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Age and Growth of *Triplophysa* (*Hedinichthys*) *yarkandensis* (Day, 1877) in the Tarim River in Xinjiang, China

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XYW drafted the manuscript. FZZ and JMG in the design of the study and performed the statistical analysis. DSC and YS conceived of the study, and participated in its design and coordination. CXX and SAC have given final approval of the version to be published. All authors read and approved the final manuscript.

Key words
***Triplophysa yarkandensis*, Tarim River, Otolith**

ABSTRACT

Influenced by water pollution and flow modification, *Triplophysa* (*Hedinichthys*) *yarkandensis* (Day, 1877) may become an indigenous endangered fish species in the Tarim River. In order to study its population characteristics and improve the conservation measures, 940 specimens of *T. yarkandensis* were collected in 2018 and 2020 from the Alar Section of Tarim River. Otoliths were chosen as the main age structure in this study. Results showed that the rings of *T. yarkandensis* formed once per year from March to May. The ages ranged from 1+ to 10+, with 2.5+ to 6.5+ predominating in the total specimens. Among them, 5+ predominated for males and 4+ to 6+ predominated for females respectively. The standard length (*SL*) of the specimens ranged from 30.0 to 195.0 mm, and the body weight (*BW*) ranged from 3.40 to 114.00 g. The predominating *SL* ranged from 75.0 to 125.0 mm, accounting for 51.81% of the specimens. Length-weight relationship was described as $W = 0.0355 \times L^{2.5916}$ ($R^2 = 0.8736$, $n = 940$). Growth was described by the von Bertalanffy equation: $L_t = 184.79\ (1-e^{-0.1026\ (t+1.0458)})$, $W_t = 68.08\ (1-e^{-0.1026\ (t+1.0458)})^{2.5916}$ for male and $L_t = 236.63\ (1-e^{-0.0900\ (t+0.1831)})$, $W_t = 129.16\ (1-e^{-0.0900\ (t+0.1831)})^{2.5916}$ for female. Age at inflection point for male and female were 8.24 and 10.39 years, respectively. The goal of this study is to better understand the population's makeup and migratory patterns in order to exploit and develop germplasm resources of *T. yarkandensis*. Subsequent efforts should be made to prevent overfishing of young fish by limiting the minimum fishing individuals, and to protect older individuals by banning fishing during the breeding period.

INTRODUCTION

Age determination is the basis for studying the biology and population ecology of fish (Yin, 1995). Age can be determined by three methods: Direct observation, statistics and analyses on catch, and structural analyses on calcification (Ye, 2002). Scales, vertebrae, otoliths, opercular, cleithra, actinosts, basioccipital bones, and hyoid bones are usually used for the observation, analysis, and estimation on age (Wu, 1975; Xie *et al*., 1994). Previous studies have found out that it is not reasonable to choose scales for those old and slow-growing fishes. Their scales are easy to get lost which results in underestimation (Shen *et al*., 2001).

For different fishes, different age structures should be utilized. High-accurate structure should be taken as the principal material and others as the supporting materials. Therefore, it is necessary and crucial to select the best and the most suitable material for age determination (Polat *et al*., 2001). Otoliths have been proved to be the most commonly accurate age structure for fish so far. Ring marks in otoliths grow steadily no matter how widely the living environment changes (Sponaugle, 2009), because it is closely correlated with the growth of fish (Casselman, 1990; Campana, 2001; Horn, 2002; Chen *et al*., 2009). Pannella (1971) found the daily increment on otolith, which laid a solid foundation for the studies on the relationship between otoliths and the growth of fish.

Triplophysa, widely spread in inland waters on plateaus in the middle parts of Asia, is indispensable to the fish fauna on Qinghai-Tibet Plateau (He *et al*., 2008), having special adaptability to life on plateau (He, 1996). *Triplophysa* (*Hedinichthys*) *yarkandensis* (Day 1877) (Cyprinidformes, Cobitidae, *Triplophysa*), is an endemic fish species in the Tarim River. Locally known as Goutou (Dog-head). Since 1960s, population structure has been disrupted by human activities, biological invasion, water

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pollution and other factors. This caused the decline in the numbers of endemic fishes (Wang, 1995; Olden *et al.*, 2010; Xiong *et al.*, 2015).

T. yarkandensis may become another indigenous endangered fish species in the Tarim River after *Aspiorhynchus laticeps* and *Schizothorax biddulphi* (Zhu, 1989; Chen, 2012). Recently, the research of *T. yarkandensis* mainly focuses on growth and reproduction, culture and toxicity test, genetic diversity and interspecific differences (Zhao, 1989; Zeng and Tang, 2010). As a plateau fish, the *T. yarkandensis* has a slower growth rate, smaller size and greater salt tolerance, and has specific economic value and unique ecological significance.

In this study, otoliths were chosen for age determination. Relationship between otoliths and body length/age was described. The von Bertalanffy growth model was fitted and its parameters were compared with results of other studies. The potential for population development and characteristics of the *T. yarkandensis* population growth in the Alar Section of the Tarim River were identified. It served as a foundation for the correct prediction on the development prospect of the population and the reasonable conservation and effective utilization of the fish resources.

MATERIALS AND METHODS

Sampling

Periodic sampling was carried out in February, May, August and November from 2018 to 2020, using a variety of gear, including drift gill net (mesh size 2cm), set gill net (mesh size 2cm) and net cage (mesh size 2cm). 940 *T. yarkandensis* individuals were collected in the Alar Section of the upper of Tarim River (Figs. 1A, B).

All specimens were measured at the site for standard-length (SL, mm), weight (W, g), gonad weight (W_G, g), visceral weight (W_V, g) and eviscerated weight (W_E, g). SL was accurate to 1.0 mm; while W, W_G, W_V, and W_E to 0.01 g. Biological dissection was performed on site. The left lapillus otoliths, and the 6th to 7th vertebrae and opercular were selected as age identification materials. All of them were washed, dried, wrapped in filter paper and put in 2-milliliter plastic centrifuge tubes. The specimens which were taken successfully for all three structures were 891 out of 940.

Preparation and examination of age structures

Otoliths

Lapillus was embedded in nail enamel and mounted on a glass slide with the convex side pointing upwards. After dried for 12 h, lapillus was ground with 1,000 to 2,000 grit wet sandpaper. Frequent microscopic observation and

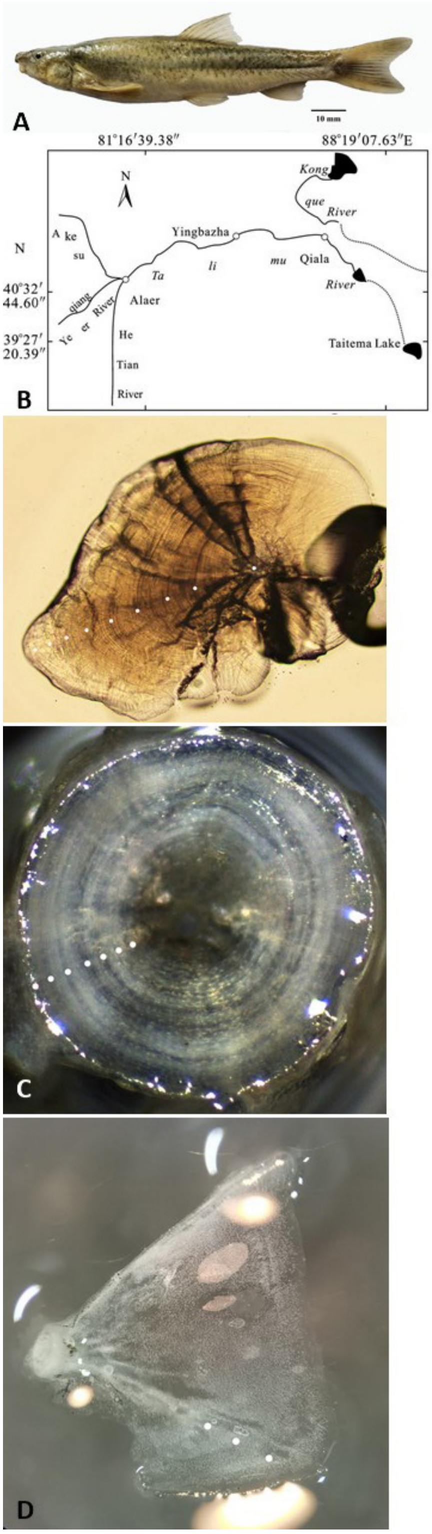

Fig. 1. A, *Triplophusa yarkandensis* in the Tarim River. B, Sample sites in the Tarim River. C, Annuli characteristics of lapillus, and vertebrae D, Operculum of *T. yarkandensis.*

adjustment were made during the grounding process until the core was reached. Then lapillus was polished. When one side was finished, acetone was applied to dissolve the nail enamel. Lapillus was turned over, mounted, dried, ground and polished in the same way (Xiong *et al*., 2006; Li *et al*., 2008; Ma, 2011). When the core plane was available, it was observed and shot under anatomical lens Leica EZ4D and Niko80i at 10×/0.3 magnification. And then the radius of lapillus at 100 um scale were measured using Image-Pro Plus 6.0 (Fig. 1C).

Previous studies have demonstrated that otolith size and weight etc. are highly associated with the growth traits. Only the radius of the sectioned lapillus was measured as the otolith of *T. yarkandensis* was extremely small.

Vertebrae

As the vertebrae of *T. yarkandensis* were relatively small, it took only 2 to 5 min to boil them in the boiling water. After the boiling, vertebrae were taken out, dried, and soaked in 1% hydrogen peroxide for 24 to 48 h. Then the 6^{th} and 7^{th} vertebrae were removed and put under anatomical lens Motic SMZ-168 for observation and taking photos. If not clear, dimethyl could be added as transparent reagent. If still not clearly read, the vertebrae could be adjusted for a better observation angle (Fig. 1C).

Opercular

Both sides of opercular were taken and put in hot water to boil for 1 min. They were cleaned for residual tissue, dried, and soaked in 1% hydrogen peroxide for 24 to 48 h. When all the above had been completed, the opercular were examined and photographed using an anatomical lens, the Motic SMZ-168.

Analysis of the formation cycle of rings

March to May is the peak breeding period of *T. yarkandensis*. The formation cycle of rings was analyzed through marginal increments (*MIR*) (Haas and Recksiek, 1995). The equation is as follows:

$$MIR = (R - R_n)/(R_n - R_{n-1})$$

Where R is the radius of the hard structure, R_n is the radius from the core to the last ring on the outside, and R_{n-1} is the radius from the core to the penultimate ring on the outside.

Photos used in the above analysis were taken under microscope Niko80i at 10×/0.3 magnification and measured at 100 um scale using Image-Pro Plus 6.0.

Age validation

Age was validated according to the ring number on the hard structure. In this study, the first ring was considered one year of age from where age increases (Massutí *et al*., 2000). Samples were assessed for age independently by two readers. If the ages assigned by each reader agreed, then the ages were considered valid. Otherwise, the two readers re-examined the structure together and reached an agreement. In case the second results still varied widely, the sample had to be abandoned (Liu *et al*., 2009).

Relationship between length and weight

In this study, a common equation was applied to analyze the characteristics of growth. Measurements of length and weight between sexes were used for Kolmogorov-Smirnov test and significance analysis (Cazorla and Sidorkewicj, 2009; Ma, 2011). A *t*-test was used to compare growth parameters *b* and 3 (Pauly, 1984) to estimate whether *T. yarkandensis* grow at a constant speed.

The Power-exponential relationship between length and weight was shown in the following equation:

$$W = aL^b$$

Growth equation

The characteristics of the growth were described by the classic von Bertalanffy growth model:

$$L_t = L_\infty(1 - e^{-k(t-t_0)}); W_t = W_\infty(1 - e^{-k(t-t_0)})$$

Where L_t is the length at age t of individual fish, W_t is the weight at age t, L_∞ is the maximum attainable length, W_∞ is the maximum attainable weight, t_0 is the hypothetical age at which length and weight were zero, and k is the curvature of the growth curve.

In order to further predict the growth trend, the growth rate and acceleration equations were obtained by first order and second order derivation of the growth equation. The growth trend of fish was fitted by the measured age data or the retrogradation age data. And the growth rate was slower when it approached an asymptotic value (Fei and Zhang, 1990; Yin, 1995; Pauly, 1981).

The growth performance index was calculated using the equation: $Ø = lgk+2lgL_\infty$ (Munro and Pauly, 1983).

Data processing

All statistical analyses were performed using SPSS 16.0 and Origin 8.0.

RESULTS

Frequency distributions of length and weight

The frequency distributions of *SL* were shown in Figure 2A. The length ranged from 30.0 to 195.0 mm and the mean SL was 96.5±17.0 mm. The predominating *SL* ranged from 75.0 to 125.0 mm, accounting for 51.81% of the whole specimens. There were 485 males whose length ranging from 65.0 to 162.0 mm, and 412 females ranging from 30.0 to 190.0 mm. The length difference between the sexes was not significant (Kolmogorov-Smirnov test, F = 0.972, $p > 0.05$).

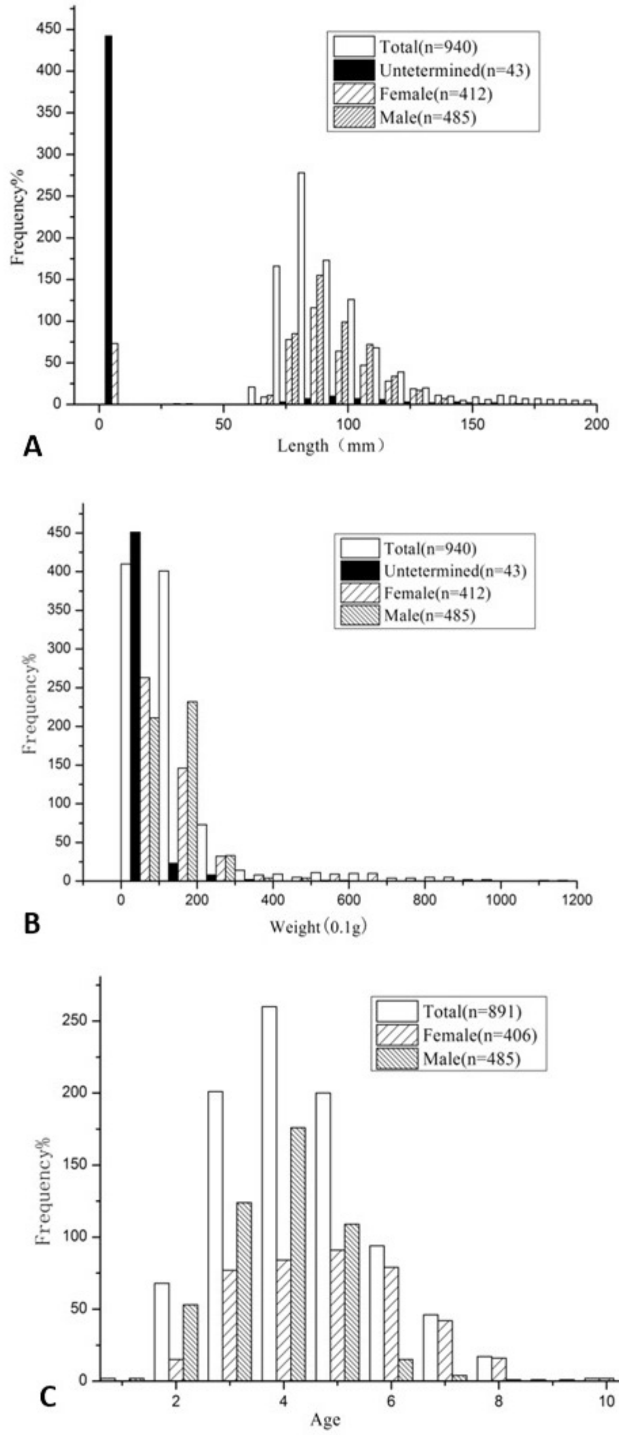

Fig. 2. Distributions of the frequencies of SL (A), W (B) and age (C) of *T. yarkandensis.*

The frequency distributions of W were shown in Figure 2B. The weight ranged from 3.40 to 114.04 g and the mean weight was 14.25±7.29 g. The visceral weight ranged from 0.07 to 12.14 g, and the evisceral weight ranged from 1.06 to 101.59 g with the mean weight of 12.77±8.52 g. The predominating weight ranged from 5.00 to 20.00 g, proportion of those above 30.00 g decreasing as their weight increases. The weight of the indeterminate ranged from 12.57 to 85.48 g. In the whole specimens, the weight of male from 3.40 to 50.99 g and female ranged from 3.59 to 114.04 g. Weight between sexes differ significantly in different ages Kolmogorov-Smirnov test, $F = 23.362$, $p < 0.05$).

Frequency distributions of age

The vertebrae and opercular were found to be less useful for determining the age of *T. yarkandensis*, especially for older fish, and were only utilized as an additional aid for identification in cases of otolith loss. 891 were successfully read for ages in the 940 specimens, and the rest 49 were unsuccessfully read (including 6 females and 43 indeterminate). Age frequency distribution of *T. yarkandensis* was shown in Figure 2C. The youngest individual in the capture population was 1 year of age, the oldest was 10 years, and the preponderant was 2.5 to 6.5 years. The predominant male was 5 years and female were 4 to 6 years of age.

Relationship between length and weight

The power-functional relationship between SL and W of the total 940 specimens were described by the following equations:

Indeterminate population: $W = 0.0376 \times L^{2.5484}$ (R^2 =0.8910, n = 43)

Male population: $W = 0.0321 \times L^{2.6362}$ (R^2 = 0.8595, n = 485)

Female population: $W = 0.0369 \times L^{2.5772}$ (R^2 = 0.8766, n = 412)

Overall population: $W = 0.0355 \times L^{2.5916}$ (R^2 = 0.8736, n = 940)

Through Kolmogorov-Smirnov test, we found that the length-weight relationship between sexes was not significantly different ($F = 0.857$, $p > 0.05$). The b value of the overall population of *T. yarkandensis* was significantly different from 3 ($t = 5.699$, $p < 0.05$), which suggesting that *T. yarkandensis* grew allometricly (Fig. 3A, B).

Relationship between otolith radius and body length/age of fish

The relationship between radius of the major axis of otolith and body length/age was analysed (Fig. 4). The fitting equation of otolith radius (OR) and body length was power function with low correlated: $OR = 7.9688L^{0.1101}$ ($R^2 = 0.0033$). While the fitting equation of otolith radius and logarithmic age (A) represented linear relationship: $OR = 293.56A+9336.6$ ($R^2 = 0.0689$). Above means otolith radius increases as body length and age increases.

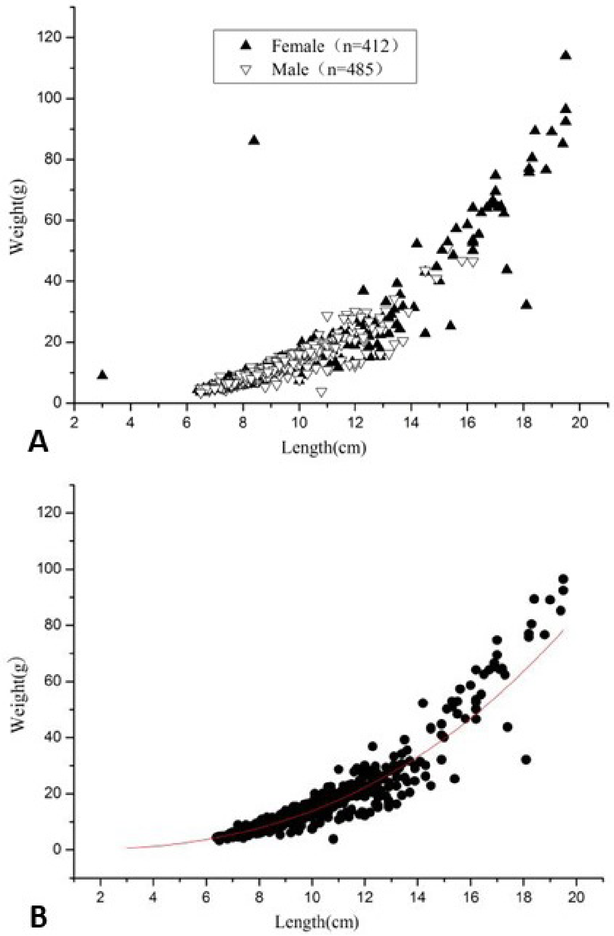

Fig. 3. Length-weight relationships of male and female (A) and overall population (B) of *T. yarkandensis*.

Growth equation

Length data of individual age classes of both sexes of *T. yarkandensis* were shown in Table I and Figure 5. Mean length between male and female in the same age class did not differ significantly (independent sample *t*-test, $p > 0.05$, except 3+ and 6+). In age class 3+ and 6+, mean length of female differed significantly ($p < 0.05$). Length differences in the same age class were also significant as *T. yarkandensis* grow older. They were used to fit growth curves.

The von Bertalanffy growth equations were fitted to measured SLs as following:

Male population: $L_t = 184.79\ (1-e^{-0.1026\ (t+1.0458)})$;

Female population: $L_t = 236.63\ (1-e^{-0.0900\ (t+0.1831)})$.

The growth equation of weight could be obtained from the above:

Male population: $W_t = 68.08\ (1-e^{-0.1026\ (t+1.0458)})^{2.5916}$;

Female population: $W_t = 129.16\ (1-e^{-0.0900\ (t+0.1831)})^{2.5916}$.

The growth performance index (Ø) of male and female were 3.6457 and 4.7106, respectively.

Table I. Number of specimens and mean ± S.D. and range of standard length at age of *T.yarkandensis.*

Age	Male (n=485)			Female (n=412)		
	n	Mean±SD (cm)	Range (cm)	n	Mean±SD (cm)	Range (cm)
X				6	10.75±1.43	7.9-13.2
1	2	7.45±0.05	7.4-7.5			
2	53	9.28±1.17	6.5-13.1	15	8.77±1.93	3.0-13.6
3	124	8.98±1.02	6.5-13.2	77	10.01±1.68	6.8-19.5
4	176	9.35±1.22	6.7-13.9	84	8.59±1.05	6.7-15.0
5	109	9.38±1.41	6.8-16.2	91	9.38±1.44	6.4-19.5
6	15	8.49±0.74	7.6-10.2	79	10.24±2.30	6.7-19.4
7	4	8.30±0.70	7.2-9.5	42	11.45±3.13	7.2-19.5
8	1	7.9	10.8-18.2	16	13.46±1.64	7.2-13.2
9	1	12.4				
10				2	13.3±5.50	7.8-18.8

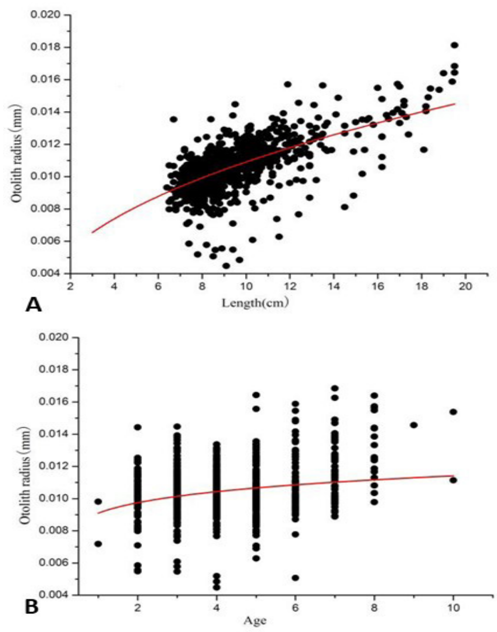

Fig. 4. Relationships between otolith radius (A) and body length/age (B) of fish.

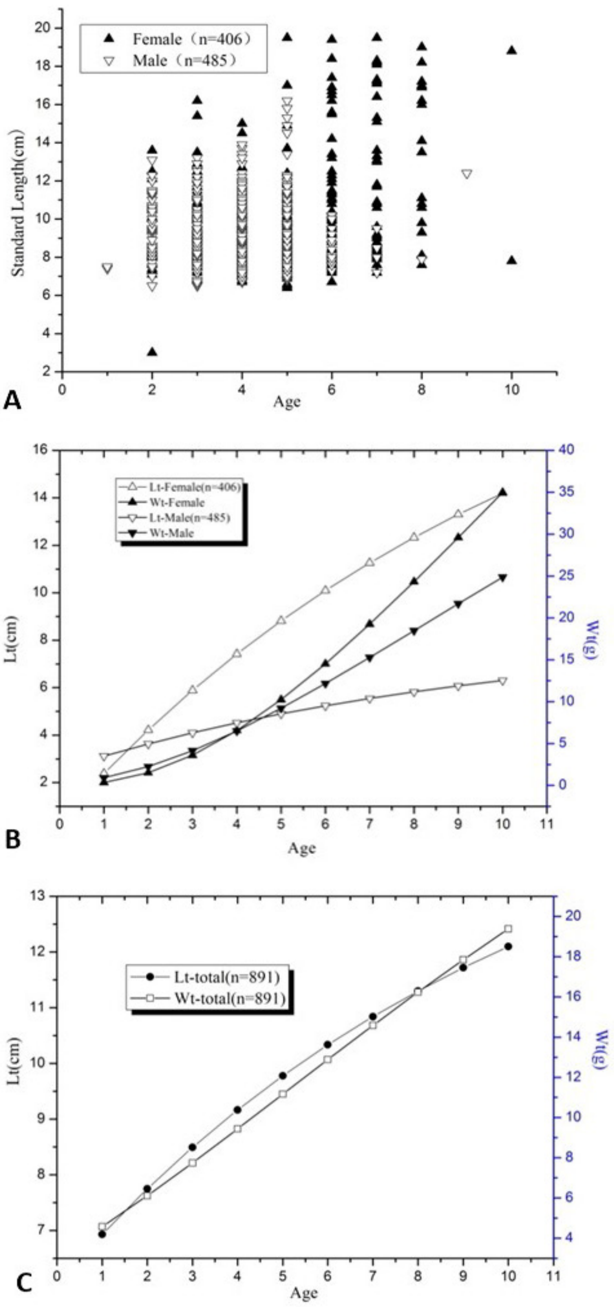

Fig. 5. Relationship of age read from otoliths and the observed standard length (A), von Bertalanffy growth curve of body length and body weight in male and female population (B) and overall population (C).

Growth rate and acceleration

Performing first-order derivative and second-order derivative based on the above length and weight growth equation. The following equations of growth rate and acceleration were obtained (Fig. 6).

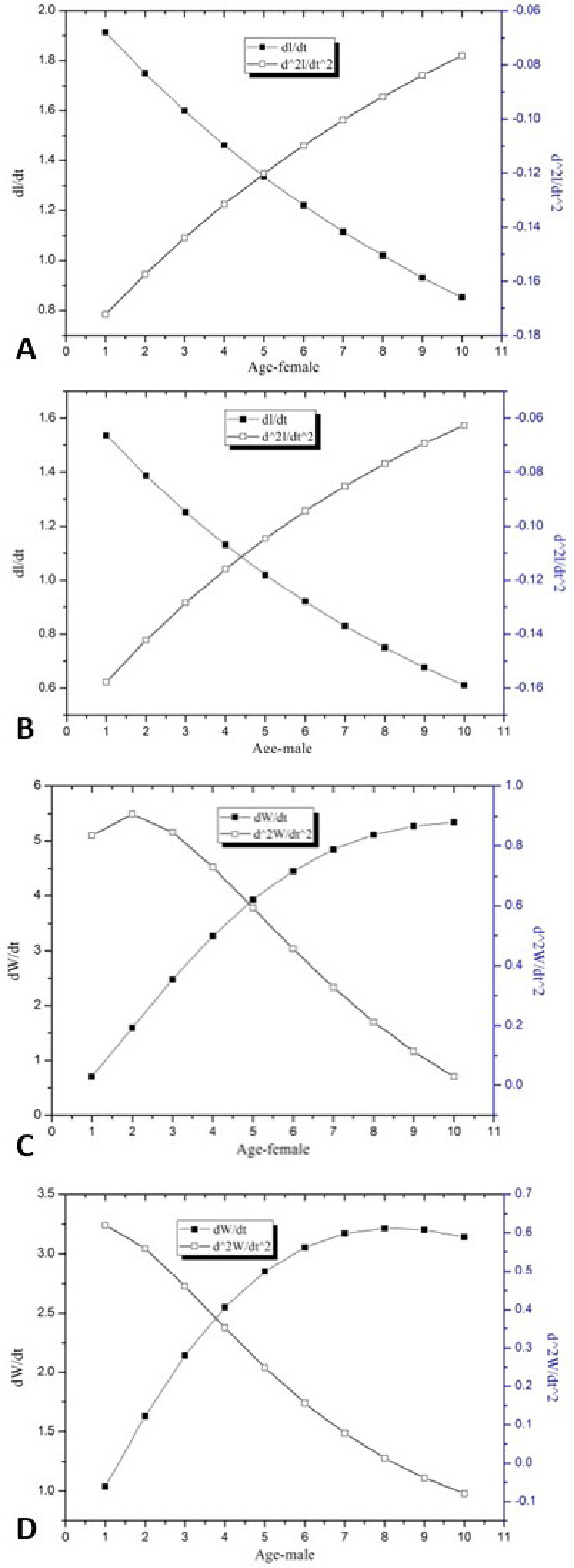

Fig. 6. Growth rate and growth acceleration of SL and W.

Male: $dL/dt = 18.96\ e^{-0.1026\ (t + 1.0458)}$;
$dL^2/dt^2 = -1.95\ e^{-0.1026\ (t + 1.0458)}$;
$dW/dt = 18.10\ e^{-0.1026\ (t + 1.0458)}\ (1 - e^{-0.1026\ (t + 1.0458)})^{1.5916}$;
$dW^2/dt^2 = 1.86\ e^{-0.1026\ (t + 1.0458)}\ (1 - e^{-0.1026\ (t + 1.0458)})^{0.5916}\ (2.5916 \times e^{-0.1026(t + 1.0458)} - 1)$;
Female: $dL/dt = 21.30\ e^{-0.0900\ (t + 0.1831)}$;
$dL^2/dt^2 = -1.92\ e^{-0.0900\ (t + 0.1831)}$;
$dW/dt = 30.13\ e^{-0.0900\ (t + 0.1831)}\ (1 - e^{-0.0900\ (t + 0.1831)})^{1.5916}$;
$dW^2/dt^2 = 2.71\ e^{-0.0900\ (t + 0.1831)}\ (1 - e^{-0.0900\ (t + 0.1831)})^{0.5916}(2.5916 \times e^{-0.0900\ (t + 0.1831)} - 1)$.

Male and female *T. yarkandensis* had similar variation trends in the growth rate and acceleration of length and weight (Fig. 5-6). Age at inflection point for male (t_i) was 8.24, length and weight of which was 113.5 mm and 19.26 g, while for female (t_i) was 10.39, length and weight of which was 145.2 mm and 36.47 g.

We found no inflection point on the curves of growth rate and acceleration of length. Growth rate and acceleration were negatively correlated with age increase.

The weight grew sustainably and the trend was flat at the inflection point. While the acceleration of weight increases first and then decreases, representing a slow decline. As for female, arriving at the maximum when the age was two, which was key to the inflection point of weight. As for male, there was no rising trend but a slow drop, leveling off when age was zero.

DISCUSSION

There has always been disputed about the relationship between the size of otolith or scale and the body length of fish (Yin, 1995). By taking measurements on otolith through different axes, we could find that otolith grow all the time but on a non-uniform rate (Fowler, 1990). For old or slow-growing fish, continuous distal deposition of otolith resulted in non-uniform growth of body length (Boehlert, 1985; Reñones *et al*., 2007; Ma, 2011), which will reduce the accuracy of back-calculation of length (Campana, 1990).

From the growth equation of *T. yarkandensis*, we found the age at inflection point was relatively old. 10+ were the most numerous in the specimens and around 9+ for male and female. The relationship between coefficient k and asymptotic length and asymptotic weight was positive, suggesting that *T. yarkandensis* is a fast-growing, long-lived *Triplophysa* although the growth is slow compared to other fish (Table II). The maximum length and weight of female were bigger than those of male, and the k value of female was smaller than that of male. This was closely related to the big change in the aquatic environment of the Tarim River and the different maturity of individual male and female.

The finding of longevity of *T. yarkandensis* was similar to that of Zhang *et al*. (2010). The reasons for different growth parameters obtained by different authors might be the following (Table II): (1) different distributions of length and weight as a consequence of different mesh sizes and number of specimens; (2) different sampling locations, areas and seasons; (3) different methods of fitting growth equations (mainly by back-calculation and field measurement of length). And studies of Cailliet and Goldman (2004) demonstrated that if there are a lack of sufficient number of samples of younger fish or older fish to apply the established model, t_0 will be instability. This can lead to inaccurate inferred growth models.

Table II. Growth parameters of *Triplophysa.*

Species	Location	L_∞	W_∞	t_0	t_i	Source
T. yarkandensis	Tarim River	184.8♂ 236.6♀	68.08♂ 129.16♀	-1.0458♂ -0.1831♀	3.4645♂ 4.7106♀	this study
T. yarkandensis	Yarkand River	166.5742	56.6625	-1.319	3.9548	Wang, 2022
T. yarkandensis	Hotan River	302.772	310.845	-0.4608	9.0710	Wang, 2022
Triplophysa bombifrons	Tarim River	633.1	585.64	-1.4371	13.71	Yao *et al*., 2018
Triplophysa orientalis	Yarlung Tsangpo River	125.19♂ 151.659♀	18.787♂ 31.496♀	-0.0695♂ -0.01786♀	5.83♂ 8.19♀	Li *et al*., 2016
Triplophysa tenuis	Kaidu	237.5	97.42	-0.7	9.14	Jin *et al*., 2020
Triplophysa stenura	Nujiang River	246.943	132.03	0.1689	18.45	Deng *et al*., 2010
Triplophysa siluroides	Beichuanhe Bssin	103.2	9.24	-0.8345	2.14	Yao *et al*., 2019
Triplophysa stewarti	Lake Chugutso	138.91	28.179	-2.895	3.65	Tian *et al*., 2022
Triplophysa markehenensis	The Dadu River	173.1241	60.7531	-0.5328	6.25	Zhang *et al*., 2010

The growth coefficient k was useful during the assessment of the potential sensitivity of fish resources (Musick, 1999; Li *et al*., 2009). This study showed that the k value of male was higher than that of female. Those slow-growing, long-lived, late-maturing fish, such as *T. yarkandensis*, were very sensitive to the environment. High mortality and fast consumption of resources make the recruitment rate slower than expected (Musick, 1999). These characteristics were probably due to the adaptation to extreme environments of low water temperature and lack of food.

CONCLUSION

In this study, *T. yarkandensis* of Tarim River was slow-growing, long lived and the individual was relatively small-sized. The fullness was asynchronous and uneven and the population structure was not stable. They cause the fish resource was limited. For those fish, it is very difficult to recover when the stock is depleted. In conclusion, as an endemic fish, *T. yarkandensis* deserves our attention and proper conservation. It was quite necessary to set up a set of scientific fisheries management measures for the conservation of *T. yarkandensis*.

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Ethical statement

This research was conducted in accordance with ethics committee procedures of animal experiments.

Statement of conflict of interest

The authors have declared no conflict of interest.

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