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An Assessment of Population and Health Status of Native Fish Species from Rivers Kabul and Swat, Khyber Pakhtunkhwa, Pakistan

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Condition factor, *Schizothorax plagiostomus*, LWR, GSI, HIS

ABSTRACT

The present study investigated the population and health status of fishes from Rivers Kabul and Swat, Khyber Pakhtunkhwa province, Pakistan. Three sites per river were sampled to cover a range of land use, from pristine areas, to sites close to highly agricultural, urbanized, and industrialized areas. Morphometric measurements, age, hepatosomatic index (HSI), gonadosomatic index (GSI), gonadal staging, and hematological parameters were assessed. A total of 128 fish comprising five native (*Schizothorax plagiostomus, S. labiatus, Cirrhinus mrigala, Tor macrolepis,* and *Clupisoma naziri*) and one introduced species (*Cyprinus carpio*) were sampled in June 2018 and in April 2019. From Swat, we were able to compare populations of the Himalayan seatrout (*S. plagiostomus*) across sites. Except for a small decline in HSI and GSI in fish from sites closer to urban and agricultural areas, we found little differences in health parameters across sites. Although similar analyses were not possible from Kabul as species differed across sites, we report health values that fall within ranges of previous studies in this region. Our study adds to the limited amount of basic physiological information available for these valuable fish species.

INTRODUCTION

Fish health is often considered a reliable proxy for overall ecosystem health (Adeniran *et al*., 2017; Simon, 2020). This is because fish are in constant contact with water and poor water quality, including presence of pollutants and pathogens, which can result in adverse health outcomes. Fish health can be assessed through a variety of measures. Basic morphometric measures such as body mass and length are easily obtainable but are variable and can change significantly with age, developmental stage, season, and sex. Two metrics are often used to standardize the relationship between body mass and length-Fulton's condition factor (K) and the length-weight relationship (LWR). The K standardizes the relationship and assumes isometric growth where the mass of an individual is proportional to the cube of its length (Nakagawa *et al*., 2007), whereas, LWR is an allometric analysis and does not assume isometric growth- it can vary with life stage and/ or across time (Singh and Serajuddin, 2017; Araújoa *et al*., 2018; Mandal *et al*., 2018). Hepatosomatic and gonadosomatic indices (HSI and GSI, respectively) are two commonly used organo-somatic indices used for estimating the status of energy reserves and reproductive condition of fish (Adeniran *et al*., 2017; Araújoa *et al*., 2018). HSI relates the mass of the liver to the overall mass of the fish with higher values suggesting greater energy reserves in an individual while GSI relates gonadal and somatic masses with higher values indicating greater reproductive potential (Nakagawa *et al*., 2007). Hematological parameters such as packed cell volume (PCV), and plasma protein levels can also be used to investigate fish health as changes in blood composition can be induced by several environmental stressors, including contaminants (Gopal *et al*., 1997; Shah, 2006; Öner *et al*., 2008; Karimi *et al*., 2013; Adeniran *et al*., 2017; Burgos-Aceves *et al*.,

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2019; Sheikh and Ahmed, 2019). For instance, fish from polluted environments have higher PCV and red blood cells (RBCs) in comparison to the fish from relatively cleaner environments (Burgos-Aceves *et al*., 2019). Blood plasma protein values can also be influenced by exposure to toxicants, however the magnitude of change can vary across fish species (Gopal *et al*., 1997; Öner *et al*., 2008; Adeyemo and Enefe, 2021).

Rivers Kabul and Swat, Pakistan, are of huge economic and ecological importance. River Kabul is the major river of Hindu Kush Highlands in Pakistan (has a drainage basin of 66,000 square kilometers) (Yousafzai *et al*., 2008; Nafees *et al*., 2011; Ahmad *et al*., 2015). River Swat, another major river (has a drainage basin of about 7,863 square kilometers irrigate about 160,000 acres (65,000 hectares) within the province, joins River Kabul at Nisatta Charsadda (Yousafzai *et al*., 2013). River Kabul and its tributaries receive untreated sewage from the adjacent regions of Swat, Malakand, Dir, Mardan, Charsadda, Nowshera, and Peshawar (IUCN-Pakistan, 1994; Khan *et al*., 2011). Similarly, industrial effluents are also discharged either indirectly or directly into River Kabul (Abrar *et al*., 2011; Khan *et al*., 2011; Ahmad *et al*., 2015). A study identified 21 and 25 contamination hotspots on Rivers Kabul and Swat, respectively, and reported levels of heavy metals (Cd, Cr, Pb and Zn) exceeding surface water quality standards set by the World Health Organization (WHO) and the Pakistan Environmental Protection Agency (Pak-EPA) (Kiran, 2021). Heavy metals have also been quantified in fish from these river systems and some (Cr, Cu, Ni, Pb, and Zn) been reported to exceed recommended dietary allowance (RDA) values for fish fillets (Ahmad *et al*., 2015). The only study that has examined accumulation of organic pollutants in fish from these river systems reported dichlorodiphenyl trichloroethane (DDT) and hexachlorocyclohexane (HCH) in tissues from four fish (Aamir *et al*., 2016).

Systematic studies assessing the population and health status of native fish species from Pakistani Rivers are lacking. This information is important because it can be used to make sound fishery management decisions. Therefore, the objective of this work was to determine the population and health status of native fish species sampled from Rivers Kabul and Swat. Several fish species were sampled and health parameters, including morphometrics and hematology, were used to assess health condition.

MATERIALS AND METHODS

Study area

This study was conducted on Rivers Swat and Kabul, Khyber Pakhtunkhwa (KP), Pakistan. Both rivers are used for irrigation and are major sites of commercial and sport fishing in this highly populated area (Rafique, 2001) (Fig. 1). River Swat originates from the Hindukush mountains and flows through the Kalam Valley, Swat, Lower Dir, and Malakand and at Charsadda it enters River Kabul. Therefore, the three sites were selected to include a range of fish habitat, from very good to pristine (Madyan) to more impacted by agriculture and urbanization in Panjigram and Landakay. In Kabul, three sampling sites were also selected, from west to east: Warsak Dam (relatively cleaner site), Akbarpura, and Akramabad (relatively polluted sites receive untreated sewage waste and industrial effluents from adjacent regions).

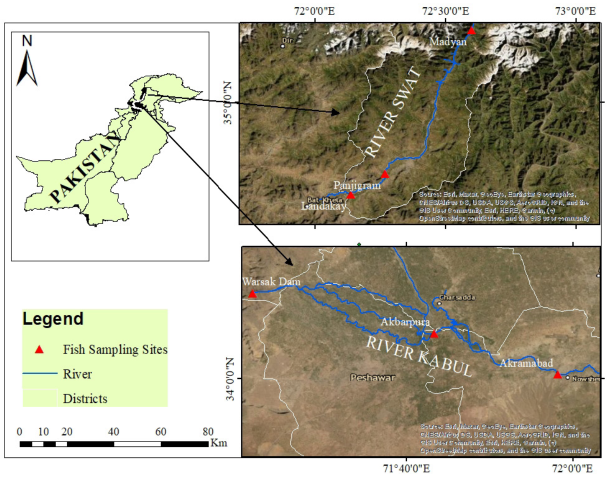

Fig. 1. Map of study area showing fish sampling sites (the river flows from northern mountainous region towards southern plains).

Fish sampling

In June 2018, fish were collected by local fishermen using gill nets. A second fish collection from River Kabul occurred in April 2019 from a single site (Akbarpura). Only fish that were alive were bled (see below). Four species were sampled from River Kabul: mrigal *Cirrhinus mrigala*; mahseer *Tor macrolepis*; catfish *Clupisoma naziri*; and the introduced common carp *Cyprinus carpio*. No overlap in species was observed between rivers as only the Himalayan seatrout *Schizothorax plagiostomus* and the Kunar snow trout *S. labiatus* were sampled from River Swat.

Morphometric measurements and hematology

Immediately upon collection, fish were anesthetized with buffered MS-222 (tricaine methanesulfonate) (150-250 mg/L) and bled from the caudal peduncle using 21-25 G 1-11/2” needles. Blood samples were collected in

2.0 ml heparinized vials. A sample of whole blood was collected using a capillary tube, sealed on one end using clay, and spun on a hematocrit centrifuge for 15,000 rpm for 5 min. Hematocrit or packed cell volume (PCV) was measured afterwards. A drop of plasma was used to measure total plasma proteins (g/dL) using a portable clinical refractometer. Subsequently, fish were euthanized (> 350 mg/L MS-222), weighed to the nearest 0.01 g using a digital balance, and measured (total length) to the nearest 0.01 cm using a measuring tape. Fish were necropsied and gonads and liver collected and weighed using a digital balance to calculate gonadosomatic index (GSI) and hepatosomatic index (HSI) using Equations 1 and 2, respectively (Rad *et al*., 2013). Fulton's condition factor (K) was calculated using Equation 3 (Mozsár *et al*., 2015). A section of the gonads was fixed in 10% buffered formalin for histological examination as described below (Saraiva *et al*., 2015).

$$GSI\ (\%) = \frac{\text{Gonad weight (g)}}{\text{Total body weight (g)}} \times 100 \quad \ldots(1)$$

$$HSI\ (\%) = \frac{\text{Liver weight (g)}}{\text{Total body weight (g)}} \times 100 \quad \ldots(2)$$

$$K = \frac{\text{Total body weight (g)}}{[\text{Total length (mm)}]3} \quad \ldots(3)$$

Gonadal staging

Gonads were dehydrated in graded ethanol solutions (50%-100% C_2H_5OH) and embedded in paraffin wax. Subsequently, gonad sections of 5 µm thickness were cut and stained using hematoxylin and eosin (H and E) (Barnhoorn *et al*., 2004; Sadekarpawar and Parikh, 2013; Saraiva *et al*., 2015). Sections were then examined under a light microscope (40–100X) (Nikon Eclipse Ni) coupled with a digital camera (Nikon DS Ri2). Gonadal stage determination was done following USEPA Guidelines (Ankley *et al*., 2006). Briefly; ovaries were categorized into 5 stages, from stage 0 (undeveloped) to stage 4 (late vitellogenic). Testes were divided into 4 stages from stage 1 (early spermatogenic) to stage 4 (spent testes having some remnant sperm).

Fish aging

Fish with scales (*Cirrhinus mrigala, Cyprinus carpio and Tor macrolepis*) were aged using scales. Fish scales were collected and preserved in paper envelopes, washed with 1% KOH and observed using a microfiche reader. Annuli were identified and counted (a representative image is given in Fig. 2) (Ujjania *et al*., 2014).

Statistical analysis

Descriptive statistics (mean ± SEM, minimum, and maximum) were calculated for body mass, total length, K, GSI, HSI, PCV, and plasma proteins for all species sampled at all sites of both rivers. Regression analyses for length-weight relationships were performed to explore potential differences in the growth patterns across fish species and size at age plots were observed by performing correlations using IBM SPSS Statistics 21. There were no species present at all sites on River Kabul, however *S. plagiostomus* were consistently collected at all sites on River Swat. Therefore, additional analyses were conducted using only *S. plagiostomus* for which sex could be determined collected from River Swat. Two-way ANOVAs were conducted in SigmaPlot version 13.0 (Systat Software Inc., San Jose, CA, USA) and used to determine differences in K, GSI, HSI, PCV, and plasma proteins between the Madyan site and the Panjigram and Landakay sites as well as between males and females within each site. For all analyses, significant differences were defined at $p \leq 0.05$.

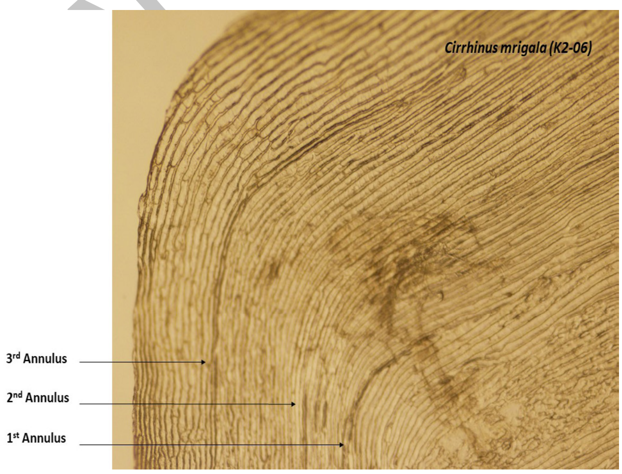

Fig. 2. Image of a scale taken from a sample of *Cirrhinus mrigala* observed under microfiche reader. This is a three-year old fish.

RESULTS AND DISCUSSION

Summaries of mean, standard error of the mean (SEM), minimum and maximum values for body sizes, K, GSI, HSI, PCV, and PP are presented in Table I for River Kabul and Table II for River Swat. As already discussed, statistical analyses were only possible for one fish species (*S. plagiostomus*) from Swat, so the following section mostly focuses on comparisons on health parameters in relation to location and sex for the Himalayan seatrout. Nevertheless, we also briefly discuss results from other fish species sampled from River Kabul.

Table I. Fish samples collected from River Kabul (n = 61).

Site on Kabul River	n		Age (years)	K	GSI	HSI	Packed Cell Volume	Plasma Proteins (g/dL)
2018, Family: Cyprinidae; Species: *C. mrigala*								
Warsak Dam	10	Mean ± SEM	2.2 ± 0.5	1.02 ± 0.02	1.58 ± 0.40	0.94 ± 0.12	38.40 ± 4.29	6.2 ± 0.5
		Range	0-4	0.93-1.14	0.05-4.69	0.39-1.44	17-56	4.4-9
Akbarpura	2	Mean ± SEM	2.5 ± 0.5	0.80 ± 0.08	1.10 ± 0.06	0.71 ± 0.004	45.50 ± 6.50	7.2 ± 1.2
		Range	2-3	0.72-0.89	1.04-1.16	0.70-0.71	39-52	6.0-8.4
Akramabad	0	Mean ± SEM	NA	NA	NA	NA	NA	NA
Species: *T. macrolepis*								
Warsak Dam	6	Mean ± SEM	1.0 ± 0.4	0.87 ± 0.06	2.50 ± 0.80	0.72 ± 0.12	27.00 ± 5.05	3.9 ± 0.7
		Range	0-2	0.72-1.14	1.04-4.69	0.70-1.44	39-56	6.0-9.0
Akbarpura	1		2	0.86	0.35	0.83	32	7
Akramabad	0	Mean ± SEM	NA	NA	NA	NA	NA	NA
Species: *C. carpio*								
Warsak Dam	0	Mean ± SEM	NA	NA	NA	NA	NA	NA
Akbarpura	7	Mean ± SEM	0.3 ± 0.3	1.33 ± 0.12	1.65 ± 0.58	1.00 ± 0.19	42.57 ± 3.21	8.6 ± 0.8
		Range	0-2	0.69-1.60	0.12-4.19	0.25-1.75	33-54	5.8-11.9
Akramabad	0	Mean ± SEM	NA	NA	NA	NA	NA	NA
Family: Schilbeidae; Species: *C. naziri*								
Warsak Dam	0	Mean ± SEM	NA	NA	NA	NA	NA	NA
Akbarpura	16	Mean ± SEM	NA	0.81 ± 0.03	1.14 ± 0.25	1.38 ± 0.09	44.94 ± 4.02	9.8 ± 0.4
		Range		0.63-1.11	0.07-3.74	0.29-1.80	18-72	6.4-12.0
Akramabad	5	Mean ± SEM	NA	0.72 ± 0.06	0.89 ± 0.25	1.21 ± 0.11	36.20 ± 6.84	8.6 ± 0.2
		Range		0.51-0.87	0.31-1.57	0.93-1.60	14-53	8.0-9.0
2019, Family: Schilbeidae; Species: *C. naziri*								
Akramabad	14	Mean ± SEM	NA	0.88 ± 0.02	1.19 ± 0.32	1.93 ± 0.18	48.21 ± 2.60	8.6 ± 0.5
		Range		0.76-1.01	0.12-3.78	0.59-3.37	32-64	5.7-11.2

Table II. Fish samples collected from River Swat (n = 61). Note that *S. labiatus* was not included in the statistical analyses.

Site on Swat River	n		Body mass (g)	Total length (cm)	HSI	GSI	K	Packed cell volume	Plasma proteins (g/dL)
2018 Family: Cyprinidae; Species: *S. plagiostomus*									
Madyan	21	Mean±SEM	60.0 ±5.4	19.0±0.5	1.25 ±0.11	3.72±0.36	0.84±0.03	57.6±3.04	9.1±0.3
		Range	26-131	15.0-24.5	0.50-2.53	0.32-4.97	0.69-1.39	30-84	6.4-11.4
Panjigram	14	Mean±SEM	117.1±15.6	23.6±0.9	0.97 ± 0.08	0.92±0.17	0.83±0.02	59.64±3.15	7.9±0.2
		Range	45-276	18.2-30.0	0.58-1.72	0.25-2.22	0.72-1.02	43-80	6.4-9.2
Landakay	18	Mean±SEM	250.1±141.7	19.7±1.6	0.87 ± 0.09	0.54±0.10	1.12±0.21	50.06±2.30	7.8±0.3
		Range	25-2000	15-39.5	0.32-1.43	0.08-1.56	0.71-3.86	28-68	6-10
Species: *S. labiatus*									
Landakay	8	Mean±SEM	102.1±28.6	21.2±1.6	1.27 ± 0.14	0.56±0.11	0.91±0.04	52.38±2.13	8.1±0.4
		Range	34-265	16.5-29	0.83-1.83	0.17-1.09	0.75-1.09	43-62	6.8-9.5

Body condition and organosomatic indices

Results on body condition (K), hepatosomatic index (HSI) and gonadosomatic index (GSI) are presented in Figure 3. There were no significant differences in K for the Himalayan seatrout sampled across the three sites, and gender did not affect this parameter. Overall, a value ~ 1 is generally an indication of good health (Adeyemo and Enefe, 2021). Akhtar *et al*. (2021) and Arafat and Bakhtiyar (2022) reported comparable K values for *S. plagiostomus* from Neelam and Jhelum Rivers, Pakistan and from the Vishav stream of Kashmir Himalaya, India.

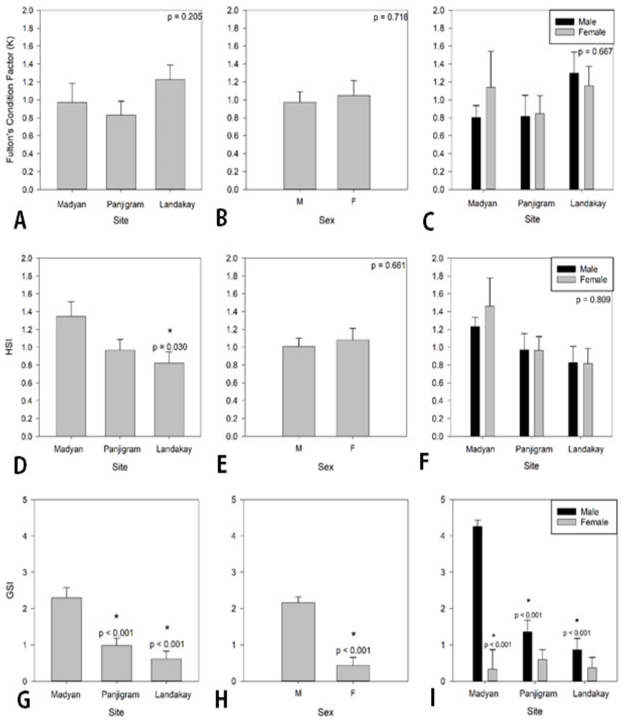

Fig. 3. Fulton's condition factor (K) (A, B, C), hepatosomatic index (HSI, %) (D, E, F) and gonadosomatic index (GSI, %) (G, H, I) in *S. plagiostomus* sampled from River Swat in June 2018. There were no significant differences in K between sites or sexes. In panel D and G, asterisks (*) denote significant differences from the control site (Madyan). In panel H, asterisks (*) denote significant differences between sexes. In panel I, asterisks (*) denote significant differences between sites within the same sex while crosses (+) denote significant differences between sex within the same site.

In relation to other species sampled from River Kabul, K values ranged from 0.51 to 1.60 (Table I). Ullah *et al*. (2022) reported mean K values of 1.30 and 1.19 for *C. mrigala* and *C. carpio*, respectively, from Urbashi Dam, Pakistan collected from October 2020 to July 2021. Similar results were reported by Vohra *et al*. (2021), for *C. mrigala* and *C. carpio* from Kori Lake, Thatta, Pakistan. Overall, these values are higher and lower than what we report here for mrigal (SEM ranging from 0.80 to 1.02) and for common carp (1.33), respectively. Hussain *et al*. (2009) reported a mean K value of 0.76 for *C. naziri* from River Indus, Pakistan, which is similar to what was found in the present study (SEM ranging from 0.72 to 0.88). The implications of a < 1 K for *C. naziri* populations are unknown, but they would suggest poor habitat/diet quality for this species at the sites sampled.

Results on HSI and GSI are also shown in Figure 3. Overall, fish from Site 3 (Landakay) had a lower HSI compared to controls: Mean of 0.87% compared to means of 1.25 and 0.97% for Sites 1 (Madyan) and 2 (Panjigram), respectively. Lower GSI were observed in fish from Sites 2 and 3 compared to Site 1 (Fig. 3G, H, I). In addition, females had lower GSI than males, and differences in GSI across sites was driven mostly by high GSI in males from Site 1.

Jan and Ahmed (2016) reported seasonal variations in HSI for *S. plagiostomus* from River Lidder, India. They observed the lowest mean HSI of male and female fish (0.622 and 1.027, respectively) in May and the highest mean (2.436 and 2.163, respectively) in November. In another study conducted in Pakistan, a maximum GSI value of 14.83% was observed in male Himalayan seatrout sampled in March, with lowest values (4.13%) in July (Jan and Ahmed, 2016). For females, a peak of 14.95% was observed in May and the lowest value observed in August (1.06%).

Our samples were collected in June and HSI values are a bit higher compared to those reported by Jan and Ahmed (2016) in May. GSI in females was low (< 1%), which supports the idea that these fish had already spawned by the time they were collected. This was confirmed at the histology level as the majority of the females examined (>90%) had ovaries with no vitellogenic follicles (Fig. 4A). Overall, our results concur with the reproductive cycle of this species, with increased liver and gonadal weights in the fall, followed by a decline after spawning in the summer. This explains the overall low liver and gonad weights observed in our fish as they were sampled in June, likely after spawning. The implication of this, is that differences in HSI and GSI across sites could in part be explained by having sampled fish at different reproductive stages (Fig. 5).

Examples of the different reproductive stages found for *C. mrigal* and *C. naziri* are summarized in Figure 4B, C. Frequency of gonadal developmental stages is shown in Figure 5.

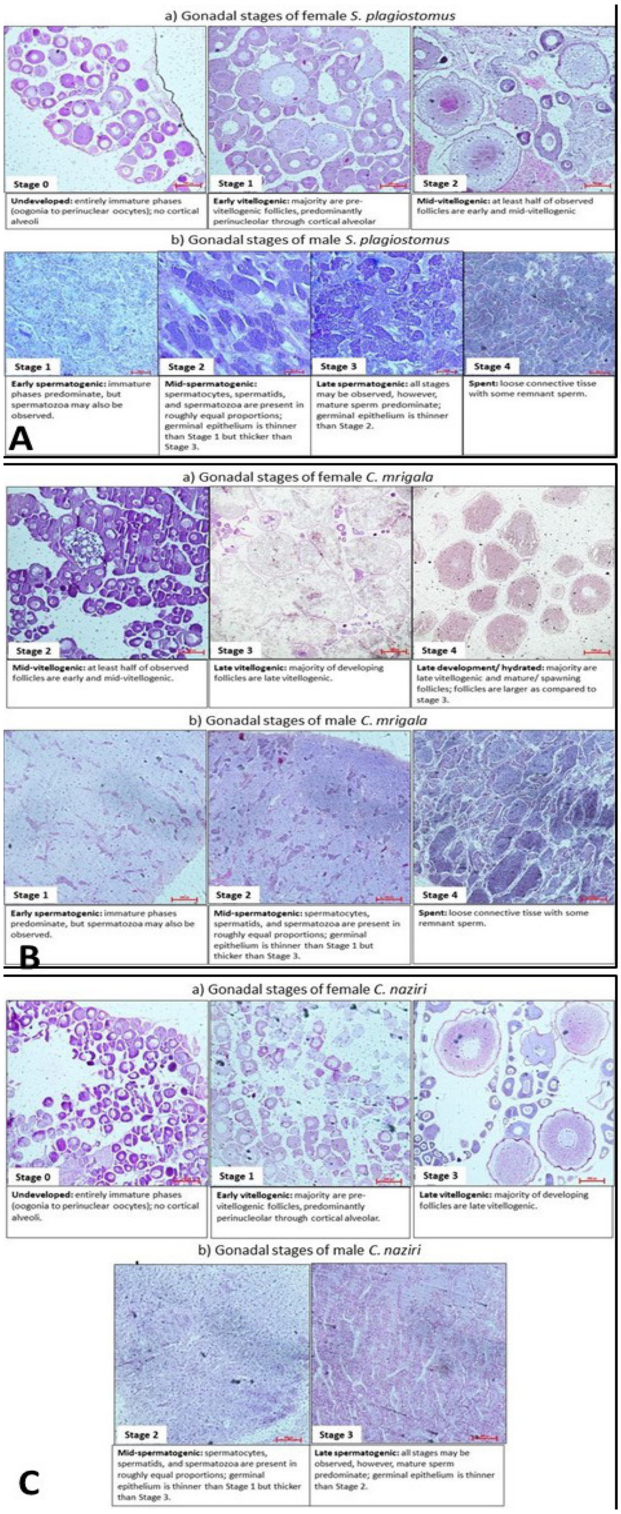

Fig. 4. Gonadal developmental stages of male and female *Schizothorax plagiostomus* (A), *Cirrhinus mrigala (B)* and *Clupisoma naziri* (C) (a and b, male and female, respectively).

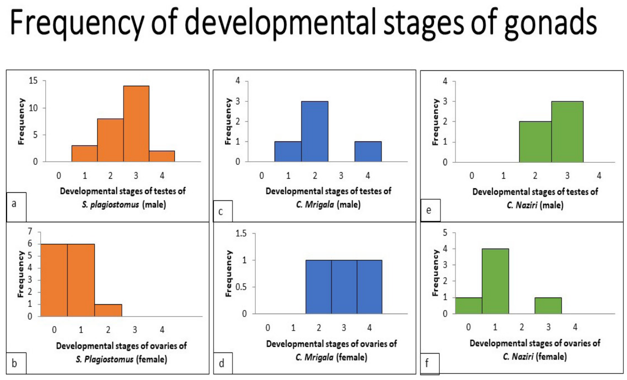

Fig. 5. Frequency of gonadal developmental stages of male and female *S. plagiostomus* (a and b); *C. mrigala* (c and d); *C. naziri* (e and f) sampled in June 2018 from Rivers Kabul and Swat.

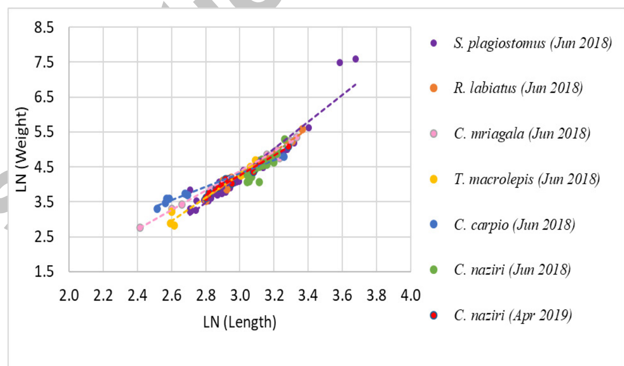

Fig. 6. Length–weight relationships for fish species sampled from Rivers Kabul and Swat.

Length-weight relationships

Length-weight relationships for the six fish species studied are presented in Table III and Figure 6. Positive allometric growth was observed for *S. plagiostomus* (n= 53) and isometric growth for *S. labiatus* (n= 8) from River Swat. Khan *et al*. (2021) reported isometric growth of *S. plagiostomus* (b= 3.03) and *R. labiatus* (b = 3.02) from River Panjkora, Khyber Pakhtunkhwa, Pakistan, with *b* values slightly lower than the present study,whereas, Mir *et al*. (2014) reported negative allometric growth of *S. plagiostomus* (b= 2.60) from Kashmir Valley, India. Variations in *b* values for the same species across geographical areas are expected and can be due to differences in food availability and environmental conditions (Kuriakose, 2017). Overall *b* values for *T. macrolepis*, *C. mrigala*. *C. naziri* and *C. carpio* ranged between 2–4, similar to previous studies with these species in this region (Mortuza and Al-Misned, 2013; Anani and Nunoo, 2016).

Table III. Length-weight relationships for six fish species from Rivers Kabul and Swat.

Family	Species	n	a	95% C.I. of a	b	95% C.I. of b	R^2	Growth pattern	$W=aL^b$
Cyprinidae	*S. plagiostomus* (June 2018)	53	0.0006	–8.32 - –6.30	3.85	3.52 - 4.19	0.95	PA	$W = 0.0006L^{3.85}$
	R. labiatus (June 2018)	8	0.0034	–7.47 - –3.86	3.31	2.72 - 3.90	0.98	IS	$W = 0.0034L^{3.31}$
	C. mrigala (June 2018)	12	0.018	–4.63 - –3.31	2.78	2.56 - 2.99	0.99	NA	$W = 0.018L^{2.78}$
	T. macrolepis (June 2018)	7	0.003	–7.60 - –3.79	3.33	2.66 -3.99	0.98	IS	$W = 0.003L^{3.33}$
	C. carpio (June 2018)	7	0.24	–2.14 - –0.63	1.90	1.62 - 2.17	0.99	NA	$W = 0.24L^{1.90}$
Schilbeidae	*C. naziri* (June 2018)	21	0.0001	–12.2 - –5.40	4.27	3.17 - 5.38	0.88	PA	$W = 0.0001L^{4.27}$
	C. naziri (April 2019)	14	0.013	–5.27 - –3.29	2.84	2.51 – 3.18	0.98	NA	$W = 0.013L^{2.84}$

a, intercept of regression line; b, slope of regression line; C.I., Confidence interval; R^2, Regression coefficient. NA, Negative allometric (b<3); IS, Isometric (b=3); PA, Positive allometric (b >3).

Hematology

PCV in *S. plagiostomus* was not affected by site or sex (Fig. 7). In contrast, PP was lower in fish from Sites 2 and 3 compared to fish from Site 1 (Fig. 7). This decline was small (from a mean of 9.1 g/dL in fish from Site 1, to means of 7.9 and 7.8 g/dL for Sites 2 and 3, respectively) and likely not biologically significant.

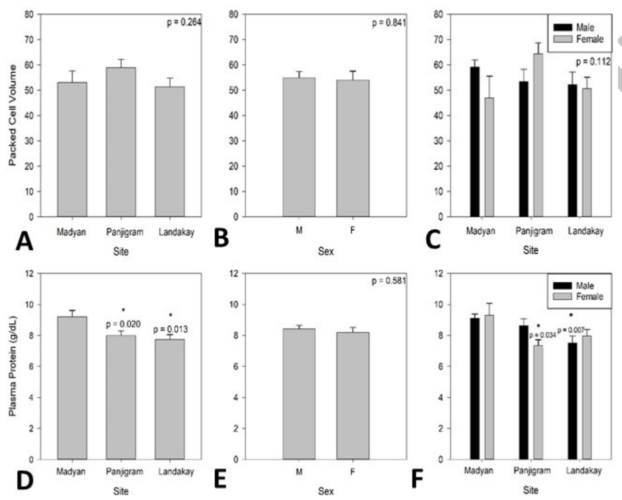

Fig. 7. Packed cell volume (PCV, %) (A, B, C) and plasma proteins (PP, g/dL) (D, E, F) in *S. plagiostomus* sampled from River Swat in June 2018. There were no significant differences in PCV across sites or between sexes. In panel D, asterisks (*) denote significant differences from the control site (Madyan). In panel F, asterisks (*) denote significant differences between sites within the same sex while crosses (+) denote significant differences between sex within the same site.

Sheikh and Ahmed (2019) reported values of total proteins in *S. plagiostomus* ranging between 4.40-6.35 g/dL and 4.33-5.76 g/dL in male and female fish, respectively. Pradhan *et al.* (2014) reported seasonal variation of plasma proteins in *C. mrigala* ranging from 3.4 to 4.15 g/dL. These values are about half of what we report in the present study and differences could be due to the use of different techniques to quantify proteins in plasma.

CONCLUSIONS

Fishes from Rivers Swat and Kabul are ecologically and commercially important, however, little information is available on the general health of native fish populations inhabiting these river systems. In this study, we sampled a total of five native and one introduced fish species from several sites, across a geographical gradient of water and habitat quality. For Swat, we were able to compare populations of the Himalayan seatrout across sites and found little differences in health parameters across sites. Although similar analyses were not possible from Kabul as species differed across sites, we report health values that fall within ranges of previous studies in this region. Our study adds to the limited amount of basic physiological information available for these valuable fish species.

DECLARATIONS

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IRB approval

This study was conducted with prior approval from Ethical Research Committee, University of Peshawar.

Statement of conflict of interest

The authors declare no conflict of interest.

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