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Feeding ecology of *Acrossocheilus yunnanensis* (Regan, 1904), a dominant fish in the headwaters of the Chishui River, a tributary of the Yangtze River

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Abstract

Feeding ecology of *Acrossocheilus yunnanensis*, a dominant fish in the headwaters of the Chishui River, a tributary of the upper Yangtze River, was studied using the analysis of gut contents. From March 2015 to January 2016, a total of 543 individuals were collected and analyzed. The results showed that *A. yunnanensis* was an omnivorous fish mainly feeding on chlorophytes, diatoms, and aquatic insects. The trophic level was 2.69±0.62 (mean±SD), signifying *A. yunnanensis* as a primary or secondary predator. Dietary shifts were found among different ontogenetic stages and seasons. Specifically, young individuals fed primarily on aquatic insects and diatoms, whereas older fish fed mainly on chlorophytes. In spring, the preferred food item was aquatic insects and in other seasons, chlorophytes became the predominant prey. Diet composition showed no differences among individuals of different sex and diel periods. The feeding intensity of *A. yunnanensis* was not affected by diel periods, suggesting this species feeds continuously. However, its feeding intensity was significantly influenced by seasons. Pairwise comparison found that the feeding intensity was higher in spring and autumn than that in summer and winter, with minimum food intake in winter and maximum in spring. Analysis on Amundsen graph and niche breadth index indicated that *A. yunnanensis* might pursue an opportunistic and moderately generalized feeding strategy, which could explain why it has become the dominant fish species in our study area.

Keywords: Dietary, Ontogenetic shifts, Seasonal variations, Natural reserve, Conservation

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Introduction

The study of fish feeding habits is essential to understand their adaptation mechanisms to their environments and to the development of conservation and management plans (Vinyard and O' Brien, 1976). The feeding habit of a species is ususlly related to its environmental characteristics (Zander, 1997). However, headwaters consist of many unique and highly diverse physico-chemical environments, which harbor many unique species that occur nowhere else in the river ecosystem (Meyer *et al*., 2007). Shallow and flowing water, long average annual sunlight hours, together with boulders and cobbles in the substratum in the headwaters (Jiang *et al*., 2016), which are conducive to photosynthesis and algal growth (Wang *et al*., 2016). Consequently, a huge biomass of periphytic algae is always found in headwaters (Yin *et al*., 2013). In addition, the large inputs of allochthonous organic detritus from surrounding forest zones (Vannote *et al*., 1980) and the high rates of primary productivity in un-shaded headwaters create an environments that is rich in food for primary consumers such as aquatic insects (Meyer *et al*., 2007). Therefore, headwaters are abundant with periphytic algae, organic detritus and aquatic insects, which determine the basic structure and function in headwater ecosystems.

Since headwaters are usually unique and important to the whole river ecosystem, they have received extensive attentions and have been established as protected areas. By contrast, the headwater ecosystem structure and function, especially fish feeding habits and adaptation mechanisms, have received very little attention, hindering the development of suitable conservation plans.

With a length of 437 km, the Chishui River (27°20′-8°50′ N, 104°45′-06°51′ E) is the last undammed primary tributary of the upper Yangtze River. It harbors approximately 160 fish species, and many of these are endemic to the upper Yangtze River (Wu *et al*., 2010). As the core region of "the national natural reserve for rare and endemic fishes of the upper Yangtze River", the Chishui River is still well protected and plays a very important role in biodiversity conservation (Jiang *et al*., 2016).

Acrossocheilus yunnanensis (Cyprinidae: Barbinae) is a fish species endemic to China, that is exclusively distributed in the upper reaches of the Yangtze and Pearl Rivers (Ding, 1994). Generally, *A. yunnanensis* lives in the headwaters (Ding, 1994). Due to dam construction, over exploitation, invasion of alien species and other human activities, the population of this species has declined dramatically in many rivers over the past few decades and has even completely disappeared from some of its original habitats (Ye *et al*., 2015). However, *A. yunnanensis* is the most dominant fish species in the headwaters of the Chishui River (Wu *et al*., 2010). In our investigations, this species accounts for 34.5 % of the local fisheries. Therefore, why this species became a dominant species and how it has adapted to the environment in the headwaters of the Chishui River have attracted attention.

The objectives of this study were to (1) analyze the diet composition of *A. yunnanensis* qualitatively and quantitatively; (2) examine the effects of ontogenetic, seasonal, diel and sexual variations on its feeding habits; (3) determine its diel and seasonal feeding intensity; (4) evaluate its niche breadth and trophic level; and (5) illustrate its feeding strategies.

Materials and methods

Sample collection and prey analysis

Fish samples were captured in the headwaters of the Chishui River (Fig. 1). The sampling was fixed within a 7 km-long area, and *A. yunnanensis* were landed quarterly from March 2015 to January 2016 (spring: March to April 2015, summer: June to July 2015, autumn: September to October 2015, and winter: December 2015 to January 2016). During each sampling, the fish were collected by electrofishing (180 volts AC, 5 A, and 50 Hz) and stationary gillnets (8 m long×1.2 m high, 5 cm mesh size) at 4-h intervals during 24-h periods (2:00, 6:00, 10:00, 14:00, 18:00, and 22:00 h).

In the field laboratory, the standard length (*SL*, 1 mm) and body weight (*BW*, 0.1 g) were measured immediately after capture. The gut length (*GL*, 1 mm) was measured, and the gut contents were fixed in a 4 % formaldehyde solution for further analysis. Samples with highly digested prey were excluded from the diet analysis. Sex of each fish was determined by examination of the gonads.

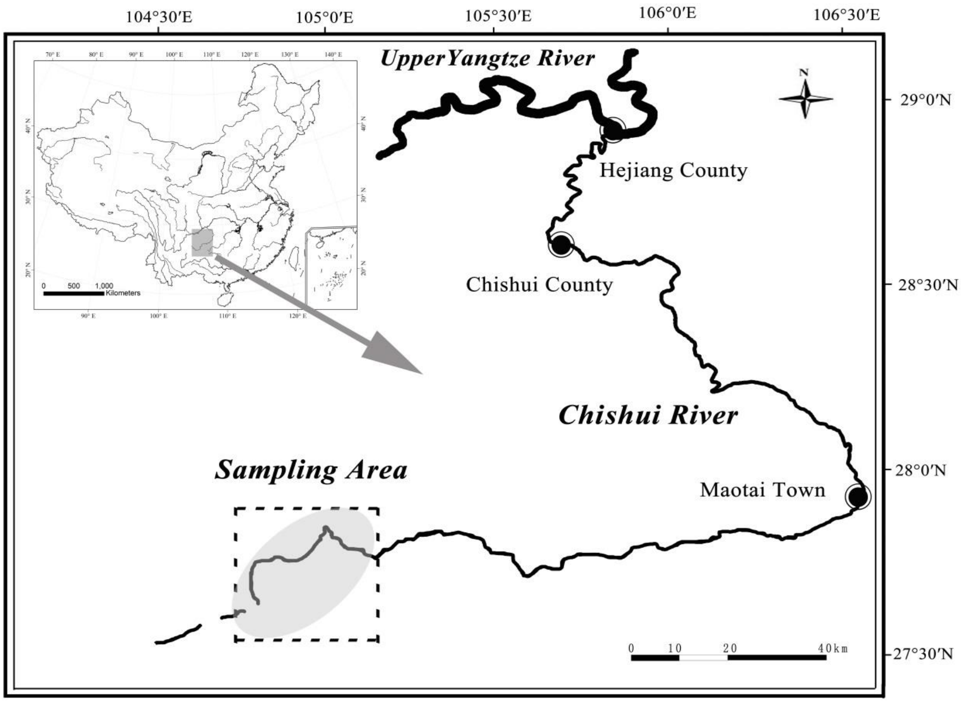

Figure 1: Map of the study region showing sampling area (dotted box) for *Acrossocheilus yunnanensis* in the Chishui River of China.

In the laboratory, the gut contents were identified to the lowest possible taxon. The utmost care was given to the identification of even small fragments to minimize the underestimation of small and soft prey. The food items were examined using a dissecting microscope and a binocular microscope and then counted and weighed to the nearest 0.0001 g.

Data analysis

To assess whether the number of fish samples analyzed was sufficient to describe the diet with respect to total samples, seasons, size groups, and sexes, cumulative prey curves (Ferry and Cailliet 1996) were constructed using EstimateS 9.1.0 (http://purl.oclc.org/estimates). The slope of the linear regression (b) of the last five subsamples was utilized for this assessment, where $b \leq 0.05$ signified sufficient samples for the dietary description (Brown *et al*., 2012).

The contribution of each prey to the diet was quantified using several indices: the average percent abundance of number and weight (*% AN*, *% AW*), the percent prey-specific abundance by number and weight (*% PN*, *% PW*), the percent frequency of occurrence (*% FO*), and the prey-specific index of relative importance (*PSIRI*). Brown *et al*. (2012) have provided detailed formulas

To investigate possible ontogenetic shifts in diet, the samples were divided into six size classes according to age (Zhao *et al*., 2009): 1st age: I (n=11), 2nd age: II (n=66), 3rd age: III (n=51), 4th age: IV (n=123), 5th age: V (n=110), and 6th age: VI (n=19). Hierarchical cluster analysis and non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity index and the *% W* data were conducted to group the six age classes (Mitu and Alam 2016). The *% W* index was selected because it can overcome the problems that digestion poses for enumerating prey items (White et al. 2004). Then, the similarity percentage (SIMPER) routine was used to assess the contribution of each prey to the dissimilarity observed between groups.

To evaluate possible seasonal dietary variation, the samples were analyzed with respect to the season. Diel dietary variation was analyzed by sorting the samples into six classes according to sampling time, namely, 2:00, 6:00, 10:00, 14:00, 18:00, and 22:00. Finally, to assess possible dietary differences based on sex, the samples were divided into an unidentified group (n=5), a female group (n=212), and a male group (n=163). Seasonal, diel, and sexual diet variations were tested by analysis of similarities (ANOSIM) through the Bray-Curtis similarity matrix based on *% W* data.

The feeding intensity related to diel period and season was determined by the gut fullness index (*GFI*), which was expressed as 100%×(gut content weight/body weight) (Grabowska *et al*., 2009). Given that the *GFI* was not normally distributed (Shapiro-Wilks test, $p<0.05$), the variations of feeding intensity were tested using non-parametric Kruskal-Wallis H test, followed by Mann-Whitney U tests for pairwise comparisons.

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The niche breadth (B_N) was measured using the standardized Levins index (Levins, 1968; Hurlbert, 1978). The Shannon-Wiener diversity index (H') (Shannon, 1948) was used to examine feeding diversity. Then, based on the *% W* of each prey, the trophic level (*TL*) and its variation in relation to season and ontogenetic group were calculated according to the formula proposed by Cortés (1999). The *TL* of animal prey was obtained from the research of Ebert and Bizzarro (2007), and the vegetable prey was defined as 1.0.

Finally, the feeding strategy was described by the Amundsen graphical method (Amundsen *et al*., 1996). The distribution of prey along the diagonals and axes of the diagram provides information about the feeding strategy, niche width contribution, and prey importance.

All the statistical analyzes were conducted in PRIMER 5 and SPSS 20 at the significance level of 0.05. The images were performed by Origin pro version 8.0.

Results

A total of 543 individuals of *A. yunnanensis* were collected and examined, with the *SL* ranging from 55 to 253 (124.3±30.4, mean±SD) mm, and the *BW* ranging from 3.5 to 339.5 (41.8±32.4) g (Table 1). Among the samples, 43 with empty guts and 380 containing prey (gut fullness equal to or greater than 20%) were used for diet analysis (Table 1). All nine cumulative prey curves reached an asymptote ($b< 0.05$) (Fig. 2); therefore, the number of samples was considered sufficient to describe the diet.

Table 1: Body size (standard length and body weight) and number of *Acrossocheilus yunnanensis* during the entire project. *N* represents the total number of samples for each class, and *n* represents the number of guts analyzed.

Classification	Standard length (mm)		Body weight (g)		*N*	*n*
	Range	Mean ± SD	Range	Mean ± SD		
Season						
Spring	85-188	136.0 ± 19.6	11.7-152.1	52.5 ± 24.5	61	37
Summer	77-190	129.1 ± 23.9	8.1-129.1	44.8 ± 25.0	153	99
Autumn	55-188	104.1 ± 23.9	3.5-113.2	23.5 ± 19.1	171	134
Winter	68-253	136.9 ± 34.6	5.2-339.5	54.5 ± 42.7	158	110
Diel period						
2:00	84-204	141.0 ± 28.5	11.2-156.2	57.4 ± 30.8	89	72
6:00	55-253	134.7 ± 32.7	3.5-339.5	53.3 ± 41.4	144	88
10:00	79-178	118.2 ± 20.5	9.4-73.6	30.3 ± 15.1	62	46
14:00	70-177	101.5 ± 19.5	6.9-113.3	22.0 ± 15.5	90	67
18:00	68-163	105.4 ± 22.8	5.2-77.8	25.5 ± 18.6	37	21
22:00	63-194	125.2 ± 28.3	5.3-152.1	42.2 ± 28.9	121	86
Total	55-253	124.3 ± 30.4	3.5-339.5	41.8 ± 32.4	543	380

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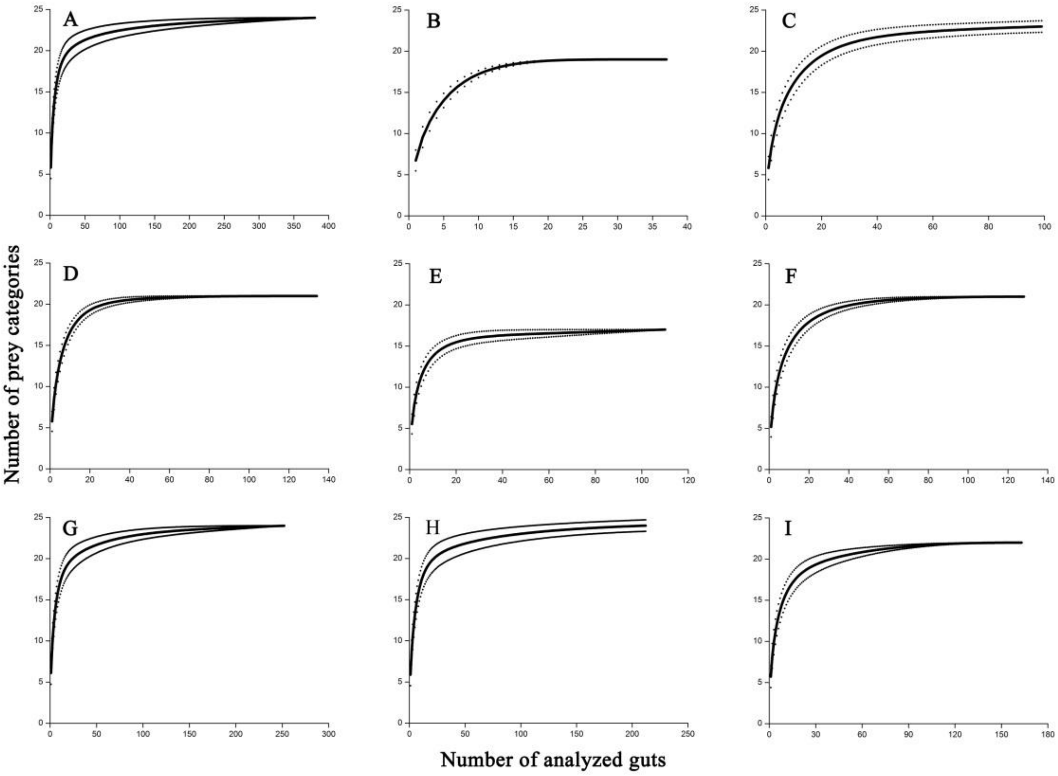

Figure 2: Cumulative prey curves (solid lines) and SD (dotted lines) for (A) total, (B) spring, (C) summer, (D) autumn, (E) winter, (F) YAG (*SL*≤110 mm), (G) OAG (*SL*>110 mm), (H) female, and (I) male samples.

Diet composition

The diet of *A. yunnanensis* contained a wide variety of algae, plants and animal prey (Table 2). A total of 93 different food taxa belonging to seven main prey categories (diatoms, chlorophytes, other vegetable prey, aquatic insects, mollusca, other invertebrates, and remains) were identified (Table 2). The most important prey was chlorophytes (*PSIRI*=41.30%), of which *Spirogyra* (one of the filamentous algae) was the most important component. The second most important prey was diatoms (*PSIRI*=28.80%), and the third was aquatic insects (*PSIRI*=21.67%). According to the identified aquatic insects, Ephemeroptera was the most important prey, followed by Trichoptera and Chironomidae larvae (Table 2).

Table 2: Diet composition of *Acrossocheilus yunnanensis*. Diet indices include average percent number (*% AN*), average percent weight (*% AW*), percent frequency of occurrence (*% FO*), percent prey-specific number (*% PN*), percent prey-specific weight (*% PW*), and prey-specific index of relative importance (*PSIRI*); * represents values < 0.01.

Prey	*% AN*	*% AW*	*% FO*	*% PN*	*% PW*	*PSIRI*
Diatoms	45.95	11.65	98.68	46.56	11.80	28.80
Melosira	15.44	4.93	87.11	17.73	5.66	10.19
Navicula	7.01	2.15	89.21	7.85	2.41	4.58
Nitzschia	1.60	0.21	68.95	2.32	0.31	0.91
Cymbella	2.38	0.24	72.37	3.29	0.33	1.31
Gomphonema	6.66	0.63	91.58	7.27	0.69	3.65

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Table 2 continued:

Synedra	0.81	0.34	56.05	1.45	0.60	0.57
Achnanthes	1.61	0.26	68.68	2.35	0.38	0.94
Diatoma	2.46	0.67	71.84	3.42	0.93	1.56
Rhoicosphenia	0.09	*	11.84	0.73	0.02	0.04
Cocconeis	3.03	1.26	79.74	3.80	1.58	2.14
Fragilaria	0.80	0.01	58.16	1.37	0.01	0.40
Gyrosigma	0.20	0.03	27.37	0.74	0.10	0.12
Pinnularia	1.19	0.27	63.95	1.85	0.42	0.73
Cyclotella	0.38	0.03	46.05	0.83	0.07	0.21
Epithemia	0.11	0.05	31.32	0.36	0.16	0.08
Surirella	1.02	0.15	49.74	2.04	0.30	0.58
Frustulia	0.56	0.31	47.37	1.19	0.66	0.44
Diploneis	0.11	0.03	24.21	0.45	0.13	0.07
Didymosphenia	0.07	0.02	15.53	0.47	0.13	0.05
Amphora	0.01	*	2.89	0.20	0.14	*
Cymatopleura	0.42	0.05	10.26	4.10	0.47	0.23
Eunotia	*	*	0.53	0.18	0.06	*
Rhizosolenia	*	*	0.53	0.17	0.07	*
Chlorophytes	51.33	31.26	76.84	66.80	40.68	41.30
Cosmarium	0.05	*	3.95	1.15	*	0.02
Mougeotia	0.01	*	1.58	0.41	0.16	*
Scenedesmus	0.01	*	0.26	2.27	*	*
Oedogonium	0.01	*	0.79	1.74	0.01	0.01
Chlorella	0.47	*	19.74	2.37	*	0.23
Ankistrodesmus	0.32	*	8.68	3.63	*	0.16
Closterium	0.09	*	3.16	2.75	0.15	0.05
Actinastrum	0.14	*	0.26	52.93	*	0.07
Crucigenia	0.01	*	0.79	1.02	*	*
Spirogyra	49.91	31.21	63.42	78.70	49.20	40.56
Cladophora	0.33	0.05	1.05	31.09	4.61	0.19
Other vegetable prey	1.52	8.24	70.00	2.17	11.78	4.88
Cyanophytes	1.14	0.06	33.16	3.45	0.18	0.60
Oscillatoria	0.24	0.05	19.74	1.22	0.25	0.15
Anabeana	0.09	*	3.16	2.73	0.01	0.04
Merismopedia	0.01	*	1.05	1.11	0.01	0.01
Spirulina	0.13	*	2.89	4.62	0.02	0.07
Phormidium	0.56	0.01	10.53	5.36	0.08	0.29
Microcystis	0.10	*	0.26	38.20	*	0.05
Aphanothece	0.01	*	0.26	2.33	*	*
Chroococcus	*	*	0.26	0.36	*	*
Dinoflagellates	0.15	0.01	18.95	0.79	0.06	0.08
Gymnodinium	0.12	*	17.37	0.69	0.03	0.06
Peridinium	0.03	0.01	3.16	0.95	0.19	0.02
Euglenophytes	0.10	*	0.26	36.47	*	0.05
Trachelomonas	0.10	*	0.26	36.47	*	0.05
Rhodophytes	*	3.37	14.21	0.01	23.69	1.68
Lemanea sinica	*	3.37	14.21	0.01	23.69	1.68

Table 2 continued:

Organic detritus	0.12	2.82	37.11	0.31	7.59	1.47
Other plant material	0.01	1.99	9.21	0.09	21.61	1.00
Vascular plants	*	0.08	1.32	0.01	6.39	0.04
Plant seeds	0.01	1.91	7.89	0.10	24.15	0.96
Aquatic insects	0.76	42.58	80.26	0.95	53.05	21.67
Odonata	*	0.01	0.26	*	3.99	0.01
Gomphidae larvae	*	0.01	0.26	*	3.99	0.01
Plectoptera	*	0.35	1.05	0.03	33.09	0.17
Perlidae	*	0.35	1.05	0.03	33.09	0.17
Trichoptera	*	2.53	15.79	0.02	16.00	1.26
Ephemeroptera	0.34	32.86	71.05	0.48	46.24	16.60
Diptera	*	2.10	24.74	0.01	8.48	1.05
Chironomidae larvae	*	1.77	22.89	0.01	7.73	0.89
Psychodidae	*	0.03	2.63	0.01	1.29	0.02
Tipulidae	*	0.25	0.26	*	94.59	0.12
Tabanidae	*	0.04	0.53	*	8.52	0.02
Coleoptera	0.04	2.02	11.05	0.41	18.31	1.03
Dytiscidae larvae	0.04	1.33	5.26	0.85	25.22	0.69
Dytiscidae adult	*	0.18	0.26	*	70.04	0.09
Hydrophilidae larvae	*	0.47	4.21	*	11.06	0.23
Hydrophilidae adult	*	0.05	1.58	*	2.95	0.02
Megaloptera	0.26	0.95	2.11	12.50	44.91	0.60
Sialidae	0.26	0.63	1.32	20.00	47.89	0.45
Corydalidae	*	0.32	0.79	*	39.93	0.16
Hemiptera	*	0.28	0.79	*	35.92	0.14
Aphelochirus	*	0.03	0.26	*	13.04	0.02
Naucoris exclamationis	*	0.25	0.53	*	47.36	0.12
Unidentified	0.11	1.49	10.26	1.10	14.47	0.80
Mollusca	*	1.56	12.63	0.01	12.39	0.78
Bivalvia	*	0.76	7.89	0.01	9.60	0.38
Limnoperna lacustris	*	0.71	7.11	0.01	10.05	0.36
Cuneopsis heudei	*	0.04	0.79	*	5.53	0.02
Gastropoda	*	0.81	5.53	0.01	14.60	0.40
Radix	*	0.77	5.00	0.01	15.31	0.38
Bellamya	*	0.04	0.53	0.01	7.92	0.02
Other invertebrates	0.43	0.71	26.58	1.63	2.66	0.57
Terricolous insects	*	0.19	3.68	0.01	5.28	0.10
Hymenoptera	*	0.19	3.42	0.01	5.46	0.09
Unidentified	*	0.01	0.26	*	2.94	*
Oligochaeta	*	0.32	0.79	0.01	40.74	0.16
Earthworm	*	0.32	0.79	0.01	40.74	0.16
Crustacea	0.02	0.19	14.21	0.16	1.31	0.10
Cladocera	*	0.09	7.11	0.02	1.26	0.05
Copepoda	0.02	0.10	8.95	0.24	1.09	0.06
Rotifera	*	*	0.26	*	0.01	*
Brachionus calyciflorus	*	*	0.26	*	0.01	*
Protozoa	0.41	*	15.53	2.65	0.02	0.21

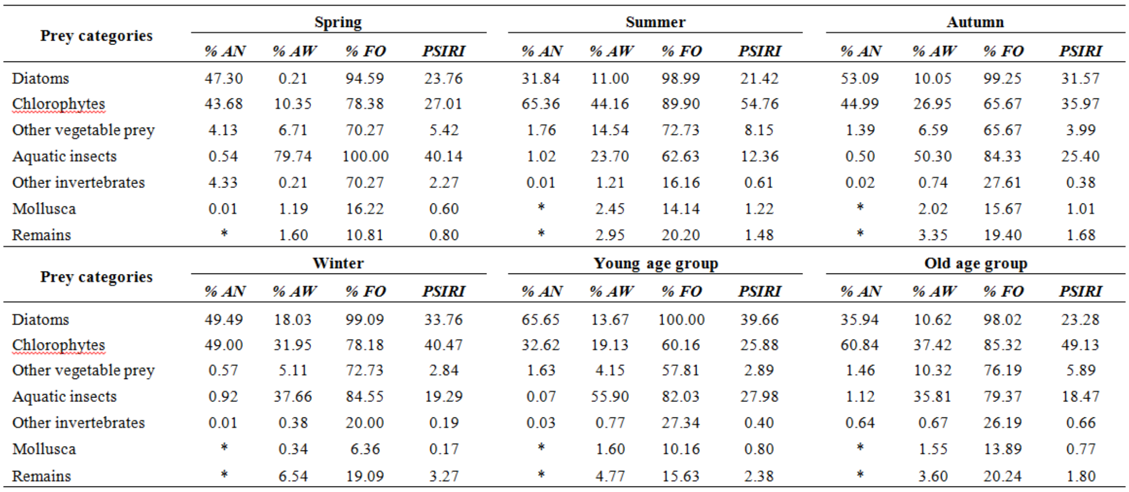
Table 2 continued:

Tintinnidium	0.01	*	0.53	1.75	0.09	*
Stentor	0.01	*	0.26	1.92	0.07	*
Oxytricha	*	*	0.53	0.47	0.10	*
Halteria	*	*	0.26	0.28	*	*
Euplotes	*	*	0.53	0.24	0.03	*
Chilodonella	0.27	*	10.79	2.53	0.01	0.14
Tetrahymena	*	*	0.26	0.83	0.01	*
Coleps	*	*	0.79	0.09	*	*
Difflugia	0.04	*	3.42	1.27	*	0.02
Arcella	0.01	*	0.79	0.99	*	*
Actinophrys	0.04	*	0.26	14.59	*	0.02
Amoeba	0.01	*	0.79	1.25	*	*
Globigerina	0.02	*	1.32	1.28	*	0.01
Vorticella	*	*	0.26	*	0.01	*
Remains	*	4.00	18.68	0.01	21.39	2.00
Feather	*	*	0.26	*	0.02	*
Woollen	*	*	0.26	*	0.75	*
Unidentified	*	3.99	18.16	0.01	22.00	2.00

Ontogenetic dietary shift

An ontogenetic shift in the diet composition was detected. The six age classes can be classified in two distinct groups through both cluster analysis (complete linkage) and an NMDS ordination plot (stress=0) (Fig. 3). The two groups were defined as young age group (YAG: I-III, *SL*≤110 mm) and old age group (OAG: IV-VI, *SL*>110 mm). The data showed that the YAG mainly consumed diatoms (*PSIRI*=39.66%) and aquatic insects (*PSIRI*=27.98%), whereas the OAG consumed more chlorophytes (*PSIRI*=49.13%) but fewer aquatic insects (*PSIRI*=18.47%) and diatoms (*PSIRI*=23.28%) than the YAG (Table 3). The SIMPER test indicated that the dissimilarity between the YAG and OAG was caused mainly by aquatic insects (33.06%), chlorophytes (26.74%), and diatoms (15.31%).

Table 3: Dietary variations of *Acrossocheilus yunnanensis* with season and size group. Diet indices include average percent number (*% AN*), average percent weight (*% AW*), percent frequency of occurrence (*% FO*), and prey-specific index of relative importance (*PSIRI*); * represents values<0.01.



Prey categories	Spring				Summer				Autumn			
	% AN	*% AW*	*% FO*	*PSIRI*	*% AN*	*% AW*	*% FO*	*PSIRI*	*% AN*	*% AW*	*% FO*	*PSIRI*
Diatoms	47.30	0.21	94.59	23.76	31.84	11.00	98.99	21.42	53.09	10.05	99.25	31.57
Chlorophytes	43.68	10.35	78.38	27.01	65.36	44.16	89.90	54.76	44.99	26.95	65.67	35.97
Other vegetable prey	4.13	6.71	70.27	5.42	1.76	14.54	72.73	8.15	1.39	6.59	65.67	3.99
Aquatic insects	0.54	79.74	100.00	40.14	1.02	23.70	62.63	12.36	0.50	50.30	84.33	25.40
Other invertebrates	4.33	0.21	70.27	2.27	0.01	1.21	16.16	0.61	0.02	0.74	27.61	0.38
Mollusca	0.01	1.19	16.22	0.60	*	2.45	14.14	1.22	*	2.02	15.67	1.01
Remains	*	1.60	10.81	0.80	*	2.95	20.20	1.48	*	3.35	19.40	1.68
Prey categories	**Winter**				**Young age group**				**Old age group**			
	% AN	*% AW*	*% FO*	*PSIRI*	*% AN*	*% AW*	*% FO*	*PSIRI*	*% AN*	*% AW*	*% FO*	*PSIRI*
Diatoms	49.49	18.03	99.09	33.76	65.65	13.67	100.00	39.66	35.94	10.62	98.02	23.28
Chlorophytes	49.00	31.95	78.18	40.47	32.62	19.13	60.16	25.88	60.84	37.42	85.32	49.13
Other vegetable prey	0.57	5.11	72.73	2.84	1.63	4.15	57.81	2.89	1.46	10.32	76.19	5.89
Aquatic insects	0.92	37.66	84.55	19.29	0.07	55.90	82.03	27.98	1.12	35.81	79.37	18.47
Other invertebrates	0.01	0.38	20.00	0.19	0.03	0.77	27.34	0.40	0.64	0.67	26.19	0.66
Mollusca	*	0.34	6.36	0.17	*	1.60	10.16	0.80	*	1.55	13.89	0.77
Remains	*	6.54	19.09	3.27	*	4.77	15.63	2.38	*	3.60	20.24	1.80

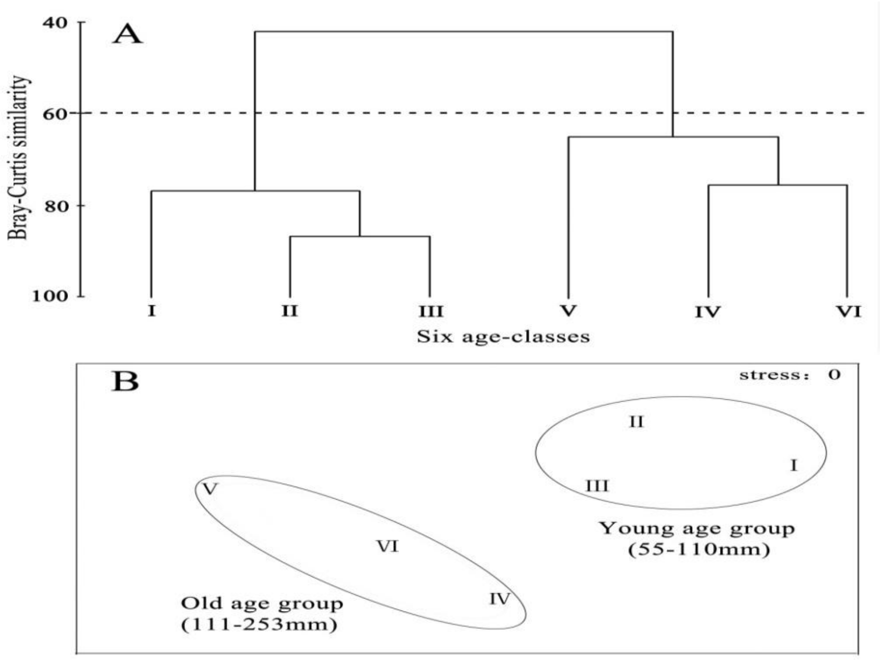


Figure 3: Hierarchical cluster analysis and NMDS based on the percent of weight (*% W*) of the six age classes. (A) The two size groups (YAG and OAG) defined at arbitrary similarity level of 60 % are indicated (dotted line); (B) NMDS showing the ordination of the six age classes into two size groups with similar diets.

Seasonal dietary variation

The diet composition varied conspicuously by season (ANOSIM, Global *R*=0.102, *p*<0.001). In spring, the predominant prey was aquatic insects (*PSIRI*=40.14%), followed by chlorophytes (*PSIRI*=27.01%) and diatoms (*PSIRI*=23.76%); notably, the *% FO* of aquatic insects was 100% in spring (Table 3). In summer, chlorophytes (*PSIRI*=54.76%) were the primary prey, whereas aquatic insects (*PSIRI*=12.36%) contributed the least compared to the other seasons (Table 3). In autumn, the most important prey was chlorophytes (*PSIRI*=35.97%), and the second most important prey was diatoms (*PSIRI*=31.57%), which had the highest occurrence (*% FO*=99.25%) compared to the other seasons (Table 3). In winter, *A. yunnanensis* fed predominantly on chlorophytes (*PSIRI*= 40.47%) followed by diatoms (*PSIRI*=33.76%). It consumed more diatoms in winter than that in the other three seasons (Table 3). The other prey items also varied with season (Table 3).

Diel and sexual dietary variations

The two-way ANOSIM results showed that there were no diel dietary variations in relation to season (Global *R*=0.005, *p*>0.05) or size group (Global *R*=0.025, *p*>0.05). Similarly, the diet composition did not differ between the sexes with respect to season (Global *R*=0.013, *p*>0.05) or size group (Global *R*=-0.012, *p*>0.05).

Diel and seasonal feeding intensity

Diel feeding intensity showed no significant difference throughout the entire 24-h periods (Kruskal-Wallis *H* test, *p*>0.05). Although no significant difference was found, enhanced feeding activity can be observed visually at 14:00 and 22: 00 (Fig. 4).

However, a seasonal difference in feeding intensity was detected (Kruskal-Wallis *H* test, $p<0.001$) (Fig. 5). Based on the Mann-Whitney pairwise comparisons, the average *GFI* values were slightly higher in spring than those in autumn, although the difference was not significant ($p>0.05$). However, the values in both spring and autumn were significantly greater than those in summer and winter ($p<0.05$). Moreover, higher *GFI* values were found in summer than those in winter ($p<0.05$).

Niche breadth, feeding diversity, trophic level and feeding strategy

The results showed that *A. yunnanensis* has a moderate niche breadth (B_N=0.38) and high feeding diversity (H'=2.17). The highest values of those indices appeared in summer (B_N =0.58, H'=2.18), whereas the lowest values appeared in spring (B_N=0.06, H'=0.95). In terms of ontogenetic groups, the OAG fish (B_N =0.39, H'=2.15) had a greater niche breadth and feeding diversity than the YAG individuals (B_N =0.22, H'=1.94) (Table 4).

Table 4: Standard niche width (B_N), Shannon-Wiener diversity index (H'), and trophic level (*TL*) of *Acrossocheilus yunnanensis* in relation to season, size group, and the total sample.

Classification	B_N	H'	*TL* (mean±SD)
Spring	0.06	0.95	3.22±0.47
Summer	0.58	2.18	2.42±0.53
Autumn	0.36	1.95	2.81±0.64
Winter	0.38	2.17	2.62±0.58
Young age group	0.22	1.94	2.90±0.62
Old age group	0.39	2.15	2.59±0.60
Total	0.38	2.17	2.69±0.62

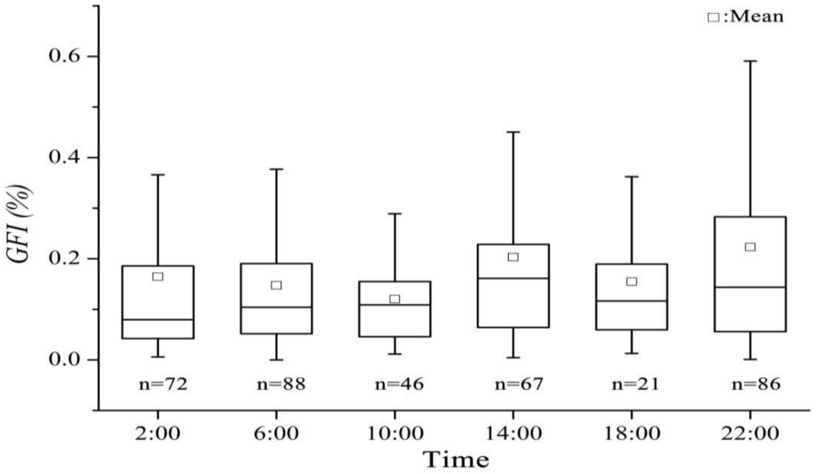


Figure 4: Boxplot showing diel variation in the mean percent gut fullness index (*GFI*).

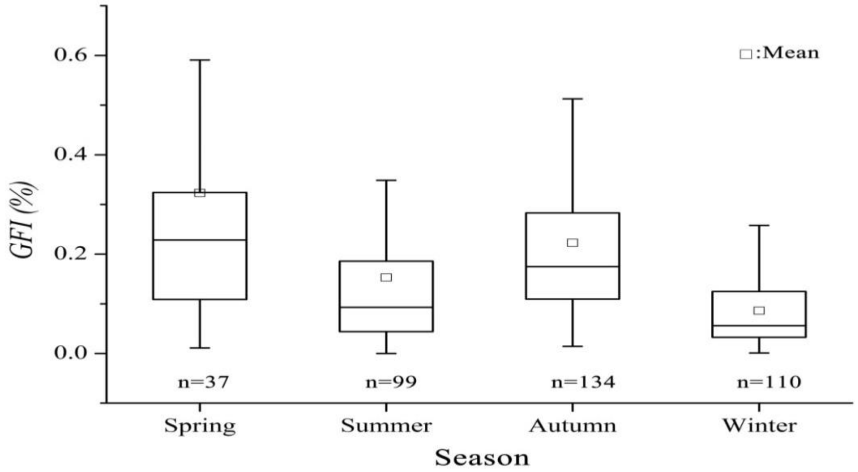


Figure 5: Boxplot showing seasonal variation in the mean percent gut fullness index (*GFI*).

The average *TL* was 2.69±0.62 (mean±SD). The highest *TL* was found in spring (*TL*=3.22±0.47), and the lowest *TL* was observed in summer (*TL*=2.42±0.53). In addition, the YAG (*TL*=2.90±0.62) had a higher *TL* than the OAG (*TL*=2.59±0.60) (Table 4).

A few prey occupied very similar positions in the Amundsen graph (Fig. 6). At the population level, all prey categories were in the lower part of the graph, signifying a generalized strategy. However, the preference of *A. yunnanensis* for chlorophytes, aquatic insects, and diatoms (*% FO*>75%) demonstrated a relatively specialized strategy. Therefore, from the perspective of the Amundsen graph and the niche breadth (B_N=0.38), *A. yunnanensis* can be considered as a moderate generalist predator. In terms of niche width contribution, all prey items lay on the lower right and under the diagonal of the graph, demonstrating the individuals utilize many common prey, none of which dominate the diet. Regarding prey importance, all prey items except those in three categories (chlorophytes, aquatic insects, and diatoms) were situated in the lower left, which manifested that they were rare or unimportant prey.

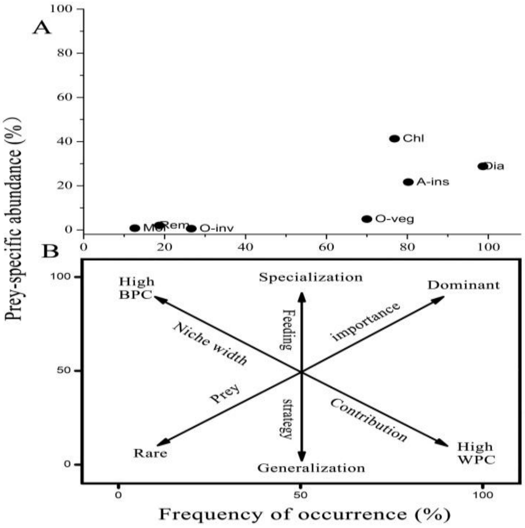


Figure 6: Feeding strategies of *Acrossocheilus yunnanensis*. (A) Amundsen graph, black dot (•) represents prey category. Dia, Diatoms; Chl, Chlorophytes; O-veg, Other vegetable prey; A-ins, Aquatic insects; O-inv, Other invertebrates; Mol, Mollusca; Rem, Remains. (B) Explanatory diagram for the interpretation of feeding strategy, niche width contribution and prey importance; BPC and WPC represent between-phenotype component and within-phenotype component, respectively.

Discussion

Diet composition

The analysis of the gut contents revealed that *A. yunnanensis* is an omnivorous feeder. The gut length (*GL*) index (the ratio of *GL* to *SL*) for this species confirmed this conclusion, with a value of 1.71±0.21 (mean±SD), which is in the range (1-3) for omnivores (Geevarghese, 1983). *A. yunnanensis* has a broad trophic spectrum (Table 2); it can take advantage of all available food resources in the environment, indicating that it is an opportunistic predator. Therefore, we can conclude that food resources are not a limiting factor for population growth and expansion in this species, which may be one of the important reasons that it has become the dominant species in headwaters of the Chishui River.

Our results revealed that the most important prey of *A. yunnanensis* were filamentous chlorophytes, diatoms, and aquatic insects. Tarkowska-Kukuryk (2013) pointed out that the diatoms and filamentous chlorophytes are usually the dominant algae groups in periphyton. Therefore, the main prey of *A. yunnanensis* were just from the abundant food resources in the environment. This might be the result of fish adaptation to its environment.

Ding (1994) briefly noted that *A. yunnanensis* mainly prey on filamentous algae, accompanied by a small proportion of fish and shrimp. Both Ding’s and our study indicated that filamentous algae were the primary food for *A. yunnanensis*. However, fish and shrimp were not found to be prey in our study; instead, aquatic insects were the third most important prey in terms of *PSIRI*. This difference between the two studies may be attributed to different habitats; the specimens investigated in the previous study were collected from a different river basin. The monkey goby (*Neogobius fluviatilis*), in the Vistula River in Poland exhibited significant spatial differences in diet composition compared to those living in the largest tributary of that river, the Bug River (Grabowska *et al*., 2009). In other words, *A. yunnanensis* is flexible in its diet and feeds on available food resources. This variation may indicate a strategy that considerably reduces the cost of seeking prey, and maximizes its net energy intake (Prejs and Prejs 1987).

Dietary variation

Ontogenetic shifts in resource use, particularly in diet, are prevalent in fish (Guo *et al*., 2013). Ontogenetic dietary shifts allow a population to share a habitat by effectively partitioning individuals into different feeding guilds or ecological roles, thereby reducing intra-specific competition (Wootton, 1990). Our results showed that the YAG fish consumed more aquatic insects and diatoms, whereas the OAG individuals preyed on more chlorophytes. This discrepancy may be due to the morphological changes and different metabolic requirements at different ontogenetic stages. In this study, the *GL* index was 1.55±0.23 (mean±SD) for the YAG and 1.75±0.18 for the OAG. The increase in *GL* for the OAG individuals enhances their digestive ability by increasing the active surface area for digestion (Akin *et al*., 2016), thus driving the OAG individuals to consume more filamentous chlorophytes, which contain a high proportion of indigestible cellulose or lignin (Wootton, 1990). In contrast, to satisfy the demands for organ development and growth, the YAG fish must feed on prey, such as aquatic insects, that contain high-energy and easily digested (Barbini *et al*., 2010). Similar phenomena have been observed for two species, *Schizopygopsis younghusbandi* and *S. oconnori*, in which younger fish tend to consume more animal prey to meet their growth demands (Yang *et al*., 2011; Ma *et al*., 2014).

The seasonal dietary variations may suggest that both abiotic and biotic factors change seasonally (Wootton, 1990). In particular, those alterations may directly reflect seasonal changes in prey abundance or availability. The lowest proportion of mobile prey (animal prey, especially aquatic insects) was observed in summer, probably because macro-invertebrates were less abundant in that season (Jiang *et al*., 2016). Moreover, higher water levels, higher water velocity and reduced transparency caused by floods in summer may impede the ability of fish to catch animal prey (Wootton, 1990). Despite the high biomass of macro-invertebrates in winter, the feeding activity of the fish decreased dramatically due to the low water temperature (10.2 °C) (Wootton, 1990; Jiang *et al*., 2016). Therefore, it is not surprising to observe a relative lower proportion of aquatic insects and higher proportion of motionless (vegetable) prey being consumed during the winter. In fact, the biomass of macro-invertebrates in spring was higher than that in autumn in our study area (Jiang *et al*., 2016); and thus, the highest proportion of aquatic insects (*PSIRI*=40.14%) was found in spring, followed by autumn (*PSIRI*=25.40%). In addition, more aquatic insects were consumed in spring, which might be due to an effort to store energy for the reproductive activity that occurs in summer (Ding, 1994).

Diel and seasonal feeding intensity

There was no apparent difference in diel feeding intensity, possibly due to the large proportion (*PSIRI*=74.98%) of low-energy food (vegetable prey) consumed (Table 2). As a rule, low-energy food is evacuated faster than high-energy food (Wootton, 1990). Thus, *A. yunnanensis* may never feel satiated and may take in food continuously. *S. younghusbandi*, a typical fish that feeds on low-energy food, feeds almost continuously and relies on a rapid turnover of the gut contents (Yang *et al*., 2011).

The feeding intensity of *A. yunnanensis* displayed a clear variation tendency. Our results showed that the minimum *GFI* appeared in winter, possibly because the low water temperature (10.2 °C) decreased the feeding activity and digestion rate. The maximum *GFI* was found in spring and could be ascribed to several factors, including (1) appropriate physical environment factors, (2) a high abundance of prey items, (3) a need to

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consume more food to recover vigour after the reduced feeding period in winter, and (4) a greater energy requirement for gonad development and to stockpile energy for the summer spawning activity. The stable hydrologic conditions and appropriate water temperature in autumn (Jiang *et al*., 2016) led to the second highest *GFI* value. In addition, eating more food in autumn was conducive to storing energy for winter (Yang *et al*., 2011). Floods and breeding activity in summer impede food intake and give rise to relatively lower *GFI* values. Olasotoca *et al*. (2000) noted that for fish in spawning or pre-spawning periods, gonad development requires a certain amount of space in the body cavity, resulting in reduced feeding intensity. Some species, such as *S. younghusbandi*, cease feeding altogether during their spawning period (Yang *et al*., 2011).

In conclusion, the current work provides detailed information on the feeding ecology of *A. yunnanensis*. The findings of this research are a valuable reference for developing management rules for conserving this endemic species and for managing the nature reserve river ecosystem. Further studies are suggested to focus on the niche partition of sympatric fish species in the same study area.

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