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REPRODUCTIVE CYCLE OF THE FRESHWATER LOACH LEPIDOCEPHALICHTHYS THERMALIS FROM THE RIVER THAMIRABARANI, TIRUNELVELI, SOUTH INDIA.

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INTRODUCTION

Lepidocephalichthys thermalis (Valenciennes) is a small, burrowing shallow-water tropical cobitid fish species of economic importance in Tamil Nadu. The fish is hardy, sensitive to minor changes in the environment and at the same time can adjust well under laboratory conditions. Despite its small size (upto 7.5 cm) it is considered to be very nutritious. These highly valued food fish are brought to the market in live condition and sold. Being a burrowing fish, *Lepidocephalichthys thermalis* use less energy for maintenance compared to other fish species.

Most of the bonny fishes reproduce in a cyclical or seasonal phenomenon and spawning occurs only during a particular stage of the reproductive cycle. Some of the fishes breed annually, and some breed at regular intervals throughout the year (Mollah, 1986). The annual reproductive cycle and spawning season of a fish can be easily determined by studying the reproductive and body indices such as gonadosomatic index (GSI) Nelson *et al*, (2018), condition factor (K), fecundity, and length at first maturity (Lp_{50}), hepatosomatic index (HSI), length and weight-frequency relationship and sex-ratio (Marcano *et al*. (2007), Sarkar *et al*. (2012), and Gupta and Banerjee (2013).

The weight and size of the gonads vary seasonally as well as at various stages of development which is used to study the sexual maturation in fishes (Delgado and Herrera, 1995; Wootton and Smith (2015)). In order to understand the energy allocation and energy stored in liver it is essential to study the seasonal ovarian and biochemical changes with regard to reproduction (Patil and Kulkarni, 1994).

Fecundity is an indicator of the reproductive potential of a fish (Maia *et al*., 2013). The estimation of fecundity and its relationship with the body measurements give an idea about the clutch size of the fish species (Hossain *et al*., 2012; Adebiyi, 2012; Wootton and Smith, 2015). Typically, increased fish size is associated with increased fecundity, and presumably smaller eggs increase the potential for high clutch size (Marcano *et al*. (2007), Sarkar *et al*. (2012), and Gupta and Banerjee (2013)). The highest fecundity indicates the spawning peak of the fish. Yet another parameter essential to identify the reproductive strategy of a fish species is size at first maturity. It indicates the maturity stage of the male and female fish, and is useful to ascertain the breeding months of the species (Marshall, 1979). Usually seasonal morphological studies are carried out to analyze the relationships between the reproductive features with relevance to seasonal sexual changes and individual variations (Katano, 1990). The sex-ratio of the population is analysed to find out whether it deviates significantly from the hypothetical distribution of 1:1 or not (Marcano *et al.* 2007). The preponderance and synchronized activity of females and males during spawning are also revealed through sex-ratio (Katano, 1990).

The onset of maturation and synchronization of spawning in tropical waters are associated with the monsoon rains, and the cyclic development of gonads is influenced by photoperiod and temperature (Marcano et al., 2007; Maia *et al.*,2013). Effects of seasonal cycles on fish, in particular on their reproductive biology, have been studied by many authors (Pen *et al*., 1993; Marcano et al. (2007), Sarkar et al. (2012), Gupta and Banerjee (2013)).

So in the present study, seasonal changes in various reproductive traits such as gonadosomatic index (GSI), condition factor, relative abundance of fish stages, fecundity, mean egg size (MES), size at first maturity, length and weight-frequency distribution of adult fish, and sex-ratio were analyzed to determine the exact spawning season and annual reproductive cycle of *Lepidocephalichthys thermalis* in relation to the seasonal climatic changes. Formerly the chosen fish was *Lepidocephalus thermalis* but now it is renamed as *Lepidocephalichthys thermalis.*

MATERIALS AND METHODS

Sample collection

Regularly fortnight samples of the freshwater loach *Lepidocephalichthys thermalis*, were collected for 18 months at random from a perennial pond fed by the Thamirabarani river at Tirunelveli (Tamil Nadu), 8.44^o N and 77.44^o E. Each sampling consisted of about 150 specimens and data such as total length, standard length, live body weight, stages of sexual maturity, and length and weight of the gonads were recorded after bringing them to wet lab in plastic buckets. The length measurements were taken to the nearest millimeter, and weight to the accuracy of 0.1 mg. Sex (male or female or immature) was determined by macroscopic observation of the gonads.

Gonadosomatic index (GSI)

The ovaries and the testes of each fish were dissected out from the body cavity and weighed to the accuracy of 0.1 mg using an electronic balance. Temporal patterns in gonad development were described using the gonadosomatic index (GSI) for each male and female *Lepidocephalichthys thermalis* according to the formula (Pen *et al*., 1993; Gupta and Banerjee, 2013),

$GSI = (W_1 / W_2)\ 100$

where, W_1 is the wet weight of the gonad in grams and W_2, the wet weight of fish in grams. Changes in GSI values plotted against the months of collection were used to determine the spawning season for each sex.

The temperature, photoperiod, and rainfall data collected daily were obtained from the meteorological unit of Tamil Nadu Public Works Department, Tirunelveli, and the mean ± S.D was calculated for each month and shown for easy understanding of the breeding season.

The criteria used to identify the stages of maturity were colour, shape and size of the ovary, diameters of the unspawned eggs and their general appearance, particularly the extent of yolk formation (Jacob and Nair, 1983; Nelson *et al,* (2018)), and to delineate the spawning season through a study of the gonadosomatic index and percentage occurrence of gonads in different stages of maturity during the different months of the year. The size and shape of the testes were also analysed and examined throughout the year, especially during the breeding months.

Condition factor

The condition factor or coefficient of condition (K) was calculated according to the equation by Tesch (1968),

$K = 100\ W / L^3$

where, W is the body weight in grams, and L is the standard length in cm.

Size at first maturity

The minimum size at onset of sexual maturity for each sex was estimated separately by plotting GSI values against the standard length, for all the fish collected within the spawning season, and validated by microscopic staging of gonadal tissue to determine the presence of vitellogenic oocytes or mature sperm (Ha and Kinzie, 1996).

Nevertheless, in order to evaluate the mean length of the fish at the onset of sexual maturity (Lp_{50}), fish were classified on the basis of gonad macroscopy as juveniles and adults. Lp_{50} was considered as the standard length class, which comprises 50% of the reproductively active population (Nelson *et al*, 2009; 2018).

Specimens were categorized into different standard length groups on the basis of their stages of maturity, to determine minimum size at first maturity. The length at which 50% of the specimens attain maturity is considered the length at first maturity (Kagwade, 1968).

Hepatosomatic index (HSI)

The hepatosomatic index (HSI) was calculated from the following formula,

HSI = (Weight of liver (g) X 100) / Body weight (g)

Fecundity

Fecundity was estimated from all the ovaries of mature fish during the breeding months. Approximately 10 mg of each ripe ovary was weighed to the nearest 0.1mg and the ova were loosened on a micro slide under the binocular microscope, and were counted to determine fecundity. Absolute fecundity (AF) is defined as the total number of mature eggs in a female prior to spawning (Hossain *et al.*, 2012; Adebiyi, 2012). It was estimated then, since the mean number of eggs 10 mg $^{-1}$ ovary and the total weight of each ovary were known (Hossain et al., 2012; Adebiyi, 2012). The relative fecundity (RF) was calculated as the number of eggs per gram wet weight (e.p.g) of the mature fish (Treasurer, 1990), by dividing the number of eggs per fish by its total body weight (Papageorgiou, 1979).

The absolute fecundity (AF) of fish was studied in relation to the total length, standard length, body weight of fish, ovary length and ovary weight.

A portion of the gravid ovary was teased apart, and the dimensions of mature ova were measured with an ocular micrometer to determine the diameter, using the following formula, Mean ovum diameter (mm) = (long axis length + short axis length) / 2.

Sex-ratio

The sex-ratio was calculated as the percentage occurrence of each sex in the total catch for every month, as well as in three different SL classes (Class I, 25-33 mm; Class-II, 34-42 mm; and Class III, 43-51 mm). Deviations in sex-ratios from an expected 1:1 ratio were tested using χ^2 goodness of fit (Ramanathan and Natarajan, 1979).

RESULTS

The seasonal variation in the gonadosomatic indices of *Lepidocephalichthys thermalis* was obtained by plotting the fortnightly GSI values against the time of collection, and was used to determine the spawning season for each sex. The mean GSI of female was 7.3 ± 3.2% in early October (2015), 15.4 ± 3.7 % in late October and 21.8±9.4% at the beginning of November just before ovulation and spawning. After spawning, in late November it was 5.9 ± 3.5 %. Then the GSI declined gradually to its basal level of 0.7 ± 0.4 % in March. In January, the mean GSI of 1.6 ± 0.8 % was relatively low because the ovaries were in spent and post-spawning condition. The fortnightly values of GSI of females for 18 months are shown in Fig. 1 a. During April and May, there was a slight increase in GSI, 3.9 ± 2.4 % and 3.1 ± 1.6% respectively marking a minor spawning peak. Among females, only 15.1% were fully mature and 17.8 % partially mature in April. But in May, 21.8 % of the females were fully mature, and moreover 7.2 % of the females were ready to spawn at the end of May.

The ovaries of *Lepidocephalichthys thermalis* were very small and immature in June through September. Then GSI increased steadily resulting in a value of 1.2±0.5 % at the end of September, and 7.2 ± 3.4 % in the early part of October (2016). At the end of October, it was 15.3 ± 5.5%, and November was the peak spawning period when the GSI was assessed to be the highest, 27.4 ± 5.2%. The maximum GSI of an individual female was 39.462%.

The mean GSI of male during every fortnight collections for 18 months is also shown in Fig. 1 b. The GSI was 0.7±0.3% in October, 0.8±0.2% in November, and 0.9±0.3% in December. Since there was not much difference in the values of fortnightly samples, the two values in each month are combined. Immediately after the spawning period, the GSI of male was reduced to 0.5±0.2% in January. During the non-breeding months of February, March (0.2±0.1%) and April, the GSI was very low, indicating that the testes had completely regressed. In late April and May the GSI of male was slightly higher (0.5 ± 0.2% and 0.5 ± 0.2% respectively), indicating a short minor breeding season. From July to September also the GSI was as low as 0.4 ± 0.2% indicating the pre-spawning period. The maximum GSI of an individual male was 1.5%.

The temperature, photoperiod and rainfall data are also plotted and shown in Fig.2. The highest range of female gonadosomatic index very much coincides with the highest rainfall of the year, the shortest light period and also the lowest temperature. So also in male, the peak level of GSI coincides with the highest rainfall during the year. The coincidence of the highest GSI value with the highest rainfall of the year indicates that the fish *Lepidocephalichthys thermalis* is a seasonal breeder, and its breeding season falls exactly between October and November. April and May are considered as the period when the fish breeds partially. Hence *Lepidocephalichthys thermalis* is considered as polytelic and iteroparous fish.

The GSI value of females showed a higher peak in October-November (major spawning season), and a minor peak in April-May (minor spawning season). The GSI of males showed the highest peak in October, November and December, but in the other months GSI was at a very low level.

The relationship between the standard length and GSI of males and females during the spawning season is shown in Fig.5a and 5b respectively. GSI value was found to increase with the increase in SL in both females and males.

Condition factor (K)

Seasonal changes in condition factor (percentage of ratio between the body weight and standard length cubed) of both male and female are shown in Fig. 3 (a) and (b) respectively. Condition factors of *L.thermalis* ranged from 0.70 to 1.45 for males, and from 0.62 to 1.64 for females. Seasonal changes in condition factor were similar between males and females. Condition factor increased from October through December, and then decreased upto March. Again there was a slight surge in April upto June, and thence declined upto September in both males and females.

Size at first maturity

By plotting the standard length (SL) against the GSI of female during the spawning season (Fig.4) it was observed that the smallest female fish with vitellogenic oocytes measured 32 mm SL. Females with ovaries containing mature oocytes had GSI values greater than 10.59. Based on these results, the size at first maturation for female fish was established in this study as 32 mm SL. Similarly the SL of males were plotted against their respective GSI (Fig.5), and the smallest mature male fish had a length of 29mm.

The results presented in Table 1 show that all female specimens of *L.thermalis* below 30 mm SL are immature. Maturity sets in from the 31 mm stage onwards. The length at which 50% of the specimens attain maturity, considered the length at first maturity, lies between 31 and 35 mm SL.

The results presented in Table 2 show that all male specimens below 25 mm SL are immature. Maturity has started from 26mm SL onwards. The length at which 50% of the male specimens attain maturity, being the length at first maturity, lies between 26 and 30 mm SL (Fig.6).

By another method, the mean size at onset of sexual maturity (Lp_{50}) in female *L.thermalis* is estimated as 32 mm SL in October and in male 27 mm SL in October (Fig.6). The smallest mature female was 28 mm SL and the smallest mature male was 32 mm SL. The smallest gravid female was 34mm in SL, and the smallest ripe male was 33mm in SL. The females were larger than the males.

Fecundity
During the spawning month (November), fecundity (Absolute Fecundity) of *L.thermalis* ranged from 182 eggs in a 21 mg ovary for fish of 41 mm total length and 339 mg of body weight, to 3426 eggs in a 372 mg ovary for a fish of 59 mm total length (48 mm SL) and 1496 mg of body weight. The maximum number of eggs per gm body weight (Relative Fecundity) of *L.thermalis* was 3742, and per gram ovary weight 9046. The absolute fecundity and the relative fecundity are shown in Table 3. The mean diameter of a mature ovum was 0.55±0.01 mm.

Hepatosomatic index (HSI)
The glycogen content in *L.thermalis* appears to show different relationship in ovary and liver, wherein the highest content was found in the ovary and the lowest in the liver during the gravid phase of ovary. The lowest glycogen level in the ovary and the highest level in the liver were, however, evident during the spent phase and recovering phase. In *L.thermalis*, a higher protein level was found in the developing and gravid ovaries, and a lower level in the post-spawning (January-March) and pre-spawning (July-September) ovaries.

Ovarian and hepatic lipids also showed an inverse relationship. The maximum amount of lipid was found in the gravid and spawning ovaries and a minimum during the resting phase. Lipid was found to increase gradually in the ovaries of *L.thermalis* during the maturing and developing phase, and attained the highest level in the gravid phase. Then it was followed by a continuous decline through subsequent phases of the ovarian cycle, to attain very low levels during the spent period.

Sex-ratio
The percentage frequencies of males and females were 45.32 / 54.68 in *L.thermalis*. This difference was not statistically significant (P>0.05), indicating that the overall sex-ratio is 1:1. The sex composition of random samples examined every month shows that the two sexes are present in more or less equal number, since the variation in the number between males and females is negligible during most months. The monthly percentage occurrence of males and females and the sex-ratio are shown in Table 4. In January and March the ratio was almost 1:1.5. But in December and February, the females were found as double the number of males in the capture, *i.e.,* 1:2.The sex-ratio was 1:1 in the SL classes I and II, but in class III it varies widely in all the months (Table 5).

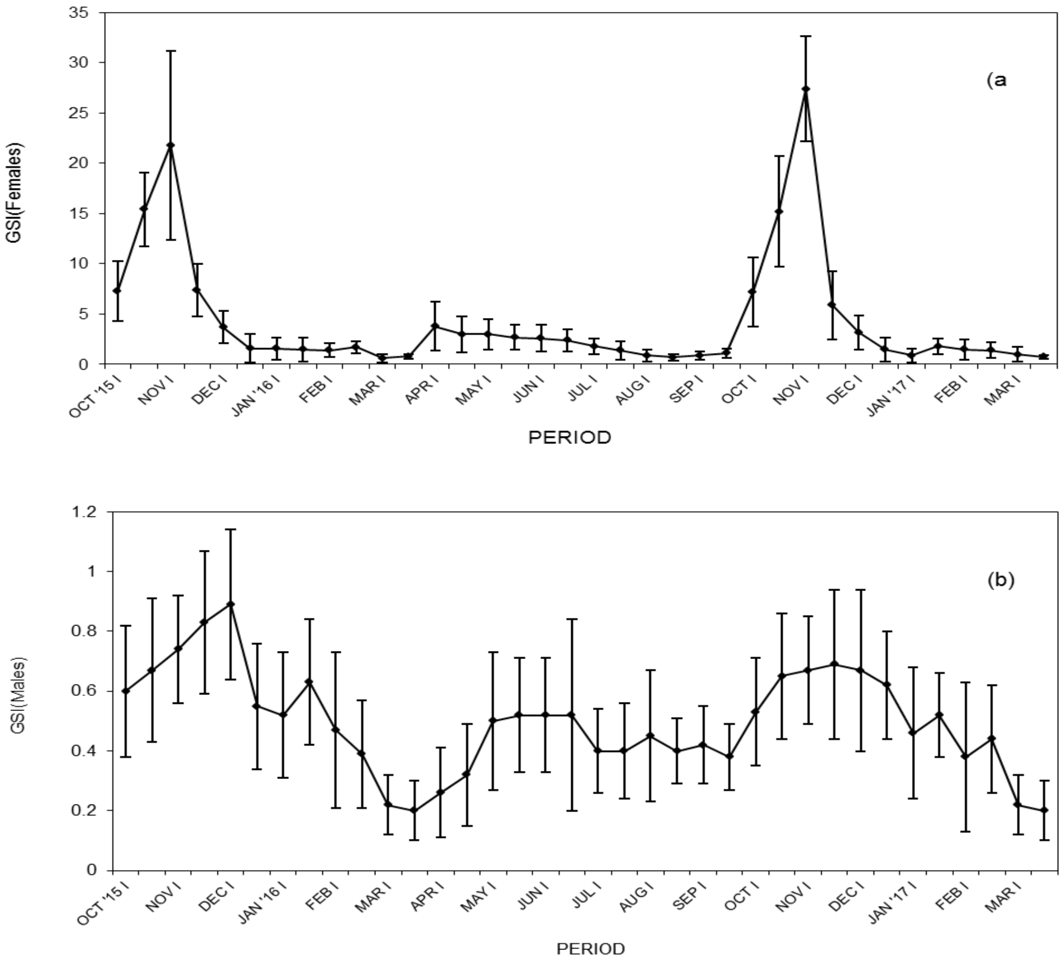

Figure 1: L. thermalis: Mean gonadosomatic index (GSI ± SE) of females (a) and males (b) during their annual reproductive cycle.

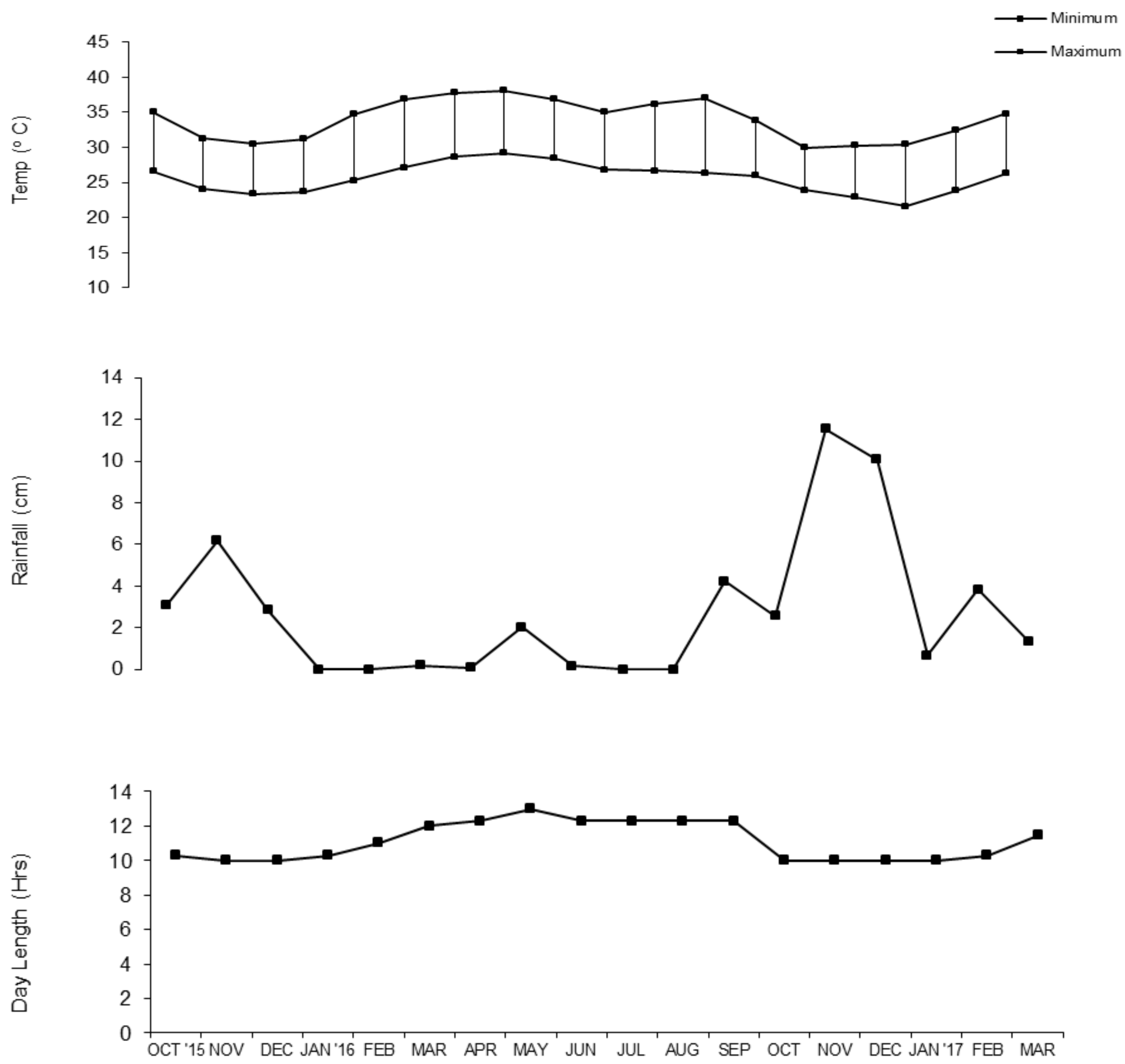

Figure 2: Seasonal variations of temperature, rainfall and day-length at Palayamkottai.

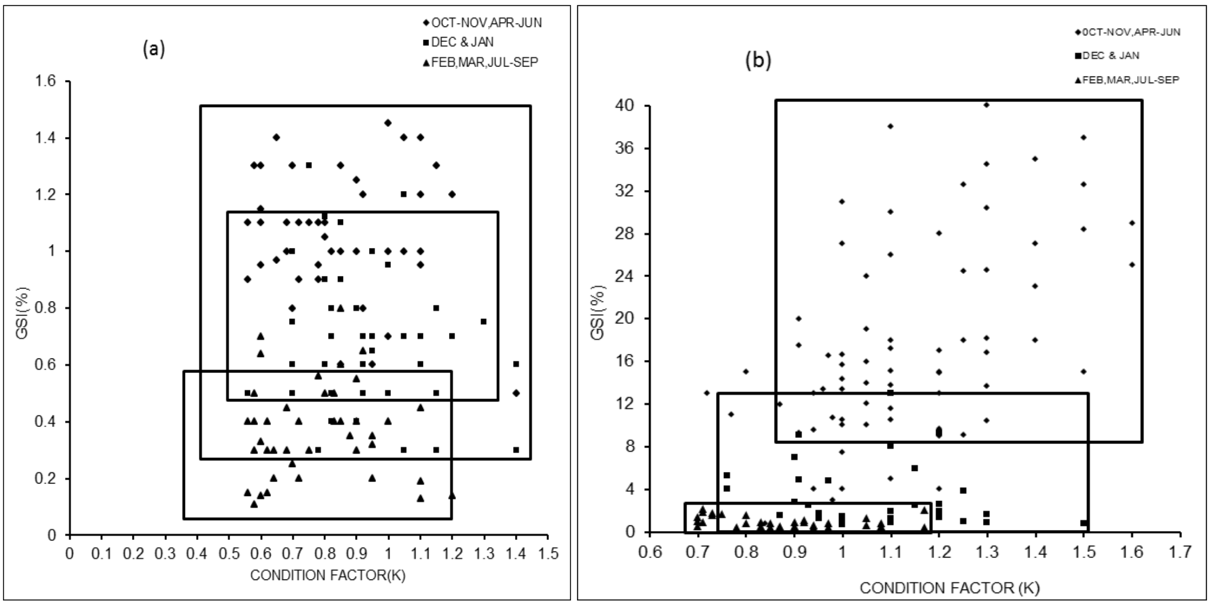

Figure-4 ***L.thermalis*****: Relationship between GSI and Condition factor (K) of (a) males and (b) females in different seasons.**

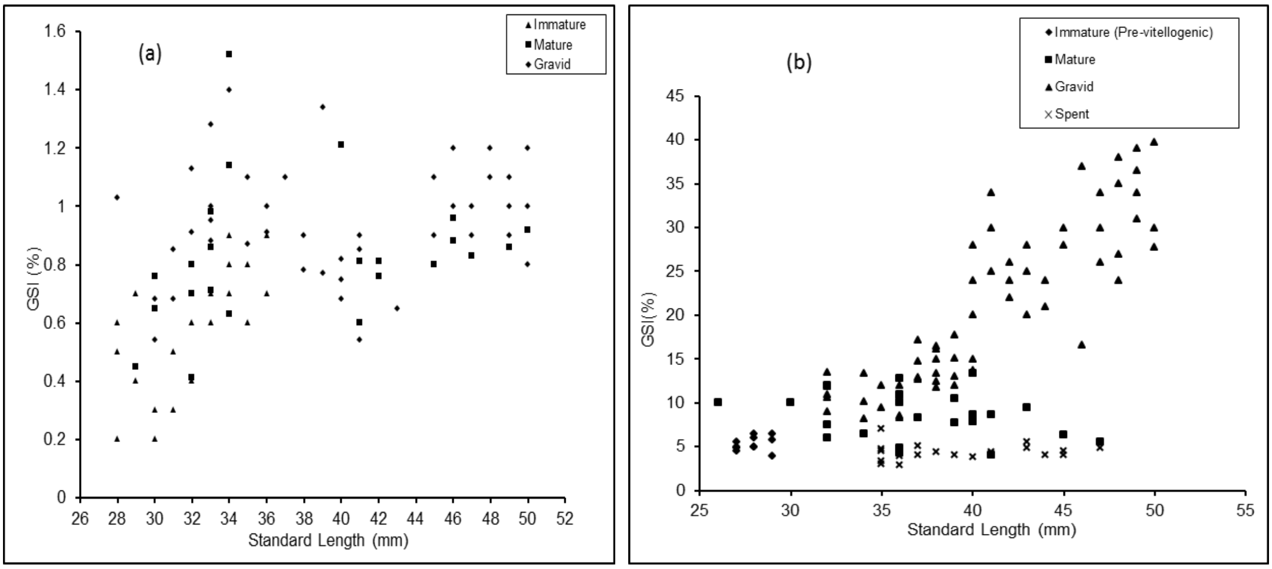

Figure 5: L. thermalis: Relationship between GSI and standard length (mm) of males (a) and females (b) in the peak spawning season.

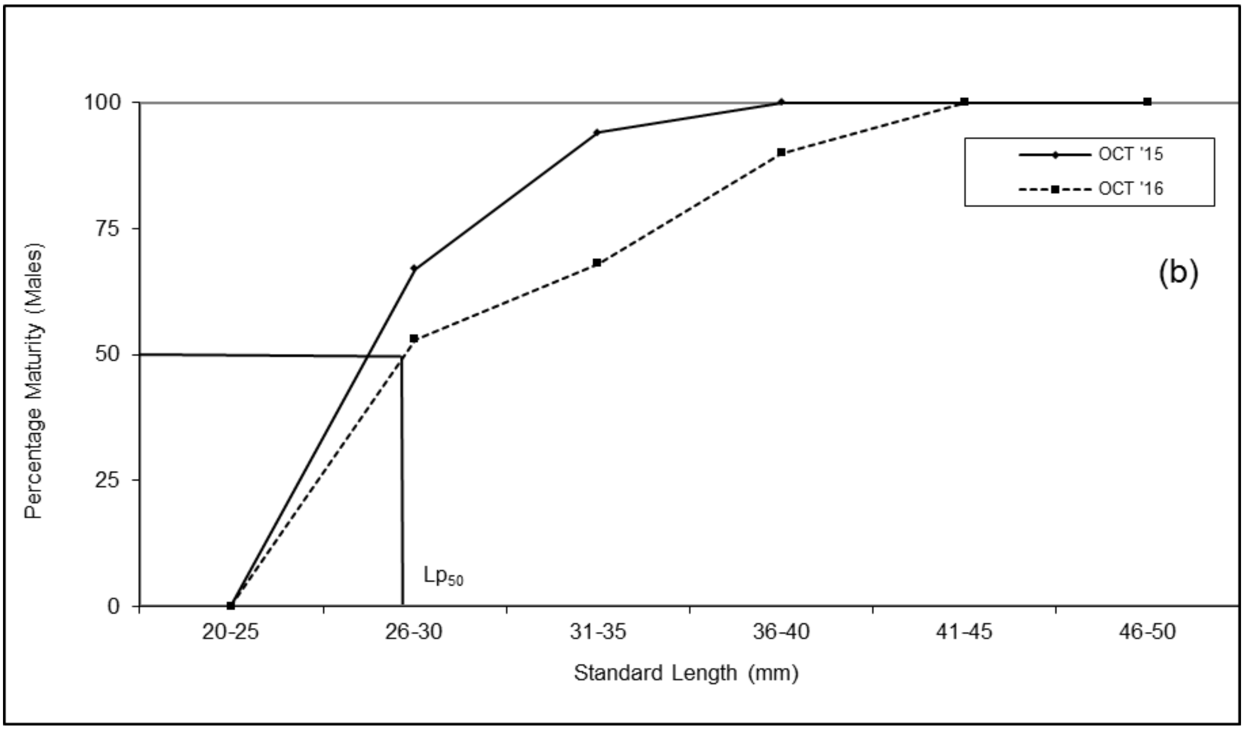

Figure 6: *L. thermalis*: Percentage of maturity of adult females and males indicating 50% length at maturity.

Table 1: *L.thermalis*: Percentage occurrence of females in different stages of maturation at various size groups before the spawning month*.

Standard Length (mm)	Immature (%)	Maturing (%)	Mature (%)
Early October ' 15			
26-30	84.62	15.38	---
31-35	41.05	48.42	10.53
36-40	15.38	61.54	23.08
41-45	---	40.00	60.00
46-50	---	25.00	75.00
Late October ' 15			
26-30	62.85	37.15	---
31-35	16.67	50.00	33.33

36-40	---	18.75	81.25
41-45	---	17.65	82.35
46-50	---	---	100.00
Early October ' 16			
26-30	100.00	---	---
31-35	33.33	51.85	14.82
36-40	15.15	24.24	60.61
41-45	---	42.86	57.14
46-50	---	66.67	33.33
Late October ' 16			
26-30	60.00	40.00	---
31-35	10.00	50.00	40.00
36-40	---	28.57	71.43
41-45	---	26.32	73.68
46-50	---	---	100.00
* During the spawning month (November), Maturity is almost 100% from 31 mm SL onwards .			

Table 2: *L.thermalis* Percentage occurrence of males in different stages of maturation at various size groups before the spawning season.

Standard length (mm)	Immature * (%)	Maturing ** (%)	Mature *** (%)
October ' 15	---	---	---
20-25	100.00	---	---
26-30	33.33	66.67	---
31-35	6.25	78.12	15.63
36-40	---	70.59	29.41
41-45	---	---	100.00
October ' 16			
20-25	100.00	---	---
26-30	47.37	52.63	---
31-35	31.79	59.25	8.96
36-40	9.52	52.38	38.1
41-45	---	---	100.00
46-50	---	---	100.00
November '15			
20-25	85.36	14.64	---
26-30	20.00	80.00	---
31-35	---	47.54	52.46
36-40	---	16.67	83.33
41-45	---	---	100.00
November '16			
20-25	80.00	20	---
26-30	12.14	71.67	16.09
31-35	10.64	51.06	38.3
36-40	---	47.69	52.31
41-45	---	7.14	92.86

* The immature are considered as males without testes or with testes upto 1 mg weight.
** Maturing males have testes upto 2.5 mg weight and
*** The mature with testes of above 2.5 mg weight.
The maximum size of testes was 19 mm long and of 10mg net weight.

Table 3: *L.thermalis* : Fecundity as a function of size during the spawning seasons. The values are expressed in mean ± S.D.

Month	SL (mm)	N	Live wt.of fish (mg)	Ovary length (mm)	Ovary weight (mg)	GSI	OI@	Absolute fecundity *	Relative Fecundity**	No.of ova / gm. Ovary	Ova diameter (mm)
Oct-15	28 ± 3	18	214.5 ± 29.56	9.81 ± 2.25	10.38 ± 4.53	4.93 ± 2.22	0.011	36 ± 33	173 ± 168	3045 ± 1217	0.45
	32 ± 2	27	348.6 ± 43.64	12.62 ± 4.11	22.79 ± 10.78	6.28 ± 2.61	0.025	152 ± 118	390 ± 274	5713 ± 2171	0.48
	36 ± 3	33	468.5 ± 56.48	13.85 ± 3.36	31.39 ± 14.17	6.52 ± 2.64	0.034	234 ± 149	469 ± 280	6414 ± 2177	0.50
	40 ± 2	29	651.6 ± 63.3	14.26 ± 2.84	43.57 ± 11.24	6.95 ± 1.83	0.033	391 ± 124	581 ± 176	7241 ± 1759	0.55
	44 ± 3	25	661.3 ± 76.4	14.63 ± 2.51	48.66 ± 15.13	7.08 ± 2.25	0.051	672 ± 295	842 ± 268	7397 ± 1057	0.55
Oct-16	28 ± 3	26	210.4 ± 38.2	7.42 ± 2.15	8.57 ± 3.62	3.81 ± 2.11	0.012	32 ± 15	122 ± 18	3296 ± 514	0.45
	32 ± 2	39	334.8 ± 50.2	11.25 ± 2.86	24.85 ± 12.96	6.88 ± 3.4	0.023	245 ± 139	672 ± 384	7118 ± 2637	0.50
	36 ± 3	41	449.2 ± 63.6	11.68 ± 1.75	27.52 ± 10.6	5.81 ± 1.96	0.032	248 ± 116	487 ± 205	7028 ± 1670	0.52
	40 ± 2	24	654.2 ± 113.3	12.45 ± 2.60	51.68 ± 21.46	7.76 ± 2.53	0.036	462 ± 218	683 ± 271	8268 ± 1083	0.55
	44 ± 3	20	795.8 ± 252.3	18.66 ± 3.57	106.4 ± 86.52	10.81 ± 6.14	0.058	991 ± 827	813 ± 320	8741 ± 339	0.55
	48 ± 2	15	1388.7 ± 116.8	25.2 ± 4.12	270.5 ± 57.86	19.3 ± 2.43	0.110	2548 ± 552	1818 ± 268	9406 ± 318	0.55
Nov '15	28 ± 3	12	212.6 ± 53.7	9.58 ± 2.47	11.26 ± 2.61	4.78 ± 2.86	0.031	67 ± 21	286 ± 165	6014 ± 1773	0.48
	32 ± 2	39	333.8 ± 63.4	13.17 ± 1.22	29.47 ± 16.59	8.29 ± 4.44	0.038	293 ± 98	837 ± 302	7854 ± 2392	0.49
	36 ± 3	110	467.6 ± 94.4	15.46 ± 2.31	58.93 ± 21.92	9.46 ± 4.15	0.048	488 ± 187	874 ± 383	8118 ± 1955	0.52
	40 ± 2	25	637.2 ± 93.9	14.85 ± 2.92	66.05 ± 24.25	10.3 ± 3.61	0.055	626 ± 286	936 ± 392	8125 ± 1987	0.55
	44 ± 3	18	682.7 ± 134.7	15.74 ± 1.56	102.6 ± 30.35	11.72 ± 4.89	0.069	825 ± 255	1127 ± 423	9512 ± 552	0.55
Nov '16	28 ± 3	15	205.5 ± 36.3	8.2 ± 2.45	9.75 ± 1.66	2.55 ± 0.56	0.010	31 ± 16	149 ± 68	3892 ± 265	0.45
	32 ± 2	20	368.7 ± 72.9	12.32 ± 2.44	23.33 ± 12.92	6.56 ± 3.38	0.026	264 ± 74	561 ± 262	7949 ± 861	0.50
	36 ± 3	77	580.7 ± 99.1	14.86 ± 2.12	102.9 ± 63.38	16.26 ± 8.36	0.092	823 ± 657	1399 ± 885	8107 ± 1377	0.50
	40 ± 2	36	774.8 ± 121.7	17.51 ± 2.29	198.9 ± 79.19	25.45 ± 9.34	0.131	1821 ± 777	2194 ± 931	9007 ± 623	0.55
	44 ± 3	29	912.3 ± 113.1	18.25 ± 3.37	204.8 ± 76.31	21.95 ± 7.18	0.144	1918 ± 768	2058 ± 741	9172 ± 599	0.55
* Total number of mature ova per individual fish					** Number of ova per gm body weight				@OI - Ovarian index		

Table 4: *L.thermalis*: Annual cycle of sex-ratio.

Month	No.of fish examined	Males %	Females %	Sex-ratio
OCT '15	273	45.05	54.95	1:1.22
NOV	354	48.87	51.13	1:1.05
DEC	293	33.79	66.21	1:1.96
JAN '16	303	39.6	60.4	1:1.53
FEB	303	36.63	63.37	1:1.73
MAR	282	38.3	61.7	1:1.61
APR	276	46.74	53.26	1:1.14
MAY	304	51.32	48.68	1:0.95
JUN	258	50.39	49.61	1:0.98
JUL	304	49.67	50.33	1:1.01
AUG	292	47.26	52.74	1:1.12
SEP	295	47.46	52.54	1:1.11
OCT	272	47.43	52.57	1:1.11
NOV	302	51.32	48.68	1:0.95
DEC	301	47.51	52.49	1:1.10
JAN '17	299	45.15	54.85	1:1.21
FEB	297	47.81	52.19	1:1.09
MAR	278	41.37	58.63	1:1.42
Average		**45.32**	**54.68**	**1:1.21**

Table 5: *L.thermalis*: Relationship between standard length classes and sex-ratio in different months.

Month	Standard length (mm)		
	25-33	**34-42**	**43-51**
OCT '15	1:1.04	1:1.48	0.3
NOV	1:1.06	1:1.03	1:2
DEC	1:1.84	1:2.05	1:0
JAN '16	1:1.56	1:1.6	1:7.5
FEB	1:1.77	1:1.59	0:3
MAR	1:1.46	1:1.18	1:0
APR	1:1.33	1:0.93	1:11
MAY	1:0.84	1:0.96	0:1
JUN	1:0.83	1:1.07	0:1
JUL	1:1.98	1:1.29	0
AUG	1:1.11	1:1.09	0
SEP	1:1.74	1:0.58	1:0
OCT	1:1.13	1:1.04	1:1.18
NOV	1:0.48	1:0.99	1:5
DEC	1:1.27	1:1.00	0
JAN '17	1:1.11	1:1.29	1:0.33
FEB	1:1.1	1:1.29	1:1.5
MAR	1:1.48	1:1.38	2:0.00

DISCUSSION

The results of monthly changes of GSI values show that *Lepidocephalichthys thermalis* has one major spawning peak in North-East monsoon period (October-November) and a minor spawning peak coinciding with South-West monsoon (April-June). Condition factor was positively correlated with GSI values (Katano, 1990; Saleh and Ali (2009); Maia *et al.*,2013; Nelson *et al*, (2018)). Sexual maturity and spawning have been shown to have remarkable bearing on the condition factor of the fishes as reported by Saleh and Ali (2009) which is also observed in *L. thermalis*. Therefore it may be said that the changes observed in the condition factor of *L.thermalis* reflect the combination of the changes in the feeding and reproductive cycle. The highest condition factor coincided with the highest GSI values during the spawning season in *L. thermalis*, as observed in *Mystus gulio* by Pandian (1970). The condition factor and GSI showed a parallel pattern throughout the annual cycle in adults in *L thermalis*. A similar result was reported in fishes like *Barbus sclateri* by Encina and Lorencio, 1997; *Epinephelus guaza* by Saleh and Ali (2009).

The relationship between body length, condition factor and GSI value differed greatly between males and females. Body size was positively correlated with the GSI value except in the post-spawning period, as shown for other fish species (Azevedo *et al*., 2017; Nelson *et al*, (2018)).

It is assumed that the period during which GSI is the highest corresponds to the breeding season of the fish. (Stoumboudi *et al.*,1993; Pasey *et al*., 2001; Wootton and Smith, 2015).

The present study demonstrates a major and a minor spawning season for *L.thermalis.* This is strongly supported by the trend in monthly GSI, which shows a major peak between October and November, and a small peak in April-May. Similarly *Cyprinus carpio* is a seasonal breeder with a biannual pattern of reproduction, *i.e.*, during the monsoon (June-August) and during winter (December-February) in the plains of West Bengal, India (Alikunhi, 1966; Nelson *et al*., 2010 in *Oncorhynchus mykiss*). Spawning in *L.thermalis* appears to occur from the middle of November, since spent individuals were caught at the end of November. A similar pattern of reproductive cycle was also reported in *Labeo bata* (Siddiqui *et al.*, 1976) in Indian rivers, lakes and reservoirs.

The fact that the fishes breed in the monsoon months is supported by a number of works. Padmanabhan (1955) stated that *Macropodus cupanus* is a perennial spawner with a noticeable increase in the frequency of spawning during the monsoon months. Chellappa *et al.,* (2009) reported fish do breed during the monsoon period in sic American Chichlid fishes. As far as the onset of maturation and synchronization of spawning are concerned, endogenous factors are the most important (Chellappa and Costa (2003); Chellappa *et al., 2003;* Chellappa and Chellappa (2004)), in tropical waters climatic changes associated with the monsoon rains may also stimulate spawning as observed in the present study in *L. thermalis*.

The liver is considered as one of the important organs of fish, which stores glycogen, protein, and lipid for utilization during the reproductive cycle (Hismayasari *et al.,* (2015). The highest glycogen level in the ovary and the lowest in the liver during gravid phase, and the reverse condition during the spent phase/resting period were also found in *Notopterus notopterus* (Patil and Kulkarni, 1994). Low level of hepatic protein during gravid phase and highest hepatic level during the spent period are probably indicative of the role of liver in the contribution of protein for vitellogenesis in the ovary, as evidenced by Piska and Waghray (1989). High protein values were found in the mature gonads, which decreased gradually in the post-spawning period (December), when protein was utilized for spawning. This was also observed in *Amblypharyngodon mola* (Piska and Waghray, 1989), and *Notopterus notopterus* (Patil and Kulkarni, 1994).

The decline in the hepatic lipid during spawning may be due to fat mobilization by high levels of estrogen and testosterone (Saleh and Ali, 2009). These observations clearly suggest the existence of relationships of various organic reserves of liver and ovary with regard to oocyte growth in *L.thermalis.*

The size at first reproduction has an important role in the understanding of the life-history adopted by a species during its evolution (Nelson *et al.*, 2009; 2018). Nonetheless, it is accepted that the optimium size for the first maturation depends upon many factors, including the relative allocation of food energy between somatic and gonad growth (Mazzoni and Caramaschi; 1995). In *L.thermalis* the two sexes appear to mature at different sizes, that is 26mm in males, and 31mm in females. In a number fish species the males and females mature at different sizes (White *et al.*, 2011; Farell *et al.*, 2012).

The phenomenon of occurrence of males and females in approximately the same proportion has been reported in a number of fish species. In *L.thermalis* the sex-ratio did not deviate significantly from the hypothetical distribution of 1:1. David *et al.,* (2015) reported sex ratio of 1:1 in *Gambusia affinis*. The overall sex-ratio approximates unity in five *Barbus* species, but in *B.vittatus* it deviates from unity (De Silva *et al*., 1985). Vicentini and Araujo, (2003) reported males slightly outnumbered females in *Micropogonias furnieri* i.e 1.3:1.0 sex ratio (Hussey *et al.*,2012). With regard to the size class in *L.thermalis*, the sex-ratio of SL class-I and II was 1:1, but in SL class III it varies widely in all months as reported for *Barbus vittatus*, *B.cumingi* and *B.bimaculatus* (De Silva *et al*., 1985). There was slight deviation in the overall sex ratio from the expected 1:1 in *L. thermalis*.

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