Seasonal Dynamics of Feeding Habits of a Cyprinid Fish, *Saurogobio dabryi* in the Downstream of the Jialing River, China

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ABSTRACT

This research studied the food composition and feeding rhythm of *Saurogobio dabryi* in the lower reaches of the Jialing River, aimed to explore the seasonal difference of its feeding habits. Gut contents of 268 *S. dabryi* in the downstream (Hechuan section) of the Jialing River were collected seasonally from September 2015 to August 2016. The feeding habits of *S. dabryi* were studied by microscopic examination, analysis of similarity, and the Amundsen graphical method. A total of 26 food categories were identified, among which algae and organic detritus were the dominant food source for *S. dabryi*. Animal prey contributed relatively low proportions to the food of *S. dabryi*. Seasonal variations of food composition of *S. dabryi* were significant (*P*<0.05). Hierarchical cluster analysis shows that the food composition in autumn was significantly different from other seasons at a similarity level of 60%. The results indicated that the feeding rhythm of the *S. dairyi* in the lower reaches of the Jialing River showed significant seasonal changes (*P*<0.05); The feeding intensity and food amount gradually increased from spring to winter, which may be related to the morphological structure, breeding period, special habitat, and seasonal changes of biological prey of *S. dabryi*.

INTRODUCTION

Fish show a variety of adaptation mechanisms in response to changing environments. They not only passively accept the environment but also actively adapt to the environment (Foote *et al.*, 1992). In recent years, an increasing amount of studies have shown many fish have corresponding alterations on growth, reproduction, and especially feeding activities to the great changes in the aquatic ecological environment (e.g., building dams, sewage discharge) (Hahn, 2007; Ferrareze *et al.*, 2014). Fish will adopt different feeding strategies to actively adapt to changes in the environment, such as increasing feeding time, expanding predation types, reducing feeding intensity, and competing for interspecific alternate feeding to maintain the continuity of the population under the

changed environmental pressure (Oliveira *et al.*, 2018; Rayner *et al.*, 2009). Therefore, studying the feeding habits of fish and their dynamic changes are of great significance for understanding fish's adaptation mechanisms to the environment and formulating fish protection and management plans (Done, 1999).

The study on the fish diet is based on the composition of the gut contents and the importance of food. The commonly used methods include the frequency of occurrence method, numerical method, gravity method, and the index of the relative importance (Hyslop, 1980). Prejis and Prejis (1987) discovered that the prey items of *Tetragonopterus argenteus* were mainly composed of crustaceans, chironomid larvae, and coleoptera through the gravimetric method, and explored the relationship between its special geographical environment and seasonal changes in prey organisms by gravity method. Grabowska *et al.* (2010) adopts a variety of methods such as the frequency method and relative importance index to study the dietary sources of racer goby (*Neogobius fluviatilis*). The results showed that there was no obvious difference in the food composition of chironomid larvae, small mollusks, and crustaceans, which may be related to its wide predation range and opportunistic predation strategy as an invasive species.

Saurogobio dabryi (Cyprinidae: Gobioninae), commonly known as boat nail, likes flowing water life. It is not only an important river fish but also one of the main economic fish in the Jialing River (Zhang *et al.*, 2018). In recent years, the original aquatic ecological environment has undergone a great change because of the construction of cascade hydropower projects on the Jialing River, which has caused adverse effects on fish who like rapids or flowing water (Zeng, 2012). However, detailed feeding research on the rapids or flowing water living fish in the Jialing River cascade hydropower project water areas were scarce. It is not known whether its food habits will change with the seasons under the new environment (slow water conditions) or not. This study selected *S. dabryi* as an example of rapids or flowing water fishes in the lower reach of the Jialing River, to explore its food composition, feeding rhythm, and to analyze the seasonal differences of their feeding habits, using combined methods of microscopic examination, analysis of similarity, and the Amundsen graphical method. The result will provide a reference for the protection and management of fish resources in the Jialing River.

MATERIALS AND METHODS

Sample collection and prey analysis

The sampling site was located at the Hechuan River section of the lower Jialing River (29°30′-29°40′N, 106°30′-106°40′E), within a range of about five kilometers (Fig. 1). The sampling point has a straight river section with abundant fish resources (Jiang and He, 2008). Sampling was performed monthly from September 2015 to August 2016, using gillnet (mesh size 30 mm), cast net (mesh size 30 mm), and ground cage (mesh size 15 mm). Water temperature, PH, and other indicators (e.g., dissolved oxygen, nitrogen, and phosphorus content) of the sampling site were measured and recorded. In the field experiment, the standard length (*SL*, 1 mm) and body weight (*BW*, 0.01 g) were measured immediately after capture (Zhang *et al.*, 2018). To prevent prey from being digested, the gut contents were fixed instantly after capture in a 4% formaldehyde solution for further analysis. The gut was divided into three parts: foregut, midgut, and hindgut according to gut morphology. The degree of filling of different gut segments was divided into six grades, which indicated that food accounted for about 0%, 25%, 50%, 75%, 100% of gut volume, and gut expansion (Yin, 1993).

To reduce the error caused by digestion, only samples with gut fullness degree ≥ 2 were selected for the diet analysis. The food prey was identified and counted by a dissecting microscope and a binocular microscope, then its weight was calculated through the length-weight relationship (±0.0001 g) (Baumgärtner And Rothhaupt, 2003). Even small fragments must be identified very carefully to minimize the underestimation of small and soft prey. During identification, two pieces were generally counted and the average value was used, aquatic insects were generally identified to the family or genera level, and algae were usually identified to the genera level (Bernal *et al.*, 2015; Didenko *et al.*, 2018).
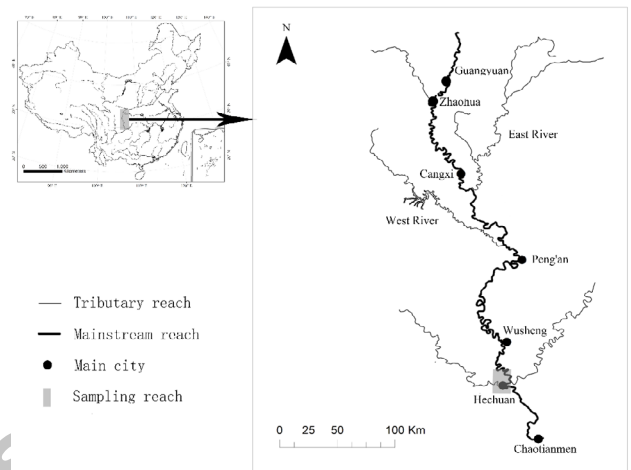

Fig. 1. Sampling site of *S.dabryi* in the lower reach of the Jialing River.

Data analysis

To evaluate whether the number of samples used for diet analysis was sufficient, non-empty cumulative prey curves were constructed by Estimate S 9.1.0 (http://purl.oclc.org/estimates) software (Espinoza *et al.*, 2013). The slope of linear regression (*b*) of the last five subsamples was used for evaluation, where $b \leq 0.5$ indicated that samples were enough for diet description (Brown *et al.*, 2012).

This research used frequency of occurrence (% *F*), percentage number (% *N*), weight percentage (% *W*), index of the relative importance (*IRI*), and percent index of relative importance (% *IRI*) (Hyslop, 1980) to quantitatively evaluate the importance of prey, the specific calculation method was as follows: % *F*= (the number of guts containing one prey item / the total number of non-empty guts) × 100. % *N*= (the number of one prey item / the total number of prey items) × 100. % *W*= (the weight of guts containing one prey item/ the total weight of prey items) × 100. *IRI*= (% *N* + % *W*) ×% *F*. % *IRI*= (index of the relative importance of one prey item / index of the relative importance of the total prey items) × 100.

The study test seasonal differences in the feeding habits of *S. dabryi* by Analysis of Similarity (ANOSIM)

(Clarke and Warwick, 2001), which was based on the Bray-Curtis similarity index. The % W index was selected because the index can overcome the problems that digestion poses for enumerating prey items (White *et al.*, 2004). In the analysis, the R statistic was used to measure the similarity of food between different seasons. At the same time, the Hierarchical cluster analysis (UPGMA) method was used to cluster analysis of food composition in different seasons.

Because the use of the gut fullness index cannot accurately reflect the feeding rhythm of fish in the natural state (Devlaming *et al.*, 1982; Cortés, 1997). the empty gut rate and the gut fullness index were used at the same time when studying the feeding rhythm of *S. dabryi*.

The empty gut rate (%) = (empty gut number/ total number of fish) ×100

The gut fullness index (K) = (gut content weight/ body weight) ×10^4

The empty gut rate showed a non-normal distribution (Shapiro-Wilks test, $P < 0.05$). Therefore, the seasonal difference of the empty gut rate was analyzed by the chi-square test (χ^2), the seasonal difference of the gut fullness index was tested by analysis of variance.

Using Amundsen's graphical method (Amundsen *et al.*, 2010) improved on Costello (1990), the frequency of occurrence and prey-specific abundance constitute a two-dimensional graph on the horizontal and vertical coordinates respectively. The distribution of the position of the scattered points to describe the feeding strategy of *S. dabryi* in the lower reaches of the Jialing River. The formula for calculating the Prey-specific abundance was as follows:

Prey-specific abundance (P_i) = ($\Sigma S_i / \Sigma S_{ii}$) × 100

P_i was the Prey-specific abundance; S_i was the weight of prey i in the gut, S_{ii} was the content weight of the stomach (gut) of the individual with prey i in the gut.

RESULTS

A total of 268 individuals (137 females and 131 males) of *S. dabryi* were collected and examined, with the *SL* ranging from 70.5 to 184.0 (112.3 ± 16.7, mean ± SD) mm, and the *BW* ranging from 3.7 to 44.4 (16.3 ± 6.8)

g (Table I). There were also significant differences in the number of samples collected in different seasons, with less in spring and winter, and more in summer and autumn.

Seasonal feeding intensity

The results of the chi-square test indicated that the empty gut rate changed significantly in different seasons ($\chi^2 = 24.24$, $P < 0.05$). The empty gut of *S. dabryi* was the highest in spring (53.33 %) and the lowest in winter (8.47 %) (Fig. 2). The results of ANOVA showed that there were significant differences in the fullness index among different seasons ($F = 7.25$, $P < 0.05$), the highest in winter (62.02 %), followed by autumn (54.40 %) and summer (47.07 %), and the lowest in spring (26.64 %). Besides, *S. dabryi* showed feeding activities throughout the year, during which its feeding activities were weakest in spring and the strongest in winter.
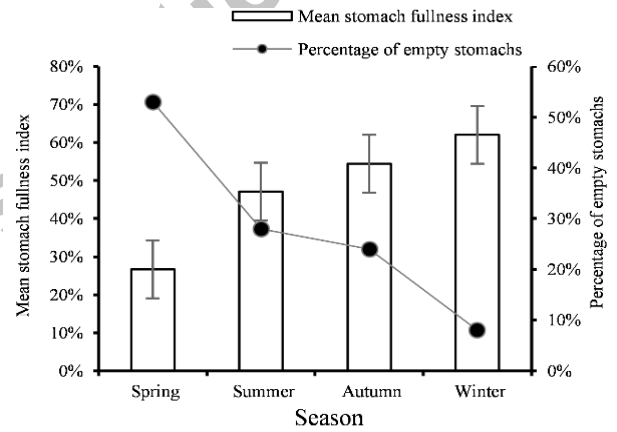

Fig. 2. Mean gut fullness index and percentage of empty gut of *S. dabryi* in each season (vertical bars indicate S.E.).

Cumulative curves

The analysis of the prey accumulation curve showed that the curve had reached an asymptote ($b < 0.5$). Therefore, the number of samples in each season was considered sufficient to describe the diet (Fig. 3).

Table I. Sampling of *S. dabryi* in the lower reaches of the Jialing River in different seasons.

Season	Specimens	Body length (mm)		Body weight (g)	
		Range	Mean±SD	Range	Mean±SD
Spring	30(14)	86.0~124.6	103.9±8.0	9.1~25.7	14.9±3.8
Summer	89(69)	70.5~136.0	105.6±16.8	3.7~31.1	14.7±7.1
Autumn	90(73)	86.0~184.0	120.6±18.4	4.9~44.4	17.9±7.9
Winter	59(54)	101.1~147.0	114.3±9.6	11.1~40.5	17.1±5.4
Total	268(210)	70.5~184.0	112.3±16.7	3.7~44.4	16.3±6.8

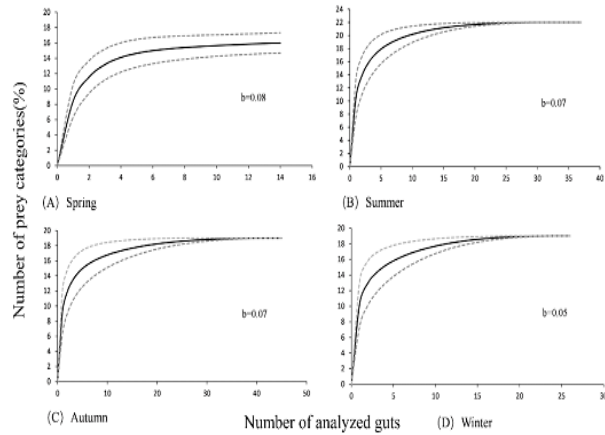Notes: the parenthesis indicates the non-empty gut sample size.

Fig. 3. Cumulative prey curves of *S. dabryi.*

Food composition and seasonal variation

The gut content of *S. dabryi* contained a wide variety of algae, plants and animal prey. A total of 26 different food taxa belonging to 10 main prey categories (e.g., Cyanophyta, Bacillariophyta, Chlorophyta, organic detritus, aquatic insects) were identified (Table II). Among them, organic detritus (% *IRI* = 28.15), Anabaena (% *IRI* = 16.92), and Cocconeis (% *IRI* = 16.74) were the most important food sources for *S. dabryi*. Although there were many kinds of animal items, the overall proportion was low (% *IRI* = 1.15).
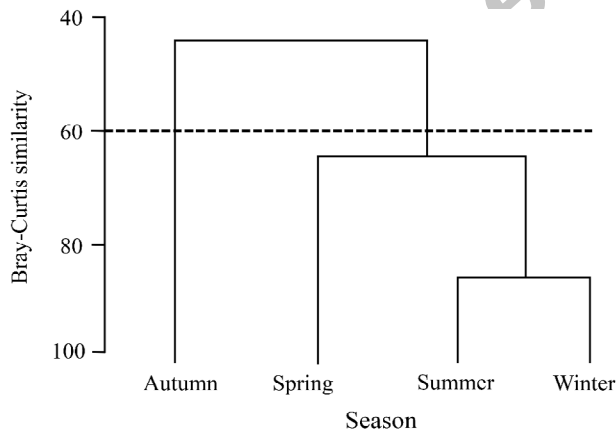

Fig. 4. Dendrogram of cluster analysis on dietary composition of *S. dabryi.*

Analysis of similarity (ANOSIM) (Fig. 4) indicated that there was a significant seasonal difference in the food composition of *S. dabryi* (R= 0.18, $P < 0.05$). The hierarchical cluster analysis shows that the food composition of *S. dabryi* in autumn was significantly different from other seasons at a similarity level of 60

% (Bray-Curtis Similarity). Among them, spring was the season with the largest consumption of animal feed (% *IRI* = 7.79) (Table II), and the contribution rate of organic detritus to the food of *S. dabryi* was also relatively high (%*IRI*=31.81). In summer, *S. dabryi* almost only consumes algae prey (% *IRI* = 89.41), and the organic detritus was the least in all seasons (% *IRI* = 10.01). In autumn, it mainly fed on algae (% *IRI* = 75.22) and organic debris (% *IRI* = 24.41). In winter, organic debris (% *IRI* = 40.85) contributes the most to the food of *S. dabryi*, while the intake of animal feed was very small.
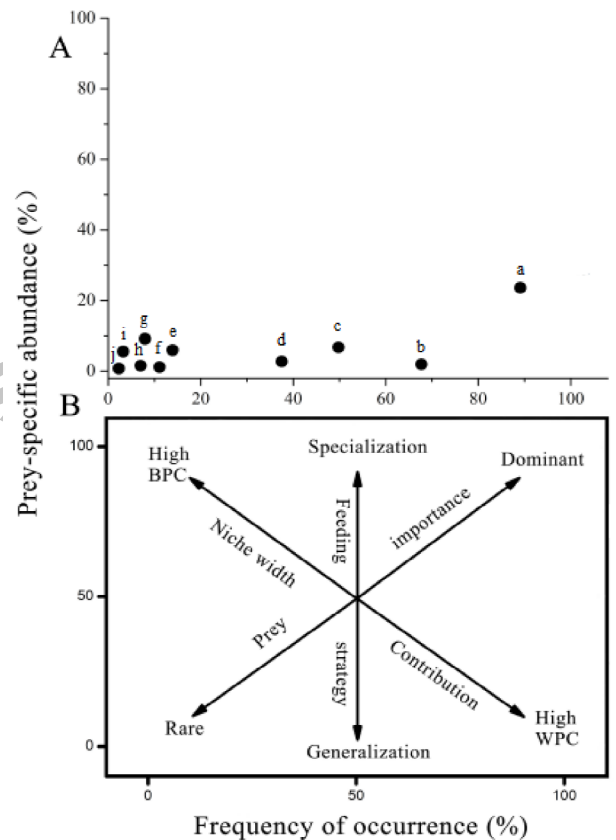
Fig. 5. Feeding strategy of *Saurogobio dabryi* (A) Amundsen graph, black dot (•) represents prey category. a: Other; b: Bacillariophyta; c: Cyanophtya; d: Chlorophyta; e: Aquatic insect; f: Oligochaeta; g: Polychaeta; h: Rotaria; i: Crustacea; j: Sarcodina; (B) Explanatory diagram for the interpretation of feeding strategy and prey importance; BPC and WPC represent between-phenotype component and within-phenotype component, respectively.

Feeding strategy

According to the Amundsen graph (Fig. 5) of the feeding strategy of *S. dabryi*, most of the prey categories were in the lower part of the graph except for a few types of food, which reflected *S. dabryi* can be considered as a

Table II. Food composition of *S. dabryi* in each season from the lower reaches of Jialing River.

Prey items 小种类			Spring				Summer				Autumn				Winter			
			%F	%N	%W	%IRI	%F	%N	%W	%IRI	%F	%N	%W	%IRI	%F	%N	%W	%IRI
Plant prey	Cyanophtya	Nostoc	55.54	4.67	6.12	3.94	93.33	8.02	27.85	19.65	93.33	7.18	7.88	8.56	100	6.86	3.65	6.19
		Aphanizomenon	63.63	2.46	1.95	1.88	40.00	1.54	1.24	0.65	86.66	5.56	6.72	6.66	93	8.33	4.56	7.08
		Dactylococcopsis	-	-	-	-	26.66	1.19	0.64	0.29	66.66	4.63	2.70	3.06	53	5.88	1.39	2.28
		Phormidium	36.36	2.46	15.48	4.36	20.00	0.85	0.88	0.20	40.00	2.78	3.76	1.64	67	3.19	2.15	2.09
		Anabaena	36.36	2.70	2.00	1.14	93.33	15.87	34.04	27.33	86.66	13.66	17.32	16.80	93	10.54	25.52	19.80
		Lyngbya	-	-	-	-	26.66	0.85	1.08	0.30	6.66	0.46	0.45	0.04	-	-	-	-
		Microcystis	-	-	-	-	66.66	4.61	3.39	3.13	73.33	4.40	16.60	9.64	80	4.90	2.36	3.42
		Colorella	-	-	-	-	40.00	2.05	1.86	0.92	46.66	2.31	2.97	1.54	40	2.21	1.50	0.87
		Aphanocapsa	-	-	-	-	46.66	3.75	2.33	1.67	26.66	2.08	1.80	0.65	27	0.98	0.74	0.27
	Bacillario-phyta	Cocconeis	90.90	33.91	9.38	26.32	100	27.30	7.92	20.67	80.00	19.21	7.78	13.52	73	11.76	2.34	6.08
		Tabellaria	63.63	4.91	2.05	2.96	46.66	2.05	0.98	0.83	33.33	1.85	1.16	0.63	20	6.62	1.83	0.99
		Rhizosolenia	90.90	18.18	12.25	18.51	99.65	15.36	6.42	12.78	93.33	12.27	7.51	11.56	93	12.99	4.34	9.51
	Chlorophyta	Scendesmus	27.27	1.47	0.92	0.44	-	-	-	-	-	-	-	-	-	-	-	-
		Schroederia	-	-	-	-	40.00	2.73	1.48	0.99	40.00	1.85	1.84	0.92	27	1.72	0.65	0.37
		Volvox	27.27	2.21	2.46	0.85	6.66	0.34	0.26	0.02	-	-	-	-	-	-	-	-
	Other	Organic detritus	100	22.35	25.19	31.81	93.33	11.09	7.20	10.01	100	20.13	18.86	24.41	100	22.30	47.14	40.85
Animal prey	Polychaeta	Nereis	18.18	0.49	0.60	0.13	-	-	-	-	-	-	-	-	6.66	0.25	0.25	0.02
	Rotaria	Rotaria	9.09	0.49	0.43	0.06	-	-	-	-	6.66	0.23	0.45	0.03	-	-	-	-
	Oligochaeta	Limnodrilus	-	-	-	-	6.66	0.17	0.26	0.02	6.66	0.23	0.49	0.03	6.66	0.25	0.23	0.02
	Sarcodina	Colpoda	-	-	-	-	6.66	0.17	0.14	0.01	-	-	-	-	-	-	-	-
	Crustacea	Cladocera	27.27	0.98	5.62	1.20	6.66	0.17	0.24	0.02	-	-	-	-	13.33	0.49	0.46	0.07
		Copepoda	-	-	-	-	13.33	0.34	0.42	0.06	-	-	-	-	-	-	-	-
		Gammaridae	-	-	-	-	-	-	-	-	-	-	-	-	6.66	0.25	0.51	0.03
	Aquatic insect	Phryganeidae	-	-	-	-	6.66	0.17	0.18	0.01	6.66	0.23	0.34	0.02	6.66	0.25	0.17	0.02
		Chironomid	27.27	0.74	0.72	0.27	20.00	0.51	0.34	0.10	6.66	0.23	0.45	0.03	6.66	0.25	0.17	0.02
		Insect appendage	54.54	1.97	14.83	6.13	33.33	0.85	0.86	0.33	20	0.69	1.31	0.25	6.66	0.25	0.21	0.02

Notes: %F means occurrence percentage, %N means numerical percentage, %W means weight percentage, %IRI means percent index of relative importance, "-" means less than 0.01.

moderate generalist predator, this will greatly preserve the continuation of the species under harsh environmental conditions. Organic debris, diatoms, cyanophytes, and chlorophytes were the main foods, all of which have a high occurrence rate and abundance of prey. However, the aquatic insects, oligochaetes, polychaetes, rotifers, crustaceans, and sarcodina were a low relatively occurrence rate and rare prey, this was consistent with the relatively low contribution rate of animal feed. Besides, the distribution position of prey indicates that the difference in food composition among different individuals of the *S. dabryi* population was small, and the degree of food overlap was high.

DISCUSSION

Diet composition

The feeding habits of fish was the result of fish adapting to changes in the living environment, the same fish may have completely different feeding habits in different environments (Zander *et al.*, 1999). Grabowska *et al.* (2009) found that the monkey goby (*Neogobius gymnotrachelus*) mainly feeds on amphipods and chironomid larvae in the invasion area (the Baltic Sea), which was quite different from the original place (the Lazim Lake) in food composition. This may be due to the lack of food resources in the invasion area, and adapting strategies of the fish, such as competing for interspecific alternate feeding and opportunistic predation to alleviate the survival pressure (Hermoso *et al.*, 2011). This study found that there were many kinds of food items of *S. dabryi*, including animal and plant prey (mainly plant prey), indicating that *S. dabryi* was omnivorous and more plant-eating fish, and its food composition contains more organic detritus and benthic items, which were compatible with the benthic life of *S. dabryi*, with thick upper and lower lips and special morphological structure of feeding organs (Lv *et al.*, 2019). On the other hand, it was also related to the unique habitat of the Jialing River and the abundance of the prey item. The suitable climatic conditions (e.g., water temperature, light.) of the Jialing River were beneficial to the growth and reproduction of algae and provide abundant plants food for *S. dabryi* (Long *et al.*, 2008). However, the feeding habits of *S. dabryi* in the Dadu River and Chishui River showed more preference for animal feed, which were quite different from those in the lower reaches of Jialing River (Li, 2016; Wang *et al.*, 2018). This may be due to the position as a dominant species of *S. dabryi* in the Jialing River, and its gut structure exhibits allometric growth pattern to adapt to different food compositions (e.g., Cladocera, Chironomid.), which

was more competitive than other fishes in obtaining high-quality animal prey resources (Zhang *et al.*, 2018).

Dietary variation

The seasonal variation of fish feeding habits was the result of fish adapting to the fluctuation of the surrounding water environment (Jakubaviciute *et al.*, 2017). Generally, most fish have significant seasonal changes in their food habits, while a few fish have no significant seasonal differences (Hanson, 2002). For example, the food composition of walleye pollock (*Theragra chalcogramma*) was an obvious seasonal variation in Alaska (Adams *et al.*, 2007), On the one hand, it was related to the seasonal decrease of its prey abundance, on the other hand, it was related to its unique living environment. However, there was no significant difference between feeding habits and seasons in the karstic dace (*Telestes karsticus*) in Croatia, this was determined by the relatively stable living environment and the abundant food supply in the local river (Marčić *et al.*, 2017).

In this study, we found that the feeding of *S. dabryi* in autumn was significantly different from that in other seasons. The main food sources of the *S. dabryi* in autumn were plant preys such as Anabaena and organic detritus. This may be related to the seasonal changes in algae food. Spring and autumn are the peak periods of algae reproduction each year. Cyanophyta and Diatoms were the dominant groups in the lower reaches of the river (the amount of bait is abundant), which are easier to be caught by the *S. dabryi* than other diets (Li, 2017; Zhang *et al.*, 2015). On the other hand, it may be related to the unique habitat of the Jialing River. In summer, the lower reaches of the river enter the flood season, when a large amount of organic matter enters the water body through scouring and gradually sinks to the bottom to become organic debris, large rainfall and fast flow velocity (Zhou *et al.*, 2008). As the water level of the Jialing River decreases in autumn, the flow rate slows down, and the transparency increases, the relatively stable water environment is conducive to the ingestion of organic debris by *S. dabry* (Deng *et al.*, 2010). Although there were little differences in the feeding habits of the *S. dabry* in spring, summer, and winter, there were different food preferences among the seasons. *S. dabry* consumes the most animal food in spring, which may be a huge energy consumption during the breeding period, and animal food can better meet the demand. However, in summer, the *S. dabry* almost only ingests algae, and the contribution rate of other preys is little, which is because the Jialing River is in the flood period in summer, the water level rises rapidly, and the water body is turbid, which is not conducive to the feeding activities of *S. dabry* and other bottom fish. Besides, in the winter, the proportion

of organic detritus ingested by *S. dabry* further increased, which was due to the decrease of water temperature, which led to the reduction of algae population and the difficulty of predation. Similar phenomena have also been reported in other fishes. Tamada (2005) found that seasonal changes in the feeding habits of *Rhinogobius giurinus* and a narrower food space (limited to food near the nest). This phenomenon was related to the water velocity in the habitat and the abundance of prey organisms, especially during the breeding period.

Feeding rhythm

The feeding activities of most fish have significant seasonal changes, which were related to changes in their physiological characteristics or environmental factors (Bolliet *et al*., 2001). For example, Olaso *et al*. (2000) found that the feeding rhythm of adult individuals of *Pogonophryne marmorata* was greatly affected by the breeding season, which gonads develop rapidly during the reproduction period (accounting for more than 25% of the body cavity), which leads to a significant decrease in feeding rhythm. The Bolliet *et al*. (2001) research indicated that the feeding rhythm of *Oncorhynchus mykiss* was more affected by the day and night (night eating) in European, this may be related to the abundance and availability of prey in the environment. The results showed that the feeding rhythm of the *S. dabryi* in the lower reaches of the Jialing River gradually increased from spring to winter, showing significant seasonal variation ($P < 0.05$), which was closely related to the breeding period. Spring was the peak period for the reproduction of *S. dabryi*, and the developed gonads will occupy most of the space in the body cavity of the fish, which leads to the phenomenon of reduced feeding activities during the breeding period. However, they reduced the proportion of plant prey and preferred to choose animal prey to meet their nutritional needs during breeding. The feeding activity of *S. dabryi* was the strongest in winter. There are two reasons for this: firstly, it may be to consume a large amount of food and convert excess energy into fat for energy storage for overwintering. Secondly, it may reserve energy for reproduction in the next spring so that gonads can develop and mature rapidly in the coming year (Yang *et al*., 2011).

In the long-term evolution of fish, a series of morphological characteristics adapted to their respective living environment and feeding patterns have been formed (Olsson and Eklov, 2005). This can not only reflect the passive adaptation of fish to changes in the living environment but also better reflect the active choice of their feeding strategy (Delariva *et al*., 2013). This study investigated the seasonal dynamics of the feeding habits of *S. dabryi* in the lower reaches of the Jialing River from 2015 to 2016. The results showed that the *S. dairyi* was omnivorous and partial plant-eating fish, mainly feeding on algae and organic debris. There were significant differences in food composition between autumn and other seasons, the morphology and structure of its feeding organs and the unique habitat of the Jialing River were the main reasons that affect the current food composition of *S. dairyi*. Besides, there were significant seasonal variations in the feeding rhythm of *S. dairyi* ($P < 0.05$). The feeding intensity and feeding amount were the lowest in spring and the highest in winter, which was related to its breeding period and seasonal variation of biological prey.

Statement of conflict of interest

The authors have declared no conflict of interest.

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