observed it to be linear (Gupta, 1968; Joshi and Khanna, 1980; Singh *et al.,* 1982; Nautiyal, 1985 and Agarwal, 1996). The findings of the present work, was found a linear relationship between fecundity and fish weight.

A close correlation is usually expected between the number of eggs and the ovarian weight. According to Chondar (1977) and Joshi and Khanna (1980) the number of egg production depends upon the ovary weight. The results differ from the investigation of Varghese (1976, 80) observed a curvilinear relationship between ovary weight-fish length and ovary weight-fish weight in *C. ramcarati* and *C. dussumeri*. But in the present study, *Trichogaster lalius* possessed a linear relation ship. The regression coefficient values of these relationships indicate the rate of increase of ovary weight in relation to the

Table 5: The Regression and Correlation Co-efficients between the dependent variant and independent variant of *Trichogaster lalius*

Dependent Variant Y	Independent Variant X	Equation Log Y = Log a + b log X	Regression Co-efficient (b)	Correlation Co-efficient (r)
Fecundity (F)	Total length of Fish (TL)	Log F = -4.0121 + 4.2866 log TL	112.32	0.954
Fecundity (F)	Total weight of Fish (FW)	Log F = 2.9442+0.7307 log FW	572.08	0.989
Fecundity (F)	Length of Ovary (OL)	Log F = 2.6135 + 0.4904 log OL	65.226	0.983
Fecundity (F)	Weight of Ovary (OW)	Log F = 3.3571 + 0.2422 log OW	4370.1	0.98
Weight of Ovary (OW)	Total length of Fish (TL)	Log OW = -30.216 + 17.571 log TL	0.0256	0.966
Weight of Ovary (OW)	Total weight of Fish (FW)	Log OW = -1.6891 + 2.9286 log FW	0.1283	0.979

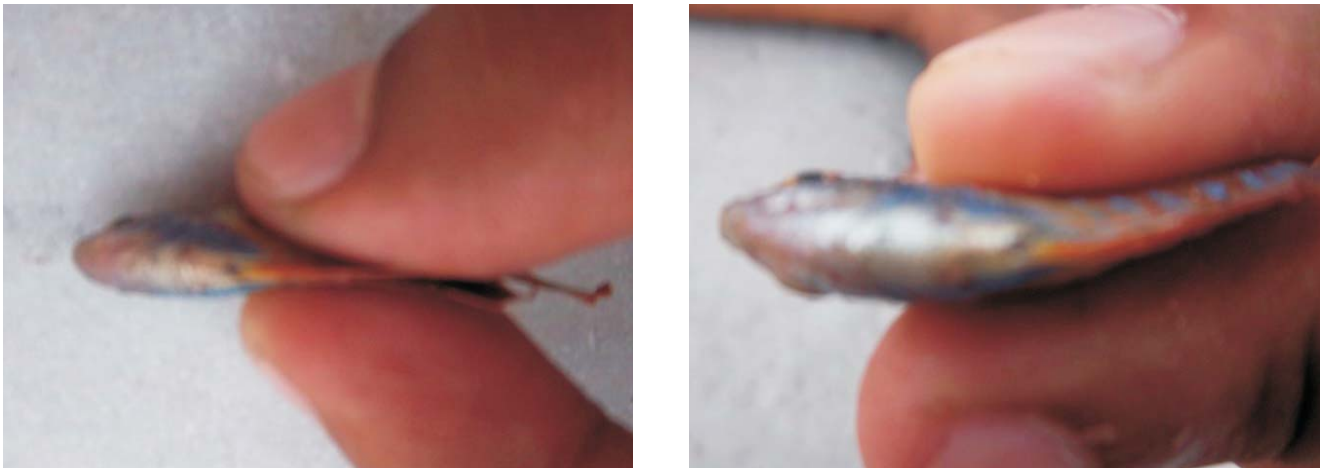

Plate VII: Abdomen and vent of male (♂) *T. lalius* during non-breeding and breeding season

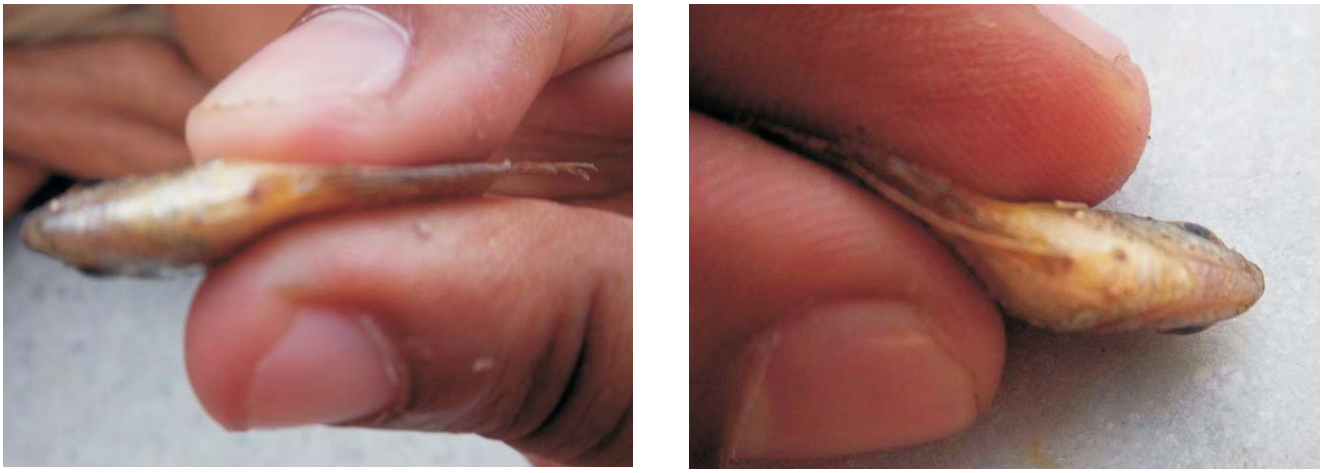

Plate VIII: Abdomen and vent of female (♀) *T. lalius* during non-breeding and breeding season

body length is greater than the rate of increase of ovary weight in relation to body weight. The exponential value and correlation coefficient of *Trichogaster lalius* was 0.0256 and 0.966 respectively. It is reflecting the made by Gupta (1968).

The relationship of fecundity (F) with length of fish and weight of fish, length of ovary and weight of ovary were found out and it has been noted that the fecundity was directly related to the length of fish (r = 0.954) and weight of fish (r = 0.989) than the length of ovary (r = 0.983) and weight of ovary (r = 0.980).

On the other hand, the relationship of ovary weight with length of fish and weight of fish were found out and here also observed that the ovary weight was related to the length of fish (r = 0.966) and weight of fish (r = 0.979).

The present investigation on *Trichogaster lalius* revealed it saverage relative fecundity as 786 which indicate that the *Trichogaster lalius* has a higher reproductive potential. On the other hand, the relative fecundity of *Trichogaster lalius* was varying from 747 to 823 numbers of ova and it decreases with growth as the size of ova increases during growth. Present finding is agreeing the observation made by Phukon and Biswas (2002).

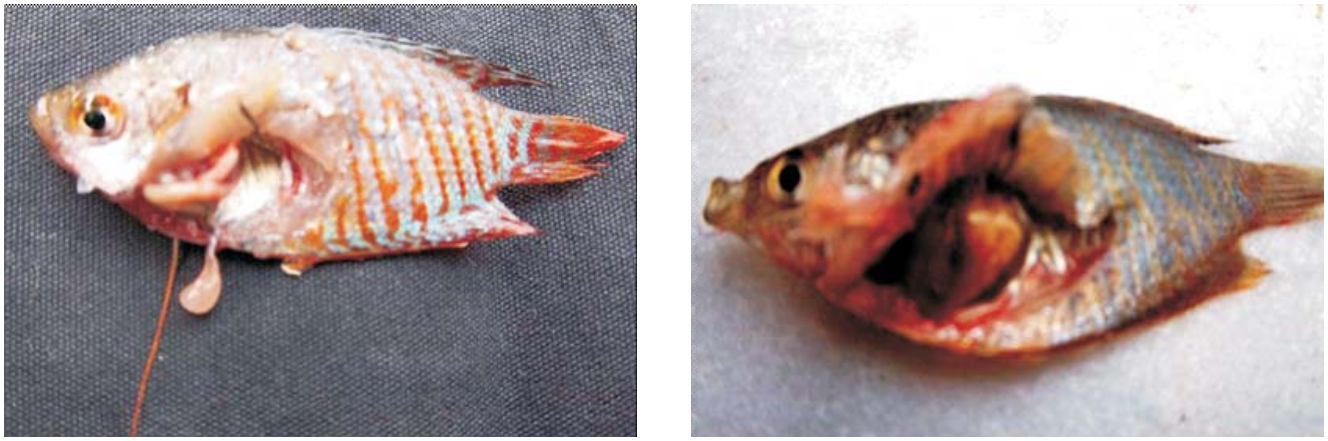

Plate IX: Locationof testis and ovary (♂) and female (♀) *T. lalius* in body cavity

The presence of only one batch of maturing/mature ova indicates that the species breeds only once in a year which is supported by GnSI values exhibiting a single annual peak in both male and female.

The present studies, concluded that *T. lalius* is a total spawner, its spawning takes place during mansoon months and its absolute fecundity is related with total length, body weight and gonad weight.

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