Journal of Oceanology and Limnology
Vol. 37 No. 2, P. 736-744, 2019
https://doi.org/10.1007/s00343-019-7372-1

Estimates on age, growth, and mortality of *Gymnocypris firmispinatus* (Cyprinidae: Schizothoracinae) in the Anning River, China*

MA Baoshan[1], NIE Yuanyuan[1, 2], WEI Kaijin[1, **], XU Bin[1], ZHU Xiangyun[1], XU Jin[1]

[1] *Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China*
[2] *Development Center for Animal Husbandry in Mountain Area in Miao Autonomous County of Songtao, Tongren 554100, China*

Received Dec. 6, 2017; accepted in principle Feb. 14, 2018; accepted for publication May 10, 2018

Abstract The age, growth, and mortality of *Gymnocypris firmispinatus* were studied by 582 individuals that collected between July 2015 and June 2016 from the tributaries of the Anning River, China. The total length (TL) ranged from 27 mm to 242 mm, total weight (W) ranged from 0.2 g to 148.2 g. The relationship of total length and weight was expressed as W=7.754×10^{-6}TL$^{3.304}$ for the total samples. The age classes of males were 1–9 a, and that of females were 1–13 a. The von Bertalanffy functions based on otolith readings and observed length data were $L_{t♂}$=198.6(1–e$^{-0.094\ (t+3.497)}$), and $L_{t♀}$=289.3(1–e$^{-0.089\ (t+1.109)}$). The total mortality (Z) of male and female *G. firmispinatus* was estimated as 0.73/a and 0.40/a, respectively. The natural mortality (M) was evaluated as 0.25/a for males and 0.20/a for females, fishing mortality (F) was 0.35/a for males and 0.16/a for females. The exploitation ratio of the population was 0.66/a for males and 0.50/a for females. It was concluded that the growth of the fish is relatively slower than other congeneric fishes. The population of *G. firmispinatus* in the tributaries of the Anning River might be in over-exploitation under the current fishing intensity.

Keyword: otolith; growth; von Bertalanffy model; mortality; *Gymnocypris firmispinatus*

1 INTRODUCTION

The Anning River Basin is located in the transitional zone of the Qinghai-Tibet Plateau, Yunnan-Kweichow Plateau, and Sichuan Basin. The transitional zone maintains high fish diversity and plays significant role in the ecological security and ecological construction of Sichuan Province, China. Eco-environmental problems, such as minerals, hydropower, agriculture and animal husbandry resources, in the Anning River Basin pose a threat to the ecological security in the region (Xian, 2011). Based on our and other investigations in the field (Ru et al., 2016; Ma et al., 2017b), fish resource in the Anning River is severely threatened by intensive human activities, including damming, sand excavation, and overfishing. Compared with the previous studies, the number of fish species reduced from 82 to 52, the fish size became smaller and smaller, and daily fish catches were less than 10% of those captured in ten years ago (Ru et al., 2016). Thus, many researchers (Ru et al., 2016; Cao, 2017) strongly suggested that conservation of fish resources in the Anning River should be carried out as soon as possible.

Gymnocypris firmispinatus Wu et Wu, 1988 is a small-size Schizothoracinae fishes, only distributes in the Jinsha River and its tributaries (Chen and Cao, 2000), and mainly resides in the Anning River and its tributaries (Wang, 2008; Ru et al., 2016; Ma et al., 2017b). *G. firmispinatus* preferentially inhabits in fast-flowing water and the spawner often moves to nearby upstream for reproduction (Cao et al., 1981).

* Supported by the Central Public-Interest Scientific Institution Basal Research Fund, CAFS (No. 2016JBF0303) and the National Non-Profit Institute Research Grant of Freshwater Fisheries Research Center, CAFS (No. 2015JBFM38)
** Corresponding author: weikj@yfi.ac.cn

Nevertheless, cascade hydropower development in the Anning River blocks the river continuity and destroys the ecological integrity, which might seriously impacts the propagative migration of adults and the feeding migration of juveniles. Therefore, *G. firmispinatus* had been listed as key protected animals in Sichuan province because of the decreased population.

In a word, the population of *G. firmispinatus* had gradually reduced with several anthropogenic activities, but some sound population management strategy has been hindered by lack of its basic biological studies. The available reports refer mostly to taxonomic characters and distribution (Chen and Cao, 2000), and acute toxicity (Xu et al., 2017). There have been few studies on the biology of *G. firmispinatus*. Ma et al. (2017a) compared three types of age identification material, and established the periodicity of annulus formation in otoliths and vertebrae. Ma et al. (2017b) reported the length-weight relationship and fecundity of the fish. *G. firmispinatus* seemed to be a small Schizothoracinae fish with some life-history characteristics of slow growing, low fecundity, and late maturity (Chen and Cao, 2000).

Accurate estimates of age, growth, and mortality are very important for understanding population dynamics and provide fundamental data for fishery management (Campana and Thorrold, 2001). Many reports have pointed out that ages of Schizothoracinae fishes (including *G. firmispinatus*) can be determined reliably by otolith sections (Ma et al., 2011, 2017a; Huo et al., 2012; Zhou et al., 2017). Although the population of *G. firmispinatus* had decreased gradually mainly owing to the dam construction for over ten years, the growth parameters and mortality rates of the species were not estimated up to now. Therefore, the main goals of this study were to 1) estimate the age and growth parameters of *G. firmispinatus* by sectioned otoliths, and compare growth characters of the species with other congeneric fishes; 2) estimate the mortality rates by several empirical equations; 3) discuss the implications of our findings for conservation management of the *G. firmispinatus* population.

2 MATERIAL AND METHOD

2.1 Study area and sample collection

This study was conducted in the Anning River, China, which is the biggest tributary in the lower reach of the Yalong River, with 326 km long at mainstream, 11 150 km^2 of the area and 975 m of the average drop. The river belongs to the subtropical monsoon climate, with the mean annual air temperature of 17–19°C and the annual rainfall of above 1 240 mm/a. The vegetation coverage along the river bank is very high (more than 80%), especially in spring and summer (Ning, 2009).

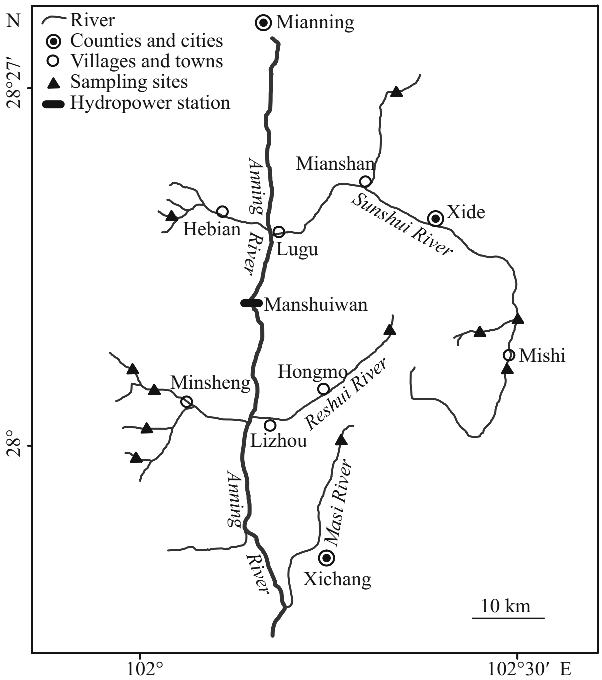

Fig.1 Sampling locations of *G. firmispinatus* from July 2015 to June 2016 in the Anning River, China

Our field investigates showed that *G. firmispinatus* seldom reside in the mainstream nowadays; instead, they mostly occur in the tributaries. Specimens were collected in the tributaries of the Anning River (Fig.1), from 1 879.2 m to 2 509.0 m ASL. Usually, *G. firmispinatus* was the dominant species in these tributaries. Specimens (n=582) were collected monthly within the period between July 2015 and June 2016. Each month more than 30 specimens were captured using backpack electro-fishing gear (HailiBao, China, 12 V). The catches were transported in icebox to the laboratory for the subsequent analysis. Fish was euthanized with MS-222 and eviscerated immediately, and then total length (TL) and total weight (W) were measured to the nearest 1 mm and 0.1 g. Specimens were sorted separately by male, female, or undetermined by visual inspection of gonads. Lapilli were removed from each fish, cleaned with distilled water, and stored dry in labeled tubes.

2.2 Otolith preparation and age estimation

A lapillus was mounted on a glass slide using transparent colorless nail polish, ground manually using wet sandpaper (1 000−2 500 grit) and polished with alumina paste (3 μm) until the core and most annuli were observed under a compound microscope. Then the mounting medium was dissolved with acetone, the otolith was remounted with the polished side facing the glass slide, and then ground and polished to expose the core and the growth increments (Ma et al., 2011). Age determination was made with an image analysis system (Jiseki ARP/W version 5.20, Ratoc System Engineering Company, Tokyo, Japan) using a microscope (Olympus BX51) and a computer connected with a CCD (Charge-coupled Device).

Each fish was assigned to an age class assuming 1 April as the birth date, which approximately corresponds to the peak spawning season and the annuli formation time (Granada et al., 2004; Qiu and Chen, 2009; Ma et al., 2017a; unpublished data). Annuli were interpreted without knowing the capture date, sex or length of all specimens. Each otolith was read twice, if the two counts differed, the lapillus was recounted, and two readings should be consistent at the end. The index of the average percent error (APE) was computed to evaluate the precision of the age estimations between two counts (Beamish and Fournier, 1981):

$$\mathrm{APE}=\frac{1}{N}\sum_{j=1}^{N}\left(\frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij}-X_j\right|}{X_j}\right)\times 100\%,$$

where N is the number of fish aged, R is the number of times each fish is aged, X_{ij} is the ith age estimation of the jth fish, X_j is the mean age computed for the jth fish.

2.3 Growth modeling

Relationships between weight and total length were evaluated using a power regression, expressed by $W=a\mathrm{TL}^b$. Differences of the slopes of the W-TL functions between sexes were detected by analysis of covariance (ANCOVA). Prior to analysis, all data were log-transformation (Cazorla and Sidorkewicj, 2008). The allometric index value (b) calculated from the equation was compared to the expected value “3” with a t-test for detecting the allometry of *G. firmispinatus* (Duan and Sun, 1999).

The von Bertalanffy growth function (VBGF) was applied to fit the otolith readings and observed length data of *G. firmispinatus* (von Bertalanffy, 1938). The formula was described as follow:

$$L_t=L_\infty(1-\mathrm{e}^{K(t-t_0)}),$$

where L_t is the length at age t, L_∞ is the theoretical maximum length, K is the growth coefficient, t is the age (a), and t_0 is hypothetical age at length 0. The growth performance index ($Ø$) was computed by the formula: $Ø=\log_{10}K+2\ \log_{10}L_\infty$ (Munro and Pauly, 1983). Differences of growth parameters between *G. firmispinatus* and other *Gymnocypris* species were performed by comparing their $Ø$ calculated in this study and other literature reported.

2.4 Mortality estimation

The mortality rates (Z) were estimated using age-based catch curve analysis (Beverton and Holt, 1957; Chapman and Robson, 1960), where the fish number of each age class was log-transformed and plotted against their corresponding age. Then Z was evaluated from the descending slope (b) of a linear regression fitted to the data of the age frequency distribution.

The natural mortality (M) was evaluated by two empirical equations: Ralston’s (1987) regression method M=0.0189+2.06K and M=-0.0021+2.5912/t_{max} (Zhan et al., 1986), where t_{max} is the maximum age in the catch.

The fishing mortality (F) was calculated by $F=Z–M$.

The exploitation ratio was estimated as:

$$E=F/Z.$$

According to Gulland (1971), the most suitable exploitation ratio for a population should be 0.5, i.e., the fishing mortality of the population should be equal its natural mortality.

2.5 Data analysis

The data are expressed as means±standard deviation (S.D.) and differences were regarded as 0.05 significant level. Analysis and image process were performed using Microsoft Excel 2010, SPSS 16.0, Origin Pro 2016, and Adobe Photoshop cc2015.

3 RESULT

3.1 Size distribution and *W*-TL relationship

The TL ranged 27−242 mm, and W ranged 0.2−148.2 g. Of the 582 *G. firmispinatus* collected, 278 were males with 55–163 mm TL, 270 were females with 58−242 mm TL, and 34 were undetermined specimens with 27−84 mm TL. The

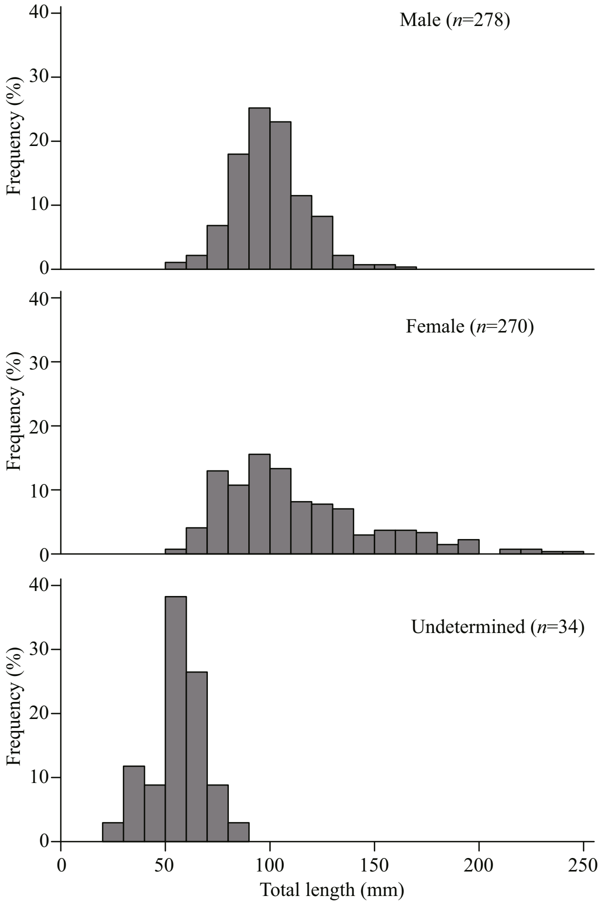

Fig.2 Distributions of the total length frequency of *G. firmispinatus* from July 2015 to June 2016 in the Anning River

proportion of males in the 80−120 mm TL group was substantially higher than that of females, whereas there were almost females in the >160 mm TL group (Fig.2).

The regression functions of W-TL relationships (Fig.3) were expressed as W=1.010×10^{-5}TL$^{2.979}$ (R^2=0.940, n=278) for males, W=7.605×10^{-6}TL$^{3.037}$ (R^2=0.977, n=270) for females and W=8.051×10^{-5}TL$^{3.017}$ (R^2=0.955, n=34) for the undetermined. No significant differences were found for W-TL relationships between males and females (ANCOVA, F=1.810, P>0.05), so the regression equation achieved from all specimens was described as W=7.754×10^{-6}TL$^{3.034}$ (n=582, R^2=0.977). The allometric index value (b) did not significantly differ from 3 (t-test, t=1.758, P>0.05), indicating a tendency for isomeric growth in *G. firmispinatus*.

3.2 Age and growth

Because of otolith fragmentation and unidentifiable annulus deposition, there were 7 otoliths discarded (accounting for 1.2%). Overall, 274 males, 268 females, and 33 undetermined specimens were successfully aged (Fig.4). The APE between 2 readings was 4.58%. The age bias for pairwise age comparisons between 2 readings was provided in Fig.5. The age range observed in all specimens varied from 1 to 13 a (corresponding to 27–242 mm TL). The fish within 1–10 a were very common, accounting

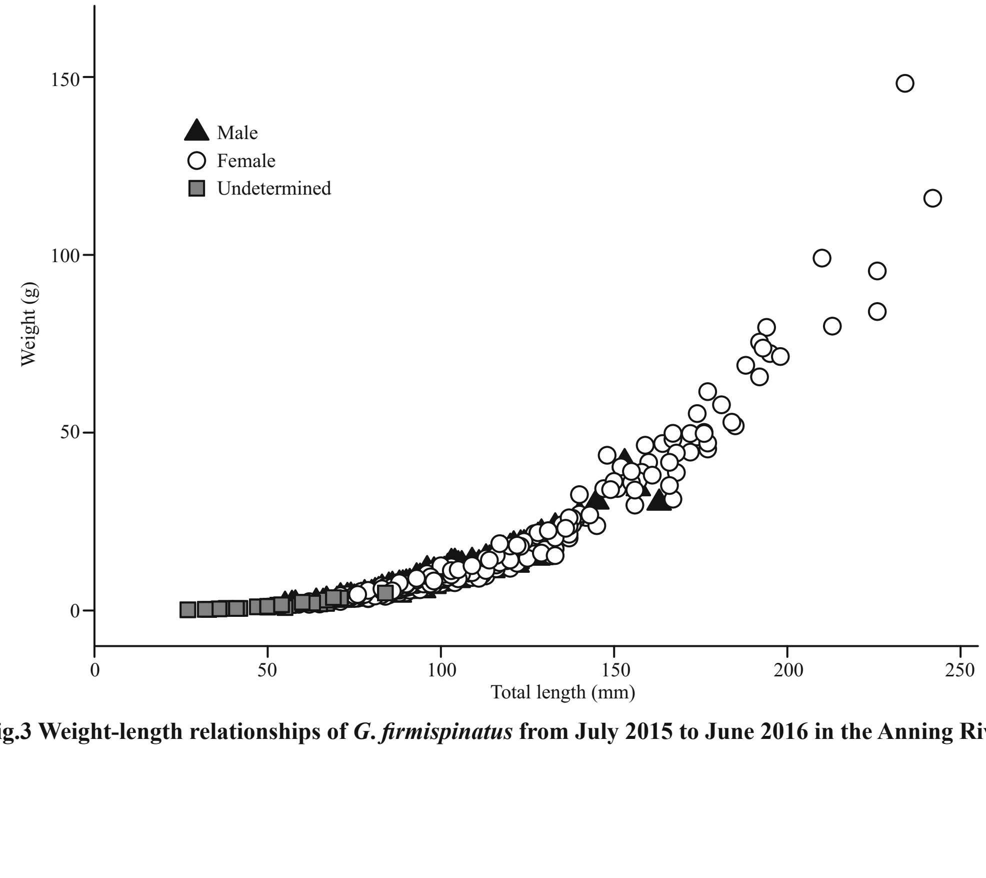

Fig.3 Weight-length relationships of *G. firmispinatus* from July 2015 to June 2016 in the Anning River

Table 1 Number of specimens and mean±S.D. and range of total length at age of *G. firmispinatus*

Age (a)	Male			Female			Undetermined		
	n	Mean±S.D. (mm)	Range (mm)	*n*	Mean±S.D. (mm)	Range (mm)	*n*	Mean±S.D. (mm)	Range (mm)
1	3	59.0±4.6	55–64	4	67.8±8.5	59–76	19	48.2±8.7	32–61
2	39	80.4±9.9	57–99	28	73.3±8.8	58–94	14	65.9±7.7	53–84
3	74	91.8±10.5	71–131	65	88.3±11.8	71–116			
4	73	100.9±10.2	81–129	64	105.1±13.5	80–139			
5	49	108.0±11.0	91–129	29	118.2±19.3	86–167			
6	23	113.4±10.9	98–132	26	129.6±22.9	93–177			
7	7	128.4±12.3	113–145	24	148.0±19.9	116–195			
8	3	147.3±13.4	132–157	10	170.7±14.1	150–198			
9	3	140.0±20.4	124–163	5	167.4±12.0	148–177			
10				6	197.2±12.1	181–213			
11				3	199.7±22.9	185–226			
12				2	234.0±11.3	226–242			
13				2	213.0±29.7	192–234			
Total	274		55–163	268		58–242	33		32–84

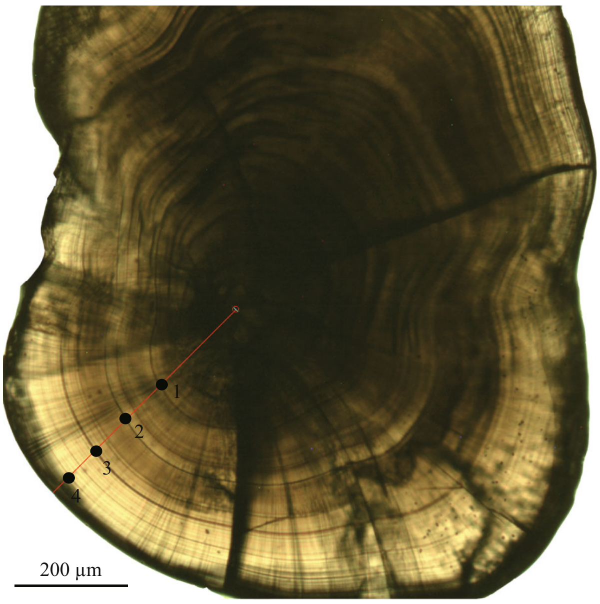

Fig.4 Sectioned lapillus under a compound microscope with transmitted light, from a 4-a-old *G. firmispinatus* with 120 mm TL

Dots indicate annuli.

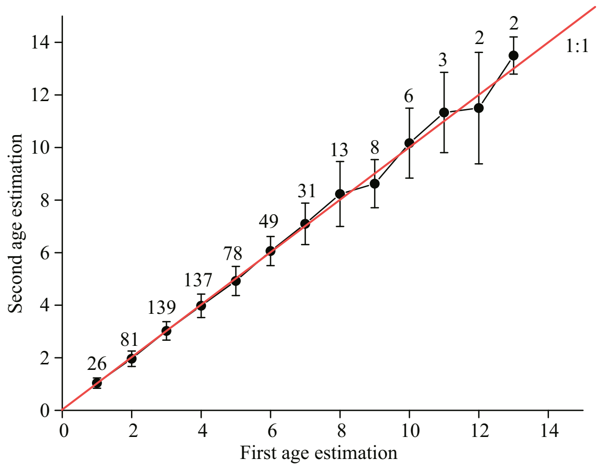

Fig.5 Age bias plot for pairwise age comparisons between two readings

for 97.6%. The maximum ages of males and females were 9 a (140 mm TL) and 13 a (213 mm TL), respectively (Fig.6).

The mean length-at-age (±S.D.) values derived from otolith observations, classified as males, females, and the undetermined, are given in Table 1. For age class 1, the mean lengths-at-age had no significant difference between sexes (unpaired *t*-test, $P>0.05$); while in age classes 2–9, the mean lengths-at-age had significant differences between sexes (unpaired *t*-test, $P<0.05$). The von Bertalanffy growth models fitted to the observed length and age data were provided as follows (Fig.7):

♂: $L_t=198.6(1-e^{-0.094(t+3.497)})$ ($R^2=0.697$, $n=274$),

♀: $L_t=289.3(1-e^{-0.089(t+1.109)})$ ($R^2=0.806$, $n=268$).

Growth parameters suggested that males grew at a relatively faster rate than females. The growth performances (Ø) of male and female *G. firmispinatus* were 3.569 1 and 3.872 1, respectively.

3.3 Mortality estimation

The estimates of *Z* from the descending slope of

Table 2 Comparison of growth characters of *Gymnocypris* fishes in different studies

Species	Location	Age structure	Sex	SL range	Age range	n	Growth parameters				Sources
							L_∞ (mm)	K	t_0	$Ø$	
G. eckloni	Szechwan Province	Anal scale	♀	-	1–12	-	565.2	0.093 7	-0.645 2	4.476 1	Tsao and Wu, 1962
G. selincuoensis	Selin Lake	Otolith	♂	34–405	1–26	121	484.2	0.068 4	0.602 8	4.205 1	Chen et al., 2002
			♀	34–430	1–29	138	485.3	0.071 0	0.567 9	4.223 3	
G. przewalskii	Qinghai Lake	Anal scale	Total	70–348	1–14	-	1103.2	0.028 7	-0.018 9	4.543 2	Zhao et al., 1975
G. przewalskii	Qinghai Lake	Otolith	♂	100–298	4–10	298	682.9	0.053 0	-0.424 0	4.393 0	Chen et al., 2006
			♀	120–300	4–11	260	551.9	0.071 1	-0.304 4	4.335 0	
G. firmispinatus	Anning River	Otolith	♂	55–163 (TL)	1–9	274	198.6	0.094 0	-3.497 0	3.569 1	Present study
			♀	58–242 (TL)	1–13	268	289.3	0.089 0	-1.109 0	3.872 1	

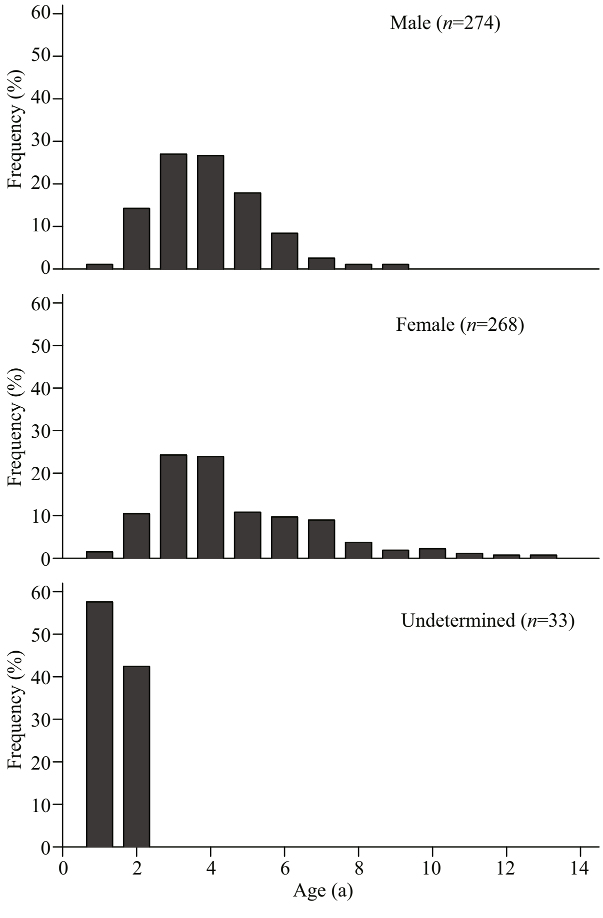

Fig.6 Age frequency composition of *G. firmispinatus* from July 2015 to June 2016 in the Anning River

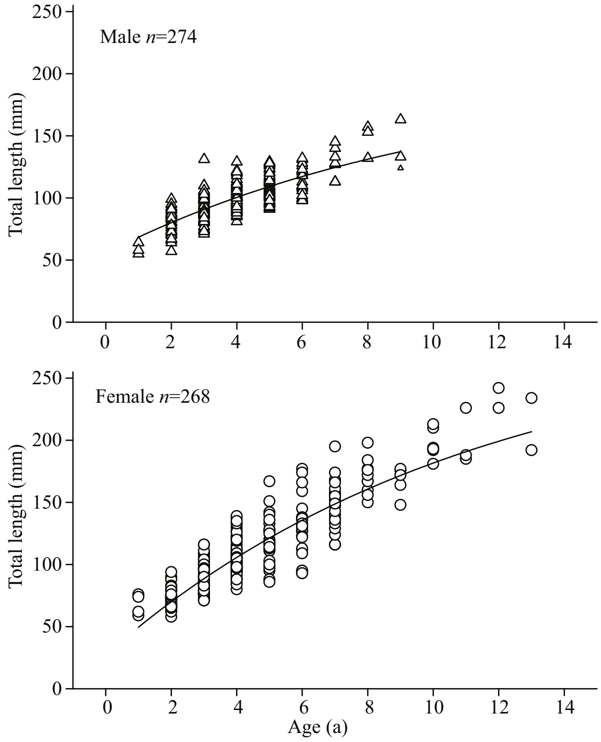

Fig.7 Von Bertalanffy growth curve fitted to total length-at-age for *G. firmispinatus* from samples captured

the catch curve were 0.73/a for males and 0.40/a for females (Fig.8). The values of M evaluated from the equation of Ralston and Zhang were 0.21 and 0.29/a for males and 0.20 and 0.20/a for females. Therefore, M was assumed to be 0.25/a for males and 0.20/a for females, the corresponding F was estimated as 0.48/a for males and 0.20/a for females. The exploitation ratio was 0.66/a for males and 0.50/a for females, respectively.

4 DISCUSSION

In this study, our results showed that the maximum ages estimated for *G. firmispinatus* were 9 a for males and 13 a for females, near with *Gymnocypris przewalskii* and *Gymnocypris eckloni*, but smaller than *Gymnocypris selincuoensis* (Table 2). When compared to other genus of Schizothoracinae fishes, *G. firmispinatus* is a shorter-lived fish [♂: 37, ♀: 40 for *Schizothorax waltoni* (Zhou et al., 2017), ♂: 24, ♀: 45 for *Ptychobarbus dipogon* (Li and Chen, 2009), ♂: 40, ♀: 50 for *Schizothorax oconnori* (Ma et al., 2010)] based on otolith observations. These long-lived Schizothoracinae fishes only inhabit in the

Yarlung Zangbo River, Tibet, where fishes were preserved relatively well due to religious sentiments. Nevertheless, the fishing intensity in the Anning River was much heavier than that in the Tibetan rivers. Thus, the exploitation level might be also a reason affecting the ecological longevity of the fish.

According to the length range of all catches, the asymptotic length (L_∞) of *G. firmispinatus* was not attained, and L_∞ for females was much higher than that of males. In addition, the results indicated that males grew smaller than females, while the *K* value for males (0.094) was slightly higher than that of females (0.089). *G. firmispinatus* grows slowly in a pattern that is shared with other *Gymnocypris* fishes (Table 2); this genus has *K* values ranging from 0.03 to 0.09. The L_∞ of *G. firmispinatus* obtained in this study was the smallest, while *k* values derived here were higher than other congeneric fishes (except for *G. eckloni*). The growth performance indices (*Ø*) of the fish were smaller than those of other congeneric fishes (Table 2), revealing that the growth of *G. firmispinatus* is relatively slower than others. The growth of *G. firmispinatus* might be affected by many factors such as food deficiency and environmental conditions in the small tributaries of the Anning River (Beamish and McFarlane, 1983).

Based on empirical evidence from comparative life history information, many methods have been reported in the last 70 a to evaluate the natural mortality rate (*M*) of a stock (Then et al., 2014). In the present study, we chose two methods to avoid the one-sided scheme of a single t_{max}-based estimator or single growth-based estimator. The exploitation ratio of *G. firmispinatus* was 0.66/a for males and 0.50/a for females, respectively. Therefore, the *G. firmispinatus* population may be over-exploited under the current harvesting strategy. The exploitation ratio of the species was similar with *Oxygymnocypris stewarti* in the Yarlung Zangbo River (Huo et al., 2015), but higher than those of *P. dipogon* in the Lhasa River (Li and Chen, 2009). At present, many Schizothoracinae fishes have been seriously threatened owing to overfishing, damming and biological invasions. Therefore, scientist and fishery department have both paid great attentions to the conservation of their natural populations (Huo et al., 2015).

Many researchers found that the anthropogenic activities mainly contributed to the declined population of *G. firmispinatus* in the Anning River (Wang, 2008; Ru et al., 2016). It seems that the species

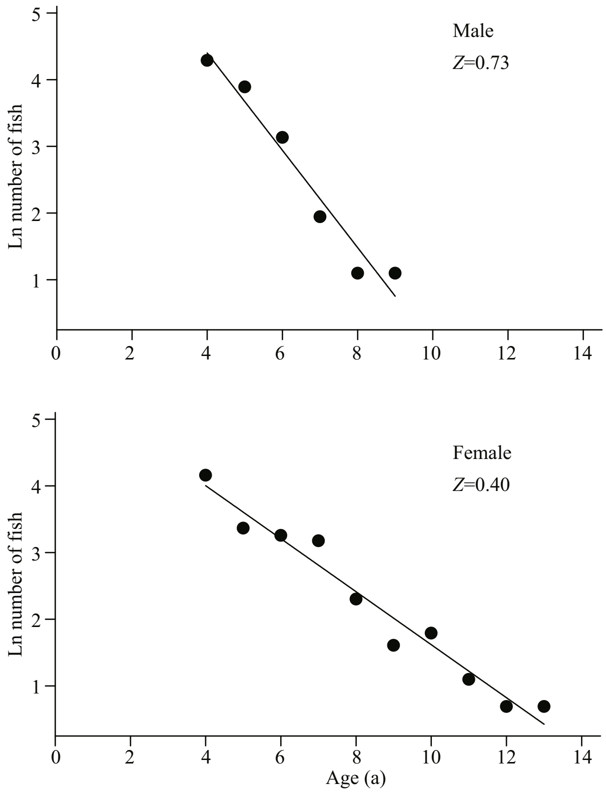

Fig.8 Catch-curve based on observed age for *G. firmispinatus* samples captured between July 2015 and June 2016

Ages 1–3 were excluded (outliers), because they were considered as not fully recruited to the electro-fishing gear.

mostly inhabited in the tributaries, rather than mainstream, because of the river fragmentation induced by the construction of hydropower stations at the mainstream. Furthermore, the populations of slow-growing fish (such as *G. firmispinatus*) would be difficult to be restored if they were expired (Musick, 1999). It is therefore essential to establish effective management strategies for *G. firmispinatus* focused on a tolerable fishing intensity and prohibiting fishing during the spawning season, to allow for its sustainability. Specifically, some small-size hydropower stations in the mainstream of the Anning River should be reduced as much as possible, to insure more suitable habitats for *G. firmispinatus*.

5 CONCLUSION

It was concluded that *G. firmispinatus* seem to be a small-size fish with relatively shorter longevity, when compared to other Schizothoracinae fishes. The growth of the fish is relatively slower than other congeneric fishes. Our results also indicated that the population of *G. firmispinatus* might be in over-exploitation under the current harvesting strategy.

6 DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are available within the article.

7 ACKNOWLEDGEMENT

We gratefully acknowledge LIANG Meng for help with the collection of the survey data. The authors also thank Prof. XIE Songguang (Institute of Hydrobiology, Chinese Academy of Sciences) for providing the otolith image analysis system.

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