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Investigations on Gonadosomatic Index and Gonad Histology of Barred Spiny eel *Macrognathus pancalus* Hamilton, 1822 from Upper Assam, India

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ABSTRACT

Background: *Macrognathus pancalus* or barred spiny eel is a highly valued ornamental and food fish found in the Indian subcontinent. Due to the burgeoning population and their associated impacts, the population of the species is in rapid decline that necessitates time-bound intervention to conserve the species. The current investigation was undertaken to study the gonadosomatic index and gonad histology of *Macrognathus pancalus* collected from upper Assam, India.

Methods: 500 samples of *Macrognathus pancalus* were collected from different water bodies such as wetlands, ponds, paddy fields, *etc*. from upper Assam, India during 2018-2019. Monthly samplings were carried out to evaluate the gonadosomatic index. The gonads were dissected out, measured and subsequently preserved for histological studies. Histological sections of preserved samples were prepared by employing accepted methodologies. The sections were then photographed using Leica DM 750 and gonadal staging was ascertained.

Result: Gonadosomatic index studies revealed that it peaked in August in both males (3.45±0.18) and females (8.85±0.35), thereby indicating its spawning season. Minimum GSI values were observed during January in males (0.39±0.04) and in December in females (1.04±0.15) indicating the culmination of the breeding period and the advent of the preparatory phase. Macroscopic and microscopic examination unearthed five different phases of gonadal maturation. Gonadal staging through microscopic and macroscopic technique showed synchronicity with GSI values.

Key words: Gonadosomatic index, Gonadal staging, *Macrognathus pancalus*, Spawning season.

INTRODUCTION

The barred spiny eel or striped spiny eel, *Macrognathus pancalus* Hamilton, 1822, is a common fish species belonging to the family Mastacembelidae (Talwar and Jhingran 1991). It is a sturdy fish inhabiting a variety of habitats including 'beels'/wetlands, small rivers, streams, canals, inundated fields, river plains and estuaries of Pakistan, Nepal, Bangladesh and India (Rahman, 1989; Talwar and Jhingran, 1991; Galib *et al*. 2009). The species commands considerable market value as both food and ornamental fish in domestic and international trade (Suresh *et al*. 2006; Abujam and Biswas, 2011; Raghavan *et al*. 2013). Their entire demands, however, are met through wild catches from their native habitats (Suresh *et al*. 2006). It has been observed that along with unregulated catching practices, the impact of different anthropogenic activities including habitat modifications, construction of dams, invasive species introduction, overexploitation, *etc*. has had a profound impact on population dynamics of many freshwater fishes around the world including *M. pancalus* (Maitland, 1995; Abujam and Biswas, 2011). The persistence of these problems highlights time-bound interventions to prevent further declination of natural stocks.

In recent years, aquaculture practices have shown to be a viable alternative to boost productivity in many extant fishes through the adoption of effective management and conservation strategies. Successful implementations, in turn, have proved fruitful in reducing pressure on the depleted stocks as well as the sustainable utilization of aquatic resources (Diana, 2009; De Silva, 2012). Nonetheless, efficacious aquaculture ventures require thorough studies on taxonomy, distribution, feeding biology, reproductive dynamics, *etc*. of target species (Maitland, 1995). Among them, the identification of spawning chronologies is a major

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contributing factor in determining life history information and the environmental variability of fish populations (Brewer *et al*. 2008). Traditionally, numerous methodologies have been employed to ascertain reproductive success in fishes where the gonadosomatic index (GSI) stands out as the most frequent indices to understand the reproductive traits in different groups of fishes. Also, it has been observed that histological interpretation of gonadal stages is effective to identify reproductive timing in fishes, especially of those that have multiple spawning or low reproductive investments (McAdam *et al*. 1999). Detailed studies on these aspects often determine the success of aquaculture practices. Many authors have carried out studies on sexual dimorphism, reproductive strategies, feeding habits, induced breeding, etc. up to some extent in *M. pancalus* (Swarup *et al*. 1972; Serajuddin and Ali, 2005; Abujam and Biswas, 2020; Borah *et al*. 2020). However, a detailed study on the histology of gonads of *M. pancalus* is still lacking. With this backdrop information, morpho-anatomical indices and validation through histological techniques were incorporated for the analysis of gonads and to ascertain the reproductive traits in *M. pancalus* which will supplement existing information to develop future management and conservation strategies.

MATERIALS AND METHODS

Sampling and Gonadosomatic index (GSI)

Samples of *M. pancalus* (n= 500, 209 males, 291 females) were collected from different water bodies (ponds, wetlands, streams, *etc*.) of Dibrugarh district, Assam, India, on monthly basis during 2018-2019. Analyses of specimens were carried out in Freshwater Biology Research Laboratory, Department of Life Sciences, Dibrugarh University, Assam. Measurements of fish length (nearest cm) and weight (nearest g) were precisely carried out before dissections of gonads. After segregating the samples into males and females through external examination, the gonads were dissected, weighed, and subsequently preserved in 10% formalin for further studies. Determination of reproductive traits and spawning season was carried out through monthly observation of GSI using the following equation:

$$GSI = \frac{\text{Gonad weight}}{\text{Total body weight}} \times 100$$

(Wooton *et al*. 1978; Htun-Han, 1978c)

Gonadal histology

Male and female gonads of *M. pancalus* were dissected out from fresh specimens every month for histological preparation. The dissected gonads were immediately cleared and subsequently fixed in alcoholic Bouin's solution for 24 hours for further processing. They were then passed through graded alcohols (ethanol) for dehydration and finally embedded in paraffin blocks. Embedded tissues were sectioned at 4-5 µm thickness. The sections were then cleared in xylene solution and then stained with haematoxylin and eosin stain following Lal (2009). Four to five slides prepared from each tissue sample were photographed using the Leica DM 750 microscope. Morphological characteristics of both ovaries and testes were examined according to colour, structure, and types of cells. Female gonads were examined according to the mature oocyte, presence of most dominant oocyte type, and atretic follicle whereas the stages in male gonads were identified according to spermatogonium, spermatocyte, spermatids and spermatozoa numbers. The maturity stages were classified following Pathak *et al*. (2012). The microscopic data were also arranged according to the five maturity stages from the macroscopic scale.

RESULTS AND DISCUSSION

The present investigation was undertaken to understand the reproductive traits in *Macrognathus pancalus* from the upper Assam region of India. It has been observed that environmental factors such as photoperiod, temperature, rainfall, food availability, *etc*. have pronounced effects on the timing of gametogenesis, vitellogenesis, and maturation in fishes (Louiz *et al*. 2009; Miranda *et al*. 2009). Variations in such factors, thus, may lead to differential breeding patterns in different geographical locations. The uniformity of studies and their interpretation regarding the overall biology of a particular species from a particular region is of utmost necessity for future management practices. The current study, thus, is an attempt to study the spawning behaviour through GSI and histology studies that may shed light on a better understanding of their reproductive traits.

GSI is an ideal and accepted tool to examine the seasonal gonadal changes and to predict the spawning season of a fish species (Htun-Han, 1978c; Barros and Regidor, 2002; Tsikliras *et al*. 2013). Studies on GSI of *M. pancalus* indicated variation in GSI values in both males and females during the study period (Fig 1). The highest value of GSI was recorded in August in both males (3.45±0.18) and females (8.85±0.35) while the lowest values were observed during January in males (0.39±0.04) and in December in females (1.04±0.15) (Fig 1). In females, a gradual increase in GSI was observed that peaked in August. Such an increase in GSI may be attributed to an increase in gonad weights during the spawning period due to the uptake of fluid by developing and ripe gonads (Htun-Han, 1978a, b). It was followed by a drastic drop in GSI values indicating the culmination of the spawning season. A similar trend was also recorded in males. The present investigation indicated an annual synchronous breeding pattern in *M. pancalus* in the study area. Similar findings were also reported by Abujam and Biswas (2020) and Faridi *et al*. (2020). Earlier studies by Abujam and Biswas (2020) revealed almost similar trends in GSI peak values in *M. pancalus* during May and June for males and females respectively. However, their observation was based on specimens collected from different habits in contrast to the present study. Furthermore, contradictory results of the spawning period were reported earlier by Zahid *et al*. (2013) in *M. pancalus*. They reported two main breeding periods in *M. pancalus*; one during February/March and the other one in July/ August. They interpreted that observed variations may be due to the influence of day length on spawning activity. The notion may hold true as the environmental conditions reported by Zahid *et al*. (2013) are in contrast to our study and that of Abujam and Biswas (2020). Variations in reproductive traits in different geographical regions and habitats, therefore, warrant further studies to confirm their semelparous or iteroparous nature in *M. pancalus*. Recently, Faridi *et al*. (2020) studied reproductive strategies on *M. aculeatus* from the Ganga River and reported peak GSI values in June for both sexes. The current investigation further revealed that the males maintain uniform GSI values for a longer period with its peak in August. Htun-Han (1978b) in this regard suggested that such tendencies in male gonads facilitate and ensures successful fertilization in fishes.

Gonad histology is often regarded as a powerful tool to analyse reproductive health and maturity in fishes (Pieterse, 2004; Flores *et al*. 2015). It is considered the most accurate method to determine maturity stages through the unambiguous interpretation of maturity status (West, 1990). In the case of Indian teleosts, a literature survey indicates four to six maturity stages (Sathyanesan, 1962; Guraya *et al*. 1975; Dey *et al*. 2004). Reports of seven to eight maturity stages have also been documented (Nagahama, 1983; Mayer *et al*. 1988; West, 1990; Treasurer, 1990; Fishelson *et al*. 1996; Unal *et al*. 1999; Verma, 2013). The current endeavour revealed five maturity stages in both sexes of *M. pancalus* based on macroscopic and microscopic observation (Table 1 and 2). Morphological examination of male and female gonads of *M. pancalus* revealed five principal stages-immature virgin, maturing or recovering spent, ripening, mature or ripe and spent stage. The gonads are paired, elongated and remain suspended in mesenteries containing the fat bodies. The testes were thin, ribbon-like, and transparent during immature stages. They developed into somewhat flattened, whitish-yellow and relatively solid objects when fish progresses through different maturity stages. The ovaries, on the other hand, resembled immature testes during immature stages. At the onset of maturity, they become progressively enlarged in length and girth and tend to be somewhat yellowish.

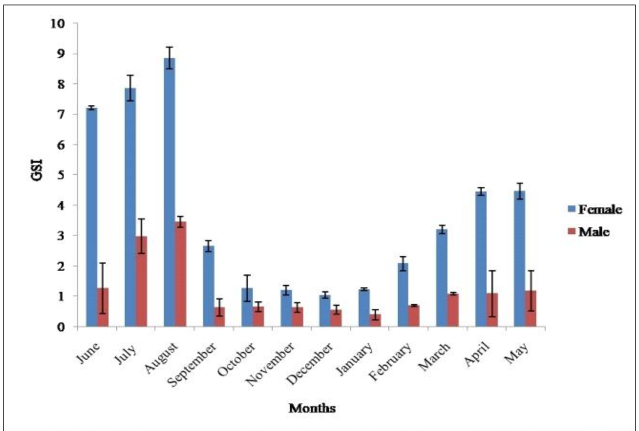

Fig 1: Monthly variation of GSI of *M. pancalus* studied in upper Assam region, India

Results showed that the cell ratios were similar at the developmental stage of the ovary (February to June) with high numbers of nucleolar cells. Numerous previtellogenic and maturing cells were observed during February and March (Fig 2). The maximum number of post-vitellogenic and mature cells was present at the advent of spawning season *i.e.* from June to August. The presence of numerous post-ovulatory follicles with numerous early and late perinucleolar cells signified the degenerative or spent stage from September to December. In most freshwater teleosts, ovarian development has been classified asynchronous or asynchronous, based on the growth pattern of the oocytes (Scott, 1987). Apart from these two growth stages, Blazer (2002) mentioned grouped synchronous growth where two groups of oocytes are observed; one developing and the other one in the previtellogenic resting stage. The study revealed that oogonia proliferated from the germ mother

Table 1: Macroscopic and histological characteristics of ovary of five developmental stages of *M. pancalus*.

Developing stages of ovary		Macroscopic description	Histological description
Stage I	Immature virgin or Rest (November to December)	Ovaries are small, thin, semi-transparent.	All the oocytes in the ovary were in the primary growth stage and oocytes well packaged. Resting females might contain late atretic oocytes and oogonia dominant in this stage.
Stage II	Maturing or recovering spent (January to February)	Ovaries elongated, swollen, light yellow in colour.	Occurrence of dominant large vitellogenic oocyte containing well-developed zona radiate and yolk globules
Stage III	Ripening (March to May)	Ovaries voluminous. Ova are with distinct yolk and are visible the naked eye.	Presence of mature follicles (MF) showing germinal vesicle migration (GVM) and yolk granules in the with cytoplasm.
Stage IV	Mature or ripe (June to August)	Ovaries massive in size and occupy a major portion of the stomach cavity.	Occurrence of MF with fused hydrated and large yolk globules. Nuclear membrane disappears in most of the MF some oocyte collapsed to atresia.
Stage V	Spent (September to October)	Ovaries flaccid, shrinking, left with only a few residual eggs under ova.	High level of residual primary oocytes. Some atresia follicles are found, disorganisation of ovary structures, numerous blood vessels absences of yolked oocytes.

Table 2: Macroscopic and histological characteristics of testes at five developmental stages of *M. pancalus*.

Developing stages of testes		Macroscopic description	Histological description
Stage I	Immature virgin or rest (November to December)	Testes are very thin, thread -like and translucent.	Abundant spermatogonia are present at this stage and primary spermatocytes are very less in no.
Stage II	Maturing or recovering spent (January to February)	Testes are slightly elongated and are white.	At this stage spermatogonia are dominant and abundant spermatocyst of primary spermatocytes are present surround the lobule.
Stage III	Ripening (March to May)	Testes are creamy-white that occupies half of the stomach cavity.	Abundant number of primary and secondary spermat-ocytes and developed cells migrate towards lumen.
Stage IV	Mature or ripe (June to August)	Testes are massive in size, creamy white. The maximum size was observed in this stage.	At this stage, spermatids can be found along with primary and secondary spermatocytes. Large no. of mature sperms is observed in the lumen of spermatocyst.
Stage V	Spent (September to October)	Testes start shrinking, flaccid, whitish to translucent, and very thin.	Lobules are contracted, sperms are shed. Distinctive blood vessel and interstitial tissue. At this stage, spermatocytes are rarely seen and spermatogonia are dominant cell types.

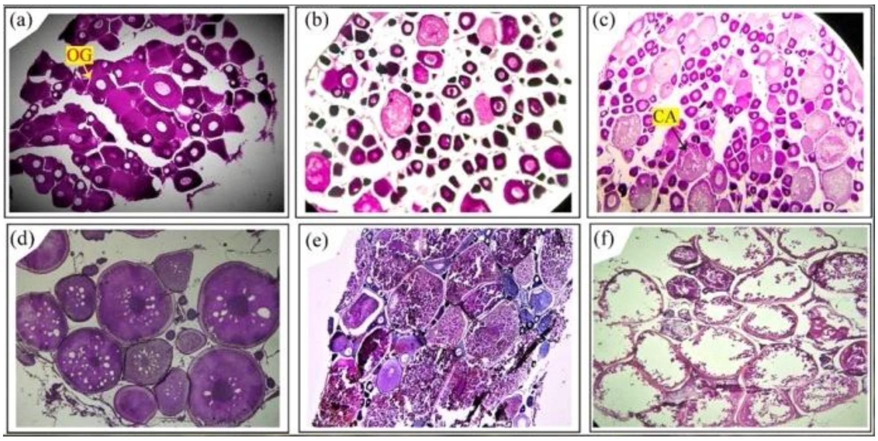

Fig 2: Six microscopic maturity stages of female gonad of *M. pancalus*.
(a) Early Perinucleolar stage (b) Late Perinucleolar stage (c) Cortical alveoli stage (d) Vitellogenesis
(e) Post-vitellogenic stage (f) Spent. OG= Oogonia, CA= Cortical alveoli.

cells of the ovigerous fold and is found in clusters. The period from October to January was observed as the period of gonadal recrudescence of oogonia. Primary oocytes were recruited and oogenesis began during this period. The growth phase of oocytes was reported from February to May. Primary oocytes developed rapidly with the incorporation of yolk. Yolk vesicles contained endogenetically synthesized lipids, glycoprotein, and increased the space for incorporation of yolk protein synthesized exogenously (Selmen *et al*. 1986). Zona radiata and follicular cell layers as zona granulose and theca appeared in this stage. Zona granulose and theca cells played important role in the synthesis and incorporation of yolk precursors and were the site for steroid hormone synthesis (Lubzens *et al*. 2010). The germinal vesicle breakdown (GVBD) and its migration towards the periphery was the major phenomenon of oocyte maturation. Yolk globules coalesced and formed a translucent yolk mass. Hydration diluted the cytoplasmic content resulting in translucent appearance and maximum size of oocytes (Foucher and Beamish, 1980). The breeding season of *M. pancalus* was predicted to be between June to August owing to the ovary attaining maximum size and the presence of mature follicles in this period. GSI also reached its peak during this period. The absence of mature and maturing follicles in September marked the completion of the spawning period. During the post-spawning phase (September-October) the post-ovulatory follicles and atretic follicles were found with residual primary oocytes. Similar cytomorphological changes in gonads have also been reported in different fish species (Chakraborty *et al*. 2007; Sivakumaran *et al*. 2003). Mature follicles became atretic with the withdrawal of gonadotrophin (Pant, 1968; Hunter *et al*. 1985). Oogenesis and oogonial proliferation began after the completion of the degeneration of yolky oocytes for recovery and repeat the cycle.

Like female gonads, male gonads too changed during the annual reproductive cycle in fishes. Observations by Sathyanesan (1959), Schulz *et al*. (2010) and Verma (2013) indicated that teleost testis exhibits similar observation in structure, spermatogenic pattern and maturation. Development

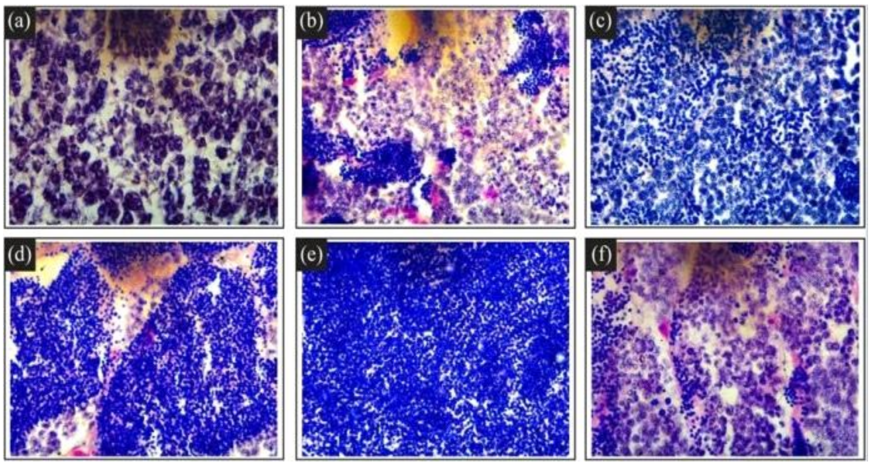

Fig 3: Six microscopic maturity stages of testes of *M. pancalus*. (a) Spermatogonia (b) Primary spermatocyte (c) Secondary spermatocytes (d) Spermatids (e) Spermatozoa (f) spent.

of sperm or milt in fishes thus comprises of multiplication stage, growth and maturation stages (Nagahma, 1983; Schulz *et al*. 2002). In the present investigation, histological assessment of male gonads of *M. pancalus* revealed five different maturity stages that showed conformity with earlier studies of Zaki *et al*. (1995), Assem (2003) and Abujam and Biswas (2020) (Fig 3). Mid spermatogenesis stage was observed from February to May. The spawning stage that had started in June continued until August. The advent of the spent stage or culmination of spawning season was observed in the latter half of September (Abujam and Biswas, 2020). In the spermatogonial stage, the testes were simple and comprised of numerous seminiferous tubules or lobules which were encased in a dainty peritoneum and had a fairly thick tunica. In the primary spermatocyte stage, the tunica was thick, and the lobular structure was available and entrenched. Histological observations of the spermatid phase revealed they had lobules filled with sperm. A couple of secondary spermatocytes adjacent to spermatids were additionally present. Microscopically, very few sperm were seen in the lumen of the lobules.

CONCLUSION

In summary, the present investigation elucidated the GSI and histology of gonads of *M. pancalus*. Based on morpho-anatomical and histological endeavours, it was observed that the species was a single spawner with a prolonged breeding season. The spawning season started at the advent of the monsoon *i.e.* May onwards and culminates at the end of the monsoon (September onwards). Histological interventions showed synchronicity of Gonad development with that of gonad development stages. Comparison with other studies on the species revealed the impact of physiological parameters on Gonad development of *M. pancalus*. The study provides new information that may fill the existing lacunas to formulate future conservation strategies.

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