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Tissue pH and gut ecomorphology in six freshwater teleosts occupying different trophic levels

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Abstract: This study investigated the gastrointestinal (GI) tract, blood, and muscle pH, and explored the characteristic of gut morphology (relative gut length) associated with trophic level in 6 freshwater teleosts. The results showed that GI pH differences among species and significant differences occurred in GI segments ($P < 0.05$). The lowest pH values were observed in the stomach of 2 typical carnivorous fish. An increased pH from proximal intestine to distal intestine was found in 4 species. A positive relationship was observed between blood and muscle pH values for these species ($P < 0.05$). Additionally, relative gut length revealed the trend of divergence depending on the feeding habits (in planktivores > omnivores > carnivores). A robust link was negatively found between relative gut length and trophic level ($P < 0.05$).

Key words: Fish, pH, gastrointestinal tract, blood, muscle, gut length

1. Introduction

The gastrointestinal (GI) tract in fish plays an important role in feeding and subsequent digestion and absorption of nutrients to provide energy for living systems. Substantial knowledge of the GI tract in fish species has been documented in freshwater and marine fish: ontogeny of digestive functionality during larval stages (Walford and Lam, 1993; Yu et al., 2010; Ma et al., 2014), biochemical properties of digestive enzymes (Bitterlich, 1985; Chakrabarti et al., 1995; Deguara et al., 2003), morphological features (Wilson and Castro, 2010), and its relation to food habits and feeding (De Silva et al., 1980; Patricio Ojeda, 1986; Karachle and Stergiou, 2010). The postembryonic development of the digestive system is a key event in the life history of fish. Moreover, adaptations to habitats and food preferences lead to divergent GI morphology and feeding strategies among fish species, reflecting their ontogeny and phylogeny. A linkage between gut morphology and feeding, however, has been considered to establish the functional morphological assessment in fish related to ecology. Information on the complexion is partially restricted to marine fish (Patricio Ojeda, 1986; Costa and Cataudella, 2007; Karachle and Stergiou, 2010), and fewer attempts have been made to link gut morphology to feeding habits in freshwater fish.

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In general, the focus on the GI tract and pH is related to digestive function and acid–base homeostasis. Some studies have targeted pH changes in the GI tract of fish larvae during the development or postprandial digestive tract (Yúfera and Darías, 2007; Gomes et al., 2014). In feeding Atlantic salmon smolt kept in fresh water and transferred to seawater, differences in GI pH have been found (Usher et al., 1990). Diets provide important ion sources to sustain hypertonic homeostasis other than facilitate growth in fish. Formulated or controlled feed shapes the gastrointestinal environment. Direct involvement of GI tract pH in the partitioning of bivalent ions has been shown in rainbow trout stomach (Bucking and Wood, 2009). In turn, the characteristics of GI and blood pH are influenced by diet and altered dietary electrolyte balance (Saravanan et al., 2013). Thus, condition-specific findings for these characteristics in a given species, to some degree, cannot be extrapolated directly to others, and specific validation studies for different species are needed. Any improvement in GI function may be only effective when complemented with detailed knowledge of their physiological characteristics. In freshwater teleosts, in particular Cyprinidae, available information on GI tract pH is extremely scant and knowledge gaps exist (Bitterlich, 1985; Chakrabarti et al., 1995). Blood pH constitutes one of the most important biological features, but few studies have been conducted on this aspect in fish (Saravanan et al., 2013). Of the fish species studied, the blood pH characteristics were recorded in this study. Accordingly, the potential importance of fundamental data regarding biochemical and physiological characteristics of digestive processes and relative physiological parameters could enhance the exploration in this field.

In this study, we investigated GI and blood pH for 6 freshwater species. In addition, we further determined muscle pH in these species. These parameters were studied in conjunction with correlation of gut morphology (relative gut length, gut length in percentage of body length, or total length) and trophic level and multiple feeding habits. We explored adaptation patterns between relative gut length and trophic status, and whether a relationship between blood pH and trophic level was related to differences in feeding habits.

2. Materials and methods

2.1. Sample collection

Fish were caught by purse net in Liangzi lake (114°36′E; 30°09′N), a typical freshwater shallow lake located in the middle reaches of the Yangtze River in China, in February 2015. A total of 62 individuals belonging to 6 species, silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), crucian carp (*Carassius auratus*), common carp (*Cyprinus carpio*), snakehead fish (*Channa argus*) and mandarin fish (*Siniperca chuatsi*), in this study were randomly collected. The sampled fish species are from three different feeding habits and belong to 3 families. A detailed description of these species is presented in Table 1.

2.2. Parameters measured

For each specimen, body length and total length (to the nearest millimeter) and body weight (to the nearest gram) were recorded. All fish were killed by a knock on the head. Blood was collected using a disposable syringe from the tail vein for pH measurement. The entire viscera were carefully dissected out. The GI was obtained by separating from other viscera, followed by uncoiling of the intestine, but unstretched, for intestinal length measurement. For species with a stomach (i.e. *Channa argus* and *Siniperca chuatsi*), measurements were made from pylorus to anus, whereas for stomach-less species (the remaining species), measurements were made from esophagus to anus. Relative gut length (RGL = GL/TL; RGL = GL/SL) was estimated in two ways according to methods of Al-Hussaini (1947) and Patricio Ojeda (1986), respectively. Trophic position (TP) data in this study for the six freshwater fish species were from the study by Zhang et al. (2013).

2.3. pH measurement

Blood pH was measured using a pH meter (STAR A221; Fisher Thermo Scientific, USA) connected to a micro combination pH electrode (MI-415, Radiometer; Microelectrode, USA). For the pH measurement of the GI tract, we made a small slit to introduce of the microelectrodes through the GI epithelium to the lumen. There was no food observed in the GI tract of the 2 carnivorous fish and so the pH measurement was carried out in the mucosa. Measurements were conducted for each segment (stomach, anterior intestine, medium intestine, and posterior intestine) three times in 4 Cyprinidae species. In the 2 carnivorous fish species without coiled intestine, the intestine segments were artificially divided according to their anteroposterior positions. Muscle pH was measured with a specialized pH meter (Testo 205; Testo, Germany) in the dorsal muscle with three replicates for each sample.

2.4. Statistical analysis

One-way ANOVA was used to analyze the significance of variations in blood and muscle pH followed by Student–Newman–Keuls (SNK) multiple comparison tests among fish species when the data conformed to homogeneity and normality, otherwise followed by nonparameter Kruskal–Wallis tests. Differences in GI tract segment pH for a species were also analyzed. These statistical analyses were

Table 1. Sample size (n) and fish size of the 6 freshwater fish species studied.

Family	Species	Feeding habits	n	SL (cm)		TL (cm)		BW (g)	
				Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)
Cyprinidae	*Hypophthalmichthys molitrix*	Planktivorous	3	37–42	39 ± 3	45–49	46 ± 2	1020–1400	1147 ± 219
Cyprinidae	*Aristichthys nobilis*	Planktivorous	3	26–32	30 ± 4	33–39	37 ± 4	400–780	647 ± 214
Cyprinidae	*Carassius auratus*	Omnivorous	24	11–21	13 ± 3	14–29	17 ± 4	53–423	104 ± 91
Cyprinidae	*Cyprinus carpio*	Omnivorous	18	23–64	40 ± 12	28–78	40 ± 12	320–4580	1973 ± 1585
Channidae	*Channa argus*	Carnivorous	10	14–33	23 ± 8	17–39	27 ± 10	89–1020	436 ± 431
Serranidae	*Siniperca chuatsi*	Carnivorous	4	18–55	28 ± 11	22–65	34 ± 14	83–2630	479 ± 686

done using SPSS 19.0. Statistical significance was set at $P \leq 0.05$ and all data are presented as mean ± SD.

3. Results

The pH values of the stomach, anterior intestine, medium intestine, and posterior intestine included in the GI tract are shown in Table 2. The pH values from stomach to posterior intestine along the alimentary tract increased gradually. All values varied from 6.38 to 7.82 in these fish. There were significant differences in pH amongst fish species ($P < 0.05$). The lowest pH value in the corresponding section occurred in the GI of *C. carpio*, whereas the highest occurred in that of *S. chuatsi.* Blood pH values ranged from 7.16 in *C. carpio* to 7.76 in *H. molitrix*. The 2 planktivorous species in this study showed the highest blood and muscle pH, while the lowest pH values were recorded in the 2 omnivorous species (Table 3). Blood and muscle pH were characterized by a positive relationship in the sampled fish (Figure 1). Relative gut length of each species is shown in Table 4. The relative gut length significantly decreased from herbivores to carnivores ($P < 0.05$), ranging from 6.86 in *H. molitrix* to 0.47 in *S. chuatsi*. Combined with trophic status estimated for these species, the latter was positively correlated with relative gut length (Figure 2). However, there was no observational correlation between blood pH and trophic status or relative gut length (not shown).

4. Discussion

In previous studies it has been demonstrated that relative gut length indicated that morphological features are correlated to feeding habits (Kapoor et al., 1976; De Silva et al., 1980; Karachle and Stergiou, 2010), and also that in a species it could vary depending on the habitat and the type of food ingested (De Silva et al., 1984). Noncarnivores in general need more time to digest food types like plants, detrital material, fragments, or small crustaceans in the extended intestine for abundant nutrient breakdown and absorption. Among the closely related fish species

Table 2. Gastrointestinal tract pH of the six freshwater fish species investigated.

Species	Stomach pH		Anterior intestine pH		Medium intestine pH		Posterior intestine pH	
	Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)
Hypophthalmichthys molitrix	nd	nd	6.25–6.82	6.58abA ± 0.29	6.79–7.15	7. 00aA ± 0.18	7.35–7.94	7.54cB ± 0.34
Aristichthys nobilis	nd	nd	6.76–6.89	6.83aA ± 0.07	7.23–7.66	7.46bB ± 0.21	7.10–7.96	7.82cC ± 0.13
Carassius auratus	nd	nd	6.48–7.50	7.12bA ± 0.26	6.58–7.86	7.44aB ± 0.29	6.90–7.89	7.38cbB ± 0.23
Cyprinus carpio	nd	nd	6.32–6.97	6.70aA ± 0.19	6.40–7.32	6.89bB ± 0.23	6.56–7.38	6.94aB ± 0.27
Channa argus§	4.92–6.91	6.38aA ± 0.61	6.64–7.18	7.03aA ± 0.20	6.68–7.62	7.09bA ± 0.26	6.61–7.41	7.08abA ± 0.32
Siniperca chuatsi§	6.49–6.74	6.59aA ± 0.15	7.35–7.82	7.47cA ± 0.23	7.40–7.91	7.54aA ± 0.25	7.29–7.61	7.41cbA ± 0.14

nd: No data available because the fish species included stomach-less animals.§ The 2 carnivorous species without coiled intestine and the partition of the intestine segments was according to its anteroposterior position artificially. Different small letters in the same column represent significant differences among species. Different capital letters in the same row represent significant differences among segments.

Table 3. Blood and muscle pH of the 6 freshwater fish species investigated.

Species	Blood pH		Muscle pH	
	Range	Mean (±SD)	Range	Mean (±SD)
Hypophthalmichthys molitrix	7.70–7.89	7. 76^{a} ± 0.11	6.03–6.11	6.07^{a} ± 0.04
Aristichthys nobilis	7.23–7.66	7.69^{a} ± 0.19	6.10–6.10	6.07^{a} ± 0.03
Carassius auratus	6.91–7.57	7.17^{b} ± 0.23	5.55–6.07	5.77^{b} ± 0.13
Cyprinus carpio	6.85–7.45	7.16^{b} ± 0.18	5.66–6.01	5.81^{b} ± 0.13
Channa argus	7.27–7.88	7.59^{a} ± 0.14	5.77–5.85	5.83^{b} ± 0.03
Siniperca chuatsi	7.45–7.77	7.49^{a} ± 0.18	5.65–6.09	5.88^{b} ± 0.14

In each column values with different superscripts are significantly different from each other.

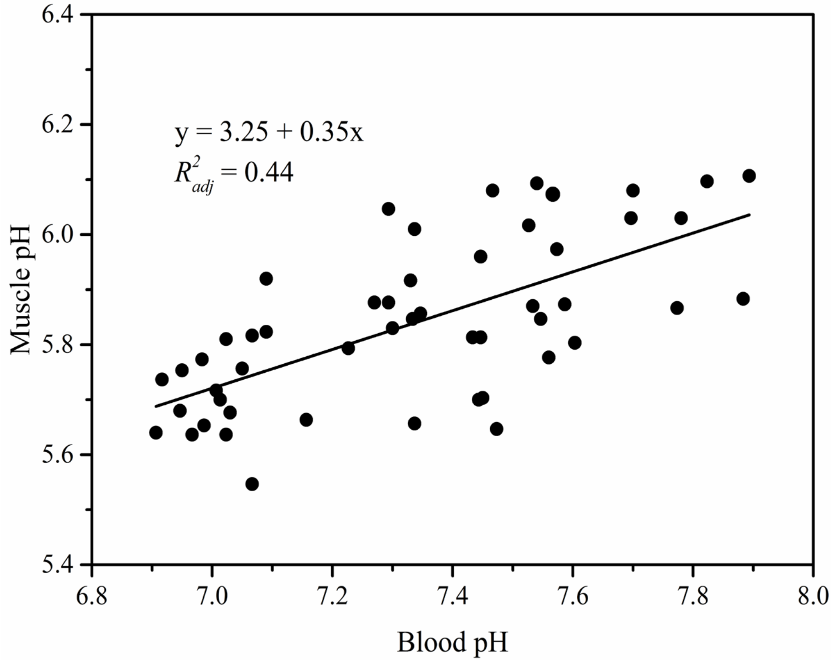

Figure 1. The line of best fit showing the relationship between blood pH and muscle pH of the 6 freshwater fish species.

Table 4. Relative gut length and trophic position of the 6 freshwater fish species studied.

Species	Relative gut length[§]		Relative gut length[*]		Trophic position (SIA)[$]		Trophic position (GCA)[#]	
	Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)
Hypophthalmichthys molitrix	5.44–5.83	$5.69^{a} \pm 0.23$	6.65–7.05	$6.86^{a} \pm 0.20$	2.3–2.8	2.6 ± 0.18	–	2.3 ± 0.30
Aristichthys nobilis	3.26–3.71	$3.51^{b} \pm 0.22$	4.12–4.49	$4.31^{b} \pm 0.19$	2.7–3.2	2.9 ± 0.17	–	2.4 ± 0.34
Carassius auratus	1.93–4.60	$3.34^{b} \pm 0.73$	2.46–5.92	$4.31^{b} \pm 0.92$	2.9–3.1	3.0 ± 0.11	–	2.5 ± 0.21
Cyprinus carpio	1.14–1.87	$1.44^{c} \pm 0.20$	1.38– 2.29	$1.74^{c} \pm 0.24$	2.6–3.8	3.0 ± 0.37	–	3.2
Channa argus	0.41–0.56	$0.46^{d} \pm 0.05$	0.41–0.56	$0.56^{d} \pm 0.06$	3.4–3.9	3.6 ± 0.26	–	3.6 ± 0.08
Siniperca chuatsi	0.33–0.42	$0.39^{d} \pm 0.04$	0.40–0.50	0.47d ± 0.05	3.5–3.8	3.6 ± 0.14	–	3.7 ± 0.13

[§] Relative gut length = gut length/total length; [*] Relative gut length = gut length/standard length; [$] SIA, stable isotope analysis; GCA, gut content analysis; [$, #] Trophic position data from the study by Zhang et al. (2013); –, no data available. In each column mean values with different superscripts are significantly different from each other.

of similar trophic status, convergent adaptation of the digestive tract is the result of processing similar diets and vice versa (Patricio Ojeda, 1986; Costa and Cataudella, 2007). Al-Hussaini (1947) reported that the relative gut length ratios decreased with elevated marine fish trophic level: 0.5–2.4 in carnivores, 1.3–4.2 in omnivores, and 3.7–6.0 in herbivores. These results are comparable with those observed in the current study. However, it is not always possible to place species in accordance with feeding category and relative gut length (Pogoreutz and Ahnelt, 2014). The differences are determined not only by the diet available, but also ontogeny and phylogeny of species (Elliott and Bellwood, 2003; Karachle and Stergiou, 2010). These observations were confirmed by relatively large differences in relative gut length between the omnivorous *C. carpio* and *C. auratus* in the present study. As suggested by Pogoreutz and Ahnelt (2014), an expected experiment of the suitability of relative gut length used to indicate trophic level in fish should correlate with gut content analysis or stable isotope analysis. Therefore, we attempted to establish the relationship between relative gut length and trophic level estimated by the data of gut content and stable

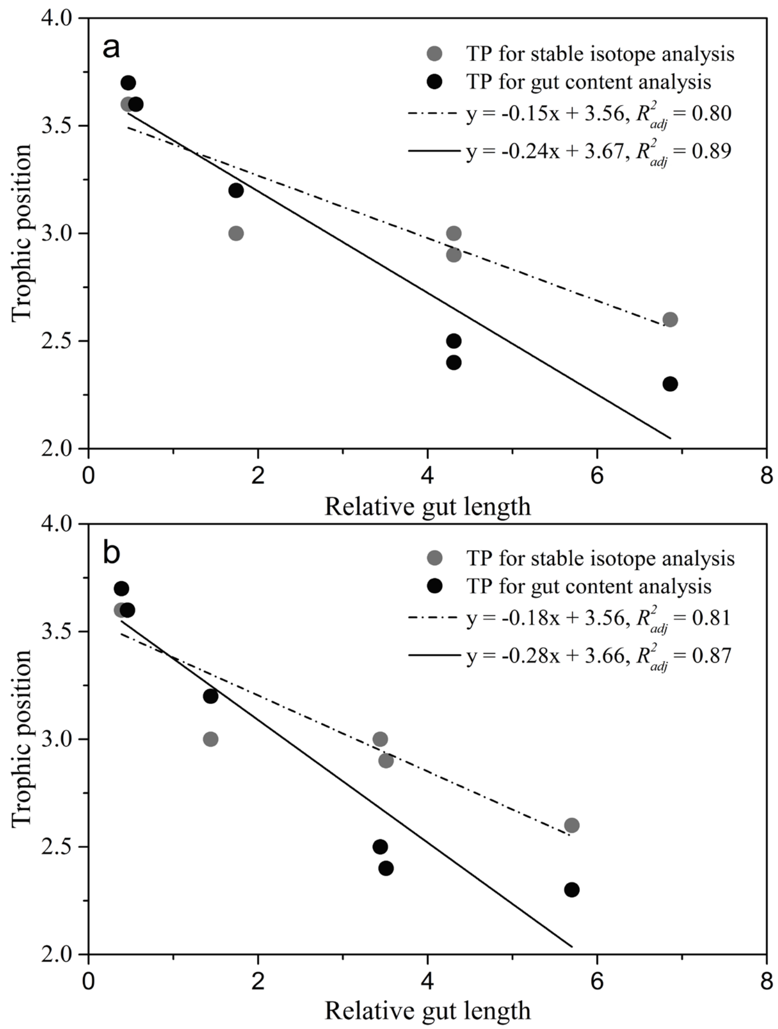

Figure 2. The relationship between relative gut length and trophic position for the 6 freshwater fish species. (a) Relative gut length is presented in percent of body length; (b) Relative gut length is presented in percent of total length. Averaged values of trophic position (TP) from the study by Zhang et al. (2013). Grey and black circle dots correspond to TP for stable isotope analysis (SIA) and for gut content analysis (GCA), respectively. Dashed line represents the linear fitting of relative gut length and TP for SIA, while solid line represents the linear fitting of relative gut length and TP for GCA.

isotope analysis (Zhang et al., 2013). Relative gut length and trophic level were significantly negatively correlated in these freshwater fish, although some differences in trophic position relying on stable isotope analysis and gut content analysis were observed.

A stomach-less digestive tract is typical in Cyprinidae. Unlike these species, carnivores are generally specialized with a stomach. It is well demonstrated that during the ingestion of a meal gastric pH is acidic for the initiation of protein digestion by the action of gastric acid secretion and pepsin (Stevens, 1991). Studies have reported that there are marked gastric pH differences, involved in multiple factors, such as fish species and ingestion time (Chakrabarti et al., 1995; Cooper and Wilson, 2008; Krogdahl et al., 2015). The two carnivorous species *C. argus* and *S. chuatsi* in this study showed no differences in gastric pH, but a trend of a neutralized gastric environment. This could be correlated to the capture season, winter, in which the two species tend to fast, as observed in their GI tract. In fish, there are two different patterns for gastric acid secretion. One is characterized by maintenance of a neutral gastric pH during fasting (Deguara et al., 2003; Yúfera et al.,

2004) and the other is characterized by continuous acid secretion and the maintenance of a low, acidic pH during fasting (Papastamatiou and Lowe, 2004; Bucking and Wood, 2009). Our results demonstrated that *C. argus* and *S. chuatsi* hold a closely neutral pH in the stomach mucosa through a long period of fasting, acidification perhaps only stimulated by the ingestion of food (Nikolopoulou et al., 2011). Moreover, intestinal luminal pH in these carnivorous species stably maintained weak alkalinity. As for the 4 Cyprinidae species, apart from *C. carpio* with weak acidic gut pH values, the others had an alkaline gut pH, but differences in luminal pH were based on species- and segments-specific intestine. This is supported by studies on different species associated with controlled diets (Nikolopoulou et al., 2011). Likewise, Sugiura et al. (2006) and Taylor and Grosell (2006) found an increased pH from proximal intestine to distal intestine, though not significantly different. Given that the prerequisite for food digestion and subsequent nutrition absorption in fish is the liquefaction or moisturization of food (extruded or pelleted feed) in the alimentary tract (Kristiansen and Rankin, 2001), it presumably means that a dynamic interaction between feed, subsequently transformed into chyme, and their alimentary tract may exist.

In this study, the blood pH values of the freshwater fish species ranged from 7.16 to 7.78. The overriding blood pH changes are subjected to external and internal challenges (Sugiura et al., 2006). The blood pH recorded from fish in previous studies also showed a wide range from 7.38 to 8.1 (Cooper and Wilson, 2008; Saravanan et al., 2013). Consequently, the blood acid-base regulation partly corresponds to the physiological activities or stressors (Iwama et al., 1989). Interestingly, the blood pH in the fish studied presently was positively related to their muscle pH. It is implied that organic tissues in fish may be interactional on systemic acid–base homeostasis. However, indirect evidence that handling stresses significantly elevated both blood and muscle lactic acids in earlier work supports the observations (Spotts and Lutz, 1981; Wood et al., 1983)

In summary, in the present study we targeted 6 freshwater teleost fish species and found a range of variations in GI tract pH among these species. Blood pH was positively correlated to muscle pH. Conversely, in these species there was a negative correlation between relative gut length and trophic level. It should be noted that, nevertheless, information is restricted to small sample size of some species, and therefore the outcome should be treated with care. Further work should include multiple species with adequate numbers of samples for knowledge of GI characteristics.

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