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Principal component analysis of alimentary canal metrics in developmental stages of the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) from Kashmir Valley, India

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Abstract

The primary objective of the research was to ascertain the variations in gut morphometry in rainbow trout *Oncorhynchus mykiss* during its life cycle spanning fry, fingerlings, yearlings, table-sized and brood-sized fish. The gut morphometric parameters viz., total length (a), standard length (b), weight (c), gut length (d), gut weight (e), intestinal length (f), liver weight (g), relative length of the gut (h), intestinal coefficient (i), relative gut mass (j), Zihlers index (k), hepatosomatic index (l) and gastrosomatic index (m) were all significantly correlated, according to the component pattern profile. The biplots that were created helped to explain the variations in the data. From the principal components PC1 and PC2, the variable i (intestinal coefficient) of *O. mykiss* in all five stages was responsible for the largest variation. This was followed by variations from PC1 that were related to the variables h (relative length of the gut), j (relative gut mass), m (gastrosomatic index), d (gut length), e (gut weight) and f (intestinal length). The largest variation in PC2 was caused by variable b (standard length), and was followed by variation caused by variable a (total length). As a result, gut length (d=0.354431), gut weight (e=0.342041), weight (c=0.333106), total length (a=0.320632), intestinal length (f=0.301986), relative gut length (h=0.241842), Zihlers index (k=0.238940), relative gut mass (j=0.247716) and gastrosomatic index (m=0.233688) all showed high positive component loading. Through the use of basic metrics to assess different gut parameters, we were able to ascertain the feed and nutritional needs of *O. mykiss* at different developmental stages. Understanding alimentary canal shape is crucial for fish digestion and for optimising fish nutrition as fish digestive systems and feeding practices advance.

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Introduction

The digestive system of fishes shows remarkable diversity in morphology and function, related to both taxonomy and different feeding habits (Al Abdulhadi, 2005). In fishes, as in other vertebrates, the digestive system is made up of the alimentary canal, varying in diameter and longitudinally divided into oesophagus, stomach, intestines and the rectum (Treer *et al.,* 1995). The alimentary canal (AC) of both vertebrates and fish shares the same strategy (Wilson and Castro, 2010). Different structures correlate with different phylogenies, ontogenies, diet compositions and environments associated with fish gastrointestinal tracts (Kozaric *et al.,* 2007; Hernandez *et al.,* 2009; Xiong *et al*., 2011a, b; Santos *et al.,* 2015a, b). Unlike other fish, carnivorous teleosts have relatively large stomachs and short intestines (Qu *et al.,* 2012). As they feed on a large number of animals as part of their diet, omnivore fish usually have a large stomach and long intestines. It is a characteristic of herbivore fishes with the longest complex intestine to have an intestinal bulb or a small gut or not have a stomach (Smith, 1991).

Under a variety of environmental conditions, the gastrointestinal tract (GIT) of fish plays a critical role in growth, nutrition and survival. Prior to passing into the oesophagus, food is captured in the mouth and processed there. Eventually, the latter connects with the stomach through a muscular and mucus-secreting channel. Food digestion takes place in the stomach (Ghosh and Chakrabarti, 2015a, b), which is the portion of the gastrointestinal tract with the most variations (Musa *et al.,* 2013). Nutrients are absorbed in the intestine (Smith *et al.,* 2000; Sanchez-Amaya *et al.,* 2007), usually aided by the slender, finger-like appendages or pyloric caeca, which are present between the stomach and large intestine. Enzymes secreted from the GIT's wall and lumen help to effectively utilise the nutrients ingested. Anus, the distal intestine and rectum are a source of mucus secreting cells that help with the expulsion of undigested food. Besides protecting the epithelium from mechanical damage, mucus facilitates the rapid elimination of pathogens as well as irritants and also forms a diffusion barrier for ions between the luminal content and epithelial lining (Veggetti *et al.,* 1999) and protects them from irritation (Reid *et al.,* 1988; Roussel and Delmotte, 2004).

Fish have a unique intestine structure that depends upon their environment, feeding habits, their body shape and weight (Davis, 1985; Barlow *et al.,* 1993). Intestine (or gut) length (GL) is thought to be a diet predictor (Kramer and Bryant, 1995a). It can be utilised for interspecific dietary comparisons in fishes (Al-Hussaini, 1947; Karachle and Stergiou, 2010a). Herbivorous species' intestines are more prolonged than omnivorous species' and omnivorous species' intestines are more extended than carnivorous species' for the same body length (Kapoor *et al.,* 1975; Kramer and Bryant, 1995b; Karachle and Stergiou, 2010a,b) As a result, the widely acknowledged pattern of fish GL variation in respect to dietary patterns is: carnivores< omnivores<herbivores<detritus feeders (Kapoor *et al.,* 1975; Ribble and Smith, 1983; Kramer and Bryant, 1995b; Karachle and Stergiou, 2010a,b). Other vertebrate classes for *e.g.*, reptiles (O'Grady *et al.,* 2005); birds (Ricklefs, 1996) and mammals (Chivers and Hladik, 1980) show a similar pattern. Sinha and Moitra (1975) investigated the functional morpho-histochemistry of the alimentary canal of *Labeo rohita* at various stages of life. Ribble and Smith (1983) studied relative intestinal length in eleven fish species and reported a significant relationship between the intestinal size and body length, and presented sufficient variations to indicate the important differences among species diets. Langer (1985) studied the food and feeding habits of *Schizothorax longipinnis* in Jhelum River and reported its feeding intensity using gastrosomatic index (GaSI).

Gut length is a valuable marker for elucidating feeding behaviour in almost all invertebrate classes, *e.g.,* fishes (Kramer and Bryant, 1995); reptiles (O'Grady *et al.,* 2005); birds (Ricklefs, 1996) and mammals (Chivers and Hladik, 1980). In addition, it can serve as a unit of comparison for interspecific comparisons in fishes. The gastrointestinal tract of fish shows marked diversity in morphology and function, which is helpful to determine the taxonomy, feeding habits and habitat (Murray *et al.,* 1996; Hellberg and Bjerkas, 2000). A mucosal specialisation exists at every segment of the digestive tract to ensure optimal efficiency of secretion, absorption, and digestive functions (Ezeasor and Stokoe, 1981). Therefore, the primary part of the fish intestine is to complete the digestive process, which starts in the stomach and to absorb the nutrients from food (Wilson and Castro, 2011). The gross histological features of the gastrointestinal tract of fishes are also different based on taxonomy, feeding habits and body shape (Al Abdulhadi, 2005). The morphological characteristics of the gastrointestinal tract of carnivorous fish diverge from that of herbivorous fish. The predatory fish tend to have shorter intestines than the herbivorous fish due to the diet's lower percentage of plant materials (Buddington and Doroshov, 1986; El-Bakary and El-Gammal, 2010). Histologically, the oesophagus of a carnivorous fish has more mucus cells than herbivorous fish (Abuzinadah, 1990).

The rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), sought after for both sport and food, are extensively commercialised. Typically, mature rainbow trout weigh between 2-3 kg, with maximum sizes of 120 cm total length (TL) and 25.4 kg weight (Froese and Pauly, 2009). These trout predominantly inhabit the upper, cold regions of rivers. Factors influencing their colour and morphology include their habitat and diet, similar to other trout species. Diverse freshwater sources host various local stocks and strains of rainbow trout, with commercially significant types often utilised in breeding programs. Enhanced versions of rainbow trout, featuring desirable traits such as ruggedness, disease resistance, increased growth rate, and reproductive capability in culture conditions, are often derived from the holotype rainbow trout population. The original habitat of rainbow trout is the freshwater sources along the Pacific coasts of North America and Asia. The main goal of the study was to investigate changes in gut morphometry throughout the life cycle of *O. mykiss.* This involved examining fry, fingerlings, yearlings, table-sized and brood-sized fish. The current research concentrates on aspects related to solving dietary issues in fish feeding. Evaluating the development of the digestive system through various gut parameters offers precise insights into the feeding and nutritional needs at different developmental stages of *O. mykiss*. This knowledge contributes to refining feeding and rearing practices, ultimately enhancing fish production and stimulating economic growth. Additionally, the findings from this research will serve as a foundation for understanding the baseline of a healthy trout gut. This baseline can aid in comprehending changes to the digestive system during different life cycle stages of trout, facilitating the development of well-informed aquaculture programs and improved trout stock management.

Materials and methods

Our study involved the collection of multiple developmental stages namely fry, fingerling, yearling, table-size and brood-size (40 specimens from each stage) of *O. mykiss* from Kokernag Trout Fish Farm, Department of Fisheries, Govt. of Jammu and Kashmir, Anantnaag. Fish samples were collected during June 2019 to February 2021, at random and were transported to the Faculty of Fisheries, Rangil in insulated boxes containing ice packs. A clean cotton cloth was used in the laboratory to dry the samples of fish, after cleaning under running tap water. Each individual's length and weight were measured with digital vernier caliper (Truesize) and electronic weighing balance (Virgo) to the nearest millimeter (mm) and gram (g) respectively. Dissecting the fish ventrally revealed the fish's alimentary canal, which was removed for further study.

Gut morphometric indices

Total length of each individual specimen was measured to the nearest 0.01 (mm) from tip of snout to the end of caudal fin using measuring scale. The fishes were cut and opened to expose their digestive system, gut of the specimen was carefully dissected out and gut length was measured by removing the entire alimentary canal from specimens, unwinding the intestine with care to minimise stretching and measuring the distance from oesophagus to anus using scale. Relative length of gut (RLG) was calculated by dividing the gut length by total length of the fish.

The relationship between gut length (GL) and total length (TL) was estimated by the formula given by Al Hussaini (1949) as:

$$\text{RLG} = \frac{\text{GL}}{\text{TL}}$$

where, GL is total length of gut (mm) and TL is total length of fish (mm).

For estimation of intestinal coefficient, standard length (SL; mm) and body weight (g) were recorded for each fish. After sacrificing the fish, a ventral incision was made to extract the digestive tract and the length (mm) of the intestine (CI; mm) was measured. Subsequently, the intestinal coefficient (CO) was estimated using the equation of Angelescu and Gneri (1949):

$$\text{CO} = \frac{\text{CI}}{\text{SL}}$$

This is also referred to as digestive somatic index (DSI), and this gut parameter represents the ratio of gut mass to the body mass, calculated by the following formula according to German *et al.* (2004):

$$\text{RGM} = \frac{\text{Gut mass (g)}}{\text{Body mass (g)}}$$

This gut parameter is a relation between the gut length and ten times the cube root of body mass and was calculated using Zihler (1981) formula:

$$\text{ZI} = \frac{\text{Gut lenght (mm)}}{\text{10 x Body mass (g)}^3}$$

The ratio of liver weight and body weight represents the hepatosomatic index and was calculated by using the formula given by Parameswaram and Liese (1974):

$$\text{HSI} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

Gastrosomatic index indicates the relation between the alimentary canal weight and weight of fish and was calculated by using the following formula of Desai (1970):

$$\text{GaSI} = \frac{\text{Total weight of the alimentary canal of fish (g)}}{\text{Total weight of fish (g)}} \times 100$$

Analysis of data

All gut morphometric measurements were subjected to Principal Component Analysis (PCA). PCA reflects the importance of the largest contributor to the total variation at each axis of differentiation (Sharma, 1998). The eigen values are often used to determine how many factors to retain. In this analysis, the first factor retaining the information were contained in the original variables. The coefficients of morphometric characters defining the five principal components of this data were estimated using appropriate statistical tools. The statistical analysis of the data was carried out by using Microsoft Excel, SPSS and SAS softwares.

Results and discussion

Forty rainbow trout (*O. mykiss*) specimens for each *viz.,* stage (fry 3.60±0.19 g; 63.31±1.32 mm) fingerling (10.56±0.78 g; 91.51±2.33 mm), yearling (45.04±1.52 g; 109.97±1.59 mm), table size (235.19±8.85 g; 258.00±3.64 mm) and brood-size (530.69±11.43 g; 341.2±2.96 mm) were taken for the experiment. A highly significant correlation was obtained between total length (a) and standard length (b) ($r=0.77$; $p<0.01$), total length (a) and relative length of gut (h) ($r= -0.79$; $p<0.01$), total length (a) and intestinal coefficient (i) ($r=-0.50$; $p<0.01$) and between standard length (b) and intestinal coefficient (i) ($r= -0.67$; $p<0.01$) (Table 1). The gut length (d) showed significant positive correlation with intestinal length (f) ($r=0.78$; $p<0.01$) and Zihler's index (k) ($r= 0.47$; $p<0.01$). The gut weight (e) showed significant positive correlation with intestinal length (f) ($r= 0.53$; $p<0.01$), relative gut mass (j) ($r= 0.47$; $p<0.01$) and gastrosomatic index (m) ($r= 0.50$; $p<0.01$). Highly significant positive correlation was recorded between intestinal coefficient (i) and intestinal length (f) ($r= 0.52$; $p<0.01$) and relative length of gut (h) ($r= 0.63$; $p<0.01$). Highly significant relationship was seen between gastrosomatic index (m) and relative gut mass (j) ($r= 0.97$; $p<0.01$). A positive significant relation was also reported in case of hepatosomatic index (l) and liver weight (g) ($r= 0.49$; $p<0.01$).

Principal component analysis

Biplots of different life cycle stages of *O. mykiss* generated through PCA explained overall variation in the data of gut morphometric parameters. The results indicated that the characters in PC1 were more likely to be associated with intestinal coefficient (i), relative length of gut (h), Zihler's index (k), relative gut mass (j), gastrosomatic index (m), intestinal length (f), gut length (d), gut weight (e) and fish weight (c) whereas PC2 was associated more with standard length (b), total length (a), liver weight (g) and hepatosomatic index (l). The analysis showed that only five principal components were included in the study as the eigen values were more than one and 80% of variation in data was contributed by the five principal components (Fig. 1). The latent vectors of all traits measured is given in table 2.

The biplots generated explained the variation in the data (Fig. 3) and from the PC1 and PC2, the highest variation was due to variable i (intestinal coefficient) of *O. mykiss* in all the five stages, followed by variation due to variable h (relative length of the gut), variable j (relative gut mass), m (gastrosomatic index), d (gut length), e (gut-weight) and f (intestinal length) included in PC1 and, in case of PC2 highest variation was due to variable b (standard length) followed by variation due to variable a (total length). Accordingly, the first principal component (Table 1) had high positive component loading from gut length (d=0.354431), gut-weight (e=0.342041), weight (c=0.333106), total length (a=0.320632), intestinal length (f=0.301986), relative length of gut (h=0.241842), Zihler's index (k=0.238940), relative gut mass (j=0.247716), gastrosomatic index (m=0.233688) (Table 2) and remaining traits in this PC1 did not contribute much; rather their effects were distributed among other PCs. The positive loading shows the presence of positive correlation trends between the components and the variables (Table 2). Therefore, the above mentioned characters, which load high positively contributed more to the diversity and they were the ones that were most differentiated. The characters in the PC1 were more likely to be associated with an intestinal coefficient

Table 1. Pearson's correlation coefficient of the traits in all the developmental stages of *O. mykiss*

	a	b	c	d	E	f	g	h	i	j	k	l	m
a	1.000	0.776	0.113	-0.0156	-0.1266	0.084	0.1744	-0.798	-0.5022	-0.0424	-0.2696	0.0005	-0.0518
b		1.000	0.275	0.065	-0.0531	0.005	0.2660	-0.455	-0.6752	-0.2023	-0.3973	0.1768	-0.2031
c			1.000	0.311	0.396	0.382	0.3299	0.254	0.2488	-0.1224	-0.3477	-0.1068	-0.0911
d				1.000	0.612	0.785	-0.1449	0.307	0.3614	0.1916	0.4711	-0.1617	0.2107
e					1.000	0.532	0.0475	0.359	0.4028	0.4713	0.1793	-0.0704	0.5056
f						1.000	-0.0387	0.218	0.5260	0.2810	0.2478	-0.1261	0.3112
g							1.0000	-0.077	-0.1151	-0.1803	-0.3146	0.4927	-0.1636
h								1.000	0.6345	0.1421	0.1083	0.0666	0.1597
i									1.0000	0.3699	0.2708	-0.1724	0.3978
j										1.0000	0.2859	-0.0470	0.9769
k											1.0000	-0.2048	0.2961
l												1.0000	-0.0261
m													1.0000

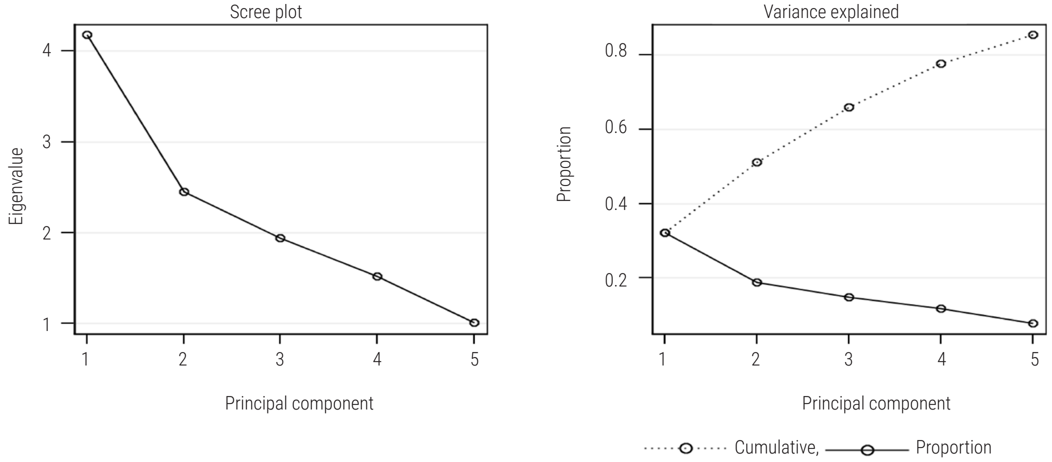

Fig. 1. Eigen value plots and variance of gut morphometry data in different life cycle stages of *O. mykiss*

(i), relative length of gut (h), Zihler's index (k), relative gut mass (j), gastrosomatic index (m), intestinal length (f), gut length (d), gut-weight (e), fish weight (c), whereas the variables with standard length (b), total length (a), liver weight (g) and hepatosomatic index (l) contributed more to second PC. In the component pattern diagram (Fig. 1 and 2); correlation between variables and the principal component depicted the dominance of each character. Claverie and Wainwright (2014) analysed the body shape variation patterns of 2,939 species of tropical Indo-Pacific reef fishes and found that body shape evolution mainly occurs along an axis of elongation across 56 family groups. An analysis of the principal components shows a major axis of shape variation that contrasts slender, elongate forms with deep-bodied species. In addition, shape changes on this axis could affect swimming, defense against predators with gapes limited, suction feeding performance and access to specific habitats. A wide range of developmental processes and functional consequences are implicated in morphological changes that underlie elongation.

Several authors have correlated intestinal length with feeding strategy of fish and derived an important gut morphometric parameter called intestinal coefficient which shows the amount of tissue dedicated to the alimentary canal. However, mean Zihler's index was dominated by size of fish and was lower in small fish (63-149 mm) and higher in larger fish (>250 mm). The majority of the discrepancies were due to differences in body weight. Available literature suggests that the gut morphometric characteristics increase with fish body size. Our results are not in agreement with German and Horn (2006) and Hani *et al.* (2018) who recorded that Zihler's index was substantially impacted by size, and was higher in small sticklebacks than in large ones. The authors observed that findings were not surprising as the structure of sticklebacks' intestine (a straight tube) governed by their elongate body shape cannot be longer than their abdominal cavity, unlike in a flat and streamlined body shape that allows looping and convolution of the intestine (Karachle and Stergiou, 2010).

Rahman *et al.* (2012) reported that the oesophagus comprises the smallest portion of the alimentary canal with rough inner surface and opens into the stomach and is suitable in structure to engulf larger amounts of food. Khulsharshtha (1975) worked on some freshwater fishes and found that the oesophagus was a short, tubular structure and was not distinguishable externally from the rest of the gut. Islam (1985) found that the oesophagus of *Mystus gulio* was short and directly opened to the stomach. The great dispensability of the oesophagus has been reported by Lagler *et al.* (1977). The structure of the stomach of fishes in this study were more or less same which were found to be large, sac-like

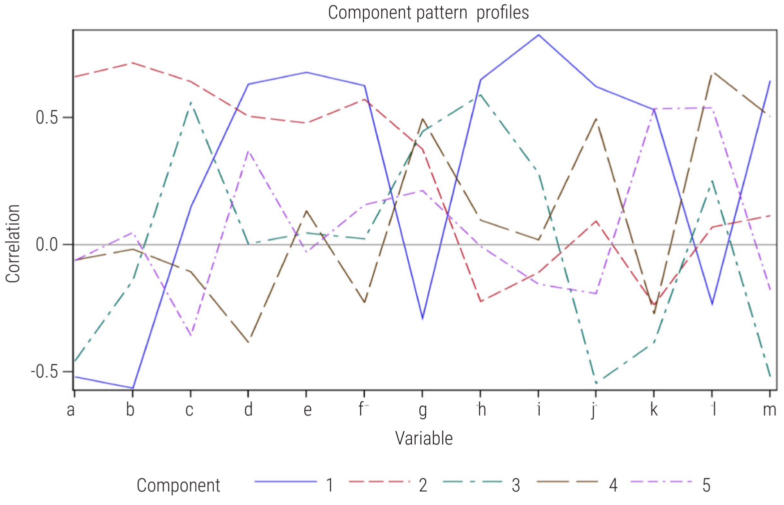

Fig. 2. Component profile of gut morphometry in different life cycle stages in *O. mykiss* generated through PCA

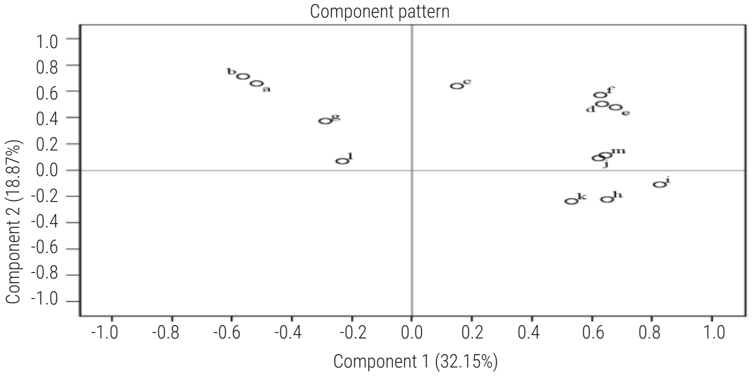

Fig. 3. Biplots of different life cycle stage of *O. mykiss* generated through principal component analysis explained overall variation in the data with gut morphometric parameters

Table 2. Latent vectors for 13 measurements of *O. mykiss*

Eigen vectors					
Characters	Prin1	Prin2	Prin3	Prin4	Prin5
a- TL	0.3206	-0.2472	-0.0853	-0.2954	-0.1061
b- SL	0.2998	-0.2798	-0.1884	-0.2577	-0.1852
c- WT	0.3331	-0.2301	0.0284	-0.2177	0.1450
d-GL	0.3544	0.1394	-0.1962	-0.0661	-.1950
e-GWT	0.3420	-0.1815	0.2241	-0.1288	0.0717
f-CI	0.3019	0.2676	-0.0411	-0.2447	0.4032
g-LWT	0.2836	-0.2491	-0.1521	0.3794	0.3438
h-RLG	0.2418	0.4088	-0.1966	0.1782	-0.2480
i- CO	0.1280	0.5116	0.0849	-.0855	0.5483
j- RGM	0.2477	0.0519	0.5574	0.2142	-0.1680
k- ZI	0.2389	0.3787	-0.2775	0.0966	-0.3981
l- HSI	0.1845	-0.2080	-0.2626	0.6616	0.1774
m- GASI	0.2336	0.0554	0.5832	0.2047	-0.1699
Eigen value	6.7760	2.5917	1.6081	1.1589	0.6206
Proportion	0.5212	0.1994	0.1237	0.0892	0.0477
Cumulative	0.5212	0.7206	0.8443	0.9335	0.9812

with thick walls and rough inner surface. Elongated structure was assumed to provide for longer passage for the food eaten for proper digestion. The thickened wall with muscular structure was most probably helped in grinding the food particles. Mookerjee and Das (1945) observed that the size of the stomach in fishes was closely related to the feeding behaviour and particularly to the size of the prey. They also stated that predators have a large mouth, short and few gill rakers, a large stomach, usually a large number of pyloric caeca and a short intestinal tract which is very similar to the current study's findings. Vladimirov (1957) stated that fishes which swallow large prey consume large amount of food at the same time and the stomach is usually large.

Intestinal length is related to feeding habits, more so in iliophagous, herbivorous and omnivorous fishes and to lesser extent in carnivorous and insectivorous fish species. The master organ, intestine involved in digestive physiology is basically a tube wherein food travel and digestion occurs in alkaline medium and finally leads to nutrient absorption (Canan *et al.,* 2012). Herbivorous fish generally have a larger relative length of gut (RLG) than omnivorous and carnivorous fish. Previous studies have shown that algae eating *Atherinops affinis* has an RLG of 1.5 (Horn *et al.,* 2006), marine herbivores

Table 3. Stage wise comparison of gut morphometric parameters of *O. mykiss*

Stages	Intestinal coefficient	Relative gut mass	Zihlers index	Hepatosomatic index	Gastrosomatic index	Standard length	Weight (g)	Gut length (mm)	Gut weight (g)	Intestinal length (mm)	Liver weight (g)	Relative length of gut (mm)
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
FRY	0.77±0.03	0.12±0.00	3.58±0.19	1.20±0.10	12.20±0.54	53.88±3.167	3.60±0.42	55.94±5.586	0.45±0.07	41.10±2.39	0.04±0.00	0.85±0.04
FL	0.86±0.04	0.15±0.01	4.09±0.12	1.11±0.12	13.69±1.38	76.57±4.605	10.57±2.10	88.05±5.859	1.58±0.45	69.02±5.29	0.12±0.02	0.95±0.01
YL	0.68±0.19	0.18±0.06	4.05±0.07	1.38±0.13	12.32±0.85	123.87±2.677	45.04±2.24	143.42±1.696	5.45±0.08	109.9±2.71	0.63±0.08	0.96±0.01
TS	1.10±0.21	0.10±0.00	4.29±0.09	1.24±0.13	10.68±0.62	214.67±6.252	235.19±22.16	262.75±3.538	25.03±2.21	196.0±11.25	2.85±0.12	1.02±0.02
BS	0.92±0.02	0.07±0.00	4.17±0.04	1.62±0.05	7.12±0.34	290.87±2.447	530.69±12.30	337.00±4.698	37.73±1.88	266.1±5.38	8.60±0.27	0.98±0.00
C.D.	N/S	N/S	0.35	0.34	2.53	12.42	34.74	13.806	4.01	19.08	0.42	0.07
SE	0.19	0.04	0.16	0.16	1.17	5.77	16.15	6.419	1.86	8.873	0.19	0.03

FRY: Fry, FL: Fingerling ; YL: Yearling; TS: Table size; BS : Brood size; CD: Critical difference; SE: Standard error

have an RLG of 1.6-17.75 (Karachle and Stergiou, 2010a, b), carnivorous fish generally have a lower RLG than herbivorous fish. Xiong *et al.* (2011b) estimated RLG value of 0.898 in carnivorous *Glyptosternum maculatum* having a preference for fish. Cephalopada and Decapoda have an RLG of 0.47-0.65 (Karachle and Stergiou, 2010). Our analysis of trout fish RLG showed a value of 0.85±0.04 (fry) to 0.98±0.00 (broodsize) which is consistent with the study of Susilo *et al.* (2021) on yellow rasbora with RLG ranging from 0.53 to 0.81. Mohammadizadeh *et al.* (2010) while studying feeding habits of sawtooth barracuda, reported RLG value of 0.34+0.002 suggesting the strong carnivorous nature of the fish. Our study recorded the maximum GaSI index for fingerling stage (13.69±0.71) owing to its faster growth while minimum value was obtained in brood stage (7.12±0.31) which may be due to development of gonads in the spawning season and consequent reduced feeding intensity. In carnivorous species, the length of the digestive tract increases with the length of the body (Kramer and Bryant, 1995). As fish increases in size the volume of their food increases which creates a need to lengthen the intestinal tract to maintain efficient digestion of food. Since mass of body tissue increases as an allometric function of linear body growth (Bagenal and Tesch, 1978) and digestive tract must provide the nutrient requirement for this tissue, the length of the intestinal tract should also increase as power function of body length. Other authors have noted that carnivore fish have relatively shorter intestines than herbivore fish (Bond, 1979; Fange and Grove, 1979). In a study of flatfish, De Groot (1971) found that intestine is shortest in Psettodidae which eat fish and large invertebrates and longest in Soleidae which eat small invertebrates. Odum (1970) found extremely rapid lengthening of gastrointestinal tract as mullet shifts from animal diet to a plant diet. This trend and our results of stage-wise comparison (Table 3) suggest that the relative length of the gut is one of the adaptive features of feeding ecology of fish. The ontogenetic development of intestinal complexity appears to represent an important functional innovation underlying the extensive trophic differentiation seen in fishes specifically facilitating the pronounced shifting away from carnivorous (including invertebrates and vertebrates) diets. The capacity to modify intestinal morphology and physiology may also be an important facilitator of trophic diversification during other phyletic radiations. Although intestinal length emerged from the phylogenetically informed analysis as a useful predictor of diet, a substantial amount of unexplained variability was also evident in the relationship. Behavioural, ecological, physiological and historical factors can interact to influence the strength of the congruence between morphological and ecological characters (Motta *et al.*, 1995). A flexible fish gut is always a reflection of adaptation to various diet patterns and shorter gut length with respect to carnivore fishes can be taken as a valid proof. A significant correlation was observed between fish weight and gut length throughout the study.

O. mykiss is an active fish, feeding voraciously and for that reason the alimentary canal structure is heavier which is reflected by relative gut mass (RGM) which is basically dedicated to evaluate the amount of tissue for digestive tract. The RGM in our study was highly correlated with GaSI and significantly related with Zihler's index because the feeding intensity increase from fry to adult stages. Relative gut mass in *Wallago attu* (Riaz and Naeem, 2020) showed positive correlation with gut weight. RGM was also found to be significantly correlated with gut weight in all the stages of *O. mykiss* in this study. Ontogenetic increases in gut length are well known in marine and freshwater herbivorous fishes (Montgomery, 1977; Zihler, 1982; Ribble and Smith, 1983; Stoner and Livingston, 1984; Benavides *et al.,* 1994; Kramer and Bryant, 1995a; Gallagher *et al.,* 2001; Drewe *et al.,* 2004). Even carnivorous fishes can increase their gut lengths with increase in standard length, but herbivores tend to show a more rapid increase (Kramer and Bryant, 1995a). RGM is used as an estimate of the amount of tissue each trout life stage was dedicating to its digestive tract. Fish feeding states can be determined by RGM (also called digestive somatic index), as individuals that are actively consuming food have heavier guts than those that are starved (King *et al.,* 1994; Lloret and Planes, 2003). Fuentes and Cancino (1990) suggested that RGM is a better indicator of diet than gut length in fishes after they showed that *G. laevifrons* altered its RGM, but not RLG, in response to diets of 1132 varying biochemical composition. In our study, higher RGM values were recorded in early developmental stages like fry, fingerling and yearling (63-149 mm) than in large ones (>250 mm) which suggest that younger stages feeding strategy maximises nutrient and energy absorption from their diet, in turn allowing them to maintain higher metabolic rates. In animals, increasing gut mass is a means of increasing calorie intake from food (Karasov and Douglas, 2013). In the present study, trout consistently exhibited the largest RGM from fry (0.121±0.00), fingerling (0.158±0.00), yearling (0.184±0.06), table size (0.107±0.00) to brood size (0.07±0.00) in all of the examined life cycle stages. This decrease in RGM in case of brood size is a result of starvation/reduced feeding strategy of the fish during reproductive phase. Whereas gut length can vary in response to intake, RGM appears to be more responsive to the biochemical composition of ingested dietary items (Fuentes and Cancino, 1990; Starck, 1999). Thus, the decrease in RGM observed in brood size fishes may have been a direct reaction to the variation of relatively high-nutrient load of the high protein artificial diet.

Yearling stage however, displayed the largest RGM in all life cycle categories, possibly in response to its active metabolism and in response to the high protein formulated diet. To our knowledge, this study presents the first attempt to compare gut dimensions in different life cycle stages of trout species and it is also one of the few investigations to compare gut lengths of life cycle stages of trout fishes in a phylogenetic context. Gut dimension parameters can vary widely among different species, especially those of different body shapes and masses, and this study shows the importance of comparing life stages in response to shape and mass to infer differences in gut dimensions.

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