



# Reproductive Traits of Invasive Amazon Sailfin Catfish (*Pterygoplichthys pardalis*) in Association with the Nutrient Concentration of the Invaded Habitat

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## Authors' Contribution

AH designed the study, collected data, analyzed the data and prepared the draft manuscript. ATRN helped in preparation of the manuscript. SA and AD performed laboratory analysis. FH helped in data collection. PKS supervised the research work and edited the manuscript. JKS supervised the research work, helped in data analysis and edited the manuscripts.

## Key words

East Kolkata Wetlands, Invasive species, Loricariidae, Nutrient enrichment, Reproduction, Sailfin catfish

## ABSTRACT

An eight-month study (May to December 2022) was conducted to evaluate the reproductive traits of Amazon sailfin catfish *Pterygoplichthys pardalis* in relation to the nutrient contents (nitrogen and phosphorus) of the invaded sites of East Kolkata Wetlands (EKW), India. Over the study period a total of 608 adult specimens of the fish and 120 water samples were collected from five distinct sites of the wetlands. Sex ratios were found highly female-biased in all study sites with no discernible site-wise variation ( $\chi^2 = 1.372$ ,  $p = 0.843$ ). However, total length at maturity showed significant variation between sites for both, male ( $p = 0.005$ ) and female ( $p = 0.002$ ). Mean gonadosomatic index (GSI) showed significant variation for females ( $F_{4,322} = 4.172$ ,  $p = 0.003$ ), while for males it was found coequal ( $p = 0.259$ ) between sites. Absolute fecundity of ripe females ranged between 2100 and 7850, but not changed significantly by sites ( $p = 0.555$ ). No significant differences by sites were also observed in relative fecundity and egg size of ripe females. Hydrological parameter analysis showed significant differences in concentration of ammonia ( $p = 0.003$ ), nitrate ( $p < 0.001$ ) and phosphate ( $p < 0.001$ ) between study sites. Analysis of pearson correlation revealed a significant correlation between nutrient contents of the invaded sites and reproductive traits of Amazon sailfin catfish. Mean GSI of mature female, gonad mass and absolute fecundity of ripe females were found to have a significant ( $p < 0.05$ ) positive correlation ( $r \geq 0.123$ ) with phosphorus concentration of the study sites. Whereas, male Amazon sailfin catfish length at maturity was found negatively correlated ( $r = -0.198$ ) with phosphorus concentration. However, only the GSI of mature males exhibited a significant ( $p = 0.035$ ) and positive correlation ( $r = 0.117$ ) with total nitrogen (ammonia + nitrate).

## INTRODUCTION

The species of the genus *Pterygoplichthys* (Siluriformes: Loricariidae), which are commonly known as plecos or janitor fish or armored sailfin catfish or South American

sailfin catfish are well-known aquarium pets. Due to deliberate release by pet fish owners or accidental escape from raising facilities, several species of *Pterygoplichthys* now have invasive population in tropical, subtropical, and warm-water habitats all over the world (Orfinger and Goodding, 2018; Wanasinghe *et al.*, 2023). *P. pardalis* (Castelnau, 1855), *P. disjunctivus* (Weber, 1991) and/or their hybrids have been reported from a number of diverse regions of the Asian countries, from freshwater rivers and ponds to wetlands, swamps and saline environment (Hussan *et al.*, 2019, 2021; Parvez *et al.*, 2023; Wanasinghe *et al.*, 2023). This shows that, these fishes have considerable plasticity in their life-history features and are thus well adapted to a range of different environments (Wei *et al.*, 2022).

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However, according to various studies (e.g. Lampert *et al.*, 2022; Wei *et al.*, 2022), environmental changes can substantially reshape the development and other life-history characteristics of a fish species across clines and geographic regions. Temperature, precipitation, the availability of resources, and biotic interactions (i.e., predators and competitors), which may vary both within and between the native and introduced ranges of a species can significantly affect their growth and ability to reproduce (Winemiller, 1989; Fox and Copp, 2014). For example, *Pterygoplichthys disjunctivus* has undergone changes in its reproductive strategies in the invaded Volusia Blue Spring, North America in response to the pressures of the novel environment. Over a period 10 year (2005–2014), the species expanded its reproductive season with increased fecundity and gonado-somatic index, while reduced its investment in individual offspring (Gibbs *et al.*, 2017). Wei *et al.* (2017, 2022) and Lampert *et al.* (2022) also documented variations of growth, maturity and reproductive traits of invaded populations of sailfin catfishes in response to the specific local environmental and hydrological characteristics. Among different factors, nutrient availability, particularly phosphorus may be a prime factor in regulating the life-history traits of loricariid fish (Tristano and Gibbs, 2023), as evidenced by the comparatively high phosphorus content in their bony coated armor plates (Capps and Flecker, 2013) compared to other fish families. However, very few studies have examined how sailfin catfish's biological traits respond to various environmental conditions in their introduced range.

The East Kolkata Wetlands (EKW), a designated International Ramsar Site and the World's Largest Integrated Wastewater Fisheries', which lies on the eastern outskirts of the city of Kolkata in India and cover 12,500 ha, are a mosaic of landforms that include 2481 ha of shallow water-bodies used as fish farms (Hussan, 2016; EKWMA and WISA, 2021). These wetlands receive over 900 million litres of nutrient-rich effluent (pre-settled sewage) from the Kolkata Metropolitan region every day and thus provide a suitable environment for *Pterygoplichthys* invasion (Hussan, 2016; Hussan *et al.*, 2019; EKWMA and WISA, 2021). *Pterygoplichthys* were first reported from the pisciculture bheries (a local term of fishery pond) of these wetlands in 2009 (DARE/ICAR Annual Report, 2008–2009) and within a decade they invaded extensively in the water bodies of these wetlands, likely supported by the nutrient rich environment, ample food in the form of detritus and a congenial environment for shelter and breeding in the form of water hyacinth cover (Hussan *et al.*, 2019). However, abundance of sailfin catfish was reported somewhat lower in the bheries of the areas of

EKW where supply of nutrient-rich effluent is low (Hussan *et al.*, 2019). Therefore, the present study was conducted to investigate the reproductive traits of sailfin catfish in correlation with the nutrient (nitrogen and phosphorus) content of the different sites of EKW. Though several authors have reported *Pterygoplichthys* present in EKW as *P. pardalis* and/or *P. disjunctivus* and/or hybrid *P. pardalis* x *P. disjunctivus* (Hussan *et al.*, 2018, 2019; Suresh *et al.*, 2019), based on genetic studies of Sahoo *et al.* (2022) and morphological identification during present study, the specimens tentatively identified as *Pterygoplichthys pardalis* (Amazon sailfin catfish) were only used in this study.

## MATERIALS AND METHODS

### Sampling sites

This study was carried out at East Kolkata Wetlands (EKW, 22°25' - 22°40' N; 88°20' - 88°35' E) of West Bengal, India. Two different locations of the EKW, viz. Bidhannagar (B) area and Kolkata leather complex (K) area were selected based on the quantity of sewage received for fish rearing and presence of *Pterygoplichthys pardalis* in the bheries (size: 5–30 ha). Sampling sites included: three sites of Bidhannagar (B) area (Narkeltala bheri, B<sub>1</sub>; Chacker bheri, B<sub>2</sub>; Jhagrasirsha bheri, B<sub>3</sub>) and two sites of Kolkata leather complex (K) area (Chacharia bheri, K<sub>1</sub>; Nalban-1 bheri, K<sub>2</sub>) (Fig. 1). Study lasted for eight months from May 2022 to December 2022.

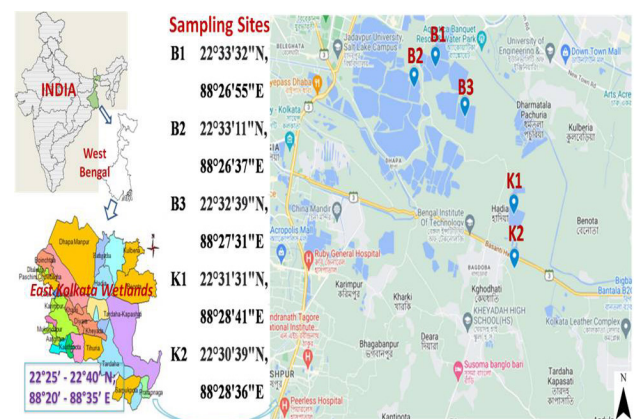


Fig. 1. Location map of East Kolkata Wetlands (EKW) and sampling sites.

### Acquisition of fish samples and estimation of reproductive traits

Monthly random samples of Amazon sailfin catfish (20–30 nos. per site; weighing > 100 g) were collected from the commercial catch (using dragnets) of valued

species and transported to the laboratory. Length was recorded as total length (TL) or standard length (SL) in cm (nearest 1 mm) using a calibrated measuring board. The whole-body weight (W) was measured to the nearest gram (0.1 g). All the fishes were then anaesthetized and dissected to examine the gonads to determine the gender and maturity status. Individuals with gonads (hereafter 'sexually mature/mature; includes both individuals with eggs or sperm and individuals with quiescent gonads) were categorized as male and female, and also females were classified into different stages of maturity based on macroscopic observation of gonad (after Gibbs *et al.*, 2008; Jumawan and Herrera, 2014). Males were defined ripe when semen was observed in the gonad, whereas females were classified as ripe when ovary occupying >50% of the body cavity, orange in color with strong vascularization and containing large yellow oocytes. Individuals without gonad and with doubtful male or female identity were excluded from analysis. Females with gonads in regression phase were also excluded from the study. Gonads of males and females were then gently removed, and the weights of the gonads ( $w_g$ ) were measured (nearest 0.01 g) using the electronic balance. Only ripe females were used for studying the fecundity and egg size.

The formula  $GSI = (\text{Gonad weight} / \text{Total body weight}) \times 100$  was used to assess the gonado-somatic index (GSI). To estimate absolute fecundity (AF) small sub-samples from the front, middle, and posterior regions of the left lobe ovary of the mature female were taken, weighed, and the eggs in the sub-samples were counted, averaged, and extrapolated to the egg count of the total ovary. Relative fecundity (RF) was calculated as  $RF = \text{absolute fecundity} / \text{total body weight}$ . To determine egg size (ES), 100 eggs were randomly selected for measurement of their diameter (nearest 0.01 mm) using a vernier caliper (Bombay Tools Center Bombay Pvt Ltd.;  $\pm 0.01$  mm). Calculated mean TL at maturity ( $TL_m$ ) were determined from the percentage of mature males and females in each one centimeter size class, following the Trippel and Harvey (1987) method.

#### Collection and analysis of hydrological parameters

Water temperature, pH, total dissolved solids (TDS) and dissolved oxygen (DO) were measured electrometrically on site by using a multiparameter portable water quality testing kit (Hanna multiparameter, USA). For analysis of other parameters, collected water samples were acidified with  $2N H_2SO_4$  and then transported to the laboratory in ice-box to store at  $4^\circ C$  until analysis. Ammonia-nitrogen ( $NH_3-N$ ), nitrate-nitrogen ( $NO_3-N$ ) and phosphorus ( $PO_4-P$ ) were determined following standard procedure (APHA, 2005). Ammonia and nitrogen are

together presented as total nitrogen (TN) and phosphorus which was measured as  $PO_4^{3-}$  (phosphate) is presented as total phosphorous (TP) for correlation analysis.

#### Statistical analysis

All data was analyzed using IBM SPSS statistical package version 23.0 (SPSS Inc., Chicago, IL, USA). All numerical data are expressed as mean and standard error (mean  $\pm$  SE). The differences between the means were compared with one-way ANOVA, and the significance was verified through Tukey's post-hoc test. Pearson correlations were performed to determine the relationships between nutrient concentrations (TP and TN) in sampling sites and reproductive traits of mature males and females. All tests were set at  $p < 0.05$ .

## RESULTS

#### Morphometrics and GSI of mature male and female

A total 923 adult individuals of *Pterygoplichthys pardalis* was obtained from five sampling sites, of which 608 were dissected (to minimize sacrifice randomly three specimen was selected from each equivalent size group, if any). Sex ratios were found female-biased in all study sites of EKW, with the highest ratio in  $B_3$  (male: female :: 1: 3.61), but not differed significantly amongst the sites ( $\chi^2 = 1.368$ ,  $df = 4$ ,  $p = 0.850$ ; Table I). No significant difference ( $t = 0.562$ ,  $p = 0.575$ ; student's t-test) was found between mean TL of mature males (mean  $\pm$  SE:  $36.92 \pm 0.32$  cm) and mature females (mean  $\pm$  SE:  $36.32 \pm 0.31$  cm) (specimens of all study sites polled together). However, length of male fish with active testis (29.3 – 44.1 cm, TL) was found narrower than the length of the female fish with the active ovaries (26.3 – 49.3 cm, TL).

**Table I. Total number of *Pterygoplichthys pardalis* specimens dissected (N), sex ratio (male: female), number of sexually mature male ( $M_m$ ) and female ( $F_m$ ) at different stages of maturity (n) with indication of the number of ripe individuals ( $Mt_R$ ) by study sites.**

Study sites	N	Sex ratio	Male ( $M_m$ )		Female ( $F_m$ )	
			n	$Mt_R$	n	$Mt_R$
Narkeltala bhery ( $B_1$ )	138	1:2.95	24	14	71	29
Chacker bhery ( $B_2$ )	143	1:2.56	32	19	82	51
Jhagrasirsha bhery ( $B_3$ )	124	1:3.61	21	8	76	37
Chacharia bhery ( $K_1$ )	114	1:3.12	17	7	53	22
Nalban-1 bhery ( $K_2$ )	89	1:3.46	13	4	45	23
Total	608	1:3.05	107	52	327	162

**Table II. *Pterygoplichthys pardalis*: Minimum and maximum total length (TL<sub>min-max</sub>, cm) and weight (W<sub>min-max</sub>, g), mean (± SE) total length (TL) and weight (W), mean (± SE) gonado-somatic index (GSI) of sexually mature male (M) and female (F); and their calculated mean TL at maturity (TL<sub>m</sub>) at different study sites.**

Study sites	N <sub>m</sub>		TL <sub>min-max</sub> (cm)		Mean TL (cm)		W <sub>min-max</sub> (g)		Mean W (g)		Mean GSI		TL <sub>m</sub> (cm)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
B <sub>1</sub>	24	71	30.9-43.5	26.3-49.2	35.98±0.67 <sup>ab</sup>	36.07±0.72 <sup>a</sup>	213.5-537.2	146.6-916	334.41±17.99 <sup>a</sup>	372.53±19.77 <sup>a</sup>	0.143±0.012 <sup>a</sup>	6.716±0.322 <sup>a</sup>	35.9	29.8
B <sub>2</sub>	32	82	31.2-44.1	28.4-48.7	37.68±0.58 <sup>b</sup>	36.18±0.65 <sup>a</sup>	261-555.5	152.3-795.5	378.06±12.85 <sup>a</sup>	367.25±17.65 <sup>a</sup>	0.115±0.009 <sup>a</sup>	6.753±0.298 <sup>a</sup>	36.3	30.4
B <sub>3</sub>	21	76	29.3-41.8	27.1-49.3	35.09±0.81 <sup>a</sup>	34.92±0.67 <sup>a</sup>	169.5-482.4	145.5-885.4	317.90±20.92 <sup>a</sup>	350.27±18.48 <sup>a</sup>	0.134±0.052 <sup>a</sup>	5.961±0.317 <sup>ab</sup>	35.8	30.1
K <sub>1</sub>	17	53	35.7-42.6	31.2-45.7	38.38±0.45 <sup>b</sup>	37.46±0.56 <sup>ab</sup>	272.4-513.9	196.7-560	346.82±15.96 <sup>a</sup>	339.64±13.28 <sup>a</sup>	0.141±0.012 <sup>a</sup>	5.138±0.291 <sup>b</sup>	37.5	34.4
K <sub>2</sub>	13	45	32.7-43.5	32.5-46.2	37.85±0.75 <sup>ab</sup>	38.93±0.51 <sup>b</sup>	244.1-531.5	210.5-615.3	359.61±20.02 <sup>a</sup>	377.95±14.76 <sup>a</sup>	0.116±0.011 <sup>a</sup>	5.731±0.422 <sup>ab</sup>	38.7	36.5

N<sub>m</sub>: Number of mature individuals; Means in a column with different superscripts indicates significant differences at P<0.05 (one-way ANOVA followed by post hoc Tukey test). Details of study sites are given in Table I.

**Table III. *Pterygoplichthys pardalis*: Average (mean ±SE) length and weight, gonad mass (GM, in g), gonado-somatic index (GSI<sub>R</sub>), absolute fecundity (AF; in numbers), relative fecundity (RF; in numbers per g female body weight) and egg size (in mm) of ripe female fishes at different study sites. SE: Standard Error.**

Study sites	N	Biological traits of ripe female fishes						
		Length (cm)	Weight (g)	GM	GSI <sub>R</sub>	AF	RF	Egg size
B <sub>1</sub>	29	36.25 ± 1.22 <sup>a</sup>	385.56 ± 32.94 <sup>a</sup>	34.04 ± 2.04 <sup>ac</sup>	8.57 ± 0.56 <sup>a</sup>	4029.11 ± 297.23 <sup>a</sup>	9.96 ± 0.24 <sup>a</sup>	2.263 ± 0.067 <sup>a</sup>
B <sub>2</sub>	51	37.03 ± 0.92 <sup>a</sup>	395.39 ± 24.86 <sup>a</sup>	30.34 ± 2.02 <sup>abc</sup>	7.73 ± 0.31 <sup>ab</sup>	4054.11 ± 184.70 <sup>a</sup>	10.09 ± 0.16 <sup>a</sup>	2.227 ± 0.054 <sup>a</sup>
B <sub>3</sub>	37	36.39 ± 1.12 <sup>a</sup>	391.36 ± 31.76 <sup>a</sup>	32.76 ± 2.27 <sup>ac</sup>	7.51 ± 0.45 <sup>ab</sup>	3982.21 ± 237.63 <sup>a</sup>	9.91 ± 0.19 <sup>a</sup>	2.220 ± 0.058 <sup>a</sup>
K <sub>1</sub>	22	38.34 ± 0.74 <sup>a</sup>	366.04 ± 20.09 <sup>a</sup>	21.82 ± 1.53 <sup>b</sup>	6.24 ± 0.41 <sup>b</sup>	3659.95 ± 202.17 <sup>a</sup>	9.29 ± 0.42 <sup>a</sup>	2.244 ± 0.094 <sup>a</sup>
K <sub>2</sub>	23	37.89 ± 0.81 <sup>a</sup>	362.65 ± 21.93 <sup>a</sup>	28.44 ± 2.22 <sup>abc</sup>	7.16 ± 0.37 <sup>ab</sup>	3595.93 ± 210.17 <sup>a</sup>	9.81 ± 0.31 <sup>a</sup>	2.202 ± 0.067 <sup>a</sup>

N: Number of ripe females; Means in a column with different superscripts within a fish group indicates significant differences at p < 0.05 (one-way ANOVA followed by post hoc Tukey test). Details of study sites are given in Table I.

Between study sites of EKW, the mean TLs of mature individuals was found significantly different both for male ( $F_{4,102}=3.942$ ,  $p=0.005$ ) and female ( $F_{4,322}=4.247$ ,  $p=0.002$ ; one-way ANOVA). Male length was found highest in K<sub>1</sub> (mean ± SE: 38.38 ± 0.45 cm; significantly longer compare to B<sub>3</sub>,  $p=0.016$ ; Tukey's post-hoc test), while female length was found greater in K<sub>2</sub> (mean ± SE: 38.93 ± 0.51 cm; significantly longer compare to all sites of location B,  $p<0.05$ ; Tukey's post-hoc test). Mean calculated TL at maturity (TL<sub>m</sub>; male: 35.8–38.7 cm, female: 29.8–36.5 cm) was also found greater at sites of location K compare to B, for both the sexes. Significant variations were also observed in the GSI of female Amazon sailfin catfishes between different sites of EKW ( $F_{4,322}=4.172$ ,  $p=0.003$ ). The GSI of female fish in K<sub>1</sub> (5.138 ± 0.291 SE) was found significantly lower ( $p<0.01$ ) compare to B<sub>1</sub> (GSI: 6.716 ± 0.322 SE) and B<sub>2</sub> (GSI: 6.753 ± 0.298 SE). Though not significant, female fish GSI in K<sub>2</sub> (5.731 ± 0.422 SE) was also found lower compare to all sites of study location B

(Table II). Greater percentage of female with ripe ovaries (stage 3) or in advance stage of maturity (stage 2) were also observed in sites of location B compare to study sites of location K (Fig. 2). However, mean GSI of male fish not differed significantly between study sites of EKW ( $F_{4,102}=1.344$ ,  $p=0.259$ , one-way ANOVA). We also didn't found any significant correlation of male and female GSI with their respective length and weight (Fig. 3). Furthermore, mean weight of mature male and female were also found indifferent between study sites (Male:  $F_{4,102}=2.091$ ,  $p=0.087$ ; Female:  $F_{4,322}=0.561$ ,  $p=0.691$ ; one-way ANOVA).

#### Reproductive traits of ripe females

The mean length, weight and other biological traits of 162 ripe female *Pterygoplichthys pardalis* are presented in Table III. No significant difference in length and weight of ripe female by sites was observed ( $p>0.05$ ; one-way ANOVA) during the study. However, significant variations were observed in the gonad mass and GSI of ripe females

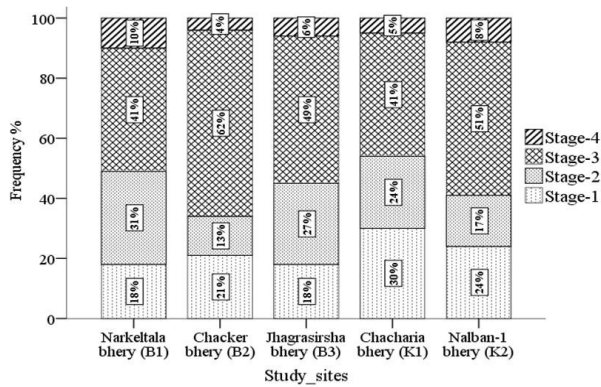


Fig. 2. Ovarian maturity stages of female *Pterygoplichthys pardalis* ( $n = 327$ ) found in different study sites of East Kolkata Wetlands, India. Stage 1 (ovary with immature follicles), stage 2 (ovary with maturing follicles), stage 3 (ovary with mature follicles/ ripe), and stage 4 (ovary with spent follicles).

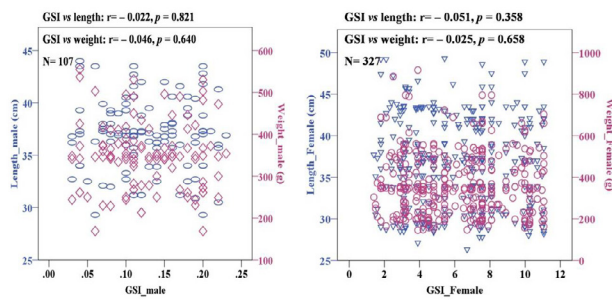


Fig. 3. Gonadosomatic index (GSI) of male (left) and female (right) *Pterygoplichthys pardalis* in relation to the total length and whole body weight of mature males and females, respectively.  $r$  and  $p$  represents the correlation coefficients and significance of correlation, respectively, for Pearson correlation analyses describing the relationships GSI with length and weight of respective gender.  $N$ , number of mature specimen.

(GSI- $F_R$ ). Gonad mass from sites of location B ( $32.02 \pm 1.24$  SE) was higher significantly ( $t_{160} = 3.104$ ,  $p=0.002$ ; Student's  $t$ -test) compare to sites of location K ( $25.21 \pm 1.43$  SE). Similarly, GSI- $F_R$  also found significantly higher ( $t_{160} = 2.685$ ,  $p=0.008$ ; Student's  $t$ -test) in location B ( $7.87 \pm 0.24$  SE) than location K ( $6.71 \pm 0.28$  SE). Absolute fecundity (AF) of ripe female fish ranged between 2100 and 7850 numbers, but not varied significantly by sites ( $F_{4,157} = 0.757$ ,  $p = 0.555$ ; one-way ANOVA). No significant difference were also observed in RF of ripe females of different study sites ( $F_{4,157} = 1.351$ ,  $p = 0.253$ ). The overall ova diameter of ripe female ranged from 1.6 to 3.1 mm, but indifferent statistically between sites ( $F_{4,157} = 0.105$ ,  $p = 0.981$ ).

#### Hydrological properties/ nutrient levels

Among the hydrological parameters significant differences by study sites were observed in pH ( $F_{4,115} = 3.537$ ,  $p = 0.009$ ), TDS ( $F_{4,115} = 10.036$ ,  $p < 0.001$ ), ammonia ( $F_{4,115} = 4.327$ ,  $p = 0.003$ ), nitrate ( $F_{4,115} = 10.820$ ,  $p < 0.001$ ) and phosphate ( $F_{4,115} = 11.476$ ,  $p < 0.001$ ), but temperature ( $F_{4,115} = 0.205$ ,  $p = 0.935$ ) and DO ( $F_{4,115} = 0.952$ ,  $p = 0.437$ ; one-way ANOVA) not varied significantly (Table IV). Mean TP concentration was found significantly higher ( $t_{118} = 5.718$ ,  $p < 0.001$ ;  $t$ -test) in the location B (mean  $\pm$  SE:  $0.445 \pm 0.019$  mgL<sup>-1</sup>) than in K (mean  $\pm$  SE:  $0.258 \pm 0.026$  mgL<sup>-1</sup>). Similarly, mean total nitrogen (ammonia + nitrate) was also found higher significantly ( $t_{118} = 6.436$ ,  $p < 0.001$ ;  $t$ -test) in B (mean  $\pm$  SE:  $0.585 \pm 0.028$  mgL<sup>-1</sup>) than in K (mean  $\pm$  SE:  $0.336 \pm 0.021$  mgL<sup>-1</sup>) (Fig. 4).

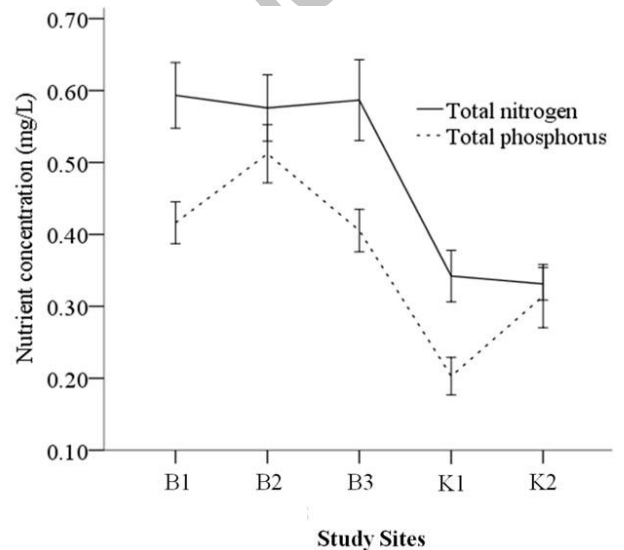


Fig. 4. Mean concentrations of total nitrogen and total phosphorus in five studied sites of East Kolkata Wetlands, India. Error bars represent standard error.

#### Correlation with nutrient levels with reproductive traits of Amazon sailfin catfish

Significant correlation between nutrient (TP and TN) concentration of the study sites and reproductive traits of Amazon sailfin catfish (Table V) were observed during present study. TP was found to have significant ( $p < 0.05$ ) positive ( $r \geq 0.123$ ) impact on GSI of mature female (GSI- $F_m$ ), and gonad mass and absolute fecundity (AF) of ripe females. Whereas, mean total length of sexually mature male (TLM<sub>m</sub>) showed a significant ( $p < 0.05$ ) negative correlation ( $r = -0.198$ ) in TP. However, TP concentrations were found not correlated significantly (all  $p > 0.05$ ) with mean total length of sexually mature female (TLF<sub>m</sub>), mean whole body weight of sexually mature male (WM<sub>m</sub>) and

**Table IV. Variations in temperature (°C), pH, dissolved oxygen (DO, mg L<sup>-1</sup>), total dissolved solids (TDS, mg L<sup>-1</sup>), ammonia (mg L<sup>-1</sup>), nitrate (mg L<sup>-1</sup>) and phosphate (mg L<sup>-1</sup>) in different study sites.**

Study site	Temperature	pH	DO	TDS	Ammonia	Nitrate	Phosphate
Narkeltala bhery (B <sub>1</sub> )	26.84±0.31 <sup>a</sup>	7.53±0.03 <sup>b</sup>	6.28±0.15 <sup>a</sup>	438.21±11.91 <sup>a</sup>	0.403±0.046 <sup>bc</sup>	0.189±0.017 <sup>a</sup>	0.416±0.029 <sup>bc</sup>
Chacker bhery (B <sub>2</sub> )	26.64±0.32 <sup>a</sup>	7.54±0.04 <sup>b</sup>	6.17±0.13 <sup>a</sup>	436.79±8.97 <sup>a</sup>	0.335±0.038 <sup>ac</sup>	0.241±0.032 <sup>a</sup>	0.512±0.039 <sup>b</sup>
Jhagrasirsha bhery (B <sub>3</sub> )	26.49±0.35 <sup>a</sup>	7.30±0.08 <sup>a</sup>	6.23±0.11 <sup>a</sup>	427.21±7.88 <sup>a</sup>	0.410±0.056 <sup>bc</sup>	0.176±0.016 <sup>a</sup>	0.405±0.029 <sup>bc</sup>
Chacharia bhery (K <sub>1</sub> )	26.83±0.31 <sup>a</sup>	7.44±0.04 <sup>ab</sup>	6.10±0.08 <sup>a</sup>	388.62±6.63 <sup>b</sup>	0.257±0.036 <sup>ac</sup>	0.084±0.007 <sup>b</sup>	0.202 ± 0.026 <sup>a</sup>
Nalban-1 bhery (K <sub>2</sub> )	26.79±0.35 <sup>a</sup>	7.41±0.05 <sup>ab</sup>	6.02±0.08 <sup>a</sup>	391.33±3.59 <sup>b</sup>	0.219±0.019 <sup>a</sup>	0.111±0.010 <sup>b</sup>	0.314±0.043 <sup>ac</sup>

The column within a group with different superscripts indicates significant differences at  $p < 0.05$  (one-way ANOVA followed by post hoc Tukey test); values are means ± standard error.

**Table V. Correlation coefficient (r) and p-values for Pearson correlation analyses describing the relationships between nutrient concentrations and biological traits of *Pterygoplichthys pardalis*. Significant effects are indicated in bold.**

Biological traits	Mean	Correlation with nutrient concentration			
		Total phosphorus		Total nitrogen	
		r	p-value	r	p-value
TL mature male (TLM <sub>m</sub> , in cm)	36.92	-0.198	<b>0.041</b>	-0.008	0.935
W mature male (WM <sub>m</sub> , in g)	349.26	0.024	<b>0.803</b>	0.035	0.723
TL mature female (TLF <sub>m</sub> , in cm)	36.32	0.076	0.170	0.082	0.140
W mature female (WF <sub>m</sub> , in g)	363.59	0.049	0.376	0.134	0.168
GSI mature male (GSI-M <sub>m</sub> )	0.126	0.175	0.072	0.117	<b>0.035</b>
GSI mature female (GSI-F <sub>m</sub> )	6.158	0.123	<b>0.027</b>	0.079	0.155
GSI ripe female (GSI-F <sub>r</sub> )	7.551	0.109	0.116	-0.072	0.365
Ripe female gonad mass (in g)	30.130	0.183	<b>0.008</b>	0.044	0.578
Absolute fecundity (AF, in nos.)	3914	0.174	<b>0.028</b>	0.097	0.162
Relative fecundity (RF, in nos./g)	9.882	0.123	0.076	0.093	0.240
Ripe female egg size (in mm)	2.230	0.071	0.305	0.024	0.732

TL, total length; W, whole body weight; GSI, gonadosomatic index.

female (WF<sub>m</sub>), GSI of mature male (GSI-M<sub>m</sub>) and ripe female (GSI-F<sub>r</sub>), and RF and egg size of ripe female. On the other hand, total nitrogen (TN) was found to have significant correlation only with GSI of mature male ( $r=0.117$ ,  $p=0.035$ ). With all other traits of both male and female correlation of TN was found insignificant (all  $p>0.05$ ), but mostly positive ( $r>0$ ).

## DISCUSSION

Populations of invasive *Pterygoplichthys* species are growing rapidly as they can consume a variety of foods, including detritus, and have ability to adapt and maintain high breeding capacity in variety of environmental conditions (Gibbs *et al.*, 2017). Further, it has been noted that many of the invaded populations of sailfin catfish have sex ratios that are skewed toward females, which

encourages more recruitment (Samat *et al.*, 2016; Wei *et al.*, 2017). Although we found that males with active testis had a narrower length range than females with active ovaries, many other studies have shown that males of loricariid catfishes reach larger sizes at first maturation and have broader length ranges than females (Wanasinghe *et al.*, 2023), supporting strong territorial behavior and parental care of the male during the reproductive season (Lampert *et al.*, 2022).

Although mature male and female TLs at different EKW sites differed in terms of minimum, maximum, and mean values, overall values were found to be consistent with the results of many previous studies. Male *P. disjunctivus* in the Marikina River, Philippines, reached sexual maturity at lengths of 29 cm SL, while the maximum recorded mature male was 45 cm SL (Jumawan *et al.*, 2016). The mature male *P. disjunctivus* minimum

and highest reported lengths in Volusia Blue Spring, Florida, were 21 cm SL (Gibbs *et al.*, 2017) and 49 cm SL (Gibbs *et al.*, 2008), respectively. Similarly, the minimum length of mature female *P. disjunctivus* from Marikina River, Philippines, and Volusia Blue Springs, Florida was 26 cm SL and 30 cm SL, respectively (Gibbs *et al.*, 2008; Jumawan and Herrera, 2014). In comparison, the smallest mature female from EKW had a much shorter length (26.3 cm TL // 19.6 cm SL). However, Wanasinghe *et al.* (2023) observed far smaller sized females of *P. disjunctivus* with functioning ovaries in the Polgolla Reservoir, Sri Lanka (16.3 cm SL// 22.6 cm TL). In addition to the above, differences in the mean size at maturity of sailfin catfishes, within a geographical region, have also been recorded (Wei *et al.*, 2017).

In present study, variation in number of mature individuals and their GSI values between different sites within EKW were observed. Though within a habitat, location specific variations in GSI of sailfin catfishes have not yet been documented elsewhere, wide differences in the GSI of different exotic populations of sailfin catfishes have been reported (Gibbs *et al.*, 2008; Jumawan *et al.*, 2016). Mean absolute fecundity ( $3914 \pm 103$  eggs/female) not differed significantly between sites of EKW, and found to be equivalent to the total fecundity of *P. pardalis* observed in the Langat River in peninsular Malaysia with  $>4000$  eggs/fish (Samat *et al.*, 2016), little higher than the total fecundity of *P. disjunctivus* reported from Mexico ( $2447 \pm 252$  eggs/fish) (Rueda-Jasso *et al.*, 2013), but far lower than what were reported for *P. disjunctivus* caught from Marikina River, Philippines ( $AF = 7705 \pm 216$  eggs/fish; Jumawan *et al.*, 2016). Ova diameter of ripe female (1.6 – 3.1 mm) observed in present study, is consistent with the previously reported maximum ova diameters for *P. disjunctivus* and *P. pardalis* of 3.6 mm and 3.3 mm, respectively (Gibbs *et al.*, 2008; Samat *et al.*, 2016). These large eggs of *Pterygoplichthys* contain more energy enabling them to develop into a specific phenotype with little or no external nourishment (Gibbs *et al.*, 2008; Samat *et al.*, 2016). Furthermore, according to a theory put forth by Balon (1999), an individual has a better chance of surviving if it is larger and more developed when external feeding starts. *Pterygoplichthys* and many other loricariids lack true larval stages, which not only lowers the cost of developing temporary organs through metamorphosis but also increases their competitiveness even in their earliest stages, making it likely advantageous for their invasion (Balon, 1999; Gibbs *et al.*, 2008).

Variations observed in the reproductive traits of Amazon sailfin catfish between different sites of EKW, might be correlated with the nutrient concentrations of the study sites, which differed significantly. Male Amazon

sailfin catfish matured at smaller size ( $r = -0.198$ ) when TP concentrations increased in the sites of EKW, but matured female length not showed any significant correlation. This findings in present study is little conflicting with the observations of Wei *et al.* (2018), where they found increased mean size of female and male at maturity with increased TP and TN, respectively. GSI of both mature male and female were found to have a very weak but mostly positive relationship with TP and TN (except thin negative correlation ( $r = -0.072$ ) of  $GSI-F_R$  with TN concentration) in present study. Significant positive correlation ( $p = 0.028$ ) of  $GSI-F_m$  with TP observed in the study, might suggests higher TP demand by female of these fishes to promote the growth of their reproductive cells (Craik and Harvey, 1984). On the other hand, significant positive correlation ( $p = 0.035$ ) of  $GSI-M_m$  with TN might indicate higher TN demand by males for reproduction, as TN can affect spermatozoa and sperm motility of males (Cheah and Yang, 2011). Although sailfin catfish can survive poor water quality, too much N (nitrogen), particularly in the form of ammonia can impinge the reproducing ability of the species (El-Shafai *et al.*, 2004), which likely describe the small negative correlation ( $r = -0.072$ ,  $p = 0.365$ ) of  $GSI-F_R$  with TN observed in the present study. Furthermore, according to Brinkman *et al.* (2009), an increase in ammonium in the water could cause severe physiological and histopathological reactions in fish, which would have a negative impact on the fishes ability to develop and mature.

Environmental-related variations in sailfin catfish growth, maturity, and reproduction were also documented by Wei *et al.* (2022), suggesting that these fish had different requirements in response to the context-specific environments in the invaded water bodies. Among the nutrients, sailfin catfishes reproductive traits were found more correlated with TP concentrations of the invaded habitat, that supports the fact of higher phosphorus demand by these fishes (Capps and Flecker, 2013; Tristano and Gibbs, 2023). Additionally, these results imply that nutrient concentrations of the invaded environment may have some influence on the colonization, life history characteristics, and population growth of *Pterygoplichthys* species (Wei *et al.*, 2018). However in EKW, all correlation of TP and TN with reproductive traits of *Pterygoplichthys pardalis* were found weak (0.20 – 0.39) or very weak (0.00 – 0.19) (Evans, 1996) indicating that, variations observed in reproductive traits of *Pterygoplichthys pardalis* in EKW might be influenced by several other environmental and correlated factors (Stearns, 1989; Wei *et al.*, 2022).

## CONCLUSION

Reproductive traits of *Pterygoplichthys pardalis* species showed location specific variations in East Kolkata Wetlands, and were found associated, though often weakly, with the concentrations of phosphorus and nitrogen of the study sites. Female gonadosomatic index, gonad mass and absolute fecundity were found correlated positively and significantly with phosphorus, while male length at maturity exhibited a negative correlation with phosphorus. Total nitrogen, however, showed a considerable favorable impact only on the male gonadosomatic index. Overall, the results of this study support the theory that nutrient enrichment may make it easier for the non-native fish like *Pterygoplichthys pardalis* to invade.

## DECLARATIONS

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### Ethical statement

All applicable institutional, national and international guidelines for the care and use of animals were followed.

### Statement of conflict of interest

The authors have declared no conflict of interest.

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