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Age and Growth of the Tibetan Catfish *Glyptosternum maculatum* in the Brahmaputra River, China

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Hong-Jing Li and Cong-Xin Xie (2008) Age and growth of the Tibetan catfish *Glyptosternum maculatum* in the Brahmaputra River, China. *Zoological Studies* **47**(5): 555-563. *Glyptosternum maculatum* was successfully aged using otoliths, opercula, and vertebrae. The maximum age obtained from vertebrae was 13 yr, while that obtained from otoliths and opercula was 12 yr. Age determinations using otoliths, opercula, and vertebrae showed good agreement with no significant difference among them. The index of the average percentage error for independent readings of 2 appraised materials was 0.11%, 0.2%, and 0.17%, respectively. Relationships between body weight (W) and total length (L) are described as follows: $W_{♀} = 5E - 06L^{3.142}$ (for females) and $W_{♂} = 5E - 06L^{3.1474}$ (for males), both of which describe isometric growth. A power relationship (females, a = 29.068 and v = 97.173; males, a = 13.402 and v = 107.88) was estimated between the total length and vertebra radius. von Bertalanffy parameters estimated from back-calculated sizes at age were $L_\infty = 342.66$ mm, k = 0.1142/yr, and $t_0 = -0.7488$ yr for females; and $L_\infty = 460.24$ mm, k = 0.0882/yr, and $t_0 = -0.2312$ for males. http://zoolstud.sinica.edu.tw/Journals/47.5/555.pdf

Key words: Age, Growth, *Glyptosternum maculatum*, Brahmaputra River, Tibet.

The Tibetan catfish, *Glyptosternum maculatum* Regan of the Sisoridae, is distributed in the mid-reaches of the Brahmaputra River and its tributaries. The highest elevation of its distribution can reach 4200 m (Chu 1988). *G. maculatum* attains 14-24 cm in total length, inhabits shallow rocky bottoms inside crevices or underneath boulders, feeds on crustaceans and small fish, and is occasionally preyed upon by other fish (Fishery Bureau in Tibet Municipality 1995). Many fundamental aspects of its biology, such as growth and reproduction, are stpill unknown.

This species is also commercially important for local fisheries in Tibet and is fished mainly by set gill nets. In past decades, the Tibetan economy has rapidly developed, the population has increased, and people's many habits and customs have changed. *G. maculatum* has the highest price among Tibet's native fish because it can be captured only in Apr. to Sept. each year in the Brahmaputra River, but is seldom caught at other times on account of lower temperatures. Due to the high price, overfishing has occurred with even illegal practices of electro-fishing and poisoning. This resource is suffering serious degradation (Zhang and Xin 1999, Li and Xie 2006).

Age estimates from band counts on aging structures are basic requirements to obtain growth rates (Beamish and McFarlane 1987). Determining the age of a catfish often involves counting increments in skeletal structures such as otoliths (Buckmeier et al. 2002), opercula (Meunier et al. 1994), vertebrae (Stumm 1984, Li et al. 2006), fin spines, and rays (Penha et al. 2004). Herein, otoliths, opercula, and vertebrae were chosen for age determination to analyze individual growth to provide basic parameters of the population dynamics. These data will be useful for the

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evaluation and future management of this valuable resource of artisanal fisheries of Tibet.

MATERIALS AND METHODS

G. maculatum specimens were collected with a gill net and sampled every 2 wk from May to Sept. 2005 in Brahmaputra River branches of the Nianchu (H4200m), Lhasa (H3000m), and Nyang Qu Rivers (H2200m). Fish were caught at depths of 0.1-3.2 m in waters with a pH of 6-7.2 and temperature of 6.5-11.5°C. In total, 219 specimens were measured to the nearest millimeter (mm) for total length (Lt) and weighed to the nearest 0.1 g for total body weight (Wt). The gonads were dissected from the fish and weighed to the nearest 0.1 g (GW), and the sex was checked with the naked eye. Otoliths, opercula, and vertebrae were removed from *G. maculatum*, labeled, and stored frozen for later age determination.

Otoliths were removed from the fish, rinsed in distilled water to remove any tissue, and then dried with a paper towel. Otoliths were stored dry in gelatin capsules, which were placed in paper envelopes on which the relevant biological information was written. Reading whole otoliths against a black background using a dissecting microscope at low magnification with reflected light proved to be unsatisfactory as the growth zones were not clearly discernable. For this reason, it was necessary to section the otoliths; otoliths were embedded in epoxy resin and sectioned transversely at a 2 mm width through the focus using a microcutter. The sectioned otoliths were polished using a grinder toward the focus, leaving a consequent thickness of 0.2 mm, and were viewed under transmitted light using a dissecting microscope.

Opercula were removed from the collection randomly and were washed in fresh water to clean their surfaces of skin and mucus. Once cleaned, they were rinsed in 70% ethanol, dried, and mounted on slides. The opercula were difficult to examine because they were too thick and opaque. The most effective method was to grind them with sandpaper. Opercula were studied using transmitted light at 2× magnification.

Cleaned vertebrae were fixed in 4% formaldehyde (for 24-48 h) and preserved in 70% alcohol. Vertebrae were embedded in polyester resin, and a section was cut with a microcutter approximately 1 mm thick to contain the nucleus. Longitudinal sections were made up of translucent (dark, narrow) and opaque (bright, broad) bands. Growth rings, defined as a band pair, were counted in each section, and distances from the focus to the margin of each narrow zone were recorded following the procedures described by Lessa et al. (2004). Vertebral radius (VR) was measured using a binocular dissecting microscope equipped with an ocular micrometer. Measurements were made at 2-3× magnification with both reflected and transmitted light (Fig. 1).

Annuli were counted without prior knowledge of the size, sex, or previous results for the individual. Counts were only accepted if counts by 2 different observers were in agreement. If the estimated number of annuli differed by 1 annulus, then the centrum was recounted. Counts that differed by 2 or more annuli were rejected (Joung et al. 2004).

The index of the average percentage error (IAPE) was used to evaluate the precision of the age determinations (Beamish and Fournier 1981). Data taken from vertebrae and their corresponding otoliths and opercula were compared using the IAPE, as calculated by the formula:

$$\mathrm{IAPE} = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|Xij - Xj|}{Xj}\right];$$

where N is the number of fish aged, R the number of times each was aged, Xij is the ith age determination of the jth fish, and Xj is the average

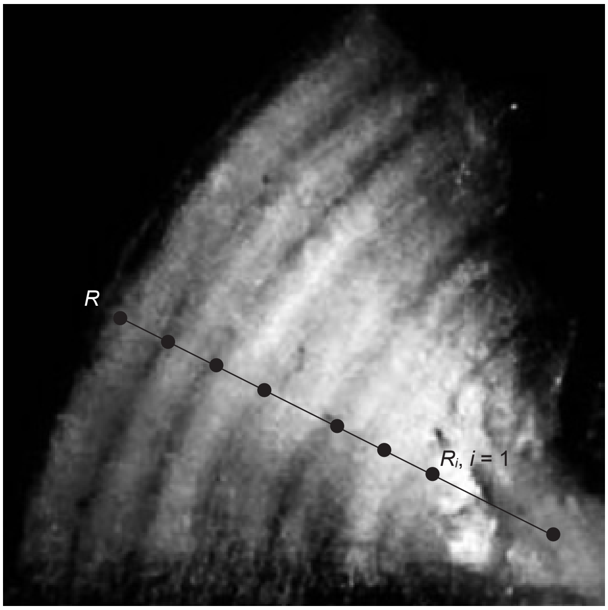

Fig. 1. Vertebra of *Glyptosternum maculatum* from Tibet. R_i is the radius of the ith band (distance from the center of the vertebrae to the outer margin of the annulus), and R is the radius of the vertebrae at capture.

age calculated for the *j*th fish. Readings were also compared using the coefficient of variation (CV) (Chang 1982).

The relationship between body weight (W) and total length was determined for both males and females. Analysis of the residual sum of squares (ARSS) was carried out to test for a difference in growth between males and female (Chen et al. 1992). The Pauly (1984) method of using paired *t*-tests on each likelihood ratio was used to compare if the exponential index, b, of the weight-length relationships differed from 3.

The relationship between vertebral radius and total length was examined using linear, power, logarithmic, and polynomial regression methods, and the sequent correlation coefficients were compared to determine the best method for back-calculation of size. Regressions were fit to the male and female data, and analysis of the residual sum of squares was used to test for differences between the 2 relationships (Francis 1990). Back-calculated lengths and observed lengths were also compared using χ^2 test.

Length-at-age data were fitted using a non-linear least-squares regression to the von Bertalanffy growth model, $Lt = L_\infty[1 - e^{-K(t-t_0)}]$ (Ricker 1973). The parameters L_∞, K, and t_0 were calculated to determine the Ford-Walford method (Kimura 1980). Back-calculated lengths and predicted lengths were also compared using χ^2 test.

RESULTS

Overall, 219 specimens of *G. maculatum* were collected and used for this study. Of the fish examined, 77 were males, 128 females, and the sex of the remaining 14 fish could not be determined macroscopically because they were immature with very thin, translucent gonads. The sex ratio differed significantly from 1: 1 (*t*-test, $p < 0.05$). Fish ranged in size from 115.0 to 320.0 mm. Males ranged in size from 147.0 to 320.0 mm. Lengths of females ranged from 115.0 to 270 mm. The total weight of fish was 16.3-233.9 g for females and 31.1-373.7 g for males. Thus, males attained larger sizes than females, and a significant difference was found in the mean size between males and females (*t*-test, $p < 0.05$).

Vertebrae of *G. maculatum* are biconcave. The surfaces of the 3rd-5th vertebra of every specimen exhibited a camber, with mild concavity and clear rings, so were suitable for examination. Under reflected light against a dark background, there were wide white translucent rings alternating with narrow dark-taupe ones around the center on the concavity of the 3rd-5th vertebrae. The readings from the 3 vertebrae were the same. As a whole, the vertebrae of 93% specimens had intact translucent and opaque rings showing legible annuli (Fig. 2A), while the other 7% had false bands and the annuli were hard to tell apart.

Otoliths were elongate, laterally compressed, curved, and very fragile. Their surfaces were irregular, with many mounts and crenellations. The external face of the otolith was concave with grooves and ridges running radially. There were 2 distinct regions on the otoliths: a central core area and an annual ring arrangement area. The annual ring arrangement area was divided into a posterior rostrum and anterior rostrum. The anterior rostrum was thick and short, and it was difficult to distinguish annuli due to their being closely arranged and overlapping. The posterior rostrum was thin and long with wide rings, and translucent rings corresponding to true annuli were indented in the post-rostrum region on the concave (distal) side of otoliths (Fig. 2B).

Opercula were almost triangular; the central regions were thick, while the outer regions were thin. Under reflected light, obvious alternating translucent and opaque bands surrounded the center and stretched along the dorsal curves of the operculum. Some translucent and opaque bands were C-shaped, while others were S-shaped. The annual rings of small fish were clear, while those of larger individuals were indistinct near the growth focus of the opercula. Most inside and outside annuli were discontinuous (Fig. 2C).

Of 219 otoliths, opercula, and vertebrae processed for age estimation, 3 vertebrae, 9 otoliths, and 17 opercula (1.36%, 4.11%, and 7.76%, respectively) were considered unsuitable for study. In total, 207 specimens were considered suitable for examination among otoliths, opercula, and vertebrae while 205 were fit for age estimation and sex discrimination.

Of the 205 *G. maculatum* aged 3-13 yr studied, 5- and 6-yr-old fish were the dominant age classes, and over 80% of fish were < 8 yr old. Ages determined by reading otoliths and opercula ranged 3-12 yr. Ages from vertebrae ranged 3-13 yr. The age precision analysis, performed on the first 2 readings, was used to evaluate the agreement between them. Counting variability indices of IAPE and CV were both quite low at 4.13% and 4.8%, respectively, indicating the goodness of the aging procedures adopted and a reasonable consistency (or reproducibility) between readings. Comparisons of readings from otoliths, opercula, and vertebrae from the same fish showed good agreement according to the IAPE. IAPE values of otoliths vs. vertebrae, otoliths vs. opercula, and vertebrae vs. opercula were 0.11%, 0.17%, and 0.2% respectively ($n = 205$). Age bias plots showed minimal variation around the 1: 1 ratio and no systematic bias. Figure 3 shows differences between readings taken from otoliths, opercula, and vertebrae and indicates that there was 98%, 96%, and 95% agreement, respectively. Opercula seemed to underestimate the age by 1 yr in 9.5% of samples. No significant differences were found between the mean ages determined using otoliths, opercula, and vertebrae (*t*-test, $p < 0.05$) indicating the goodness of the aging procedures adopted and a reasonable consistency among the materials examined.

Relationship between body weight (W) and total length (L) for females is described as follows (Fig. 4):

W♀ = 5E-06 L 3.142 ($r^2 = 0.9638$);

while that for males is

W♂ = 5E-06 L 3.1474 ($r^2 = 0.9742$).

There was a significant difference in the relationship between total length and weight for males and females (ARSS, $p < 0.01$); therefore, the equations for these relationships are reported separately. According to Pauly (1984), using t^2-tests for each likelihood ratio, weight-length relationships between the exponential index, b,

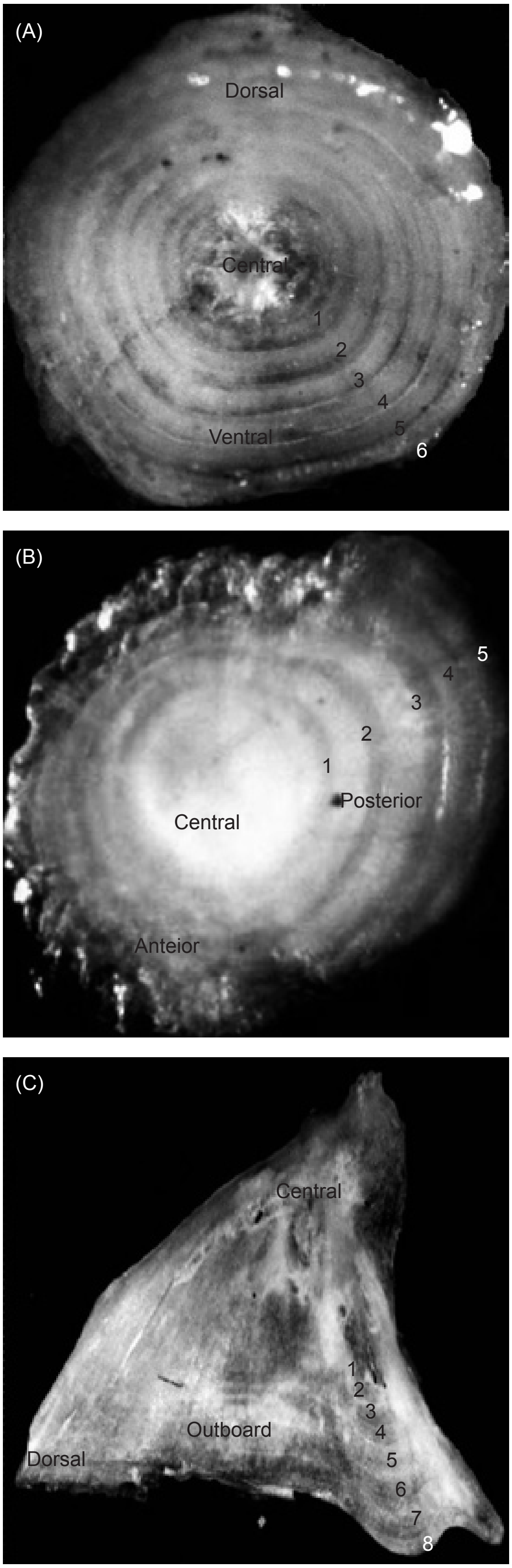

Fig. 2. Annuli in a vertebra (A), otolith (B), and opercula (C) of *Glyptosternum maculatum* from Tibet. Numerals indicate annuli.

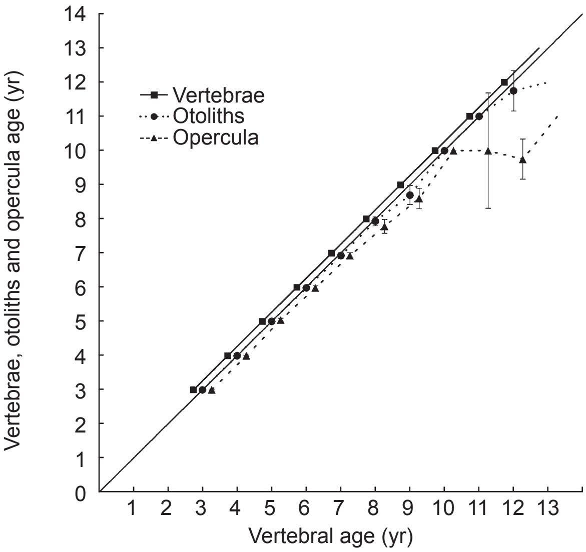

Fig. 3. Relationship among ages determined from vertebrae, otoliths, and opercula of the same fish. Deviation from the 1: 1 line shows the extent of the aging bias.

and 3 were compared with *t* values of 1.8829 for females and 1.7963 for males ($t_{0.05} = 1.96$). This result implies that the growth of *G. maculatum* is isometric.

Vertebrae radii ranged 0.93-2.88 mm. The regression analysis for the relationship between vertebrae radius and total length showed that significant differences occurred in both the intercept and slope for both sexes by the ARSS ($p < 0.01$) (Chen et al. 1992). The data were thus treated separately for each sex. The 4 regression functions provided a good fit to the data (Table 1). The linear regression function had a higher correlation r^2 making it statistically more robust. Therefore, the linear model was chosen for back-calculations (Fig. 5):

♀: $L_{♀} = 97.173Rc + 29.068$ ($r^2 = 0.879$) and

♂: $L_{♂} = 107.88Rc + 13.402$ ($r^2 = 0.9612$).

The relationship between vertebral radius and total length was linear, indicating isometric growth between the vertebral centrums and total length (Fig. 4). Back-calculated total lengths at the end of each year of life by age group for all individuals are shown in tables 2 and 3. No significant differences were found between back-calculated lengths and observed lengths (♀: $\chi^2 = 4.2021$, *d.f.* = 9, $p < 0.8976$; ♂: $\chi^2 = 1.094411$, *d.f.* = 10, $p < 0.9997$).

The back-calculated size at the time of annulus formation was used to provide lengths at age data unbiased by differences in sampling date and to estimate the von Bertalanffy equation.

Back-calculated lengths for ultimate annulus formation at different ages were also used to calculate the predicted lengths of the von Bertalanffy growth function (VBGF). The back-calculated total lengths (*L*) were fitted to the von Bertalanffy growth equation as follows:

$Lt_{♂} = 460.24[1 - e^{-0.0882(t+0.2312)}]$, and

$Lt_{♀} = 342.66[1 - e^{-0.1142(t+0.7488)}]$.

Von Bertalanffy curves produced from the back-calculated data were close to the observed data (Fig. 6). No significant differences were found between back-calculated lengths and predicted lengths ($\chi^2_{\mathrm{F}} = 2.9304$, *d.f.* = 12, $p < 0.9960$ and $\chi^2_{\mathrm{M}} = 1.067843$, *d.f.* = 13, $p < 0.9999$), therefore VBGFs can be used to decide the model of growth.

DISCUSSION

Age estimations from otoliths, vertebrae, and opercula can be inconsistent in some cases. In our study, ages estimated using opercula for the

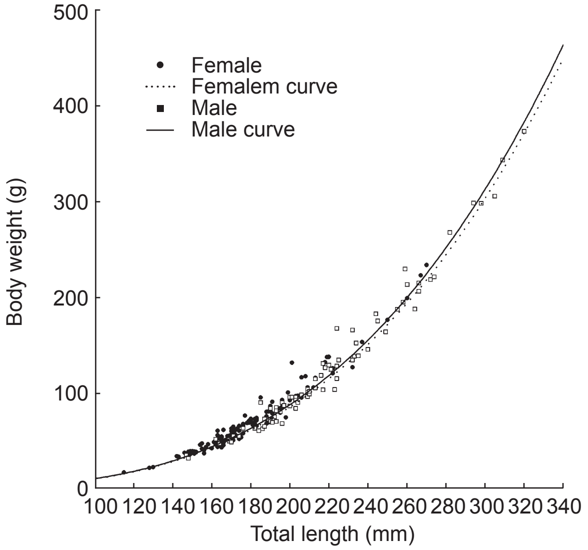

Fig. 4. Relationship between the body weight (W) and total length (TL) of *Glyptosternum maculatum*.

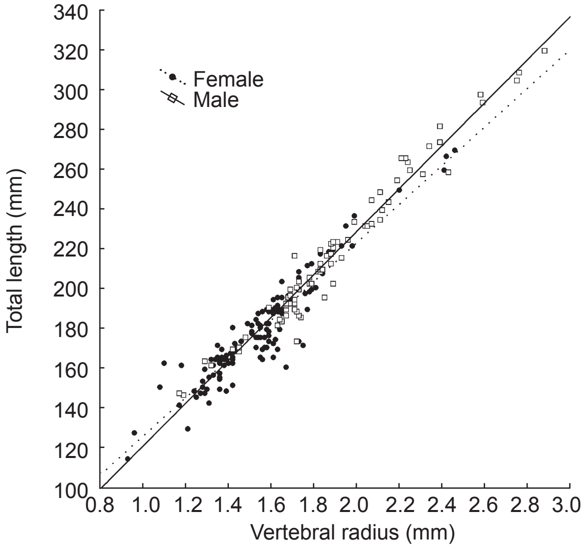

Fig. 5. Relationship between the total length and vertebral radius of *Glyptosternum maculatum*.

Table 1. Values of the correlation r^2 describing the relationship between total length and vertebra radius in males and females of *Glyptosternum maculatum*

	Linear	Power	Logarithmic	Polynomial
Female	0.879	0.8552	0.8455	0.8599
Male	0.9612	0.9535	0.9355	0.9516

age group between 3 and 9 yr coincided with those of otoliths and vertebrae. However, for samples over 10 yr old, age estimates from otoliths and vertebrae were significantly higher than that from opercula. Because otoliths of *G. maculatum* are irregularly shaped and much smaller than those of other species with similar body lengths, observing and processing this tissue were not easy. In contrast to otoliths, vertebrae had regularly formed rings and were more reliable, easier to process, and easier to handle. They were used for age determination of Siluriformes fishes in some studies. Many studies have indicated that the reliability and consistency of vertebrae for age determination are relatively higher compared to other materials (Appelget and Smith 1951, Wu 1975, Xie 1986, Duan and Sun 1999, McAuley et al. 2006). Accordingly, age determinations of *G. maculatum* from otoliths, opercula, and vertebrae in this study had acceptable consistency. Overall inter-reader comparisons showed good agreement with IAPE values of < 10%, which suggests reasonable precision. Finally, we determined that it is feasible to use vertebrae to determine the age of *G. maculatum*.

Changes in alternating opaque and translucent bands of the 3 kinds of age-determination materials should parallel the periodicities in the habitats of *G. maculatum*. At the same time, disturbances in the habitats can possibly result in the appearance of false bands, i.e., bands that do not completely encircle the centra, especially in vertebrae and opercula. Those disturbances seemed to include cessation of feeding, inhibited spawning, and failure to engage in physiological activity. There are several possible explanations for annulus formation. A shortage of food, deprivation caused by migration, and changing temperatures may all be factors affecting its formation, which can cause calcium deposited in the bone to be reabsorbed and reutilized, called the Crihton effect (Sullivan et al. 2003). Considering the special geographical environment of the Qinghai-Tibet Plateau, the appearance of false bands should most possibly be due to insufficient food availability and thermal changes in the water.

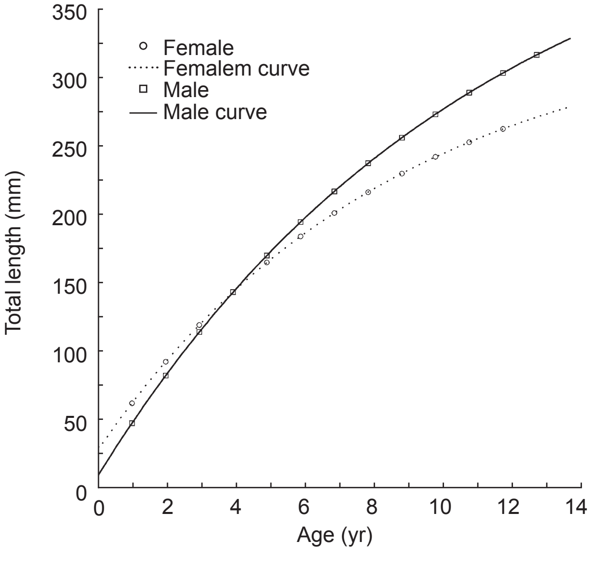

Fig. 6. Von Bertalanffy growth curves fitted to the length-age data of *Glyptosternum maculatum*.

For the present investigation of age and growth of fish, a large number of vertebrae of fish were used to back-calculate the growth history of the fish, and a high correlation was found between total length and vertebra radius for both sexes (Natanson et al. 2007). Back-calculated lengths for age were in close agreement with observed lengths. The results obtained with the back-calculation method were very satisfactory. As fish length and vertebra size are closely correlated, using measurements of previously formed marks to back-calculate the growth history was judged to be valid.

The relationship between total length (mm) and body weight (g) was calculated for each sex. Values of the allometric coefficient, b, were 3.1474 and 3.142 for males and females, respectively. There was no significant difference between the values of b and 3, indicating that the growth of *G. maculatum* is isometric. Therefore, the Bertalanffy equation can be used to describe the growth of *G. maculatum*.

Negative values of t_0 are frequent among species with rapid growth during the 1st year and reduced growth rates in subsequent years (Peres and Haimovic 2004). There are no data on 1- or 2-yr-old *G. maculatum* in the study area, but the results indicated that the lengths of 1-yr-old *G. maculatum* should be around 54.08 mm for males and 66.4 mm for females. For the age group of 2-4 yr, the rate of increment was 30 and 27.48 mm/yr, while those for the 5-12-yr age group were 14.33 and 19.99 mm/yr for females and males, respectively. The majority became sexually mature at the age of 3 and 4 yr. The growth rate significantly differed between sexes. Females were smaller than males, attained their maximum length faster, and appeared to have a longer lifespan than males. Several factors might be responsible for this growth difference between males and females, such as physiological changes influenced by temperature changes, feeding regimes, and reproductive cycles (Utagawa and Taniuchi 1999, Newman et al. 2000, Morales-Nin and Ralston 1990).

Branstetter (1987) categorized k values as 0.05-0.10/yr for slowly growing species, 0.10-0.20/yr for species with average growth, and 0.20-0.50/yr for rapidly growing species. Based on these criteria, *G. maculatum* in the Brahmaputra River has a moderate growth rate, has a faster growth rate than *Gymnocypris cuoensis* (0.0291) (Yang et al. 2000) and *Selincuo schizothoracini* (♂ 0.06839, ♀ 0.0710) (Chen et al. 2002) in the same area, and has a slower growth rate than *Pelteobagrus fulvidraco* (0.2476) (Li et al. 2006), *Glyptothorax fukiensis* (0.2541) (Sheng et al. 2005), and *Silurus meridionalis* (♂ 0.20337, ♀ 0.14743) (Xie 1986) in the same order but distributed at lower elevations. There are several possible explanations: a shortage of food, deprivation caused by migration, and changing temperatures may all be factors affecting its growth.

Generally, when the water temperature decreases with increasing elevation, the longevity of fish lengthens, the body length increases, and the growth rate is reduced (Mills 1988, Gaspar et al. 1999, Gordon et al. 2004). At the same time, under less fishing pressure, the body length of fish is shorter and the average age of individuals and the maximum age increase in the reproductive population. In this plateau river, the surface water temperature of the Brahmaputra River

Table 2. Backed-calculated total length (mm) of male *Glyptosternum maculatum*

Age (yr)	Number	Back-calculated total length (mm)											
		TL_1	TL_2	TL_3	TL_4	TL_5	TL_6	TL_7	TL_8	TL_9	TL_{10}	TL_{11}	TL_{12}
3	1	54.33	93.2	119.43									
4	5	68.91	90.29	136.93	127.21								
5	41	66.97	93.2	105.84	159.28	164.14							
6	50	66.97	92.23	130.13	146.64	160.25	187.46						
7	20	64.05	103.39	133.04	137.9	166.08	193.29	199.12					
8	5	65.99	79.6	113.61	143.73	154.42	170.94	211.75	214.66				
9	2	66.97	84.46	114.56	137.9	172.88	181.63	203.98	217.58	235.07			
10	1	62.11	84.46	102.92	142.76	158.3	183.57	197.18	224.39	230.21	245.76		
11	1	65.02	90.29	121.38	146.64	170.94	181.63	204.95	220.5	229.24	242.85	256.45	
12	2	66.97	96.12	120.41	162.19	183.57	193.29	210.78	228.27	243.82	251.59	258.39	263.25
Weighted average		66.4	93.59	121.67	148.54	162.94	187.76	202.35	219.08	236.21	247.95	257.74	263.25

Table 3. Backed-calculated total length (mm) of female *Glyptosternum maculatum*

Age (yr)	Number	Back-calculated total length (mm)												
		TL_1	TL_2	TL_3	TL_4	TL_5	TL_6	TL_7	TL_8	TL_9	TL_{10}	TL_{11}	TL_{12}	TL_{13}
3	1	53.31	87.84	120.2										
4	1	50.08	93.23	116.97	145.02									
5	5	55.48	86.76	114.81	141.78	170.91								
6	23	50.08	83.52	115.89	146.09	178.46	198.96							
7	23	55.48	83.52	122.36	147.17	170.91	195.72	221.61						
8	9	60.87	96.47	133.15	133.15	176.3	201.13	230.24	243.18					
9	8	55.48	75.97	106.18	147.17	173.06	197.88	215.13	244.26	265.84				
10	2	46.84	81.37	125.59	148.25	163.35	200.03	223.76	249.66	266.92	279.87			
11	2	51.16	86.76	124.52	139.62	160.12	193.56	212.98	238.87	260.44	284.18	298.21		
12	2	56.55	91.08	115.88	138.54	174.14	191.4	211.9	230.24	269.08	282.02	297.12	313.31	
13	1	54.39	98.63	111.57	148.25	175.22	202.19	228.08	255.05	270.16	286.34	299.28	314.38	319.78
Weighted average		54.08	85.06	119.25	145.06	173.76	197.76	221.61	243.14	265.98	282.64	297.99	313.67	319.78

is 0.8-14.8°C, and higher than 9.4°C only from Apr. to Sept. The phenomenon is complicated due to the slow growth and age structure of *G. maculatum*. Schizothoracid fishes also showed this phenomenon in the same area (Yang et al. 2000, Chen et al. 2002).

The findings on age and growth of *G. maculatum* from this research will help elucidate the distribution with age of fish and their sustainable management. Moreover, studies on otoliths, opercula, and vertebrae provide an important basis for further studies on the age and growth of related taxa.

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