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RESEARCH PAPER

Carbon, Nitrogen, And Phosphorus Stoichiometry of Three Freshwater Cultured Fishes in Growth Stage

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Abstract

Fish is important in the nutrient cycling of ecosystem, but little is known about how nutrients of cultured fish vary with growth. Whole body samples of three freshwater cultured fish species, grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) were collected from May to October in 2015. The contents of carbon (C), nitrogen (N) and phosphorus (P) were measured, and their ratios were analyzed, aiming to characterize the body stoichiometry of these fish species during farming season. The main results are as follows: the concentrations of C, N, P and their ratios varied across species, while the P content in *O. niloticus* was significantly higher than that in *C. idella* and *C. carpio* ($P < 0.05$). The correlation analysis showed that body stoichiometry of the three fish species varied with size and growth significantly during the farming season. The relationship between growth rate and P content showed varied and complex relationship. Results indicated that the three fish species in aquaculture ponds did not follow strict homeostasis and the growth rate hypothesis, and bone rather than RNA was the dominant P pool. Body morphology, scale type, and food source were the main causes of interspecific differences.

Keywords: Cultured fishes, growth stage, ecological stoichiometry, homeostasis, growth rate hypothesis.

Introduction

Intensive freshwater pond culture plays an important role in aquaculture industry of China (China Fisheries Statistical Yearbook, 2014). The stocking density is continuously increased to maximize commercial profit, and large quantities of artificial formulated feed are released into aquaculture ecosystems (Zhou & Wen, 2004), threatening the sustainable development of aquaculture. Currently, the utilization efficiency and accumulation of C, N, and P are important indicators of pollution level and effectiveness of aquaculture model (Zhang, Wang, & Dong, 2011; Liu, 2012). Fish generally occupy relatively high trophic positions in aquatic ecosystems and play an important role in nutrient cycles of the ecosystem (McIntyre & Flecker, 2010). Although scholars have reported the impact of pond cultured fish on nutrients fluxes at the sediment-water interface (Zhong, Wang, Dong, & Khairnar, 2015) and purification of N as well as P (Xia, Gao, Dong, Shin, & Wang, 2013) in aquaculture ecosystems, little attention has been paid to the accumulation of elemental nutrients in cultured fish during the farming season.

Ecological stoichiometry (ES) has been applied to study how the accumulation of nutrients in fish varies with growth, and most research products about fish stoichiometry are based on homeostasis theory and growth rate hypothesis (GRH). Some scholars supported the homeostasis theory and stated that organisms could maintain relatively constant body composition in the face of variable environments (Sterner & Elser, 2002), while other studies showed that intraspecific variation in fish body stoichiometry varied with body size, ontogeny and morphology (Pilati & Vanni, 2007; Vrede *et al*., 2011). There was also a controversy on GRH; for example, Boros, Sály, and Vanni (2015) found that fathead minnow (*Pimephales promelas*) and sheepshead minnow (*Cyprinodon variegatus*) followed the GRH during ontogeny and specific growth rate correlated significantly with RNA contents, in contrast, many scholars suggested that fish does not follow the GRH due to investment in P-rich bone tissue (Pilati & Vanni, 2007; Vrede *et al*., 2011). These controversies showed that the ES theory is still incomplete and needs to be further studied. The previous studies concerning fish stoichiometry focused on wild fish, compared to natural ecosystems, most N and P come from artificial feed in artificial aquaculture system (Liu, 2012). Nevertheless, scarce information can be obtained how the body

stoichiometry vary with growth and whether cultured fish follow homeostasis and GRH.

Grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), and tilapia (*Oreochromis niloticus*) are three important freshwater commercial fish species. We collected the whole body samples of the three fish species in different months of the farming season and measured C, N, and P contents in the samples. The purpose of this study was to (1) characterize the body stoichiometry of the three freshwater pond-cultured fish species during the farming season; and (2) explore whether cultured fish follow strict homeostasis or the GRH. This study is aimed to enrich the ES theory and provide a reference for the management of aquaculture production.

Materials and Methods

Sample Collection and Nutrient Determination

This study was conducted in three aquaculture ponds in the village Zhaodian, Shandong Province, China (37°04′N, 117°33′E), from May to October 2015. The ponds, located in the north of China, were rectangular, with an area of approximately 0.25 hm^2, and an average water depth of approximately 1.8 ±0.5 m. Each pond was equipped with a feeding machine and a 2000-watt aerator. *C. idella*, *C. carpio*, and *O. niloticus* were stocked in the ponds in monoculture (Table 1). Artificial feed was supplied to the fishes daily at 5% of total cultured fish biomass. In addition, *C. idella* and *C. carpio* were supplied with the same artificial feed (C: 40.51 ± 0.17%; N: 4.48 ± 0.11%; P: 1.08 ± 0.08%), while *O. niloticus* was supplied with a different feed (C: 45.40 ± 0.32%; N: 5.12 ± 0.22%; P: 1.25 ± 0.12%). Fish were collected randomly with a fyke net on the 18th of each month, and three healthy fish were selected arbitrarily. After 24 h of starvation, the fish were sacrificed following the method by Blessing, Marshall, and Balcombe (2010). Standard length (L_S) of each selected fish was measured to the nearest 0.1 cm and wet mass to the nearest 1 g, and then the three individuals, were used for determination of the whole body stoichiometry. The samples were placed immediately on ice and transported to the laboratory, where they were freeze-dried to constant weight and ground into fine powder using a grinding mill to obtain homogeneous mixture. C and N contents were measured with a CHONS elemental analyzer (Elementar Vario III, Hanau, Germany), while P content was measured according to the method by Zhou, Zhang, Yang, Zhang and Ma (2003). Each fish was taken as a sample. The element contents were expressed as a percentage composition of dried samples, and the ratios were expressed as molar ratios of elements.

Data Calculation and Statistical Analysis

Specific growth rate (SGR) was calculated as follows: $SGR = [(\ln W_{i+1} - \ln W_i)/30] \times 100$, where W_i and W_{i+1} are the average total wet mass (g) at the i and i+1 month. Statistical analysis of the data was performed using a statistical package (SPSS 19.0; IBM Corp., Armonk, NY, USA). Intraspecific differences in elemental stoichiometry at different sampling months and interspecific differences were analyzed using one-way analysis of variance (ANOVA) and multiple comparisons were performed by Duncan test with a significance level of 0.05. Pearson correlation coefficient was calculated between chemical variables and sample size (L_S and wet mass) for trends in nutrients. Homogeneity of variances among the groups was verified before one-way ANOVA and Pearson correlation analysis.

Results

Elemental Composition of the Three Fish Species

A total of 54 individuals from three species were analyzed for whole body nutrient content. The concentrations of C, N, and P varied across species: for C $F_{2, 51} = 11.332$, $P < 0.001$; for N $F_{2, 51} = 25.939$, $P < 0.001$; for P $F_{2, 51} = 397.826$, $P < 0.001$ (Table 1). The ratios of the three nutrients showed a significant variation: C/N $F_{2, 51} = 40.579$, $P < 0.001$; C/P $F_{2, 51} = 251.809$, $P < 0.001$; N/P $F_{2, 51} = 164.036$, $P < 0.001$ (Figure. 1).

In *C. idella*, the L_S and wet mass varied from 26.3 cm to 51.1 cm and 386 g to 2588 g, respectively (Table 1). Its SGR fluctuated during the farming season, with higher values detected in June to July and August to September and the lowest levels in September to October (Figure. 2). The mean body content was 47.52 ± 2.57% C (43.30%–51.42%), 9.40 ± 0.73% N (8.52%–11.72%), and 2.01 ± 0.08% P (1.84%–2.14%) (Table 1). C content increased as experiment progressed, and C contents in September and October were significantly higher than that in other months ($P < 0.05$). N content fluctuated greatly and no obvious trend was observed, while P content maintained relatively constant and there were no significant differences among months ($P > 0.05$). The molar ratio of C:N:P was 61:10:1. The mean C/N was 5.92 ± 0.50 (4.94–6.84), the mean C/P was 61.04 ± 2.71 (56.80–67.09), and the mean N/P was 10.36 ± 0.86 (9.12–12.78). C/P showed declining trend as experiment progressed, while C/N and N/P fluctuated dynamically with no definite trend during farming season (Figure. 1).

In *C. carpio*, the L_S varied from 23.4 cm to 41.2 cm, and the wet mass ranged from 328 g to 1528 g (Table 1). Its SGR fluctuated during the farming season and it was much higher from June to July than in the rest of the year (Figure. 2). The mean body content of the elements was 47.11 ± 1.78% C (43.57%–49.72%), 9.39 ± 0.63% N (8.49%–10.85%), and 1.87 ± 0.16% P (1.62%–2.11%) (Table 1). C and P contents showed increasing trend during farming season, while N content fluctuated and no obvious

Table 1. Body size and the element content of three fish species during the growth stage. Data was expressed as mean ± SD.

Species	Grass carp	Common carp	Tilapia
L_S (cm)			
5 May	29.23±2.59^{Ca}	24.13±0.64^{Ba}	14.73±0.45^{Aa}
6 Jun	33.53±0.55^{Cb}	27.20±1.49^{Bb}	18.80±0.79^{Ab}
7 July	39.83±1.50^{Cc}	30.20±1.21^{Bc}	21.60±1.22^{Ac}
8 Aug	41.87±0.32^{Cc}	32.13±1.21^{Bc}	23.73±0.64^{Ad}
9 Sept	45.07±1.00^{Cd}	35.03±1.61^{Bd}	27.17±0.42^{Ae}
10 Oct	50.67±0.51^{Ce}	39.50±1.48^{Be}	28.83±1.22^{Af}
Wet mass (g)			
5 May	505.33±103.37^{Aa}	372.67±76.50^{Ba}	107.00±16.82^{Ba}
6 Jun	681.00±22.61^{Cb}	438.33±88.00^{Bb}	197.00±5.57^{Aa}
7 July	1033.67±3.06^{Cc}	849.00±126.37^{Bc}	348.00±49.03^{Ab}
8 Aug	1348.00±83.35^{Cd}	989.00±178.68^{Bd}	485.33±74.70^{Ac}
9 Sept	2159.33±50.62^{Ce}	1253.00±58.40^{Be}	689.67±72.54^{Ad}
10 Oct	2503.67±85.01^{Cf}	1478.00±66.14^{Bf}	772.00±56.20^{Ad}
Carbon (%C)			
5 May	43.93±0.69^{Aa}	44.51±1.15A^{Aa}	51.56±0.73^{Ba}
6 Jun	45.01±0.19^{Aa}	45.94±0.18^{Bab}	49.94±0.53^{Cabc}
7 July	47.51±0.85^{Ab}	46.89±1.36^{Abc}	49.38±1.32^{Aabc}
8 Aug	48.02±0.18^{Ab}	48.61±0.51^{Ac}	49.15±1.91^{Abc}
9 Sept	50.39±1.16^{Ac}	48.35±1.19^{ABc}	51.32±0.81^{Bab}
10 Oct	50.26±1.00^{Ac}	48.37±1.26^{Ac}	48.85±1.22^{Ac}
Nitrogen (%N)			
5 May	9.05±0.09^{Bab}	10.23±0.54^{Cd}	7.53±0.16^{Ca}
6 Jun	9.06±0.36^{Bab}	9.46±0.13^{Bbc}	8.12±0.34^{Ab}
7 July	9.97±0.15^{Cbc}	9.29±0.20^{Bbc}	8.65±0.12^{Ac}
8 Aug	9.45±0.30^{Cabc}	8.52±0.38^{Ba}	7.81±0.01^{Aab}
9 Sept	8.69±0.15^{Aa}	9.79±0.14^{Bcd}	8.66±0.24^{Ac}
10 Oct	10.20±1.32^{Bc}	9.05±0.49^{ABab}	7.87±0.13^{Aab}
Phosphorus (%P)			
5 May	1.93±0.08^{Ba}	1.67±0.05^{Aa}	2.91±0.07^{Ca}
6 Jun	1.97±0.10^{Aa}	1.71±0.07^{Aa}	3.05±0.21^{Bab}
7 July	2.03±0.04^{Ba}	1.82±0.08^{Ab}	3.11±0.06^{Cb}
8 Aug	2.06±0.04^{Ba}	1.92±0.04^{Ab}	3.23±0.06^{Cbc}
9 Sept	2.05±0.10^{Aa}	2.06±0.05^{Ac}	3.33±0.07^{Bc}
10 Oct	2.03±0.05^{Aa}	2.03±0.05^{Ac}	3.41±0.04^{Bc}

trend was observed. The molar ratio of C:N:P was 65:11:1. The mean C/N was 5.89 ± 0.55 (4.68–6.95), the mean C/P was 65.45 ± 4.34 (58.44–72.54), and the mean N/P was 11.23 ± 1.44 (9.45–13.45). C/P and N/P increased during farming season, while C/N showed decreasing trend (Figure. 1).

In *O. niloticus*, the Ls and wet mass varied from 14.3 cm to 29.9 cm and 88 g to 817 g, respectively (Table 1). Its SGR declined continuously as the experiment progressed (Figure. 2). The mean body content was 50.03 ± 1.47% C (47.44%–52.38%), 8.11 ± 0.47% N (7.52%–8.92%), and 3.17 ± 0.19% P (2.81%–3.45%) (Table 1). P content showed increasing trend during farming season, while C and N contents fluctuated and no obvious trend was observed. The molar ratio of C:N:P was 41:6:1. The mean C/N ratio was 7.22 ± 0.47 (6.59–8.06), the mean C/P ratio was 40.91 ± 3.24 (35.94–47.48), and the mean N/P ratio was 5.67 ± 0.38 (4.95–6.30). C/P and N/P showed decreasing trend during farming season, while C/N fluctuated and no obvious trend was observed (Figure. 1).

Statistical analysis (all samples included) revealed that the C content and C/N ratio in *O. niloticus* were significantly higher than those in *C. idella* and *C. carpio* ($P < 0.05$), but the N content was significantly lower than that in *C. idella* and *C. carpio* ($P < 0.05$); there was no significant differences in C and N contents and the C/N ratio between *C. idella* and *C. carpio* ($P > 0.05$). The P content and C/P and N/P ratios were significantly different among the three fishes ($P < 0.05$), and the P content in *O. niloticus* was significantly higher than that in *C. idella* and *C. carpio* ($P < 0.05$); the C/P and N/P ratios in *C. carpio* were significantly higher than those in *C. idella* and *O. niloticus* ($P < 0.05$).

Pearson Correlation Analysis of the Element Content, Ratios, And Fish Size

The Pearson correlation analysis of the element content, ratios, and fish size in the three species is shown in Table 2. The C content and C/P ratio were significantly positively correlated with L_S and wet mass in *C. idella* ($P < 0.01$); in *C. carpio*, the C content, P content, and C/N ratio had a significant

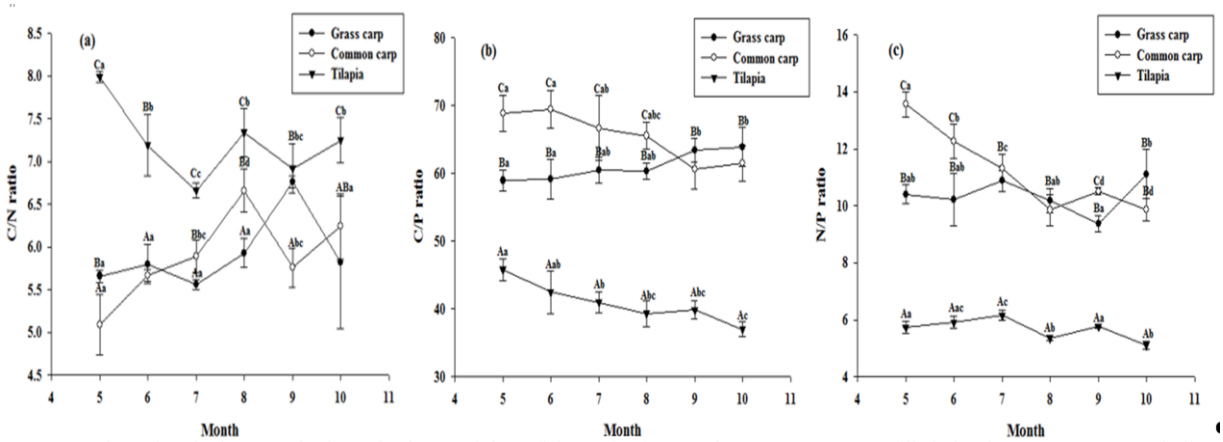

Figure 1. The ratio of C/N (a), C/P (b) and N/P (c) of three fish species in growth stage: grass carp (filled circular), common carp (hollow circular), tilapia (filled triangle). Data was expressed as mean ± SD. Lowercase letters (a, b, c and d) indicated differences of different sampling months within one fish species, and uppercase letters (A, B and C) indicated differences of three fish groups in same month. Error bars represent conditional 95% C.I.

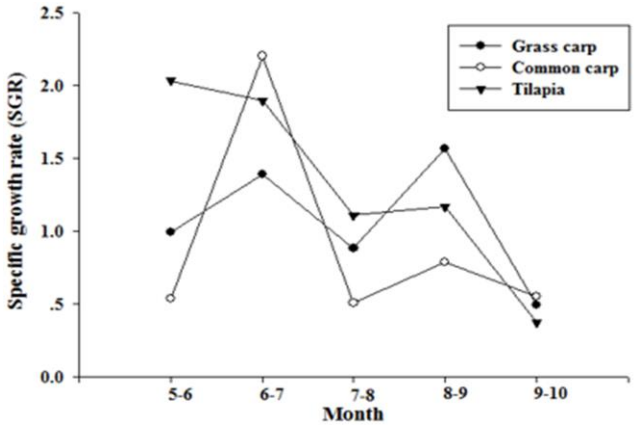

Figure 2. Specific growth rate (SGR) of three fish species in growth stage. Data was expressed as mean.

positive correlation with L_S and wet mass ($P < 0.05$), while the C/P and N/P ratios were significantly negatively correlated with L_S and wet mass ($P < 0.01$). In *O. niloticus*, the P content had a significant positive correlation with L_S and wet mass ($P < 0.01$), while the C/P and N/P ratios had a significant negative correlation with L_S and wet mass ($P < 0.05$); other correlations were not significant ($P > 0.05$).

Discussion

ES emphasizes the relationships among elements in organisms, especially C, N, and P, and considers the balance between energy and multiple elements in a biological system (Hessen, 1997; Elser *et al*., 2000). The body stoichiometry of individual organisms is a taxon-dependent trait and the result of evolutionary pressures, and it is one of the central questions of the ES theory (Williams, 1997; Sterner & Elser, 2002). Most organisms live in a complex and ever-changing environment, and organisms are able to maintain relatively stable body composition in the face of variable food nutrient content (Hessen & Anderson, 2008; Hessen, Elser, Sterner, & Urabe, 2013). Certain cell organelles, such as golgiosomes, have a specific elemental composition (Elser, Dobberfuhl, MacKay, & Schamplel, 1996), which ensures normal life activities in cells and organisms. However, the whole body stoichiometry of the three fish species fluctuated significantly, indicating that these three fish species did not follow strict homeostasis during the farming season. These results support the statement that "strict homeostasis is a simplifying assumption about a complex reality." (Hendrixson, Sterner, & Kay, 2007). Nakazawa (2011) also pointed out that the assumption of a constant body elemental composition is only an approximation and simplification used for model development and that the ES theory needs to be further improved.

Different macromolecules contain elements in different quantities (Sterner & Elser, 2002). Lipids are the essential C storage pool (Fagan, Koops, Arts, & Power, 2011), in addition, muscle tissue stores

Table 2. Pearson correlation analysis of fish size (standard length and wet body weight), and three nutrients (C, N and P) as well as their ratios of three fish species during the growth stage

Species	Size	%C	%N	%P	C/N	C/P	N/P
Grass carp	L_S (cm)	0.929**	0.338	0.513	0.342	0.692**	0.077
	Wet mass (g)	0.914**	0.214	0.466	0.455	0.713**	-0.017
Common carp	L_S (cm)	0.714**	-0.429	0.876**	0.576*	-0.734**	-0.836**
	Wet mass (g)	0.761**	-0.396	0.911**	0.575*	-0.754**	-0.845**
Tilapia	L_S (cm)	-0.319	0.351	0.869**	-0.479*	-0.804**	-0.478*
	Wet mass (g)	-0.27	0.286	0.88**	-0.396	-0.789**	-0.544*

**Statistically significant at $P < 0.01$ level.

considerable amounts of N as proteins (Vrede *et al*., 2011), while RNA and bone tissue are the main reservoir of P (Hendrixson *et al*., 2007). The mass and relative proportion of different tissues and biochemical compounds may change dynamically with growth (Boros *et al*., 2015). Therefore, fish body stoichiometry varies with size, growth, and development, which might be the reason why these three fish species did not follow strict homeostasis. Studies indicated that stoichiometry of fishes might be species-specific. For example, Take N:P stoichiometry as example, the N:P ratio in the larger bluegill (*Lepomis macrochirus*) decreased (Davis & Boyd, 1978) and that in the back bullhead (*Ameiurus melas*) increased with the increase in body size (Hendrixson *et al*., 2007), whereas N:P of some fish showed "n" function, (Sun, 2014). However, Tanner, Brazner, and Brady (2000) found no particular tendency in most of the fish species they studied. In this study, both the C level and C/P ratio had a significant positive correlation with L_S and wet mass in *C. idella* ($P < 0.01$); the increasing C and its peak at the end of the farming season might have resulted from the accumulation of lipids from spring to autumn. The levels of C and P and the C/N ratio in *C. carpio* increased, while the C/P and N/P ratios decreased as body size increased, which might be due to greater synthesis of lipids and growth of bone tissue as compared to the growth of muscles. The size of *O. niloticus* was significantly and positively correlated with P ($P < 0.01$) but significantly and negatively correlated with C/P and N/P ratios ($P < 0.01$), which indicated that much more P was allocated to the bones and diluted lipid and muscle. Growth can also influence fish stoichiometry (Pierce, Wissing, Jaworski, Givens, & Megrey, 1980; Deegan, 1986; Sweeting, Polunin, & Jennings, 2006). For adult fish, however, age may have less effect on body nutrient content, for example, elemental composition among 3- to 7-year-old bream (*Abramis brama*) changed little (Penczak & Tátrai, 1985). In this study, *C. carpio* and *O. niloticus* reached sexual maturity at the end of the farming season, while *C. idella* was still in the fast growing stage. The P content of *C. carpio* and *O. niloticus* increased as they reached sexual maturity, which indicated that much more P was allocated to bone tissue. Similar results were reported for rainbow trout (*Oncorhynchus mykiss*) by Shearer (1984).

The growth rate hypothesis states that growth requires large amounts of P-rich RNA for transcription and translation, so fast growing organisms generally have higher P content and lower N:P ratio than slow growing organisms (Elser *et al*., 1996; Elser *et al*., 2000). Therefore, the GRH combines growth, P content and RNA together. However, whether or when RNA is the dominant P pool in an organism is important for the establishment of GRH. In this study, the SGR of *C. idella* fluctuated during the farming season, while the P content remained relatively constant throughout the experiment. In *C. carpio*, the SGR fluctuated during the farming season but the P content increased, whereas in *O. niloticus*, the SGR as well as the C/P and N/P rates decreased with time. Therefore, the varied and complex relationship between the SGR and P content suggest that bone rather than RNA is the dominant P pool in this three fish species in aquaculture ponds. Boros *et al*. (2015) found that RNA is a considerable P pool in post-embryos, while bone-associated P was the dominant body P pool in later stages. Therefore, time scale should be a potentially important factor when considering the importance of the GRH.

It is generally recognized that fish body stoichiometry vary greatly at the species level, especially the P levels (McIntyre & Flecker, 2010). In the present research, P content was more fluctuant than that of C and N, which corroborated previous studies. Body morphology and scale type could partly account for the interspecific variation of P. Fish with lower P content, such as Cyprinidae and Salmonidae, tend to be elongated, with soft-rayed fins and cycloid scales, while the fish with higher P content, such as Amblycipitidae, tend to be laterally compressed and spiny-rayed (Tanner *et al*., 2000; Hendrixson *et al*., 2007; McIntyre & Flecker, 2010; Sun, 2014). *C. idella* and *C. carpio* studied herein belong to Cyprinidae, Cypriniformes, which have elongated body and cycloid scales, whereas *O. niloticus*, is from Cichlidae, Perciformes, which are laterally compressed, with ctenoid scales. This may explain the

higher content of P in *O. niloticus* compared to that in *C. idella* and *C. carpio*. In addition, interspecific variation in stoichiometry can also be the consequence of food source (Cross, Benstead, Rosemond, & Bruce Wallace, 2003; Naddafi, Eklöv, & Pettersson, 2009; Sun, 2014). In this study, the three fish species were mainly fed artificial feed. In addition, *C. carpio* could prey on plankton as well as macrozoobenthos (Zhao, Dong, Zhang, & Zhang, 2001), while *O. niloticus* might feed on some plankton (Diana, Dettweiler, & Lin, 1991). Therefore, the body stoichiometry of the three fish species could also be the result of the different food source, which warrants further studies under controlled laboratory conditions.

Conclusion

The whole body stoichiometry of three fish species fluctuated significantly, which indicated that these three fish species did not follow strict homeostasis during the farming season. Changes in the relative amount of macromolecules resulted in the variation of C:N:P stoichiometry, and the fish body stoichiometry varied with size and growth, which might be the reason why these three fish species did not follow strict homeostasis. The relationship between growth rate and P content did not follow the GRH, and bone rather than RNA was the dominant pool of P in the three fish species in aquaculture ponds. Body morphology, scale type, and food source might be the main factors leading to the significant variation in fish body P stoichiometry at species level.

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