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Age and growth characteristics of *Schizopygopsis younghusbandi* Regan, 1905 in the Yarlung Tsangpo River in Tibet, China

By Y.-J. Duan[1], C.-X. Xie[1], X.-J. Zhou[1], B.-S. Ma[2] and B. Huo[1]

[1]*College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, China;* [2]*Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan, China*

Summary

To better understand the biology of *Schizopygopsis younghusbandi* Regan, 1905 and its relationship with management considerations, this study describes the relationships between otolith size and fish length and age, verifies annual periodicity of otolith annulus formation, and estimates the *S. younghusbandi* growth parameters. These age and growth characteristics were studied using 694 specimens collected from August 2008 to August 2009. Otoliths grew asymmetrically throughout the range of standard length (SL) studied, showing a clear pattern of alternating translucent and opaque bands. Marginal increment ration (MIR) analysis of specimens up to 6 years of age indicated that one opaque band and one translucent band were laid down each year. Maximum observed age was 17 years, corresponding to a female of 35.8 cm SL and 589.1 g body weight (BW). The *SL-BW* relationship was described as $BW = 1.122 \times 10^{-5}\ SL^{3.030}$ for sexes combined. The von Bertalanffy growth function was used to model back-calculated lengths as $L_t = 338.4\ (1{-}e^{-0.233\ (t\ +\ 0.403)})$ for males, and $L_t = 433.9\ (1{-}e^{-0.194\ (t\ +\ 0.397)})$ for females. Growth performance of *S. younghusbandi* was relatively higher than those of other *Schizothoracinaes* inhabiting the same river.

Introduction

The Yarlung Tsangpo River, the largest river in Tibet, flows west to east and begins at the highest altitude of any river in the world. The unique geographical environment of this river results in the presence of many endemic fishes, with those of the genus *Schizothoracinae* the most abundant. These species exhibit slow growth, low fecundity and late sexual maturation as an adaptation to their rigorous environment (Chen and Cao, 2000). These life-history characteristics make them especially vulnerable to intense exploitation (Buxton, 1993). Before the 1950s, fishes in this area were protected because of religious sentiments. Traditionally, Tibetans did not eat fish, which are the embodiment of the dragon god in Tibetan Buddhism. However, subsequent immigration from the inland areas caused lifestyle changes and resulted in the growth of fishing, placing endemic fishes in a dangerous situation. Since the 1990s, native fish species have been threatened by the invasion of *Cyprinus carpio, Carassius cuvieri* and *Pseudorasbora parva*. The conservation of natural populations of Schizothoracinae fishes has become of increasing concern.

Schizopygopsis younghusbandi, belonging to the subfamily Schizothoracinae, family Cyprinidae, is an endemic species found only in the middle reaches of the Yarlung Tsangpo River, and is one of the most important commercial fishes in this area (Bureau of Aquatic Products, 1995). Despite the limited distribution of *S. younghusbandi* and its importance to the fishery, little is known about its biology and ecology. At present, development of a management plan is difficult because of a lack of adequate biological data, especially accurate age and growth information. The rigorous geographical environment and traditional native customs generate challenges to research, in particularly when obtaining samples of small, young specimens. Chen et al. (2009) estimated the age and growth of *S. younghusbandi* by using otoliths and scales. However, because of the difficulties in collecting a sample of smaller specimens, the minimum observed age was 3 years. The youngest and oldest fish are often the most difficult to age accurately, but are most influential in estimates of growth, mortality or longevity (Campana, 2001). Accurate estimates of age and growth are prerequisites for understanding population dynamics and setting sustainable harvest limits in fisheries (Campana and Thorrold, 2001). From 2008 to 2009, a study was initiated to better define the biological characteristics of the *S. younghusbandi* captured by the commercial fisheries of the Yarlung Tsangpo River.

Materials and methods

Collection of samples

Floating gillnets (mesh size 7.5 cm), bottom gillnets (mesh size 6.5 cm), and trap nets (mesh size 1.5 mm) were used to collect *S. younghusbandi* from the Yarlung Tsangpo River from August 2008 to August 2009 (Fig. 1). During the study samples were collected once a month for a total of 694 fish. Standard length (SL) and body weight (BW) of each specimen were measured to the nearest 1 mm and 0.1 g, respectively. Specimens were macroscopically classified as male, female, or undetermined. Lapillus otoliths were extracted from each fish, washed with 95% ethanol, dried, labeled, and stored in plastic tubes.

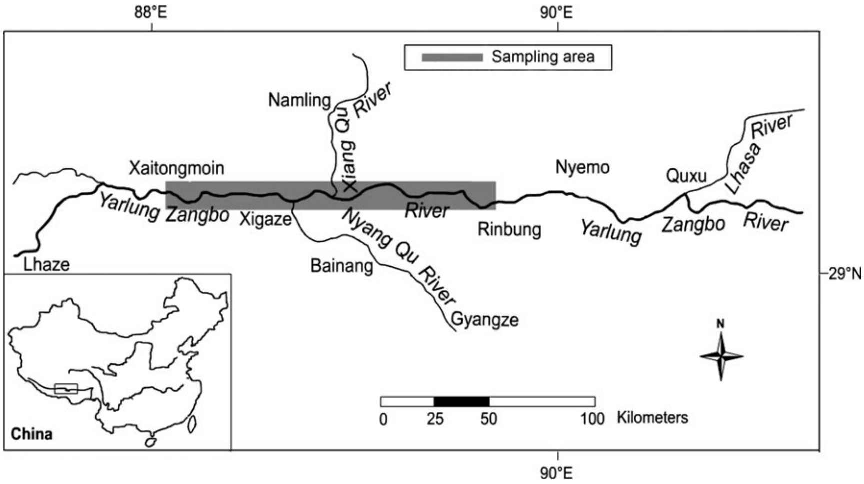

Fig. 1. Sampling locations of *Schizopygopsis younghusbandi*, Yarlung Tsangpo River, 2008–2009

Otolith preparation

To determine the relationship between otolith size and fish length and age, the length and weight of the left lapillus of 597 fish were measured. Otolith length (OL) was measured along a common axis using IMAGE-PRO PLUS software (version 6.0; Media Cybernetics) after taking photos with a LEICA APPLICATION SUITE (version 15; Leica, Germany) under a dissecting microscope linked to a CCD video camera (La Mesa et al., 2009). Otolith weight (OW) was measured to the nearest 0.1 mg with an electronic balance, after being dried over 24 h at 60°C to constant weight. Relationships between these metric sizes and fish lengths were fitted using a regression analysis. The otoliths were ground and polished following Ma et al. (2010).

Age estimation

For *S. younghusbandi*, sagittal otoliths were acicular and frangible, the asteriscus otoliths were flaky, and only the lapillus otoliths with a clear pattern of alternating zones could be used for age determination. Each fish was assigned to an age-class assuming 1 March as the designated birth date, approximately corresponding to the peak spawning season. A new ring mark found on the otolith of a fish captured before 1 March was not considered to be an annulus in the age assignment, whereas when a fish sampled after the assumed birth date had no new ring mark, an annulus that was supposed to have formed was considered in the age estimation (Granada et al., 2004).

All readings were made without reference to the length or weight of the fish, its date of capture or to the previous reading. Opaque increments were counted, the increments appearing as dark in transmitted light. Annulus counts were performed twice; if they differed, a third count was made. The aging precision between the two readings was measured by calculating the average percentage error (IAPE). The equation is expressed as:

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

Where N is the number of fish aged, R is the number of times each fish is aged, X_{ij} is the ith age determination of the jth fish, and X_j is the mean age calculated for the jth fish.

Growth

A marginal increment ratio (MIR) analysis was used to verify the period of opaque band formation in the otoliths. The marginal increment (MI, 0.01 mm) was measured as the distance from the inner margin of the outermost translucent ring and the periphery of each otolith. Measurements were always made along the longest axis of the otolith. Monthly variations of the MIR (1–6 annuli) were established using the equation: MIR = $(R-R_n)/(R_n-R_{n-1})$, where R is the otolith radius, R_n is the distance from the focus to the outer edge of the last annulus formed, and R_{n-1} is the distance from the focus to the outer edge of the penultimate complete annulus (Haas and Recksiek, 1995).

The relationship between weight and standard length was described by $BW = a\ SL^b$, where a, b are parameters. Variations in the *SL-BW* relationships between males and females were analyzed through an analysis of covariance after log-transformation (Cazorla and Sidorkewicj, 2008). The allometric index value (b) obtained was compared to the expected value by using a t-test for allometry (Pauly, 1984).

The function used for back-calculation was: $L = a + bR$, where L is the standard fish length, a is the intercept, b is the slope, and R is the radius of the otolith. The radius and the distances between adjacent annuli of each otolith section on the photos were measured in a consistent direction and then converted to the factual lengths. The traditional von Bertalanffy growth function (Von Bertalanffy, 1938) was used to fit the back-calculated lengths of *S. younghusbandi:* $L_t = L_\infty (1 - e^{-k(t-t_0)})$, where L_t is the expected length at age t years, L_∞ is the asymptotic maximum length, k is the von Bertalanffy growth constant, and t_0 is the theoretical age at zero length. Growth curves were fitted separately to males and females. Growth performance indices ($Ø = log10\ k + 2log10\ L_\infty$) were calculated to compare results obtained in this study with those published elsewhere (Munro and Pauly, 1983).

Data analysis

Analysis was carried out using SPSS 16.0 (Chicago, IL), ORIGINPRO 8.0 (Originlab, Northampton, MA), Microsoft Excel 2003 (Redmond, WA), and Photoshop CS4 Extended (Adobe, San Jose, CA). Statistical significance was accepted when $P < 0.05$.

Results

Length-frequency distributions

Of the 694 *S. younghusbandi* sampled, 442 were females at 74–423 mm SL, 164 were males at 78–337 mm SL, and 88 were indeterminate at 26–251 mm SL (Fig. 2). Captured fish were mainly 150–350 mm SL (66.7%).

Relationships between otolith size and fish length and age

Otolith length was linearly related to fish length (Fig. 3a), $R^2 = 0.918$), while a curvilinear relation was detected between the otolith weight and fish length (Fig. 3b), power function fit $R^2 = 0.909$). This goodness of fit was high, despite variation in the otolith weight–length relationship in fish over 200 mm SL.

Otolith weight and fish age also show a curvilinear relationship (Fig. 4, logarithmic function fit $R^2 = 0.664$). It is clear that otoliths continue to increase in weight throughout life, although they grow more slowly from 8 years of age when the otolith is 10 mg or more in mass.

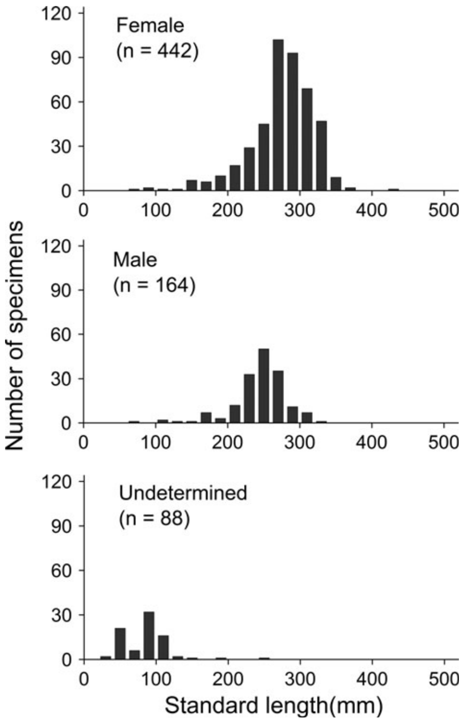

Fig. 2. Distributions of standard length frequency of *Schizopygopsis younghusbandi*, Yarlung Tsangpo River, 2008–2009

Annual periodicity

Otoliths showed a regular pattern with alternated opaque and translucent bands (Fig. 5). Monthly changes of the MIR increased gradually from May to January, and appeared to peak at 0.716 in January. Subsequently, the MIR kept a lower level between March and May, with a minimum of 0.339 in April (Fig. 6). These results indicated that the opaque band of the otoliths was laid down once a year, from March to May.

Age structure

Of the 694 otoliths examined, only 10 (approximately 1.4%) were discarded. The reliability of the age estimates had low IAPE (0.8%), reflecting concordance among aging attempts. Estimates of age were made from the otoliths of 694 fish that ranged from 26 to 423 cm. There were 17 age classes for females and 12 for males. The oldest fish was not the largest fish. The sample was dominated by specimens aged 4–7 years (74.93%), with only five fish estimated to be >10 years old. Mean and standard deviations of the standard length at age for age classes 1–17 are given in Table 1.

SL-BW relationships

SL-BW relationships were calculated separately for males, females, and undetermined (Fig. 7). The regression equations were described as $BW = 1.187 \times 10^{-5}\ SL^{3.023}$ ($R^2 = 0.963$, n = 164) for males; $BW = 2.052 \times 10^{-5}\ SL^{2.923}$ ($R^2 = 0.955$, n = 442) for females; and $BW = 2.045 \times 10^{-5}\ SL^{2.885}$ ($R^2 = 0.988$, n = 88) for undetermined. No significant differences were found in SL-BW relationships between sexes (ANCOVA, n = 694, $P > 0.05$). Therefore, the regression equation fitted by pooled data was: $BW = 1.122 \times 10^{-5}\ SL^{3.030}$ ($R^2 = 0.992$, n = 694). The allometric index value (b) obtained from the function significantly differed from 3 (t-test, $t = 2.3376$, $P < 0.05$). Therefore, *SL-BW* relationships indicate a tendency for allometric growth in *S. younghusbandi*.

Growth

The mean back-calculated length did not differ significantly between sexes for age classes 2–3 (unpaired t-test, all $P > 0.05$). The back-calculated length data of indeterminate specimens (except for one 5-year-old individual) were included in the von Bertalanffy models for both sexes. Sample sizes beyond 10 years of age of each sex were negligibly small. Back-calculated lengths for males and females are given in Tables 2 and 3, respectively. The von Bertalanffy growth function fitted to the back-calculated lengths are: $L_t = 338.4\ (1-e^{-0.233\ (t\ +\ 0.403)})$ for males, and $L_t = 433.9\ (1-e^{-0.194\ (t\ +\ 0.397)})$ for females (Fig. 8). Growth performances indices ($\emptyset$) of *S. younghusbandi* were 4.4260 for males and 4.5616 for females.

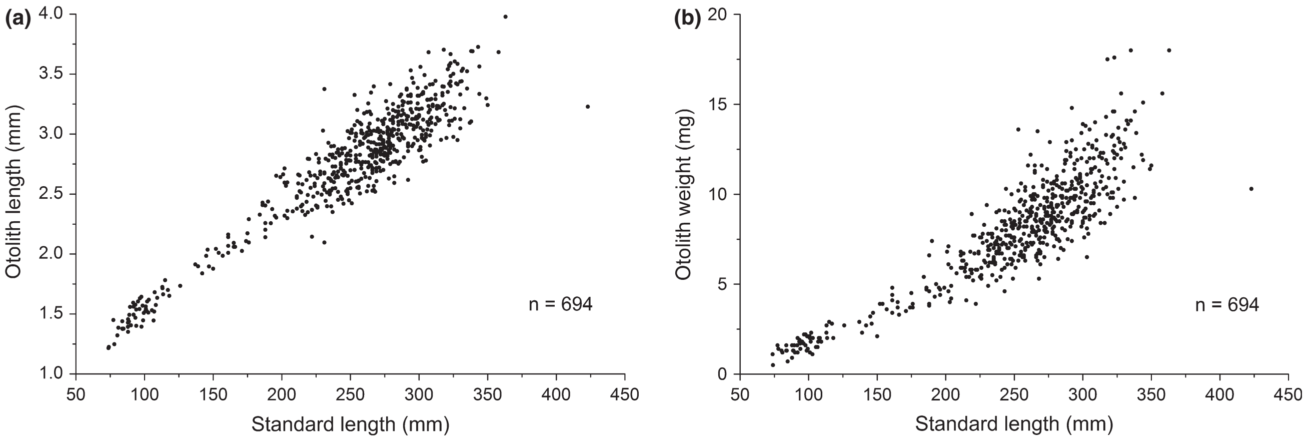

Fig. 3. (a) *Schizopygopsis younghusbandi* otolith length vs standard length, Yarlung Tsangpo River, 2008–2009. (b) *Schizopygopsis younghusbandi* otolith weight vs standard length, Yarlung Tsangpo River, 2008–2009

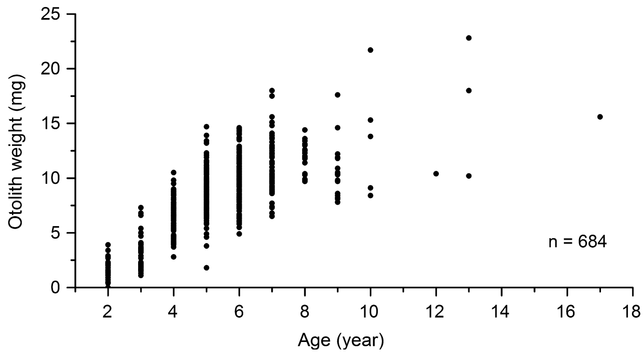

Fig. 4. *Schizopygopsis younghusbandi* otolith weight vs age determined from samples captured in Yarlong Tsangpo River, 2008–2009

Fig. 5. Lapillus of *Schizopygopsis younghusbandi*, captured in the Yarlong Tsangpo River, 2008–2009, estimated as age 16 under transmitted light using a microscope. Dots = annuli. Scale bars: 200 μm

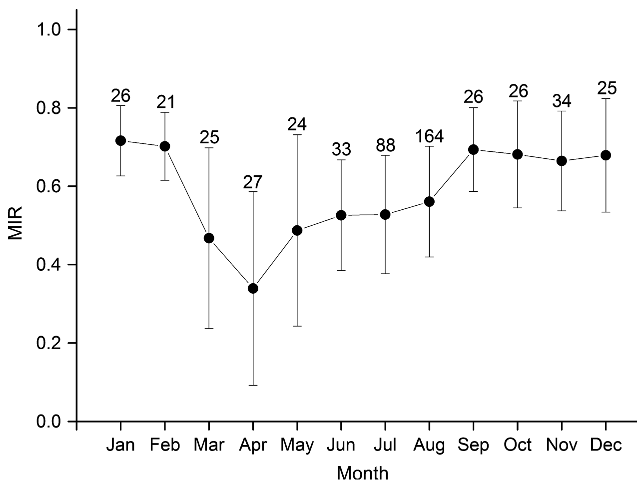

Fig. 6. Mean monthly marginal increment ratio for *Schizopygopsis younghusbandi* otoliths with 1–6 annuli determined from samples captured in the Yarlong Tsangpo River, 2008–2009. Numbers at data points = number of samples; error bars = standard deviation

Discussion

Otoliths show asymmetrical growth through time, but nevertheless grow throughout the life of a fish (Fowler, 1990). Similarly, *S. younghusbandi* otoliths grew symmetrically along the different axes and the relationship between otolith size and fish length was highly correlated, but the growth in OL and OW slowed as fish gained a few years in age. Experiments have shown that models based on the otolith-to-fish size relationship can predict the age for groups of fish. Pawson (1990) examined the relationship between otolith weight and fish length in samples of sardines, and the measurements were used to determine age. However, in the present study the variability of OW in *S. younghusbandi* decreased significantly beyond the age of 8 years. Thus, OW is an imprecise predictor of age in *S. younghusbandi*.

Evidence of the annual band formation is an integral component of age and growth studies using otoliths to assess age. The processes controlling otolith growth are still not completely understood. Annulus formation is associated with a variety of factors, including seasonal variations in water temperature, photoperiod, feeding, and reproduction (Beckman and Wilson, 1995; Morales-Nin, 2000; Tserpes and Tsimenides, 2001). The annual periodicity of increment formation in *Schizothoracinae* otoliths has been described and discussed in Ma et al. (2010) and Huo et al. (2012), where

Table 1
Number of specimens, mean ± standard deviation, and standard length range by age, *Schizopygopsis younghusbandi*, Yarlung Tsangpo River, 2008–2009

	Male			Female			Undetermined		
Age (in years)	N	Mean ± SD (mm)	Range (mm)	n	Mean ± SD (mm)	Range (mm)	n	Mean ± SD (mm)	Range (mm)
1							3	28.6 ± 2.2	26–31
2	3	103.3 ± 22.0	78–117	6	106.3 ± 26.4	74–147	63	76.5 ± 26.0	34–152
3	7	162.0 ± 12.4	137–176	14	174.0 ± 26.6	142–236	21	104.1 ± 23.5	80–188
4	33	224.2 ± 25.3	161–266	81	244.2 ± 32.7	146–306			
5	52	249.8 ± 19.3	204–300	134	272.8 ± 29.7	188–350	1	251.0	251
6	39	259.1 ± 24.2	228–337	115	292.8 ± 23.4	223–349			
7	16	258.3 ± 16.2	230–295	50	303.8 ± 23.1	201–344			
8	4	288.5 ± 27.1	262–317	15	314.3 ± 20.2	287–339			
9	6	285.3 ± 20.6	263–313	11	330.1 ± 36.3	295–423			
10	2	280.5 ± 30.4	259–302	3	332.0 ± 30.2	300–360			
11									
12	1	264.0	264						
13				3	342.7 ± 30.2	308–363			
14									
15									
16									
17				1	358.0	358			

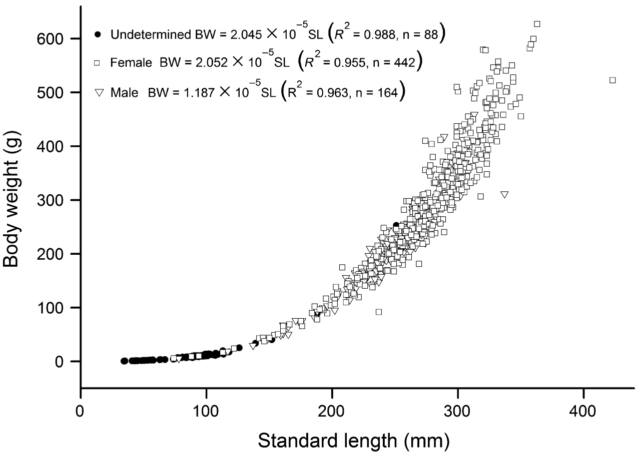

Fig. 7. Relationship between *Schizopygopsis younghusbandi* standard length and body weight, Yarlong Tsangpo River, 2008–2009

they concluded that translucent bands were formed once per year throughout the life of Schizothoracinae. For *S. younghusbandi,* marginal increment analysis demonstrates that one annulus – consisting of one opaque band and one translucent band – is formed annually. Spring, when fish are growing rapidly, is the time of opaque band formation; winter is the time of translucent band formation when fish are growing slowly. From February to June the differences in average water temperature is high, up to 13°C around the Xigaze (Li et al., 2010). The abrupt fluctuation in water temperature could change the metabolic activities in *S. younghusbandi* and possibly be associated with the formation of an opaque band. From November to February, water temperature is below 5°C, with ice floating in the Yarlung Tsangpo River in December and January. *S. younghusbandi* often hide inside crevices or underneath boulders and rarely leave these areas to feed. Formation of the translucent band may be attributed to the cold temperature and food deprivation.

Comparing results of the growth of *S. younghusbandi* with those of Chen et al. (2009), the growth performance index (Ø) value obtained in our study was larger (Table 4). Differences among all estimated parameters could be attributed to several factors: (i) different sampling locations, (ii) different types of sampling gear, and (iii) difference in age groups applied to fit the growth function. Moreover, the growth

Table 2
Back-calculated standard length, male *Schizopygopsis younghusbandi*, Yarlung Tsangpo River, 2008–2009

Age (in years)	Number	L1 (mm)	L2 (mm)	L3 (mm)	L4 (mm)	L5 (mm)	L6 (mm)	L7 (mm)	L8 (mm)	L9 (mm)
2	54	49.23								
3	28	51.93	101.89							
4	33	50.50	110.37	164.56						
5	52	48.09	105.85	161.34	209.53					
6	39	45.11	100.42	154.03	199.96	234.04				
7	16	43.25	95.70	143.16	187.16	223.94	253.99			
8	4	42.37	87.90	134.74	180.3	217.09	245.12	269.78		
9	6	35.99	79.89	126.25	164.67	196.45	227.99	256.68	295.43	
10	2	40.76	88.74	131.58	169.90	207.65	234.89	254.56	274.78	292.31
Weighted average (mm)		47.85	102.53	155.77	199.48	226.46	245.79	260.69	290.27	292.31

Table 3
Back-calculated standard length, female *Schizopygopsis younghusbandi*, Yarlung Tsangpo River, 2008–2009

Age (in years)	Number	L1 (mm)	L2 (mm)	L3 (mm)	L4 (mm)	L5 (mm)	L6 (mm)	L7 (mm)	L8 (mm)	L9 (mm)
2	57	54.21								
3	35	57.2	114.18							
4	81	54.82	119.26	178.82						
5	134	52.22	115.74	176.17	227.44					
6	115	49.67	111.23	171.31	224.82	267.61				
7	50	45.56	102.9	159.91	213.31	259.31	295.99			
8	15	46.50	98.97	151.58	198.32	240.85	277.75	305.33		
9	11	42.64	95.66	149.99	200.18	242.93	281.00	314.79	342.77	
10	3	44.68	100.46	160.20	207.99	245.64	278.04	308.45	331.52	347.88
Weighted average (mm)		51.54	112.48	171.62	221.94	261.66	289.76	306.1	340.35	347.88

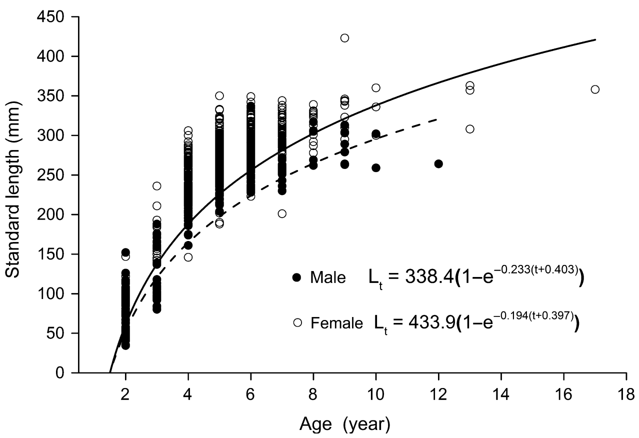

Fig. 8. Relationship between *Schizopygopsis younghusbandi* standard length and age determined from samples captured in Yarlong Tsangpo River, 2008–2009

performance index (Ø) of *S. younghusbandi* was larger than those of *Schizothorax o'connori*, while smaller than *O. stewartii,* indicating that the growth rate of *S. younghusbandi* was faster than that of *S. o'connori* and slower than that of *O. stewartii* (Table 4). These phenomena might be related to dietary variations in that omnivorous *S. younghusbandi* might have less energy than piscivorous *O. stewartii*, yet have more energy than herbivorous *S. o'connori*.

Cailliet and Goldman (2004) reported that growth model estimates are greatly affected by the lack of very young individuals. Due to the absence of small fishes in the samples, the t_0 values of the VBGM obtained for *Polyprion* species were strongly negative: −12.48 and −16.56 years for the USA wreckfish *P. americanus* (Vaughan et al., 2000) and −4.06 to −5.75 for New Zealand hapuku (Francis et al., 1999). For the present study, juveniles collected from traps provided a meaningful estimate of the t_0, which was less negative.

Schizopygopsis younghusbandi have been listed as data deficient by the International Union for the Conservation of Nature (IUCN), however, they are known to be exploited at an increasing rate. We found that the low water levels during the spawning season from March to June resulted in fishermen easily catching the spawning fish and affecting the recruitment rates. Slow-growing, long-lived fishes tend to be particularly vulnerable to excessive exploitation and exhibit rapid stock collapse, after which the population turnover may be lower than expected, with slower than predicted responses to rehabilitation measures (Musick, 1999). Therefore, it is essential to establish sound management strategies for this species to allow its sustainability. Specifically, more scientific work should be rigorously conducted to collect fundamental biological data. According to these data, new fishery regulations that focus on the sustainable fishing effort, mesh size limit, closure of fishing and proper methods for prevention of overfishing, should be established; simultaneously, monitoring mechanisms for these stocks should be

Table 4
Growth characteristic comparisons of Schizothoracinae fishes

Species	Region	Age material	Sex	L_∞(mm)	k (year^{-1})	t_0	Ø	Sources
Schizothorax o'connori	Yarlung Tsangpo River	Otolith	F	492.4	0.1133	−0.5432	4.4389	Yao et al. (2009)
			M	449.0	0.1260	−0.4746	4.4049	
Oxygymnocypris stewartii	Yarlung Tsangpo River	Otolith	F	618.2	0.106	0.315	4.6076	Huo et al. (2012)
			M	526.8	0.141	0.491	4.5926	
Schizothorax waltoni	Yarlung Tsangpo River	Otolith	F	691.1	0.056	−2.466	4.4273	Qiu and Chen (2009)
			M	689.8	0.051	−3.257	4.3850	
Schizothorax younghusbandi	Yarlung Tsangpo River	Otolith	F	471.4	0.0789	0.2	4.2439	Chen et al. (2009)
	Lhasa River		M	442.7	0.0738	−1.4	4.1603	
	Yarlung Tsangpo River	Otolith	F	433.9	0.194	−0.397	4.5616	Present study
			M	338.4	0.233	−0.403	4.4260	

built in order to assess the effectiveness of these new regulations. In addition, local governments should properly guild local customs such as 'Release Day' in order to control biological invasions that lead to the competition between native and alien species.

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Author's address: Cong-Xin Xie, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei 430070, China.
E-mail: xiecongxin@mail.hzau.edu.cn