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Age and Growth of a Long-Lived Fish *Schizothorax o'connori* in the Yarlung Tsangpo River, Tibet

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Bao-Shan Ma, Cong-Xin Xie, Bin Huo, Xue-Feng Yang, and Hai-Ping Huang (2010) Age and growth of a long-lived fish *Schizothorax o'connori* in the Yarlung Tsangpo River, Tibet. *Zoological Studies* **49**(6): 749-759. In total, 1126 individuals of *Schizothorax o'connori* were sampled during Aug. 2008 to Aug. 2009, from the Yarlung Tsangpo River, Tibet, and 2 tributaries to estimate the age and growth by an otolith analysis. The standard length (*SL*) ranged 33-553 mm and the body weight (*W*) ranged 4.3-2982.6 g. The *SL*-*W* relationship was described as $W = 2.034 \times 10^{-5} SL^{2.940}$ for the combined sexes. Furthermore, relationships of otolith dimensions (length, breadth, thickness, and weight) and fish length/age were described. Otoliths continually grew in size with *SL*, but growth became asymmetrical over time. Otolith weight was the only variable which synchronously increased with age. The index of the average percentage for age estimation of 2 independent readers was 3.24%, and 3.3% of otolith sections were considered illegible. The maximum observed ages were 40 yr for males and 50 yr for females. The mean length-at-age had no significant difference between sexes up to age 8 yr, while females attained larger lengths-at-age than males thereafter. The von Bertalanffy function was used to model the observed length-at-age data as $L_t = 499.7\ (1 - e^{-0.095\ (t + 0.896)})$ for males and $L_t = 576.9\ (1 - e^{-0.081\ (t + 0.946)})$ for females. Females grew at a slower rate but attained a larger size than males. http://zoolstud.sinica.edu.tw/Journals/49.6/749.pdf

Key words: Age, Growth, Otolith, *Schizothorax o'connori*, Tibet.

Most Schizothoracinae fishes are endemic to China and are mainly distributed in plateau lakes and rivers on the Qinghai-Tibetan Plateau. Schizothoracinae fishes are characterized by slow growth, low fecundity, and late sexual maturation as adaptations to their rigorous environment (Chen and Cao 2000). These life-history characteristics make them particularly sensitive to intense exploitation. Biological invasions (such as *Carassius auratus* and *Pseudorasbora parva*) have also increased since the 1990s, and so native fishes were placed in a dangerous situation (Chen 2009). Conservation of their natural populations has therefore become a primary concern.

Schizothorax o'connori (Cyprinidae: Schizothoracinae) is one of the endemic species in the Yarlung Tsangpo River (Chen and Cao 2000), and commercially one of the most important species in Tibet. Despite the limited distribution of *S. o'connori* and its importance to fishing, little is known about its biology and ecology. There have been few studies on the age and growth of *S. o'connori*. He (2005) and Yao et al. (2009) estimated the age and growth of *S. o'connori* by otolith readings and back-calculation. However, the maximum observed age was only 17 and 24 yr, respectively, because of difficulties in collecting sufficient samples of larger specimens and the need to develop appropriate methods for aging older fish. This aging problem often led to age underestimation, resulting in overly optimistic estimates of growth and mortality rates, which can

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contribute to overexploitation of a population or species (Campana 2001).

Accurate estimates of age and growth are prerequisites for understanding population dynamics and maintaining sustainable yields in fisheries (Campana and Thorrold 2001). Some recent studies demonstrated that age and growth of schizothoracine fish can reliably be estimated from sections of lapillus otoliths (Chen et al. 2009, Li and Chen 2009).

Some researchers focused on relationships of otolith size with fish size and age, and suggested that age can be estimated from the otolith weight and fish length (Boehlert 1985). Experimental studies using fish of known ages showed that for juveniles, the otolith growth is in fact isometric with somatic growth (Secor and Dean 1989), but for slowly growing individuals, otoliths grow asymmetrically through time, having relatively heavy otoliths for their body size, which might be attributed to an increase in otolith thickness (Reznick et al. 1989).

In this study, we attempted to estimate the age of *S. o'connori* by reading sectioned otoliths, and describing relationships of otolith dimensions with fish length and age. The study also aimed to compare parameters of fitted von Bertalanffy growth equations with those obtained by other authors.

MATERIALS AND METHODS

Collection of samples

In total, 1126 *S. o'connori* individuals were collected from the Yarlung Tsangpo River (98.9%) and its tributaries (Xiang Qu and Nyang Qu; 1.1%) during monthly sampling efforts from Aug. 2008 to Aug. 2009 (Fig. 1). At least 30 fish were collected each month using trammel nets (with an inner mesh of 7.5 mm and an outer mesh of 18 mm). In addition, a small sample consisting of juvenile *S. o'connori* was collected by trap nets (with a mesh size of 1.5 mm) in Nov. 2008-Aug. 2009. The standard length (*SL*) was measured to the nearest 1 mm using a tapeline, and body weight (*W*) was measured to the nearest 0.1 g with an electronic balance. Specimens were classified as male, female, or undetermined by macroscopic examination of gonads.

For *S. o'connori*, sagittal otoliths are acicular and fragile, and asteriscus otoliths are flaky, so only the lapilli with a clear pattern of alternating zones could be used for age estimation. Lapillus otoliths were removed, cleaned, and stored dry in labeled tubes.

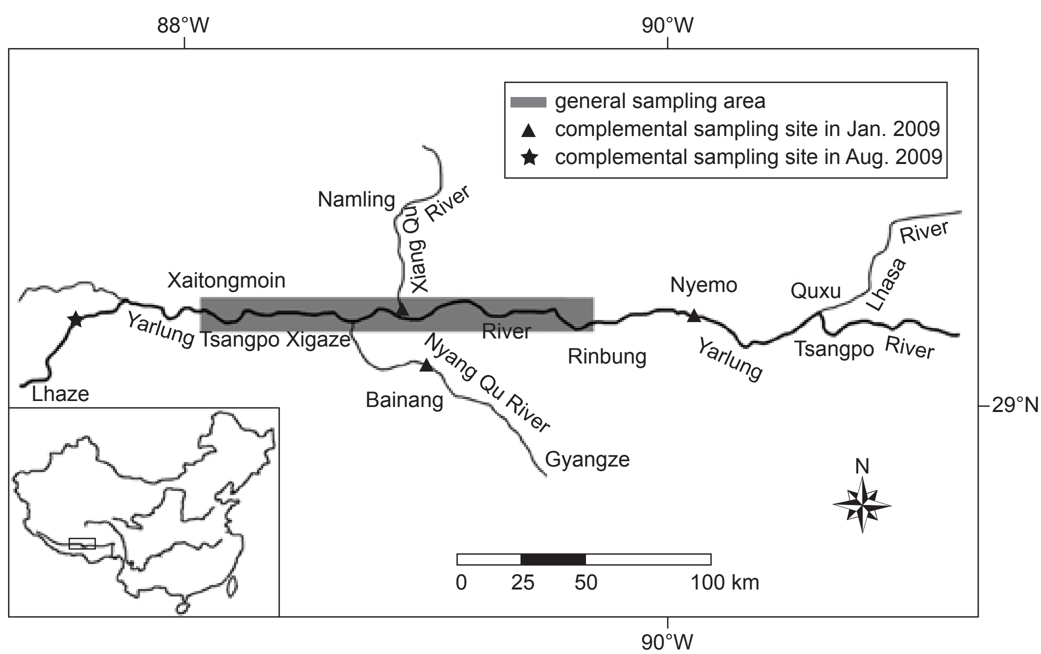

Fig. 1. Sampling locations of *Schizothorax o'connori* in the Yarlung Tsangpo River, Tibet.

Otolith preparation

The terms anterior, posterior, dorsal, ventral, proximal, and distal faces refer to the position of the otolith corresponding to its original orientation in the fish (Reñones et al. 2007, Fig. 2). To study otolith size and growth, the following measurements were made in a sample of 874 whole otoliths (Fig. 2): length (OL: maximum anterior-posterior distance), breadth (OB: dorsal-ventral distance at the widest point), thickness (OT: distal-proximal maximum distance), and weight (OW: measured after being dried at 60°C for 24 h) (Reñones et al. 2007). OL and OB were measured using Image-Pro Plus software (vers. 6.0; Media Cybernetics, USA) after taking photos with Leica Application Suite (vers. 15; Leica, Germany) under a dissecting microscope linked to a CCD video camera (La Mesa et al. 2009). The OT was measured to the nearest 0.01 mm with digital calipers and OW to the nearest 0.1 mg with an electronic balance. Relationships of otolith dimensions with fish length and age were studied by a regression analysis.

A lapillus was mounted with the proximal face on a glass slide using nail polish, ground from the distal face using wet sandpaper (600-2000 grit) and polished with alumina paste (3 μm) until the core was visible under a compound microscope. The section was re-affixed with the polished surface down, ground, and polished until the core was again exposed (He et al. 2008). Annuli were counted along the axis (Fig. 3) using an image analysis system (Ratoc System Engineering, Tokyo, Japan) with a direct data feed between the compound microscope and the computer.

Age estimation

Each fish was assigned to an age class assuming 1 Jan. as the designated birthday (Massutí et al. 2000). Annuli were counted without prior knowledge of the size, sex, or capture date of the individual. Each otolith was interpreted by 2 independent readers, and scored for readability on a 5-point scale: 1, excellent; 2, good; 3, acceptable; 4, poor; and 5, unreadable (Paul and Horn 2009). If the 2 counts differed, the otolith was recounted, and the final count was then accepted as the agreed-upon age (Liu et al. 2009). The index of the average percentage error (IAPE) was calculated to assess the precision of the age determinations between 2 readers. The equation (Beamish and Fournier 1981) is expressed as follows:

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

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

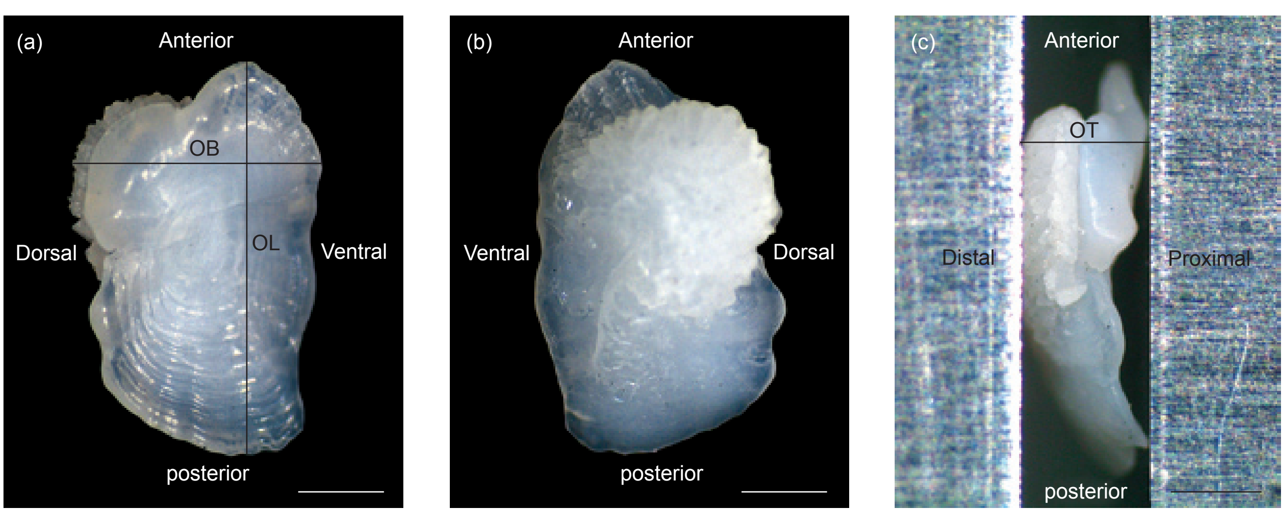

Fig. 2. Proximal (a), distal (b), and dorsal (c) faces of a whole lapillus under a dissecting microscope with reflected light, from a 13-yr-old *Schizothorax o'connori* (343 mm standard length (*SL*)). The axes along which the otolith length (OL) and breadth (OB) were measured are indicated. The otolith (c) was put inside digital calipers to measure the thickness (OT). Scale bars = 0.5 mm.

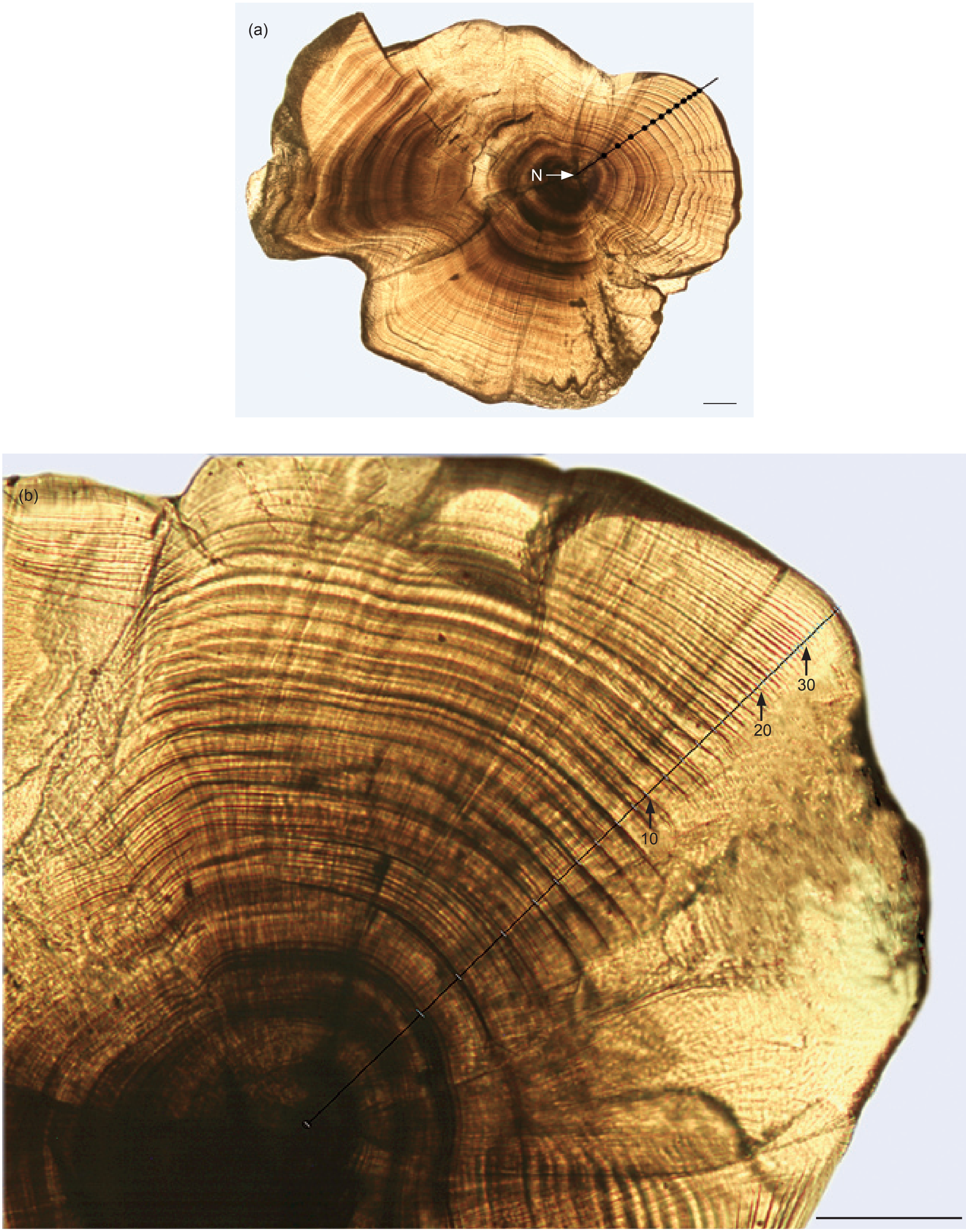

Fig. 3. Sectioned lapilli under a compound microscope with transmitted light, from a 12-yr-old (a, 390 mm standard length (*SL*)) and a 39-yr-old (b, 422 mm *SL*) *Schizothorax o'connori*. The curved arrow indicates the nucleus, the blank line indicates the axis for counting, and dots and blue lines indicate annuli. Scale bars = 200 μm.

SL-*W* relationship

Relationships between *W* and *SL* were estimated using a power regression analysis, described by $W = a\ SL^b$. An analysis of covariance (ANCOVA) was used to compare *SL*-*W* relationships between sexes 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).

Growth

The traditional von Bertalanffy growth function (von Bertalanffy 1938) was used to fit the observed length at age of *S. o'connori*: $L_t = L_\infty(1\text{-}e^{-k(t-t_0)})$, where L_t is the length at age *t*, L_∞ is the asymptotic length, *k* is the growth coefficient, *t* is the age (year from birth), and t_0 is the age at length 0. The growth performance index was calculated by the equation of Munro and Pauly (1983): $Ø = \log_{10}k + 2 \log_{10}L_\infty$. Ø was used to compare growth parameters obtained in this study with those reported by others.

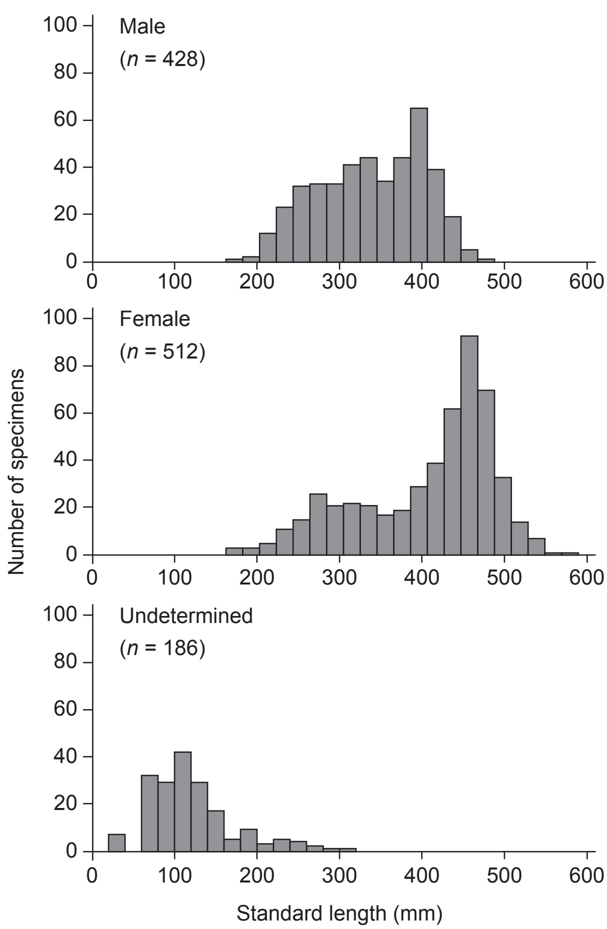

Fig. 4. Distributions of the standard length frequency of *Schizothorax o'connori*.

Data analysis

The data are presented as the mean ± standard deviation (S.D.). Differences were regarded as significant when $p < 0.05$. The analysis was conducted using SPSS 16.0 (SPSS, Chicago, IL, USA) and Origin 8.0 (Microcal, USA).

RESULTS

Length-frequency distribution and *SL*-*W* relationship

The *SL* ranged 33-553 mm, and *W* ranged 4.3-2982.6 g. Of the 1126 *S. o'connori* collected, 428 were males with 178-460 mm *SL*, 512 were females with 177-562 mm *SL*, and 186 were undetermined specimens with 33-309 mm *SL* (Fig. 4).

The *SL*-*W* relationships were plotted separately for males, females, and the undetermined (Fig. 5). The regression equations were described as $W = 8.327 \times 10^{-6}\ SL^{3.090}$ ($R^2 = 0.969$, $n = 428$) for males, $W = 8.897 \times 10^{-6}\ SL^{3.080}$ ($R^2 = 0.979$, $n = 512$) for females and $W = 2.089 \times 10^{-5}\ SL^{2.942}$ ($R^2 = 0.989$, $n = 186$) for the undetermined. No statistically significant differences were detected for *SL*-*W* relationships between sexes (ANCOVA after log-transformation, $n = 940$, $p > 0.05$). Therefore, the regression equation derived from pooled

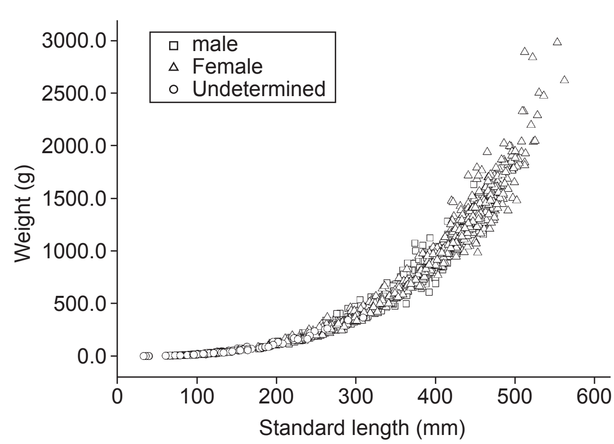

Fig. 5. Length-weight relationships of *Schizothorax o'connori*.

data was $W = 2.034 \times 10^{-5}\ SL^{2.940}$ ($R^2 = 0.994$, $n = 1126$). The allometric index value (b) obtained from the function significantly differed from 3 (t-test, $d.f. = 1124$, $p < 0.05$).

Relationships between otolith dimensions and fish length/age

The relationships between otolith dimensions (OL, OB, OT, and OW) and fish length/age are shown in figure 6. The lines of best fit between OL/OB and *SL* were slightly curved, and the growth rate of OL and OB declined as *SL* increased. The OL and OB increased with *SL* at a fixed rate up to a size of 250 mm, and at a little slower rate thereafter. The *SL*-OT relationship showed an inverse trend, and an exponential model presented the best fit. A piecewise regression fitted to the data demonstrated that a shift from slow to fast OT growth occurred at 380 mm *SL*. The goodness of fit between OW and *SL* was high for the power function. OL was the best predictor of fish length ($R^2 = 0.945$). OL and OB increased linearly with log-transformed age, whereas increases in OT and OW with age were described by power functions. The slope of the relationship between OW and age ($b = 1.015$) did not significantly differ from unity (t-test, $n = 874$, $p > 0.05$), indicating an isometric increase between the 2 parameters. Otolith dimensions were more highly correlated with fish length than age, with the exception of OT. Variability in the data significantly increased for all variables beyond 380 mm *SL* or 15 yr.

Age and growth

Of the 1126 otoliths examined, only 37 (approximately 3.3%) were discarded due to natural deformations and unidentifiable annulus deposition. Relatively more otolith sections from males were scored as having excellent or good readability, while relatively more from females were scored as being acceptable. In addition, otoliths from individuals of aged 1-20 yr had higher readability than those from older fish (Table 1). The IAPE between the 2 independent readers was 3.24%.

Overall, 416 males, 493 females, and 180 undetermined specimens were successfully aged (Fig. 7). The estimated age range varied from 1 to 50 yr (corresponding to 33-553 mm *SL*), although fish older than 28 yr were not common. Seven small fish (33-40 mm) were considered to be young-of-the-year (YOY) fish, but were nevertheless assigned to age class 1, because they were captured after the designated birthday. The maximum age observed was 40 yr (422 mm *SL*) for males and 50 yr (480 mm *SL*) for females.

The mean length-at-age (± S.D.) values obtained from otolith readings, sorted separately by males, females, and the undetermined, are provided in table 2. For age classes 3-8, the mean length-at-age did not significantly differ between sexes (unpaired t-test, all $p > 0.05$); in age classes 9-28, females attained a significantly larger mean length-at-age than males (all $p < 0.05$, except for 26). There was a large variation in the *SL* of individuals with the same age, and the variation increased with elapsed years.

The length-at-age data of undetermined specimens were included (except for a 9-yr-old individual) in models fitted for both sexes. Growth models fitting the observed length-at-age data were calculated by the least-square methods for each sex. The VBGFs fitted to length-at-age data were: $L_t = 499.7\ (1 - e^{-0.095\ (t + 0.896)})$ for males and $L_t = 576.9\ (1 - e^{-0.081\ (t + 0.946)})$ for females (Fig. 8). The asymptotic weight based on VBGFs were estimated as $W_t = 1748.1$ g for males and $W_t = 2666.2$ g for females. The growth performances (Ø) of *S. o'connori* were 4.3751 for males and 4.4307 for females.

DISCUSSION

It was argued that otolith size is highly correlated with fish size, since they are both controlled by the same metabolic processes (Gauldie 1988). Otoliths, as measured along various axes, show asymmetrical growth through time, but nevertheless grow throughout the life of the fish (Fowler 1990). Initially, *S. o'connori* otoliths grew symmetrically along the 3 axes, but the growth rate slightly declined in length and breadth after a few years of life and then increased in thickness. Asymmetries occur in otolith growth because the new material is mainly deposited on the distal face of the otolith which thickens the structure, particularly in slow-growing, long-lived species (Boehlert 1985, Reñones et al. 2007). Such a growth pattern might affect the application of back-calculation which assumes that otolith growth is isometric with somatic growth through time (Campana 1990).

In recent years, otolith weight was reported to be correlated with the age of some fish (Pino et al. 2004), and it may be a useful, simple tool for

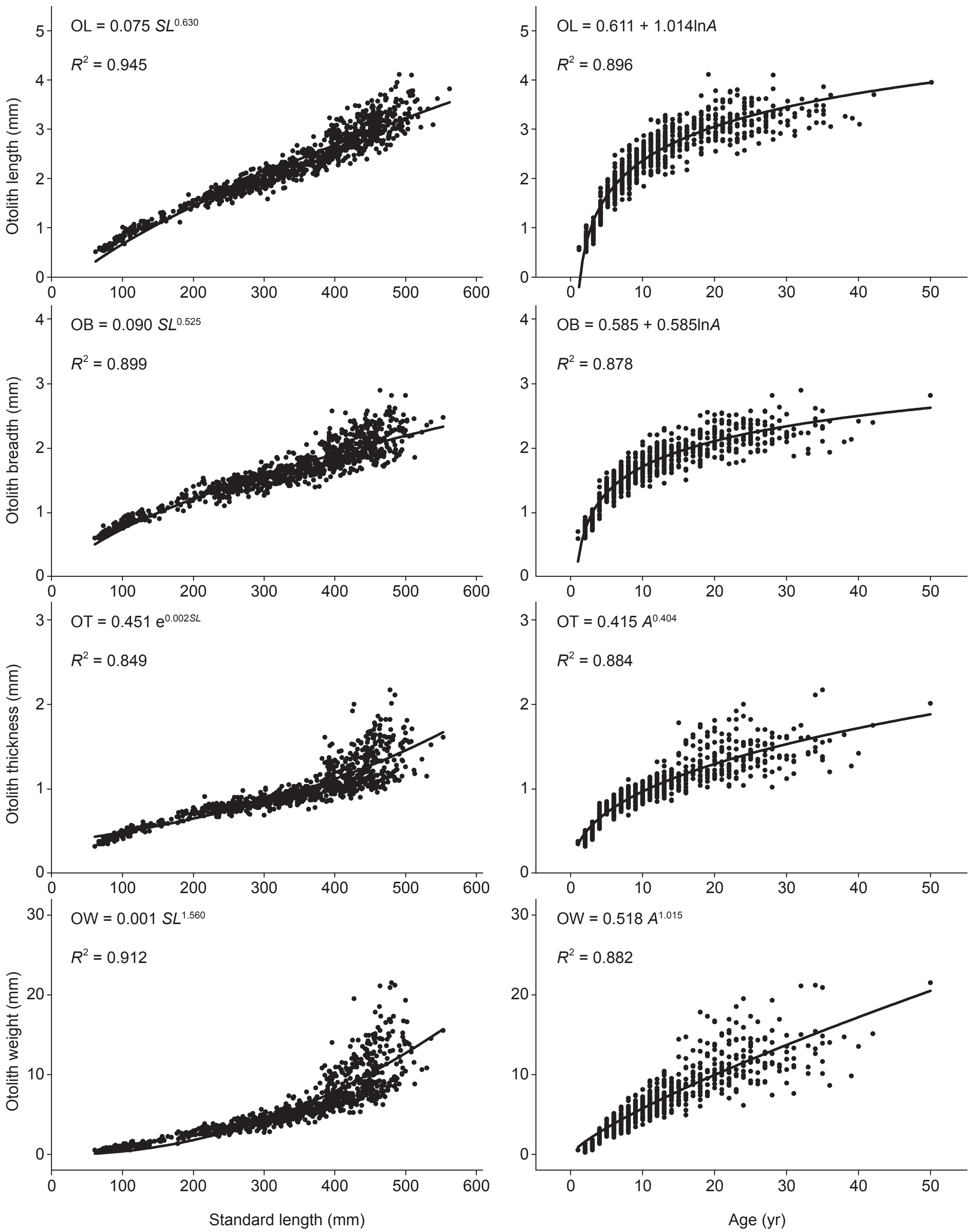

Fig. 6. Relationships between otolith dimensions (length, breadth, thickness, and weight) and fish length/age of *Schizothorax o'connori*, $n = 874$.

rapidly estimating the age of individuals (Araya et al. 2001). However, in some studies, the increased variability of OW in older fish indicated that this variable may produce less-reliable age estimates in older fish (Tuset et al. 2004). Gunn et al. (2008) pointed out that OW was an imprecise predictor of age in *Thunnus maccoyii* that was older than 10 yr. In the present study, a similar trend was observed, with variability of OW in *S. o'connori* significantly increasing beyond 380 mm SL or 15 yr.

Application of the precision measure (IAPE) presented satisfactory consistency in age determination between 2 readers, because the estimated value was below the precision point of 5.5% established by Campana (2001) for aging studies.

Regarding the estimation of VBGF parameters, a low estimate of *k* and a high L_∞ indicated that *S. o'connori* is a slow-growing, long-lived fish. Females attained a bigger size (L_∞) at a lower rate (*k*) than males. The results are similar to parameters of *Ptychobarbus dipogon* reported by Li and Chen (2009).

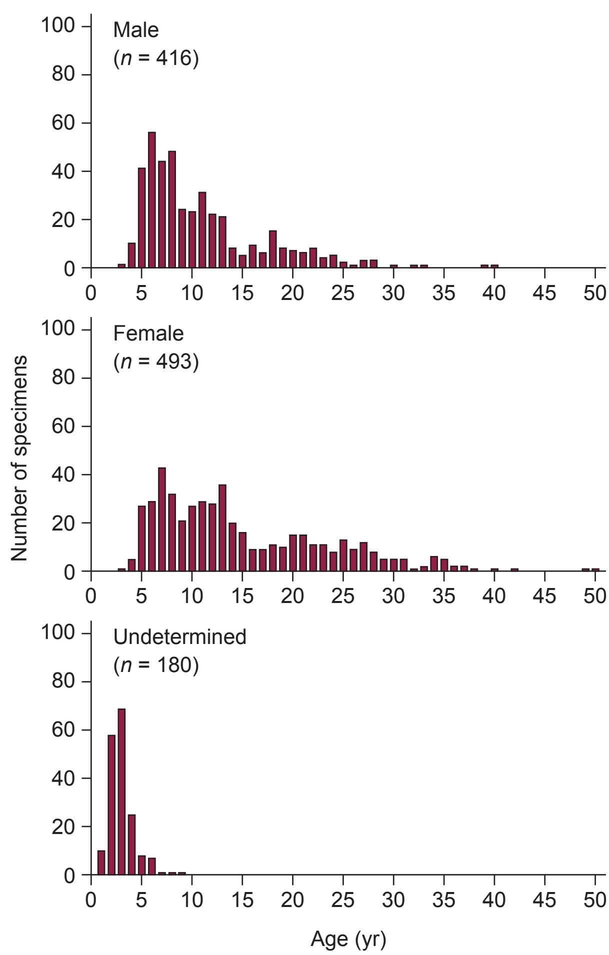

Fig. 7. Age frequency composition of *Schizothorax o'connori*.

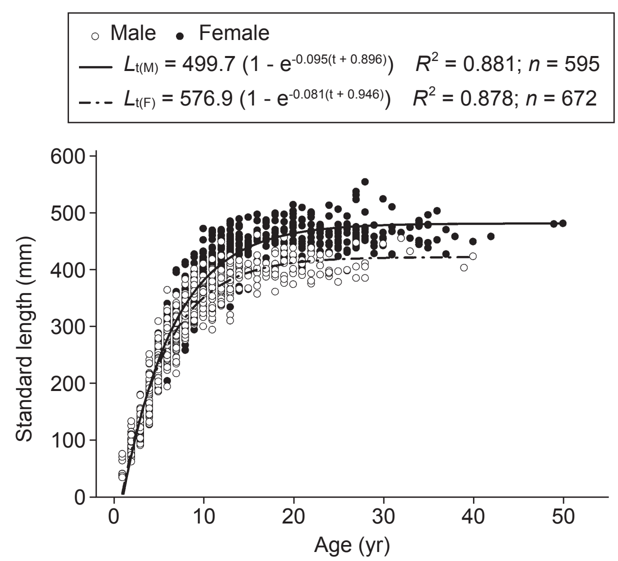

Fig. 8. The von Bertalanffy growth curve of *Schizothorax o'connori* with the observed standard length at age estimated from otoliths. The length-at-age data of the undetermined specimens were included in models fitting both sexes (except for a 9-yr-old individual, *n* = 179).

Table 1. Distribution of readability scores for otolith sections of *S. o'connori*, the values are percentages of the sample size

Sort	Sample size	Readability scores				
		1	2	3	4	5
Male	421	4.8	79.6	11.9	2.6	1.2
Female	504	5.8	74.6	15.5	2.0	2.2
Age 1-20	927	5.6	84.0	8.8	1.5	
Age > 20	162	1.2	55.6	38.9	4.3	

Cailliet and Goldman (2004) reported that growth model estimates are greatly affected by the lack of very young or old individuals. Juveniles (age classes 1-4) collected from traps provided a meaningful estimate of the VBGF parameter, t_0. More data points from juvenile fish would tend to shift t_0 values higher and closer to 0 (Paul and Horn 2009). Since the mean length-at-age exhibited no significant difference between sexes up to an age of 8 yr, the length-at-age data of undetermined specimens were included in models fitting both sexes (Peres and Haimovici 2004). Moreover, the presence of several specimens of > 500 mm *SL* also provided a reliable estimate of the

Table 2. Number of specimens and mean ± S.D. and range of standard length at age of *S. o'connori*

Age (years)	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							10	47.0 ± 15.4	33-75
2							58	86.2 ± 15.5	61-132
3	1	178		1	179		69	116.2 ± 16.0	90-155
4	10	218.8 ± 17.4	195-250	5	196.8 ± 23.1	177-233	25	159.3 ± 20.8	126-196
5	41	244.3 ± 22.8	184-308	27	243.0 ± 26.0	190-287	8	217.1 ± 27.6	186-264
6	56	272.4 ± 30.1	223-363	29	274.4 ± 30.7	203-339	7	227.7 ± 18.4	193-248
7	44	292.4 ± 31.0	216-354	43	304.2 ± 32.8	258-398	1	261	
8	48	322.4 ± 24.9	275-382	32	336.2 ± 37.9	257-412	1	290	
9	24	340.6 ± 29.8	302-400	21	366.6 ± 35.0	293-426	1	309	
10	23	353.4 ± 25.1	299-409	27	396.0 ± 37.7	316-471			
11	31	360.4 ± 30.5	293-417	29	417.2 ± 28.2	347-468			
12	22	375.4 ± 29.1	335-448	28	425.6 ± 34.2	342-469			
13	21	380.3 ± 29.5	309-423	36	442.2 ± 31.2	333-484			
14	8	394.6 ± 15.7	365-415	20	438.6 ± 33.4	361-488			
15	5	391.8 ± 16.3	366-409	16	430.3 ± 31.1	364-491			
16	9	399.3 ± 30.7	356-460	9	446.8 ± 36.9	393-496			
17	6	399.5 ± 9.7	386-411	9	461.0 ± 22.9	419-486			
18	15	401.7 ± 18.8	360-432	11	465.5 ± 25.2	431-498			
19	8	411.0 ± 28.1	375-455	10	451.6 ± 26.2	419-496			
20	7	405.0 ± 21.9	373-437	15	464.1 ± 34.1	392-513			
21	6	405.8 ± 10.8	389-415	15	453.0 ± 30.9	402-508			
22	8	406.0 ± 24.7	374-437	11	471.3 ± 22.7	440-500			
23	4	409.5 ± 15.2	390-425	11	456.0 ± 15.9	431-478			
24	5	399.4 ± 15.9	387-427	8	463.5 ± 27.1	430-512			
25	2	386.5 ± 13.4	377-396	13	454.9 ± 21.7	422-505			
26	1	398		9	454.3 ± 30.5	397-493			
27	3	411.3 ± 31.6	384-446	12	474.2 ± 35.0	437-536			
28	3	399.3 ± 13.6	384-410	8	477.5 ± 34.4	450-553			
29				5	449.4 ± 18.8	427-477			
30	1	444		5	467.2 ± 32.3	441-523			
31				5	460.0 ± 35.4	425-509			
32	1	454		1	464				
33	1	431		2	463.5 ± 13.4	454-473			
34				6	463.7 ± 19.0	443-485			
35				5	466.6 ± 24.2	437-495			
36				2	476.5 ± 36.1	451-502			
37				2	447.5 ± 30.4	426-469			
38				1	457				
39	1	402							
40	1	422		1	448				
42				1	457				
49				1	479				
50				1	480				

other VBGF parameter, L_∞ (576.9 mm for females, which was larger than the maximum observed size of 553 mm).

Comparing results of the growth of *S. o'connori* with previous studies (Table 3), the L_∞ obtained in this study was largest. *k* values achieved here (0.095 for males and 0.081 for females) were similar to that (0.0943 for both sexes) obtained by He (2005) in the Lhasa River, and lower than those (0.1260 for males and 0.1133 for females) by Yao et al. (2009) in the Yarlung Tsangpo River. Differences among all of the estimated parameters could have been a consequence of several factors: (1) different methods used (previous studies used back calculation), (2) different size distributions (probably caused by different types of sampling gear), and (3) different sampled locations. Nevertheless, values of t_0 and Ø found in the present study were similar to those of the 2 previous studies.

The growth coefficient (*k*) is a useful index for estimating the potential vulnerability of stocks to excessive exploitation (Musick 1999). Li and Chen (2009) compared the parameters of some Schizothoracinae fishes, and considered that they were slow-growing species with *k* values of around 0.1/yr. Slow-growing, long-lived fishes tend to be particularly vulnerable to excessive mortalities and rapid stock collapse, after which population turnover may be lower than expected, and its response to recovery plans slower than predicted (Musick 1999). The growth traits of *S. o'connori* may be adaptations to cold water temperatures and food deficiencies on the Qinghai-Tibetan Plateau (Chen et al. 2002). Therefore, it is necessary to establish science-based management of this resource to guarantee its sustainable use. Fishery regulations for *S. o'connori* should aim to prevent growth overfishing through minimum landing sizes, and also prevent recruitment overfishing by protecting the oldest population components especially in the spawning season.

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Table 3. Comparison of growth characters of *S. o'connori* in different studies, the ages all estimated from otoliths

Location	*SL* range	Age range	*n*	Sex	Growth parameters				Sources
					L_∞ (mm)	*k*	t_0	Ø	
Lhasa River	169-483	3-17	125	Total	554.0	0.0943	-0.8749	4.4615	He 2005
Yarlung Zangbo River	53.1-421.6	2-18	219	Male	449.0	0.1260	-0.4746	4.4049	Yao et al. 2009
	53.1-492.3	2-24	176	Female	492.4	0.1133	-0.5432	4.4389	
Yarlung Zangbo River	33-460	1-40	596	Male	499.7	0.0950	-0.8960	4.3751	Present study
	33-553	1-50	673	Female	576.9	0.0810	-0.9460	4.4307	

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