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Population estimation, proximate analysis and physico-chemical analysis of brown trout and rainbow trout in Swat River, Khyber Pakhtunkhwa, Pakistan

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Abstract

The present study was planned to find out the population status of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) along 50 Km long belt of river Swat, from Madayan to Mahoodhand village. Four sampling sites (SSs) namely Madayan (SS1), Mainkiyal (SS2), Kalam (SS3) and Mahoodhand (SS4) were fixed, visited fortnightly and fish samples were captured through cast nets. Netting index, Percent relative abundance, physico-chemical analysis (temperature, alkalinity, hardness, pH and dissolved oxygen) and proximate analysis were conducted. Heavy metal concentrations were also recorded. It is suggested that human interference, overfishing through illegal ways and reasons causing destruction of spawning ground and brood stock should be overcome by population awareness and implementations of rules by the Government so that population of trout in this area may be uplifted for the benefit of mankind.

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Introduction

There is great variation in landscape and environmental conditions in Pakistan in general and Khyber Pakhtunkhwa in specific. In the province, there are great variations in temperature and other climatic factors between winter and summer seasons. The rivers and streams are deep or shallow, clear or muddy, cold or warm, fast or slow and may have stony, sandy or muddy bottom and rich vegetation along the banks (Yaqoob, 2002). Swat valley is situated 35° north latitude and 72° and 30° east longitude and is enclosed by the mountains. It is a small district having total area of 5,337 km² and total population of 1,257,602. Chitral and Gilgit–Baltistan are situated in the north, Dir in the west, and Mardan in the south. River Swat originates from Mahoodhand village at a height of about 3,000 meters and runs through valley of Swat to Chakdara making a 160 km long belt. The main tributaries which fall into the river at different sites are Ushu, Mainkial, Ghurnai, Kedam, Daral, Beshigram, Pia and Shin. River Swat is habitat for a number of cold water fish species, two of these the brown and rainbow trout inhabit the upper mountainous part and play their role in improving the economy of the area by creating employment opportunities entertaining the communities and tourists with a proteinicious healthy diet. Mahoodhand snow covered mountains on melting produce clear water which is ideal habitat for brown trout. Maho stands for 'fish' and Dhand for 'lake' in Pashto, means the water body containing abundant fish resources (Hayat, 2007).

Because of high nutritional value, fish is an important part of human nutrition (Gladyshev *et al.*, 2006) that supplies essential amino acids (Pariser and Wallerstein 1980; Novikov *et al.*, 1997; Tocher *et al.*, 2003; Osibona *et al.*, 2009). Proximate composition of fish is the indicator of fish quality which is affected by different factors such as species, size, food resource, fishing season, water salinity and temperature (Shirai *et al.*, 2002; Sovik and Rustad, 2005; Toppe *et al.*, 2007; Erdem *et al.*, 2009).

The natural aquatic systems may extensively be contaminated with heavy metals released from domestic sewages, industrial and other man-made activities (Velez and Montoro, 1998). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.*, 2004). The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood (Canli, 1995). Heavy metals have greater densities than 5 g/cm^3. The accumulation of heavy metals in an aquatic environment has direct consequences to man and to the ecosystem. Although metals such as Cu and Zn are generally regarded as essential trace metals in view of their valuable role for metabolic activities in organisms, other metals like Cd, Pb, Ni and Hg exhibit extreme toxicity even at trace levels (Merian, 1991). There is no large scale industry in swat to pollute water of river swat. Solid waste disposal, hotels sewage, domestic's sewage, agriculture runoff and vehicles service stations are majors sources which contaminate water of river swat. The main objective of this study is to estimate the population of two trout species (brown trout and rainbow trout) and measuring different physico-chemical parameter; proximate analysis, concentration of different metals and heavy metals effecting their life along the length of Swat River Pakistan.

Materials and methods

Site selection

The present study was planned from August, 2012 to October, 2012 along 50 km long belt of river Swat from Madyan to Mahoodhand village (Fig. 1). Four sub areas (SSs) i.e. Madyan (SS_1), Mainkiyal (SS_2), Kalam (SS_3) and Mahoodhand (SS_4) having smooth water flow were selected. The harvesting was done at the selected sites with the help of local fishermen from on fortnightly basis. Local made cast nets made up of silk were used for netting and at each sampling site the net was thrown 50 times. The number of fishes captured in 50 attempts, locality, sex, weight and length of captured fish species were recorded.

Fig. 1. Showing sampling sites of the study area.

The % relative abundance and netting index for each species were calculated using following formulae;

% *relative abundance* = ni/N ×100

Where ni = Number of fishes captured of each species.
N = Total number of fishes captured.

Netting index = (ni/total net area × hrs) 100
The physico-chemical parameters of water from all the four sub-areas were recorded on fortnightly basis. Temperature and dissolved oxygen were taken by 970 Do_2 portable Jenway meter while pH was recorded through 370pH portable Jenway meter, total hardness by HI 3812 and alkalinity by HI 3811 water analysis Kits following (AOAC, 2006).

Proximate analysis
To know moisture content, fish samples were weighed, wrapped in aluminum foil and kept in oven at 60°C for seven days. After drying, the fish samples were weighed again and the net difference in weight indicates moisture content. Moisture % was determined by the following formula;

Moisture % = wet weight of sample –dry weight of sample/wet weight of sample × 100

For ash content, crucible were washed, dried and weighed. One gram of grounded sample was taken in the pre weighted crucibles and was kept in Blast furnace at 600°C for 24 hours. The crucibles were then kept in dissector for cooling to room temperature. The samples were again weighed and ash % was determined by the following formula.

$$\text{Ash } \% = \frac{\text{ash}}{\text{wt of sample}} \times 100$$

Weight of ash = weight of Crucible and ash – weight of Crucible

For fat content, fat were extracted by n-hexane in Soxhlet apparatus following AOAC (2006). One gram of moisture free samples were wrapped in already weighted filter papers and placed in thimbles of Soxhlet apparatus for further processing. Fat were extracted by evaporating n-hexane through the samples the process took 6 hours to complete. The samples were dried in oven and the crude fat was determined according to the following formula.

$$\text{Fat\%} = \frac{\text{weight of fat}}{\text{weight of sample}} \times 100$$

Weight of fat = weight of sample + weight of filter paper – weight of sample after processing

For crude protein estimation, one gram of sample, 8 grams of digestion mixture (copper sulphate and potassium sulphate 1:9) and 20ml of H_2SO_4 were taken in Kjeldhal flask. The flasks were kept on heater under hood for 4 hours. After digestion the sample were diluted in 100ml flask up to 100ml volume. Ten ml diluted sample were taken in to the upper side of titration apparatus. 10ml of 40% NaOH solution were used to distillate the 4% boric acid with the help of

Methyl red indicator containing in a small beaker. Distillation was carried out until the volume reach up to 30-35 ml and color turns yellow. The ammonia produced during distillation was collected as NH_4OH in the beaker containing Boric acid. Sample was titrated with N/10 HCl until pink color appears the volume of acid used was noted and percent crude protein was determined by the following formula.

Crude protein (%) = 6.25× % N

$$\% N = \frac{S \times N \times 0.0014 \times D \times 100}{\text{weight of sample} \times V}$$

Where,

S = Sample titration reading

N = normality of HCl

D = Dilution of sample

V = volume of sample taken for distillation

0.0014 = Milli equivalent weight of nitrogen

Heavy metals determination

Water samples for heavy metals determination were collected in clean sampling bottle rinsed with de-ionized water. Beaker was rinsed with de-ionized water making it free from contamination. 20 ml of water sample and 10 ml of concentrated HNO_3 were added to the beaker and kept for ten minutes, the sample was then filtered and kept at room temperature for detection of heavy metals by PERKIN-ELMER 2380 Atomic absorption Spectrophotometer (Bernhard, 1976; Alper *et al.*, 2003; APHA, 2005).

To find out heavy metals in fish muscles, the fish muscle samples were iced packed and transported to the laboratory. 2 gm of sample was weighed and kept in oven at 65°C for 24 hours. Then the samples were kept in blast furnace at 700-1000 °C for ninety minutes. Five ml of concentrated nitric acid was added and heated for two hours. After two hours, double distilled water was added making the volume up to 50ml. Heavy metal concentrations in the resulting solution were determined using PERKIN-ELMER 2380 Atomic absorption spectrophotometer (Honda *et al.*, 1983; Yaramaz, 1986; AOAC 2000).

Statistical analysis

The data thus obtained were subjected to Analysis of Variance (ANOVA) through SAS 9.1 statistical software and Duncan's Multiple Range Test (DMRT) was applied to compare means.

Results and discussion

Two hundred and sixteen trout fish samples belonging to two species; *Oncorhynchus mykiss* and *Salmo trutta* were captured from the study area. These samples ranged in size from 15cm to 36cm in length and 47g to 304g in weight. Similar studies conducted in Tutshi Lake, Columbia resulted in capture of 96 trout samples that ranged in length from 13.0cm to 88.4cm while the ages ranged from 5 to 40 years (Hatlevik, 1987). A total of 4 *Oncorhynchus mykiss* were captured from SS_1 (Madyan). The average body weight of these four *O. mykiss* was 130.25 ± 45.02g, average length 21.75 ± 2.5cm, netting index 0.75 and % relative abundance was 100% at SS_1. From Mainkiyal (SS_2), 42 *Salmo trutta* and 4 *O. mykiss* were captured, average weight and average length of *S. trutta* was 117.69 ± 64.79g and 20.84 ± 4.60cm, respectively while netting index and % relative abundance were 0.67 and 89%, respectively. The average body weight and length of *O. mykiss* were 113.5 ± 81.17g and 20 ± 5.71cm, respectively. Netting index and % relative abundance of *O. mykiss* was 0.082 and 10.86%, respectively. From SS_3 (Kalam) out of 63, 55 were *S. trutta*, 7 were *O. mykiss*, the average body weight, average length, netting index and % relative abundance of *S. trutta* were 141.80 ± 76.44g, 22.85 ± 5.44cm, 0.67 and 88.88%, respectively while from the same site the average body weight of *O. mykiss* was 110 ± 39.88g, average length was 20.83 ± 2.78cm, netting index was 0.08 and % relative abundance was 11.11%. From SS_4 out of total 103 fish samples, 90 were *S. trutta* and 13 were *O. mykiss*. Mean body weight, average length, netting index and % relative abundance for *S. trutta* captured from SS_4 (Mahoodhand) was 162.37 ±

75.04g, 24.23 ± 5.27cm, 0.66 and 87%, respectively while average body weight of 13 *O. mykiss* was 194.85 ± 86.94g, mean body length was 26.64 ± 6.046cm, netting index was 0.09 and % relative abundance was 12.62% at SS_4 (table 1).

Table 1. Average length, weight, sex, netting index and % relative abundance of fish captured from study area.

Site	N	Species	Average length (cm) (Mean ± SD)	Average weight (g) (Mean± SD)	Sex	Netting index	% Abundance
Madyan	4	*O. mykiss*	21.75±2.5	130.25±45.02	1♂,3♀	0.75	100%
Mainkiyal	42	*S. trutta*	20.84±4.60	117.69±64.79	9♂,32♀	0.67	89%
	4	*O. mykiss*	20±5.71	113.5±81.17	1♂,4♀	0.0825	10.86%
Kalam	56	*S. trutta*	22.85±5.44	141.80±76.44	15♂,41♀	O.765	88%
	7	*O. mykiss*	20.83±2.78	110±39.88	2♂,5♀	0.084	11.11%
Mahoo	90	*S. trutta*	24.23±5.278	162.37±75.04	22♂,68♀	0.663	87%
dand	13	*O. mykiss*	26.64±6.046	194.85±86.94	3♂,10♀	0.09	12.62

The greater amount of fishes were captured from SA_4 (Mahoodhand) as this is origin of river Swat having ice caped mountains making suitable environment for cold water fishes like *Oncorhynchus mykiss* and *Salmo trutta*. The site is also protected from humans as the mountains are covered with snow in winter season, so making hunting of fishes difficult. Sustainable capture fishery production is only achieved when stock is not over-exploited (Yaqoob, 2002). Similarly low catch from SS_1 (Madayan) indicates human interference in the area, as this area has greater human populations as compared with other selected areas, the reason might be overfishing through illegal ways and recent flood (July, 2011) in river Swat that may have affected the fish fauna by destroying their spawning ground and brooding stock. A total of 4 *O. mykiss* and no *S. trutta* were captured from the SS_1 throughout the study period, the reason might be over exploitation by humans and the presence of *O. mykiss* may be due to the presence of government and public hatcheries and farms of *O. mykiss* from which it may escape to the natural water bodies because it sometimes escapes from culture and enters open waters (Hussain, 1994).

The physico-chemical parameters recorded during present study are presented in table 2. Decreasing temperature trend was observed from SS_1 to SS_4. Baig *et al.* (2010) studied six sampling stations (Mastuji station SS_1, Kuragh station SS_2, Koghuzi station SS_3, Shoghor SS_4, Chitral station SS_5 and Drosh Station SS_6) of river Chitral and observed increasing order from SS_1 to SS_6. Water was slightly alkaline at SS_1, SS_2 and SS_3 while pH at SS_4 indicates acidic water at this site. The pH of water samples collected from various sampling stations along river Chitral showed that the water was alkaline (Baig *et al.*, 2010).

An increasing trend in total alkalinity of water was observed from SS_1 to SS_4. Iqbal *et al.* (2004) observed higher alkalinity values at Dhoak Pathan Bridge, river Soan during month of January. The values of total hardness were lowest at SS_3 and highest at SS_1. Zeb *et al.* (2011) observed seasonal variations in hardness of water in river Siran, Pakistan. Lower hardness values were recorded during winter season and higher during summer. During present study an increasing trend in dissolved oxygen from SS_1 to SS_4 was observed which may be due to the presence of water falls in the mountains of SS_4 (Mahodand) and the other reason might be the lower temperature of the site. The lower the water temperature more will be the dissolved oxygen (Nepal *et al.*, 2002; Zeb *et al.*, 2011).

Table 2. Summarizing water parameters from study area.

Site	Temperature (ºC) Mean ±SD	pH Mean ±SD	Alkalinity (mg/L) Mean±SD	Hardness (mg/L) Mean±SD	Dissolved oxygen (mg/L) Mean±SD
Madayan	11.67±2.100	7.35±0.379	81.63±8.21	123.63±7.174	6.79±0.815
Mainkiyal	10.49± 1.596	7.39±0.253	97.79± 8.983	106.79±30.353	7.88± 0.638
Kalam	9.38± 1.129	7.09±0.646	122.75±16.416	101.13±8.639	8.07 ±0.736
Mahoodhand	7.08± 0.843	6.74±0.248	142.13±15.060	102.83±11.278	10.56± 0.548

Proximate analysis of *Oncorhynchus mykiss* from various sampling sites showed 73.93 % moisture, 18.4% protein, 5.77 % fats and 1.40% ash. These values are close to the percentages given by Celik (2007) i.e. 71.65 % moisture, 19.60 % protein, 4.43 % fats and 1.36% ash, respectively. Proximate analysis of *Salmo trutta* from the study area showed 71.25 % moisture, 19.72 % protein, 5.49% fat and 1.33% ash (Table 3).

Table 3. Increase in length, weight and proximate analysis of fish samples captured from study area.

Site	Species	Length cm	Weight g	Dry weight	Moisture content	Moisture %	Protein %	Fat %	Ash %
Madayan	*O. mykiss*	16	55.36	14.61	40.75	73.60	18.03	6.33	1.84
Mainkiyal	*O. mykiss*	24	155.5	32.32	123.18	75.30	19.04	3.63	1.53
	S. trutta	22	118.86	33.62	85.24	71.71	19.61	8.11	1.55
Kalam	*O. mykiss*	28.2	165.66	41.63	124.03	74.87	16.63	6.53	1.27
	S. trutta	19	78.12	21.92	56.02	71.94	18.96	7.52	1.40
Mahoo dand	*O. mykiss*	21	116.26	32.61	83.65	71.95	19.67	6.59	0.98
	S. trutta	30	236.45	70.64	165.81	70.12	20.59	6.84	1.04

Mean concentrations of Cu, Zn, Fe, and Mn in muscle of *Oncorhynchus mykiss* were 0.0265 ± 0.008 mg/kg, 0.0815 ± 0.04 mg/kg, 0.3142 ± 0.237 mg/kg and 0.0244 ± 0.253 mg/kg, respectively. Celik *et al.* (2007) reported 8.19 ± 0.490 mg/kg Cu, 5.45 ± 0.156 mg/kg Zn, 4.15 ± 0.484 mg/kg Fe and 1.91 ± 0.132 mg/kg Mn in muscle of *O. mykiss* caught from the Ataturk dam lake in Turkey.

During present study, mean concentrations of Cu, Zn, Fe and Mn recorded in muscle of *Salmo trutta* were 0.029 ± 0.004 mg/kg, 0.144 ± 0.117 mg/kg, 0.0283 ± 0.124 mg/kg and 0.098 ± 0.09 mg/kg, respectively (Table 4). Minerals reported by Hei and Sarojnalini (2012) for *Neolissochilus stracheyi* purchased from market of Manipur were 2.565 ± 0.01 mg/kg Cu, 0.4375 ± 0.00 mg/kg Zn, 8.000 ± 0.08 mg/kg Fe and 1.000 ± 0.01 mg/kg Mn.

Heavy metal concentrations in muscle of *Oncorhynchus mykiss* were recorded 1.41 ± 1.067mg/kg Pb, 2.00 ± 3.066mg/kg Cr, 0.135 ± 0.129mg/kg Ni and 0.04 ± 0.033mg/kg Cd (Table 5). Study on fish captured from Ibiekuma stream revealed Cr 4.8 ±1.62 mg/kg, Ni 13.6 ±4.82 mg/kg and Cd 1.15 ± 0.42mg/kg (Obasohan, 2008). Mean concentrations of Pb, Cr, Ni and Cd detected in muscle of *Salmo trutta* were 1.55 ± 0.917mg/kg, 6.38 ± 3.458, and 0.15 mg/kg ± 0.142 and 0.013 ± 0.09 mg/kg, respectively (Table 6). Indrajith *et al.* (2008) detected levels of heavy metals in muscle of *Etroplus suratens*, results revealed mean Pb concentrations 0.03 ± 0.001 mg/kg, Cr 0.07 ± 0.003 mg/kg, Ni 0.05

± 0.004 mg/kg and Cd 0.011 ± 0.001 mg/kg. Mean concentrations of heavy metals in water samples collected from all the four sampling stations were Pb 0.052 ± 0.022mg/L, Cr 5.85 ± 2.310mg/L, Ni 0.05 ± 0.035mg/L and 0.07 ± 0.077mg/L. Farooq *et al.* (2012) analyzed water from Indus river where mean concentration of Pb was 1.163 ± 0.694mg/L, Cr 0.077 ± 0.041mg/L, Ni 0.25 ± 0.176mg/L and Cd was 0.090 ± 0.486mg/L.

Table 4. Mineral composition of all the fish species captured from the study area.

Site	Sr. No.	Species	Cu	Zn	Fe	Mn
Madayan	**1.**	*O. mykiss*	0.026	0.138	0.166	0.086
Mainkiyal	**2.**	*O. mykiss*	0.034	0.032	0.666	0.217
	3.	*S. trutta*	0.024	0.268	0.184	0.064
Kalam	**4.**	*O. mykiss*	0.031	0.049	0.254	0.064
	5.	*S. trutta*	0.031	0.131	0.423	0.028
Mahoodhand	**6.**	*O. mykiss*	0.015	0.107	0.171	0.610
	7.	*S. trutta*	0.033	0.034	0.243	0.203

Table 5. Heavy metal accumulation in the muscle of fish samples captured during present study.

Site	Species	Lead	Chromium	Nickel	Cadmium
Madayan	*O. mykiss*	0.65	0.13	0.007	0.046
Mainkiyal	*O. mykiss*	2.18	0.41	0.273	0.017
	S. trutta	2.35	2.56	0.041	0.022
Kalam	*O. mykiss*	2.48	0.90	0.215	0.023
	S. trutta	0.76	9.30	0.315	0.015
Mahoodhand	*O. mykiss*	0.36	6.58	0.045	0.090
	S. trutta	0.76	7.28	0.113	0.004

Table 6. Showing average level of heavy metals in water from selected area.

Sampling site	Lead (Mean ± SD)	Chromium (Mean ± SD)	Nickel (Mean ± SD)	Cadmium (Mean ± SD)
Madayan	0.0845±0.005	6.43±0.537	0.05±0.028	0.0605±0.002
Mainkiyal	0.026±0.001	7.54±0.127	0.0075±0.002	0.031±0.001
Kalam	0.045±0.001	2.2±0.184	0.0525±0.004	0.005±0.004
Mahoodhand	0.055±0.002	7.26±0.049	0.0975±0.004	0.193±0.004

Conclusion

It is concluded that the greater amount of fishes were captured from SS_4 (Mahoodhand) as this is origin of river Swat due to suitable environment for cold water fishes like *Oncorhynchus mykiss* and *Salmo trutta*. This site is also protected from humans as the mountains are covered with snow in winter season. Similarly low catch has been observed from SS_1 (Madayan) indicates human interference, overfishing through illegal ways and recent flood in river Swat causing destruction of spawning ground and brood stock. Physico-chemical parameters indicate decreasing temperature trend while an increasing trend in total alkalinity and dissolved oxygen of water from SS_1 to SS_4 while pH indicates acidic water at SS_4 whereas the total hardness value was lowest at SS_3 and highest at SS_1 showing seasonal variations. Proximate analysis of *Oncorhynchus mykiss* showed 73.93 % moisture, 18.4% protein, 5.77 % fats and 1.40% ash while *Salmo trutta* showed 71.25 %

moisture, 19.72 % protein, 5.49% fat and 1.33% ash. Heavy metal concentrations in muscle of *Oncorhynchus mykiss* were recorded 1.41 ± 1.067mg/kg Pb, 2.00 ± 3.066mg/kg Cr, 0.135 ± 0.129mg/kg Ni and 0.04 ± 0.033mg/kg Cd while in *Salmo trutta* were 1.55 ± 0.917mg/kg, 6.38 ± 3.458, and 0.15 mg/kg ± 0.142 and 0.013 ± 0.09 mg/kg, respectively.

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