Reproductive biology of paradise threadfin *Polynemus paradiseus* (Linnaeus, 1758) from Shibsa River in Bangladesh

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Abstract. The paradise threadfin, *Polynemus paradiseus* is one of the commercially important brackishwater finfish of the family Perciformes in Bangladesh. The present study aimed to investigate the reproductive biology of paradise threadfin from Shibsa River in Bangladesh. The fish samples (66) were collected from May 2021 to April 2022. The relationship between body weight (BW) and total length (TL) of sampled fish were analyzed with a non-linear power equation where $R^2 = 0.915$ and 'b' value (3.22) indicates a positive allometric growth pattern. Linear relationship indicates gonad weight (GW) increases with the BW and fecundity increases with BW, TL, GW and gonadosomatic index (GSI). The highest GSI was recorded in June (11.10±0.91), whereas, the lowest in November (0.44±0.08). The maximum fecundity was found in June (32410±7790). Ovarian histology displayed different stages of oocyte development. Late yolk granule stage (LYGS) was found in the months of September-October and May-July, respectively. Based on the GSI, fecundity and ovarian histology, it was revealed that the spawning season of *P. paradiseus* was from May to October with a major peak in June and minor one in September. The knowledge on different aspects of reproductive biology of paradise threadfin would be helpful for planning captive breeding and to design sustainable management policies for conservation of this species in the south-west coast of Bangladesh.

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1 Introduction

Paradise threadfin (*Polynemus paradiseus*) a species from Perciformes order and Polynemidae family is one of the commercially important fishes in the south-west coastal region of Bangladesh. This fish is locally known as *taposi*, *tapsi*, *muni*, *rishi*, *bairrage* in Bangladesh [1]. Paradise threadfin is distributed widely throughout the Indian subcontinent including India, Pakistan, Srilanka, Bangladesh, and the Indo-Pacific Ocean confluence of the Bay of Bengal [2-6]. This species is carnivorous in nature and fed mainly crustaceans (especially shrimps), small fishes, and benthic organisms [7]. Paradise threadfin mainly lives over the sandy bottom of the sea but in the spawning season they migrate to freshwater for reproduction [8].

This fish attracts consumer's special preference as the flesh of this species is quite oily and flavourful that makes it appealing and appetizing for human consumption [9]. The high protein content (15.97 %) of this species indicates as a good source of animal protein [10]. Because of its commercial importance in the south-west coastal region of Bangladesh, this fishery is regarded as vital as the *hilsa* fishery [3, 11]. In the south-west coastal mangrove habitats of Bangladesh, this high valued species is still available but the stock is declining. Overfishing has reduced the availability of this species in recent years [12]. According to IUCN Red List Category & Criteria [13], this species is under least concern.

To understand the potential of fish populations to recover, successful fisheries management, including practical aquaculture, requires an accurate evaluation of fecundity along with other parameters of reproductive biology [14]. Moreover, knowledge of reproductive characteristics such as gonadosomatic index, size at first sexual maturity, maturation stages, etc. is crucial for efficient management of fish in both culture and capture fisheries [15]. Furthermore, GSI is an excellent measure of reproductive activity [16]. Fecundity is an essential component of fish biology that must be studied in order to explain population fluctuations and to make attempts to improve the quantity of fish harvested. Proper calculation of fecundity is required to determine the population status of any species. Information on reproductive biology is crucial especially for the conservation of vulnerable fish species. In addition, reproductive biology studies can provide essential and fundamental information on a species gonadal maturity, breeding potential, and spawning season [17].

The effectiveness of a breeding program relies heavily on information about reproductive biology. In Bangladesh, there is a scarcity of information regarding significant fish species stocks, habitats, behaviour, reproduction, spawning, and other factors that are necessary for their conservation, sustainability, and management in the wild. Few studies have been carried out in different aspects of *P. paradiseus* but the knowledge on the reproductive biology of this fish species is yet unavailable in Bangladesh. Therefore, considering the significance of this species, a thorough investigation of the reproductive biology was carried out as a first attempt to develop the artificial breeding technique and proper management of this population in the south-west coast of Bangladesh.

2 Materials and methods

2.1 Study site

A total 66 fish samples were collected once every month from local fishermen during May 2021 to April 2022 from the Shibsa River adjacent with the mangroves of south-western part of Bangladesh.

2.2 Measurements

Total length was measured from the tip of the snout to the end of the caudal fin and fish body weight was estimated by an electric balance (And Gulf FZE, EK 3200I) with 0.01 accuracy in Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna.

2.3 Calculation of Gonadosomatic Index (GSI)

The gonadosomatic index (GSI) was calculated as the ratio of Gonad Weight (GW) and Body Weight (BW) in percentage by the following equation [18]:

$$GSI = GW/BW \times 100 \quad \text{---------- (1)}$$

2.4 Estimation of fecundity

In order to determine the peak spawning season, fecundity estimation was done throughout the spawning season. The fishes were randomly sampled, transported to the laboratory and after a longitudinal ventral incision, eviscerated, and three sub-samples from the middle, anterior and posterior parts of the ovary were placed in steamed distilled water and shaken periodically to loosen the oocytes. The external connective tissues were removed from the surface of each pair of ovaries. The moisture of the ovaries was removed with the help of blotting paper. The average number of eggs in 0.1 g of sub-sample was estimated and the total numbers of oocytes (N) in both ovaries were calculated using the following formula [19]:

$$N = W_t/W_s \times N_s \quad \text{---------- (2)}$$

Where, W_t = total weight of two ovaries, W_s = weight of subsample, and N_s = No. of counted oocytes (ova) in the subsample.

2.5 Histological observation of female gonad

After isolating selected fish gonads, it placed into Bouin's solution for 6 hours. Then preserved into 70% ethanol solution for the histological study. A histological study was conducted in the Anatomy and Histology Laboratory of Bangladesh Agricultural University, Mymensingh- 2202 following the 'animal tissue technique' method [20]. Tissue dehydration was performed by an automated tissue processor, Leica ASP300 S (Leica Biosystem, Germany), with a series of increasing ethanol concentrations of ranges from 70% to 100%, xylene clarification (two changes) and molten wax infiltration (two series). Paraffin-embedded blocks (2 µm thick) were cut with a rotating microtome (Leica RM2255, Leica Biosystem, Germany), and the sections were placed in a pre-heated (40°C) water bath (Paraffin Bath-Leica Model HI1210, Leica Biosystem, Heidelberger, Germany). The sections were then placed on a glass slide to keep overnight. Afterwards, the sections were cleaned with xylene, rehydrated with alcoholic series stained with haematoxylin and eosin stains. The stained sections were mounted with Canada balsam and covered with a cover slip. A light microscope was used to examine the slides (OLYMPUS BX 53), equipped with a camera and photographs were taken for further observation. Software 'ImageJ' was used to determine the oocytes diameter.

2.6 Relationship among different parameters

The mathematical relationship between Fecundity vs TL, BW, GW and GSI and the relationship between GW and BW was established as following equation [21]:

$$Y = a+bX \quad \text{---------- (3)}$$

Where, Y = Independent variable, X = Dependent variable and 'a' and 'b' = regression parameters.

The length-weight relationship was established as follow [22]:

$$Y = a.X^b \text{ ---------- (4)}$$

Where, Y= total body-weight in grams, X = total length in centimetres, 'a' and 'b' are the parameters.

2.7 Statistical analysis

The collected data on paradise threadfin were compiled, categorized, computed and tabulated using a computer program, Microsoft Office Professional Plus 2016. Furthermore, some statistical tests were executed by Microsoft Excel 2016 and Statistical Product and Service Solutions (SPSS) ver. 25 to determine the linear and non-linear relationships and coefficient of determination (R^2) of fecundity with TL, BW, GW and GSI; GW vs BW and the relationship between total length and body weight. The analyzed data are presented in tabular and graphical forms to describe them elaborately for extracting the information accurately.

3 Results

3.1 Relationships between different parameters

The body weight (BW) of sampled fish and total length (TL) were analyzed with non-linear power equation ($Y = a.X^b$). Here growth parameter 'b' was 3.22 and 'R^2' was 0.915. The 'b' value indicates a positive allometric growth pattern. The relationships between fecundity and gonad weight was the best-fitted model among 5 equations where the 'R^2' value was 0.938. The relationship between gonad weight-body weight and fecundity-total length showed a moderate positive correlation where 'R^2' was '0.680' and '0.632'. However, the fecundity-body weight relationship exhibited a high positive correlation with the R^2 value of '0.769'. The fecundity-gonadosomatic index relationship was positively correlated and the 'R^2' value was '0.343' (Table 1 and Figure 1).

Table 1. Relationship between BW vs TL, GW vs BW, fecundity vs TL, BW, GW and GSI of *Polynemus paradiseus* in the Shibsa river of Bangladesh (n = 66)

Equation	a	b	R^2
$BW = aTL^b$	0.0033	3.22	0.915
GW = a+bBW	-2.7332	0.1227	0.680
Fecundity = a+bTL	-33997	2804.9	0.632
Fecundity = a+bBW	2369.5	349.87	0.769
Fecundity = a+bGW	5905.9	3172.7	0.938
Fecundity = a+bGSI	3908.2	2074.5	0.343

Here, a, b = regression parameter and R^2= co-efficient of determination.

3.2 Gonadosomatic index of female fish

The gonadosomatic index (GSI) of the species gives an idea about the seasonal progression of gonad development, sexual maturity and spawning season of a fish. The highest GSI value for the female was recorded in the month of June (11.10±0.91), indicating the major/primary spawning season of this species. Thereafter, the GSI value sharply declined in the July and then gradually rose to a second peak spawning season in the month of September (7.31±3.57). The findings of this research indicate an extended spawning season from April to October

with two peaks in June and September. The lowest GSI was recorded in the month of November (0.44±0.08). After that the GSI value gradually increased from December to reach a maximum value in June. GSI value went a little down in July then gradually increased from August to give a second peak in September. With a gradual decrease in October, the GSI value drastically fallen down to bottom in November and December (Figure 2).

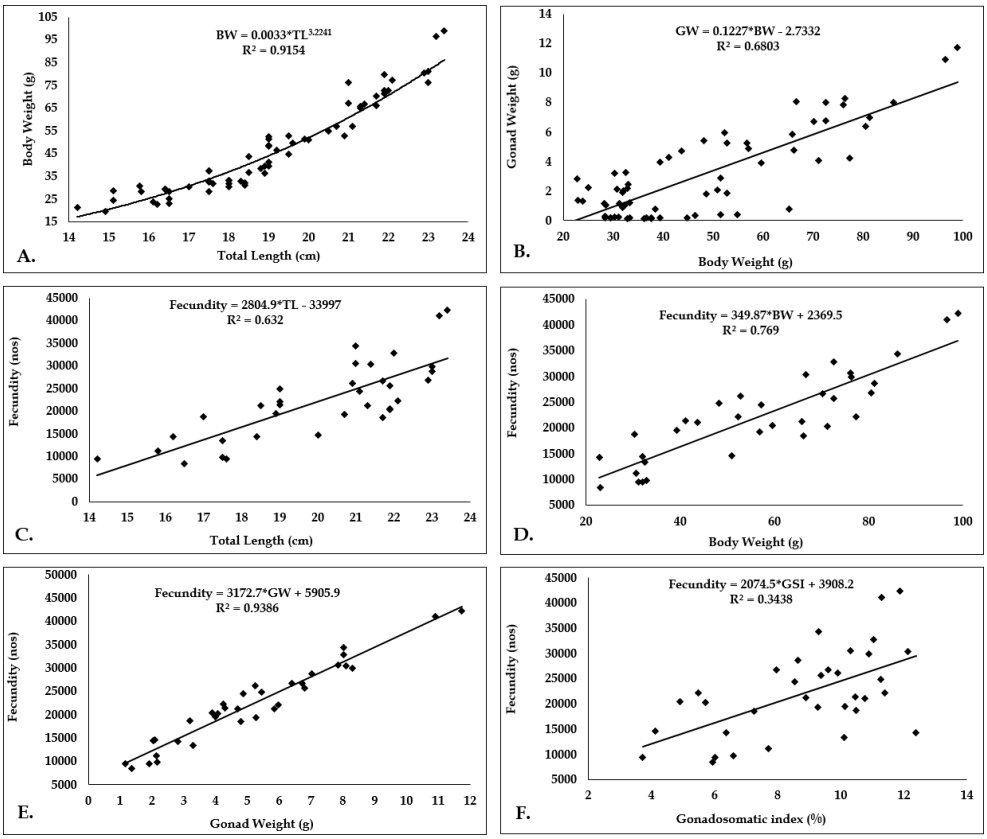

Figure 1. Relationship between (A) Body Weight and Total Length (B) Gonad Weight and Body Weight (C) Fecundity and Total Length (D) Fecundity and Body Weight (E) Fecundity and Gonad Weight (F) Fecundity and Gonadosomatic Index of *P. paradiseus* in the Shibsa river of Bangladesh

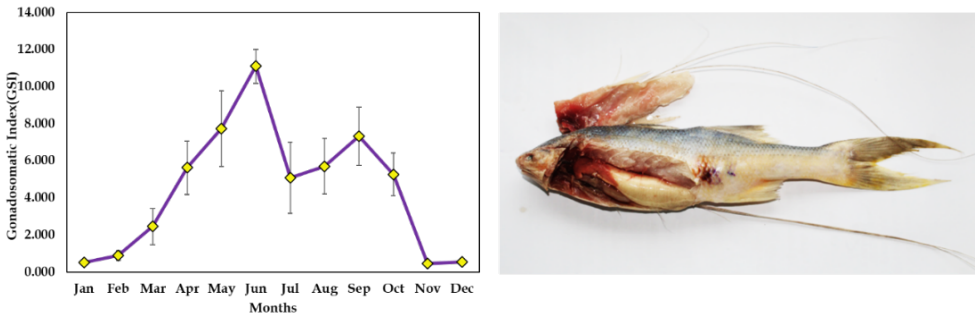

Figure 2. Graphical presentation of the monthly variation of GSI (Mean±SD) and fully gravid female *P. paradiseus* in the Shibsa river of Bangladesh

3.3 Estimation of fecundity

Absolute fecundity was found to be highest (42300) in the month of June (primary peak spawning season) with a mean value of 32410±7790 ova while, in the month of September (secondary spawning season) fecundity was found 22361±9073 ova (Table 2).

Table 2. Recorded fecundity range of *P. paradiseus* during the spawning season

Month	No. of Fish Examined	Fecundity Ranges	Fecundity (Mean±SD)
April	5	14630-26750	20068±4788
May	4	19300-25680	22670±2921
June	**7**	**21160-42300**	**32410±7790**
July	4	8450-26700	14834±8308
August	4	9500-19500	14675±5058
September	**6**	**9450-30600**	**22361±9073**
October	4	14300-26180	20172±4973

3.4 Histological observation of ovary

The development of oocytes of paradise threadfin can be divided into different developmental stages. Paired ovaries with mature oocytes were observed during the mature stage of ovary. In the present study, month-wise oocyte developmental stages were studied histologically in female *P. paradiseus*. Fully mature oocyte founds from the month of April to October indicates the spawning period of this species. Actual development of oocytes starts from the month of December to April which is confirmed by the chronological presence of CNO, EPNO, LPNO, YVO, EYGO and LYGO (Figure 3) indicates the occurrence of spawning which was found in the month of July.

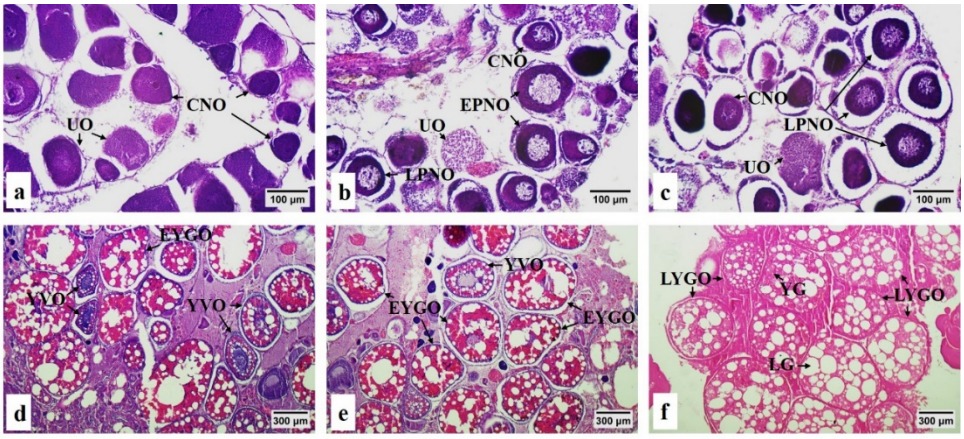

Figure 3. Micrographs showing Haematoxylin & Eosin stained ovary of *P. paradiseus* monthly (a) CNS= Chromatin Nucleolar Stage (b) EPNS= Early Perinucleolar Stage (c) LPNS= Late Perinucleolar Stage (d) YVS= Yolk Vesicular Stage (e) EYGS= Early Yolk Granular Stage (f) LYGS= Late Yolk Granular Stage. **Note:** a, b, c at 40x and d, e, f at 10x magnification, UO= Undeveloped oocyte; CNO= Chromatin nucleolar oocyte; EPNO= Early perinucleolar oocyte; LPNO=Late perinucleolar oocyte; YVO= Yolk vesicular oocyte; EYGO= Early yolk granular oocyte; LYGO= Late yolk granular oocyte; LG= Lipid Globule; YG= Yolk Granule.

3.5 Mean diameter and characteristics of oocytes at different stages of paradise threadfin

Immature oocytes were found mainly in the month of November to January with average diameter was 0.09±0.02 mm. February to April was the month of oocytes development and increased its size (0.31±0.07 mm). Presence of mature egg indicates the spawning period of this species. In the month of from May to October, mature egg was in its highest (0.57±0.11 mm) in diameter which indicated this species spawn almost six months in nature (Table 3).

Table 3. Ovarian stages, macroscopic and microscopic ovarian characteristics and oocyte diameter of paradise threadfin (*P. paradiseus*).

Ovary Stages	Observational characters		Oocyte diameter (mm)	Month
	Direct Eye	Microscopic		
Immature	Ovaries are slender, mostly reddish and transparent. Oocytes are not visible	Small size oocytes are seen. Mostly presence of UO, CNO and EPNO	0.09±0.02	Mostly in November to January
Maturing	Ovaries are getting larger and whitish in color. Oocytes are visible.	Oocytes size are increasing. LPNO, YVO are the main indicator in this stage	0.31±0.07	Usually February to April
Mature	Yellowish ovary cover most of its body cavity. Large size oocytes are visible through transparent membrane.	Size of oocyte increased to its maximum and EYGO, LYGO are prominent	0.57±0.11	Whole spawning period. Mainly May to October

4 Discussion

Reproductive parameters show an overview of the reproductive biology of the species. The relationship between fecundity and gonad weight was the best-fitted model among 5 equations where the R^2 (0.938) value was most significant [23]. The relationships between gonad weight-body weight and fecundity-total length showed a moderate positive correlation where 'R^2' was '0.680' and '0.632' respectively. However, the fecundity-body weight relationship exhibited a high positive correlation with the R^2 value of '0.769'. The fecundity-GSI relationship was positively correlated and the 'R^2' value was '0.343'. This results agree with *Pomadasys hasta* [24], *Labeo victorianus* [25], *Sardinella aurita* [26] and *Ompok pabda* [27]. Monthly mean values of GSI was obtained from 0.82 (December) to 5.44 (June) [28]. Variation in fecundity, GSI, total length, body weight, and gonad weight of paradise threadfin was found across the spawning season (April-June) [1]. The findings of these studies are in line with the present study.

A close relative species of paradise threadfin from the Polynemidae family *E. tetradactylum*, is a double breeder one from February to March and another from July to August [29]. GSI for *E. tetradactylum* was recorded 1.04 to 18.33 which is similar with the findings of our study. The estimated fecundity of *P. paradiseus* was reported as 42,000 eggs and suggested an extended spawning season for this species, from January to June which is quite dissimilar from the present study due to the different geographical locations and others environmental factors [2, 30]. The mean fecundity of *P. paradiseus* was found as 11383.33±343.35 in a partial study on reproductive indices [1]. Another species of the same

genus, *P. indicus* were determined as two spawning from April-June and October-December where, April-June was found to be the major spawning season [31].

Another similar species, *P. heptadactylus* spawn throughout the year but with two peak periods during March-June and August-November and estimated fecundity was 65,423 eggs which are closely related to the present study [32]. *P. stridens* spawns within the month December-March in the Persian Gulf [33]. Whereas, spawning season of freshwater fish *P. sophore* vary from March-July [34]. Higher fecundity was observed in *L. parsia* than *P. paradiseus* due to dissimilar order groups [23]. *L. parsia* is seen to spawn for several months having two spawning peaks one in December and other in February. Paired ovaries with mature oocytes were observed at the mature stage of paradise threadfin. The six stages of oocyte developments was found in *E. aeneus* [35]. In the present study, month-wise ovarian maturation stages were observed histologically for female *P. paradiseus*.

The chronological stages of ovarian maturation include CNO, EPNO, LPNO, YVO, EYGO, and LYGO. The LYGO was detected most of the individuals during May-October and their ovary was fully matured, this indicates the spawning season of this species. Some found to be getting maturing stages during this extended spawning time. Similar ovarian developmental stages were found in the present study that observed in a research for *P. hasta* [24]. Oocytes diameter differs with the research of the same species due to variation in time, places and environments [36] but found similar with another research regarding *P. hasta* [24].

5 Conclusion

This study suggested that, *P. paradiseus* has a distinct yearly reproductive cycle and an extended spawning season with two annual peaks. The current study of reproductive biology will be a milestone to study the reproductive cycle of other related species. Moreover, it would be helpful for planning captive breeding and to design sustainable management policies for conservation of this species in Bangladesh coast.

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