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Maturity and Spawning Season of Burmese Loach, *Lepidocephalichthys berdmorei* (Blyth–1860)

Makamguang Kamei, Sukham Munilkumar, Ch. Basudha, Subrata Dasgupta, Paramita Banerjee Sawant, Prakash Sharma

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ABSTRACT

Lepidocephalichthys berdmorei (Blyth 1758) is an important fish species that has got both food as well as ornamental value. This fish is mainly harvested from wild resources. Due to tremendous fishing pressure, the fish population has dwindled significantly. It is high time that efforts may be made to study the life traits of the species to initiate breeding programs to move toward aquaculture. A study was conducted to determine the maturity and breeding season in its natural habitat in Manipur, India. It was observed that the maximum value of the Gonadosomatic Index was recorded in March (15.16 ± 0.61) and the minimum in October during the cold season (0.58 ± 0.12). The result was further supported by the histological studies, which showed the presence of previtellogenic oocytes and oocytes in various stages of vitellogenesis during March. They were filled mainly with previtellogenic oocytes but also with many postovulatory follicles and oocytes at various stages of atresia from August onwards.

Keywords Maturity, Spawning, Gonadosomatic index, *Lepidocephalichthys berdmorei.*

INTRODUCTION

Lepidocephalichthys berdmorei (Blyth 1758), belongs to the family Cobitidae (order: Cypriniformes), is a freshwater species of good demand in the ornamental fish industry, and a high-value local food fish of North-eastern states. The fish can thrive in moderate currents and hill streams with sandy and rocky bottoms and lakes with clear water and the low-lying depression of paddy fields (Rahman 2005). They are distributed in Asia: Bangladesh (Rahman 2005), India and Myanmar (Talwar and Jhingran 1991), peninsular Thailand, and Mekong basins (Kottelat 1992). They are also found native to the North-Eastern region of India, mainly in the water system drains to the basins of the Chindwin and Barak Rivers of Manipur. Loaches are generally found in many freshwater areas, but most predominantly in ditches, rice paddy fields, streams, and places with mud and soft earth (Fujimoto *et al.* 2008). *Misgurnus anguillicaudatus* loach was

Makamguang Kamei[1], Sukham Munilkumar[1]*, Ch. Basudha[2], Subrata Dasgupta[3], Paramita Banerjee Sawant[1], Prakash Sharma[4]

[1]PhD Scholar, ICAR-Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Mumbai 400061, India

[1]Principal Scientist, ICAR-Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Mumbai 400061, India

[2]Senior Scientist, ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal 795004, India

[3]Principal Scientist, ICAR-Central Institute of Fisheries Education, Kolkata Center, 32 GN Block, Sec V, Salt Lake City, Kolkata 700091, India

[4]Senior Scientist, ICAR-Directorate of Coldwater Fisheries Research, Anusandhan Bhawan, Industrial Area, Bhimtal 263136, India

Email : munilkumars@cife.edu.in

*Corresponding author

farmed in earth ditches to concrete ditches in Japan (Saitoh *et al.* 1989, Tanaka 1999). *M. anguillicaudatus* was widely cultured in the Jiangsu province of China, bringing great profit to their economy in recent years (Zhang *et al.* 2012).

Lepidocephalichthys berdmorei, commonly known as *Ngakijou* locally in Manipur, populations vary in color pattern and size depending on location and habitat. The genus *Lepidocephalichthys* includes 20 species that are distributed in different parts of the world. It has nocturnal and omnivorous feeding habits, living in ditches and fine and soft gravel. These fishes have good value in ornamental trade and highly esteemed value in traditional medicinal purposes and as nutritious food for local consumers (Milton *et al.* 2018). The fish is of immense economic and cultural importance in South Asian countries like Myanmar, India, and Bangladesh.

In Manipur, India, it is popularly well-known as "Ngakijou."It has good commercial, and food value as the price of live fish is worth Rs1000/kg in the local market. Previously this fish species was abundant in the rivers, streams, and seasonal low-lying floodplains. Due to the increased market demand, the fish has been over exploited, destroying its natural habitat and causing a vast depletion of the wild population. The insecticides used in paddy fields, upland rivers siltation, stones and sand lifting from the river beds, and building of flood control dams, changes in ecology, over-exploitation, destruction of breeding ground, and lack of proper management result in the loss of habitat. However, before making any conservation strategy and practical implementations, the life traits of the species need to be studied thoroughly.

The Gonadosomatic Index (GSI) assesses a fish's reproductive season and condition (Arruda *et al.* 1993). Many researchers have considered the GSI value of different fish species for determining the spawning season and frequency (Islam *et al.* 2008, Ghaffari *et al.* 2011, Kingdom and Allison 2011, Jan *et al.* 2014). The type of spawning and the period of the reproductive season is also determined by the histological structure of an ovary during the reproductive period (Rinchard and Kestemont 1996). Juchno and Boron (2006) conducted histological studies to determine the age, reproduction, and fecundity of the spined loach *Cobitis taenia* L. (Pisces, Cobitidae).

Fish fauna in the northeastern states of India, including Manipur, is highly diverse; however, information on their distribution, biological life traits, and growth are scarce (Munilkumar *et al.* 2021). Hence, the present study was conducted to determine the spawning season of *L. berdmorei*, which will fill the existing knowledge gap and help adopt a captive maturation and breeding program for conservation.

MATERIALS AND METHODS

Study area

Fishes were caught using traps and Chinese dip nets against the water current of canals and irrigation drainage system from the stream passing along the field from January 2019 to December 2019. The fish was identified as per Arunkumar (2000), having 4–7 V-shaped bands at the rounded caudal fin, relatively long anterior rostral barbels, and 2 lobules at the mouth. The dorsal fin is inserted behind the origin of the pelvic fin, usually a mid-lateral series of 10–18 spots on mid-lateral lines. The fishes were collected from different districts of Manipur, such as Phayeng (24º51'02.2" N and 93º49'22.5" E) in Imphal east district, Nambol (24º40'50.5" N and 93º46'22.8" E) in Bishnupur district, Khoupum Dam (24º39'40.0" N and 93º32'30.4" E) and Iyei river (24º51'12.0" N and 93º37'40.7" E) in Noney district, Barak River, Karong (25º18'32.9" N and 94º09'00.8" E) in Senapati district and Komlathabi (24º25'19.9" N and 94º00'33.0" E) in Chandel district.

Gonadosomatic (GSI and Gastrosomatic Indices (GaSI) estimation

To determine the GSI and GaSI, only female specimens were studied. Females and males were separated by examining their gonads. Immediately after collection, a subsample of fish was cut open and fixed in a 10% neutrally buffered formalin solution. In the laboratory, preserved fish were weighed and the ovaries were removed for histological analysis. Using the formula of Afonso-Dias *et al.* (2005), GSI

was calculated, and GaSI was calculated as follows.

GSI = (Weight of gonad/Weight of fish) × 100

GaSI = (Weight of stomach/Weight of fish) ×100

Histological analysis

The histological preparations of gonads were made according to Stevens and Bancroft (1990). Once the samples were collected, these were fixed the tissues in 10% neutral buffered formalin were taken and passed through the process of dehydration, blocking, sectioning, staining, and observation. The tissues were dehydrated in 90% alcohol for an hour and then three times in absolute alcohol for 45 minutes each separately. The tissues were cleared twice in xylene for 30 minutes and embedded in paraffin thrice for 45 minutes. The tissues were then blocked and allowed to cool. Tissue sections of 5 µm thick were cut on a rotary microtome and mounted on a microscopic slide. The sections mounted were dewaxed in xylene and dehydrated serially in alcohol. The slides were then washed in tap water for 1 minute, stained in haematoxylin for 12 minutes, washed in running tap water, dipped in 2% acid alcohol, and washed in running tap water for water substitution. The sections were dehydrated through 50%, 70%, and 90% alcohol for 2 minutes, stained in eosin for 4 minutes, and dipped in absolute alcohol for 1 minute. The stained sections were cleared in xylene for 5 minutes and mounted with DPX. The DPX-mounted slides were observed under a phase contrast microscope (RXLr-5-50 Radical Instruments equipped with Jenoptik imaging software). Germ cells at different developmental stages of both males and females were identified according to (Ciji *et al.* 2021, Grier *et al.* 2009), and the classification of gonadal phases is based on (Brown-Peterson *et al.* 2011).

RESULTS AND DISCUSSION

The Physico-chemical parameters of water from the natural habitat of the fish are shown in the Table 1. where the fish adapt to its environment.

Identifying the spawning chronologies of riverine fishes is an important component in determining basic life-history information of fish (Schlosser 1990). GSI, a ratio of gonad weight to body weight, is a commonly used indicator of reproductive periods. The reliability of GSI in determining reproductive status has varied among species with different reproductive strategies (Brewer *et al.* 2008).

Table 1. Physico-chemical properties of water from natural habitat (different rivers of Manipur, India) of *Lepidocephalichthys berdmorei.*

Parameters	Mean ± SD
Temperature (°C)	20.67±4.64
pH	6.76±0.79
Dissolve oxygen (ppm)	5.70±1.09
Alkalinity (ppm)	24.92±5.95
Total hardness (ppm)	71.92±9.27
Ammonium-nitrogen (ppm)	0.06±0.02
Nitrite-nitrogen (ppm)	0.09 ±0.07
Nitrate-nitrogen (ppm)	0.12±0.08
Phosphate (ppm)	0.52±0.46

Only female fishes were subjected to the determination of GSI (See Fig.1). The GSI value increased gradually from January to March. The maximum value of GSI was recorded in March (15.16 ± 0.61) and the minimum in October during the cold season (0.58

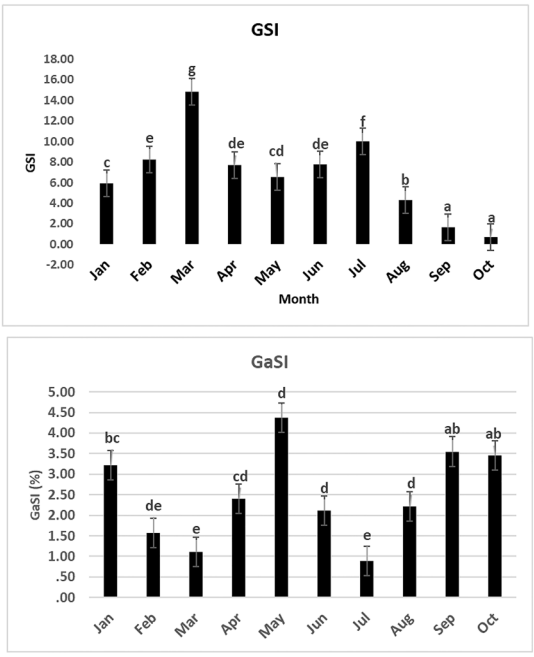

Fig. 1. Gonadosomatic (GSI) and gastrosomatic (GaSI) indices of *Lepidocephalichthys berdmorei* in different months, collected from different rivers of Manipur, India.

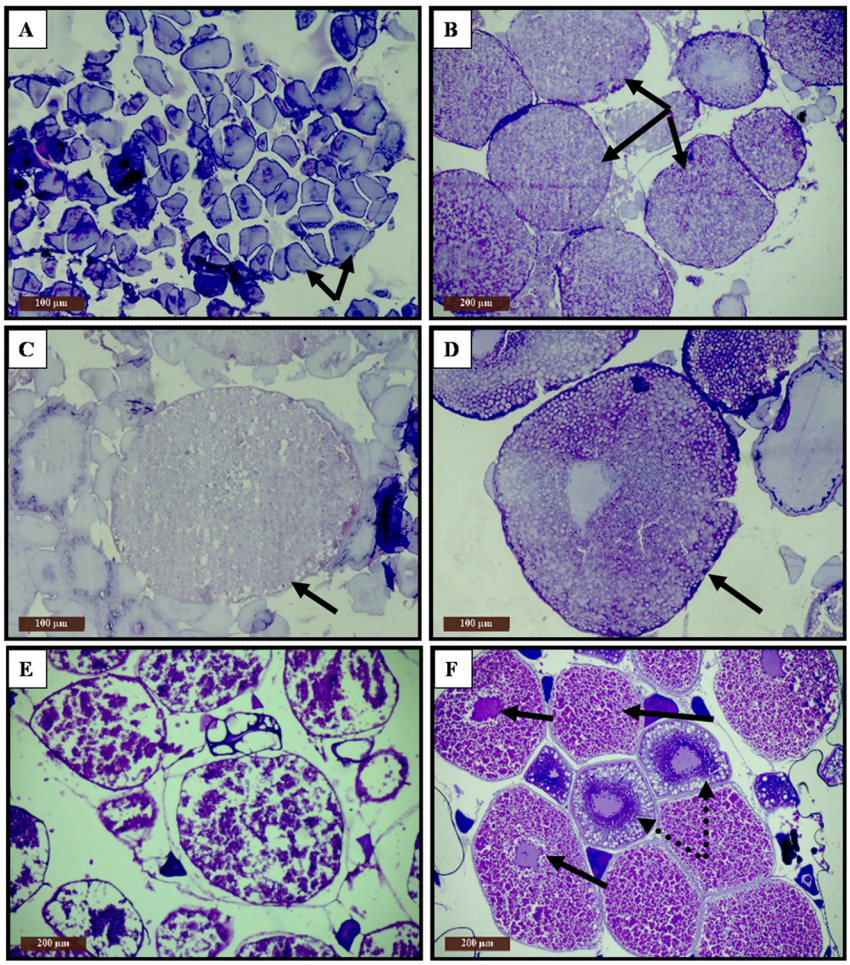

Fig. 2. Photomicrographs-A represents the ovary of *Lepidocephalichthys berdmorei* (sampled in February) in the regressed phase, indicated by follicles in the primary growth stage (perinucleolar oocytes; solid arrows). Photomicrograph-B represents another ovary (sampled in February) in the spawning capable phase, indicated by fully matured post vitellogenic oocytes with no germinal vesicle (i.e., after the breakdown of germinal vesicle; arrows). Photomicrographs-C represents the ovary (sampled in March) in the spawning phase indicated by matured oocytes (arrow) and some primary growth oocytes. Photomicrographs-D represents the ovary (sampled in April) in the developing phase, indicated by a few late vitellogenic oocytes (arrows). Photomicrographs-E and F represent the ovary (sampled in May) in the developing phase indicated by matured follicles with late vitellogenic oocytes. Arrows indicate migrating germinal vesicle (eccentric) in maturing oocytes. Immature follicles in cortical alveolar stages have centric germinal vesicles (indicated by broken arrows).

± 0.12) (Fig. 1). After March, the GSI value decreased gradually, increasing again till July and gradually decreasing in October. This suggests that *L. berdmorei* reached a breeding condition peak in March and showed a synchronous breeding behavior in July. Also, most males were found in oozing condition, with sharp barbels during the same month. Similar traits were observed in *Misgurnus anguillicaudatus,* which in their native range, spawn multiple times per year during a spawning season lasting from mid-April until mid-October (Yamamoto *et al.* 2000). Generally, seasonal gonadal recruitment is classified following either macroscopic or microscopic approaches. In the former system, external gonadal appearance, their size, and GSI are used, and in the latter histological assessment of germ cell development is used to classify reproductive phases (West 1990, Lowerre-Barbieri *et al.* 2011, Brown-Peterson *et al.* 2011). Macroscopic

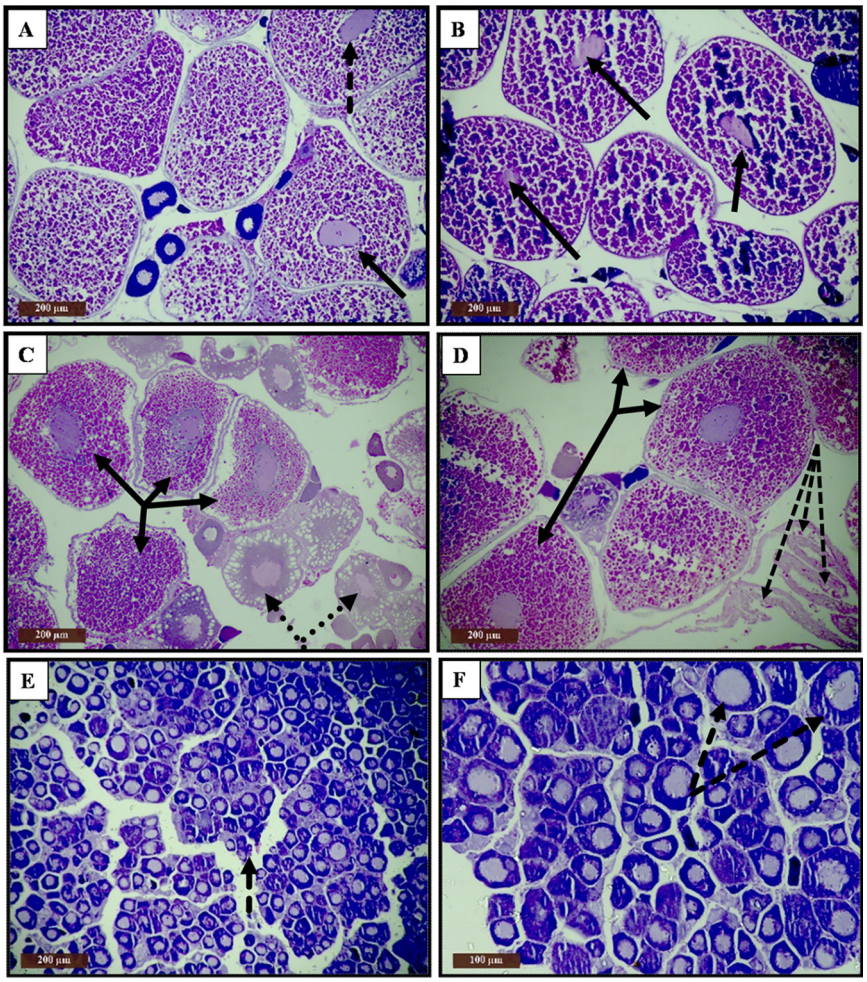

Fig. 3. Photomicrographs-A and B represent the ovary of *Lepidocephalichthys berdmore*i (sampled in June) in the developing to spawning capable phase indicated by matured follicle, with late vitellogenic oocytes. Solid arrows indicate migrating germinal vesicle (eccentric) in maturing oocytes. Immature follicles in tertiary vitellogenic stages have centric germinal vesicles (indicated by broken arrows). Photomicrograph-C represents the ovary (sampled in July) in the spawning phase indicated by maturing and late vitellogenic follicles (solid arrows). Immature follicles in cortical alveolar stages have centric germinal vesicles (indicated by broken arrows). Photomicrograph-D represents the ovary (sampled in August) of spawning capable ovary with late vitellogenic oocytes (bold arrows) and post-ovulatory follicle complexes (broken arrows). Photomicrographs-E and F are from a female representing the ovary in the regressed phase indicated by the presence of only primary growth oocytes (perinucleolar oocytes; broken arrows).

approaches are easy to carry out but are not accurate enough. They hold subjective predisposition, especially for multiple spawners, because these tools fail to distinguish phases between active spawning with regenerating and developing (Kjesbu 2009).

The GaSI depicts the intensity or rate of feeding in various months. High feeding intensity was observed during May, when the GaSI was 0.438±0.00207 and most of the stomachs were full, containing a considerable amount of food. The feeding intensity was generally low during July (GaSI 0.0089±0.00166) when stomachs contained a low amount of food, which was associated with the peak of the gonads' maturity period: during the development of the gonads, most fish are affected by a starvation period. The occurrence of low feeding in other fish species, during their breeding period peak, has been reported

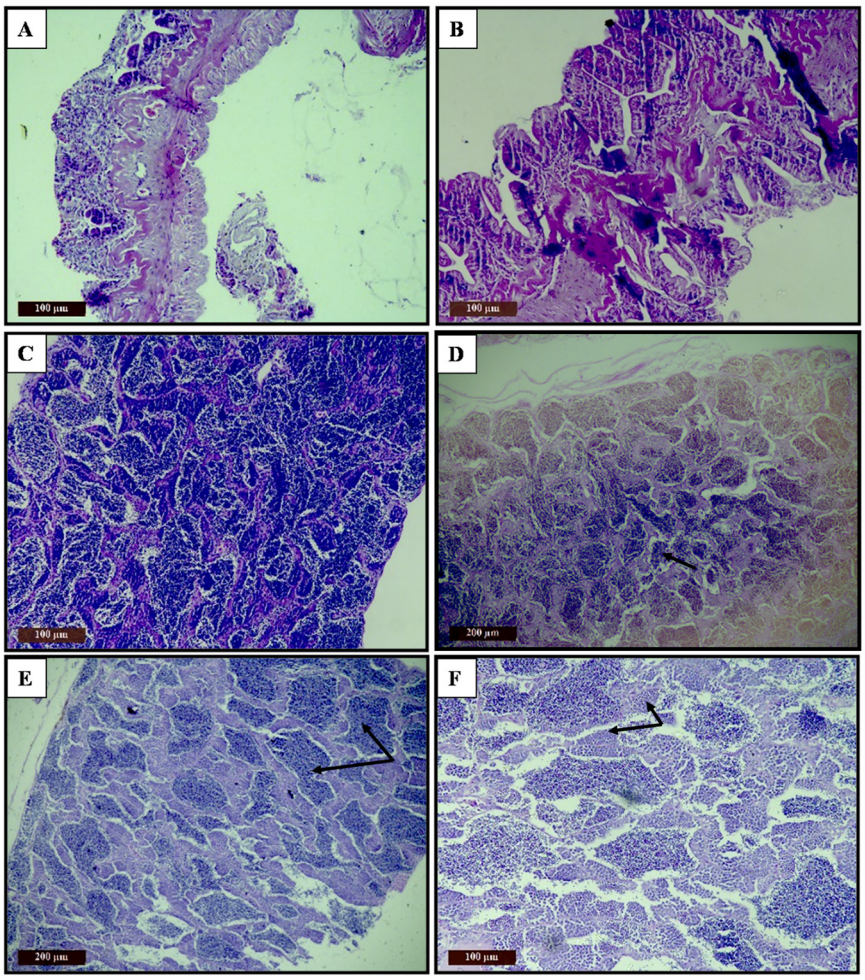

Fig. 4. Photomicrograph-A from a male *Lepidocephalichthys berdmorei* (sampled in January) is in a regressed phase, wherein active spermatogenic cysts are no longer visible, and the released spermatozoa are no longer abundant in the ductular region. Photomicrographs-B from another male (sampled in January) is in the late spawning capable phase, which is confirmed by abundant spermatozoa but no spermatogenic cysts. Photomicrographs-C from the male (sampled in June) is in spawning capable phase, having abundant spermatozoa but no spermatogenic cysts. Photomicrographs-D from the male (sampled in July) are in the spawning capable phase, with active spermatogenic cysts in the germinal epithelium and released spermatozoa in their lumen. Testicular ducts in the central region have abundant spermatozoa (indicated by the arrow).

by several authors (Lanthaimeilu and Bhattacharjee 2018, Alam *et al.* 2016, Dadzie *et al*. 2000, Saikia *et al.* 2015). The highest GaSI value during May might be associated with feeding in the pre-spawning period. A considerable increase in the rate of feeding intensity in the pre-spawning period has already been reported (Mushahida-Al-Noor *et al.* 2013, Bahri-Shabanipour and Mohammadizadeh 2010).

Mature ovaries with fully matured post vitellogenic oocytes with no germinal vesicle were predominately from fish collected in March-July (Fig. 2. C, D, E, F; 3: A, B, C, D). March appeared to be the onset of spawning season with maximum ovarian GSI and more abundant matured germ cells. At the beginning of the reproductive period (March, April), oocytes began or continued their exogenous vitellogenesis (intensive yolk accumulation) (Juchno and Boron 2006). Fig. 2C represent the oocytes stage during March in this figure, the ovary is in the capable

spawning phase, which is indicated by fully matured post-vitellogenic oocytes with no germinal vesicle. In Fig. 3: A, B, C, and D, the oocytes are in maturing/matured, and tertiary vitellogenic stage. Solid arrows (in Fig. 3: A and B) indicate migrating germinal vesicle (eccentric) in maturing oocytes. Immature follicles in cortical alveolar stages have centric germinal vesicles. Fig. 3 (C) represent the gonadal stage during July. In ovarian samples from July, some immature follicles, possibly late developing, were seen in cortical alveolar stages and they had centric germinal vesicles. The ovary in the developing to spawning capable phase is indicated by matured follicles with late vitellogenic oocytes. They were filled mainly with previtellogenic oocytes but also with many postovulatory follicles (Fig. 3; C and D) and oocytes at various stages of atresia. Fig. 3: E and F represent a female's ovary in the regressed phase, indicated by the presence of only primary growth oocytes. Fig. 4: C, E, and F represent the male gonads in the late spawning capable phase, with abundant spermatozoa but no spermatogenic cysts the testis is in the capable spawning phase, with active spermatogenic cysts in tubules and released spermatozoa in their lumen. Also, Fig. 4 (C) represents the testicular phase during June of the male is in the regressed phase. Active spermatogenic cysts are no longer visible, and the released spermatozoa are no longer abundant in the ductular region. During the spawning period, the ovaries of the females contained previtellogenic oocytes and oocytes in various stages of vitellogenesis as well as postovulatory follicles – an indication that a batch of eggs had been laid. During the post-spawning period (July and August), the ovaries of all the loaches were at the active spawning phase.

CONCLUSION

Estimation of Gonadosomatic index along with a histological examination of gonads could be used to pinpoint specific periods when spawning occurs. To capture accurate peaks in spawning, sufficient sampling must occur before and following the presumed spawning period of fish. However, the present study indicates that *L. berdermorei* starts maturing and breeding during March which progresses through July with intermittent spawning. In due course of time, concerted efforts are required for domestication, captive maturation, and seed production of different native fishes to conserve the dwindling natural population as well as to initiate farming practices of the species.

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