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Reproductive Biology of *Triplophysa* (*Hedinichthys*) *Yarkandensis* (Day) in the Tarim River, China

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Authors' Contribution
SC designed this study. XW and NY conducted the experiments. CW and YS analyzed the data. XW and NY wrote the manuscript. SC and CX were in charge of writing, reviewing, and editing the manuscript. JG and FZ collected samples. All authors have read and agreed to the published version of the article.

Key words
Tarim River, *Triplophysa (Hedinichthys) yarkandensis* (Day), Reproductive biology, Gonadal development, Reproductive population

ABSTRACT

Triplophysa (*Hedinichthys*) *yarkandensis* (Day) is one of the most common fish in the Tarim River and is commercially important for the local fishing industry. In this study, we investigated the reproductive biology of *T. yarkandensis*. Totally, 940 individuals were sampled during January 2018 to December 2020.*T. yarkandensis* specimens (n = 940) were collected. The female to male ratio was 1:1.18. The standard length, weight and age of the females at minimum maturity were 82 mm, 7.4 g and 3 years old, respectively, and those of the males were 65 mm, 3.4 g and 2 years old, respectively. From June to August, the egg diameters exhibited a unimodal distribution, indicating that the eggs followed a pattern of one-time spawning. The fecundity of 88 females in stages IV-V was calculated; these calculations included the standard length (30 to 195 mm), weight (3.59 to 114.04 g), absolute fecundity (1101 to 56320; 9944 ± 5487), relative fecundity (824 to 1140; 982 ± 158) and population fecundity (4,034,600). The TW and TL of *T. yarkandensis* were positively correlated with absolute fecundity, meaning that its absolute fecundity increased with increasing TW and TL. This study provides much information about the reproductive biology of *T. yarkandensis*, which can be used for fish population protection and artificial reproduction research.

INTRODUCTION

The Tarim River is the longest inland river in China. However, in the past few decades, due to the increase in human activities, land reclamation along the Tarim River has become increasingly serious, leading to desertification, water and soil pollution, habitat destruction and biodiversity loss, resulting in a series of potential ecological and environmental risks. These processes further lead to the serious degradation of river fishery resources and pose a threat to the water ecological security of the Tarim River (Liu and Yin, 2020).

Triplophysa yarkandensis, belonging to Cyprinidformes, Cobitidae, Nemachilinae is widespread in the Tarim River system and constitutes one of the fishes at the world's highest altitude (Chen *et al*., 2020; Ning *et al*., 2020). Due to a sharp decrease in resources, *T. yarkandensis* may become the third most endangered fish after *Aspiorhynchus laticeps* and *Schizothorax biddulphi* in the Tarim River system (Zhu, 1989; Chen, 2012). In recent years, there is little information available for *Triplophysa* as well as on fishery research on the Tarim River system. Studies focus on new species, morphological characteristics, feeding, and growth (Chen, 2012; Wang, 2022).

In this study, it is urgent to conduct studies on the reproductive biology of *T. yarkandensis* to explore the life history of *Triplophysa*, provide a theoretical basis for the protection of the reproduction and fry production of *Triplophysa* and collect basic data for the conservation and development of fishery resources in the arid, salinized, and alkalized inland areas.

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MATERIALS AND METHODS

Field and time sampling

The reproductive parameters of *T. yarkandensis* (Fig. 1) in the Alar section of the Tarim River (N80°95′00″, E40°49′07″~N82°34′47″, E40°92′99″) were assessed (Fig. 2) , and using drift gill nets (2a = 2.0 cm), fixed gill nets (2a = 2.0 cm), ground cages (2a = 2.0 cm), small lifting nets and other fishing gear, 940 specimens throughout the different seasons were collected monthly from January 2018 to December 2020. The total length (TL, to the nearest 0.1 mm) ranged from 30.0 to 195.0 mm, and the total weight (TW, to the nearest 0.01 g) ranged from 3.40 to 114.04 g. The fish were dissected, the gonad weight (GW, to the nearest 0.001 g) of each individual was removed and weighed, and the ovaries that were removed were fixed in 10% formalin.

Fig. 1. *T. yarkandensis* in the Tarim River.

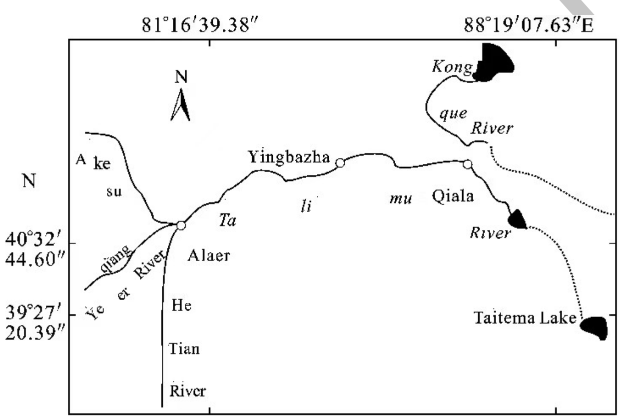

Fig. 2. Sampling sites.

Age determination

Age was validated according to the ring number on the otolith (Ma *et al*., 2010; Mohamed, 2010). The age of the samples was assessed independently by two readers. If the ages assigned by each reader were in agreement, the ages were considered valid; otherwise, the two readers re-examined the structure together until reaching an agreement. If the second results still varied widely, the sample was abandoned (Downey *et al*., 2018).

Length-weight relationship

Exponential regression was used to estimate the length-weight relationship according to the equation TW = a TL^b (Ricker, 1975). where TW is the total weight in g; TL is the total length in mm; a is the condition factor, which reflects the environmental conditions (Lin, 1999); and b is the allometric growth factor, which reflects uneven growth and development (Froese, 2006). The value of b was tested using student's *t* test ($P < 0.05$) to determine if b = 3.0. The relationship between male and female population curves was compared by analysis of covariance (ANCOVA).

Reproductive characteristic

A χ^2 test was used to determine whether the proportion of males and females was significantly different from 1:1; the mean sizes of the males and females were compared using Student's *t* test (Costa *et al*., 2019; Dinh *et al*., 2020).

The ovary diameters at 100% maturity were randomly selected from each sample. The following developmental stages of oocytes (fixed in 4%) were observed in the histological analyses: stage I (early developing phase), stage II (late developing phase), stage III (maturing phase), stage IV (mature phase), and stage V (spent phase). A transverse section from the central part of each gonad was dehydrated in alcohol and subsequently embedded in paraffin wax (Paraplast). The gonads were sectioned transversely, mounted on glass slides and stained with hematoxylin and eosin (H–E) (Downey *et al*., 2018; Costa *et al*., 2020). The preparations were observed and photographed using a compound microscope (Leica EZ4D), and the developmental stages of the ovaries were described according to the terminology proposed by Yamamoto (1956).

Furthermore, a morphometric study on the oocytes sectioned through the nucleus was conducted using a 10× magnification microscope coupled to a system consisting of a high-resolution video camera, monitor and software package (Image-Pro Plus 6.0). A minimum of 100 oocytes were randomly selected from each ovary and measured using a stereoscopic microscope.

The size/frequency distribution of the oocytes was analyzed per sampling date using the NORMSEP method (Hasselblad, 1966; Pauly and Caddy, 1985) included in the FISAT software package (Gayanilo *et al*., 1994). The percentage of oocytes to be spawned for each batch was estimated by considering those oocytes in development stages III and IV using modal progression analysis (Hunter *et al*., 1985). The gonadosomatic index (GSI) was calculated from the equation GSI = 100 GW/TW.

The absolute fecundity (F) = Number of egg grains/ Weight of the ovaries (g) × Weight of the entire ovary (g)

The relative fecundity (RF)=F/TW

The population fecundity (Fp)= $\Sigma N \times F_x$

Where, *x* is the age and *N* is the number of females.

Regression analysis was used to assess the relationships between fecundity or batch fecundity and body length and weight, GSI and gonad weight and the models with the best fit were selected (Dinh *et al*., 2020).

RESULTS

Sex ratio

A total of 940 fish were sexed, with 412 females (43.83%) and 485 males (51.60%); however, 43 fish (4.57%) were of undetermined sex macroscopically (Table I). The smallest TL recorded for the males and females were 82.0 and 65.0 mm, while TW is 7.40 and 3.40 g. The dominant ages recorded for males and females were 3^+ and 2^+, respectively.

Table I. Number of specimens, mean ± S.D., and range of standard length/weight related to the age of *T. yarkandensis*.

Age	Male (n=485)			Female (n=412)		
	n	Length (cm)	Width (g)	n	Length (cm)	Width (g)
x				6	10.75±1.43 (7.9-13.2)	17.02±5.44 (8.10-22.80)
1	2	7.45±0.05 (7.4-7.5)	6.60 (6.60)			
2	53	9.28±1.17 (6.5-13.1)	12.21±5.21 (3.40-30.30)	15	8.77±1.93 (3.0-13.6)	10.43±5.32 (5.10-24.30)
3	124	8.98±1.02 (6.5-13.2)	11.41±5.13 (4.73-27.78)	77	10.01±1.68 (6.8-19.5)	14.87±13.24 (4.63-114.04)
4	176	9.35±1.22 (6.7-13.9)	12.23±5.00 (4.26-30.12)	84	8.59±1.05 (6.7-15.0)	9.91±6.47 (4.31-43.10)
5	109	9.38±1.41 (6.8-16.2)	13.06±9.30 (3.87-50.98)	91	9.38±1.44 (6.4-19.5)	13.66±12.26 (3.59-92.44)
6	15	8.49±0.74 (7.6-10.2)	8.61±2.90 (5.36-14.98)	79	10.24±2.30 (6.7-19.4)	19.98±20.41 (4.53-89.37)
7	4	8.30±0.70 (7.2-9.5)	9.07±2.36 (6.71-12.34)	42	11.45±3.13 (7.2-19.5)	25.72±24.03 (5.30-96.44)
8	1	7.9 (10.8-18.2)	7.245	16	13.46±1.64 (7.2-13.2)	41.08±28.61 (7.01-89.04)
9	1	12.4	29.85			
10				2	18.30±0.71 (17.8-18.8	73.40±4.53 (70.20-76.60

x, Not identified.

The sex ratio for the entire set of samples was 1:1.18 (M:F), with the proportion of females significantly higher than that of males based on a sex ratio of 1:1 ($P < 0.05$; Fig. 3).

The assessment of the distribution of sexes by size class showed a significant predominance of males in specimens smaller than 12 cm in TL ($P < 0.05$); the sex ratio was 1:1 in the size class with TL from 12 to 14 cm, and individuals in size classes larger than 15 cm were mostly female ($P < 0.001$) (Fig. 3).

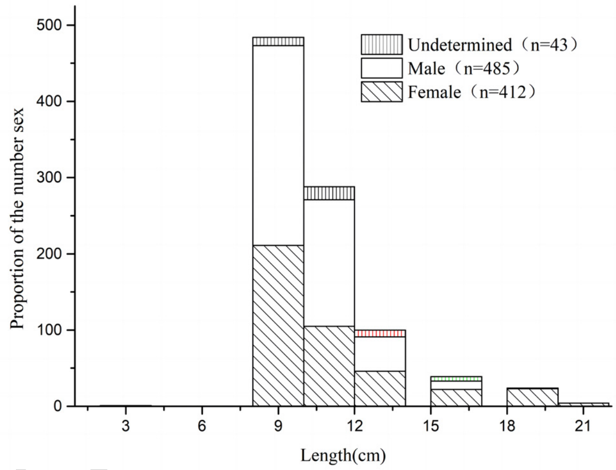

Fig. 3. Changes in the percentage of males and females *T. yarkandensis* according to length classes.

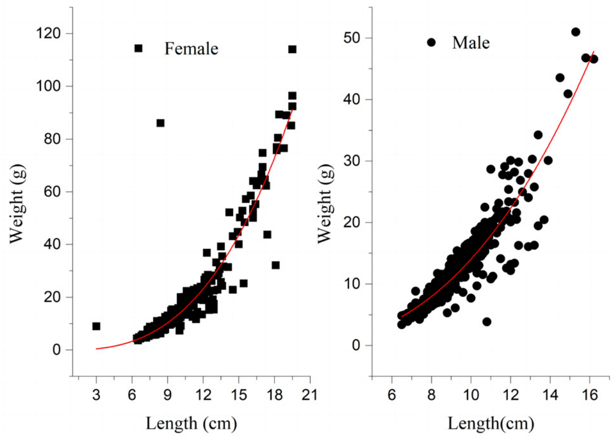

Fig. 4. Length weight relationships of *T. yarkandensis*.

The relationship between body length and weight

A length-weight curve was drawn for the distribution of male and female individuals of *T. yarkandensis*, and the fitted growth curve showed a functional relationship. As shown in Figure 4, the regression equation for the female population was $W= 0.0369\ L^{2.5772}$ ($R^2= 0.8910$), the regression equation for the male population was $W=0.0321\ L^{2.6362}$ ($R^2= 0.8595$). The covariance analysis (ANCOVA) of the relationship between the female and male populations showed that there were no significant differences between the female and male populations (F = 0.857, P < 0.05).

General reproductive pattern

During the reproductive period, the rostral end and eye socket area of female *T. yarkandensis* bulge out, the pectoral fin is circular, the rostral end is relatively pure, the ruddy genital pore evaginates, and eggs flow out when the belly is gently pressed. The rostral end of a mature male has no mastoids, the pectoral fin is sharp, the rostral end is relatively tapered, the genital pore caves inside, and milky white semen flows out when the belly is gently pressed.

Spawning pattern

Spawning time

Gonadal development was accompanied by the onset of spawning for *T. yarkandensis*. At the beginning of the season (March to June), a portion of the mature females that were histologically analyzed were classified as recovering with oocytes exhibiting primary growth, whereas other females were developing and presented early stages of vitellogenesis (I, early developing; II, late developing; III, maturing; IV mature; Fig. 5).

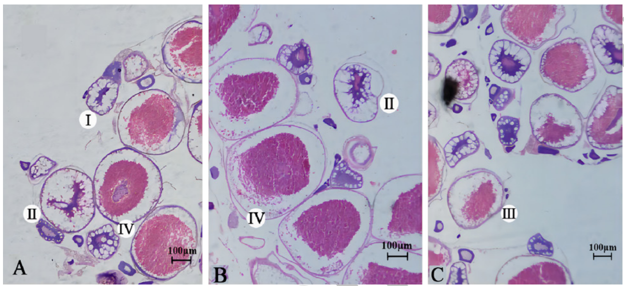

Fig. 5. *T. yarkandensis* developmental stages through egg formation.
The images show the development stages to eggs for *T. yarkandensis* in May. The figure shows the early stages (I, early developing; II, late developing; III, maturing; IV mature) of vitellogenesis in the developing female. The figure also shows that there are more individual cells in the mature stage.

Although there was some variability in GSI among years, the general seasonal pattern was repeated over the study period (Fig. 6). In all years, the mean monthly GSI was low from December to January (GSI = 0.3~0.5), increased in March (GSI = 0.7~0.9), and peaked between April and August. It can be known combined with field investigation that between May and June, the females in both the recovering and spawning phases co-occurred but varied in proportions. For males, the spawning season lasted from late March to September. *T. yarkandensis* spawned from March to June (female) over a broad region of the Tarim River and from July to September (male) from the middle and lower reaches of the Tarim River.

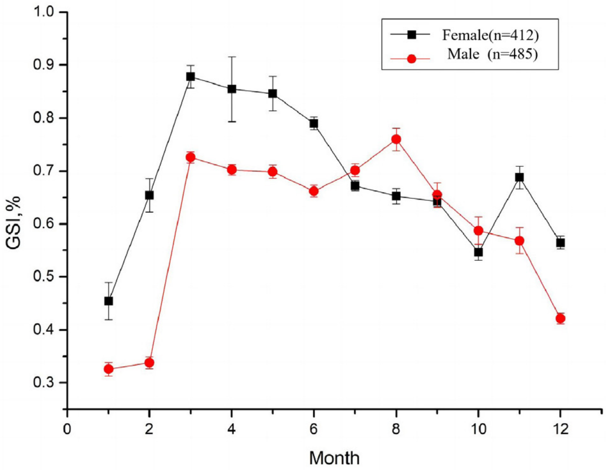

Fig. 6. Seasonal changes in gonadosomatic indices (GSI) for *T. yarkandensis*.

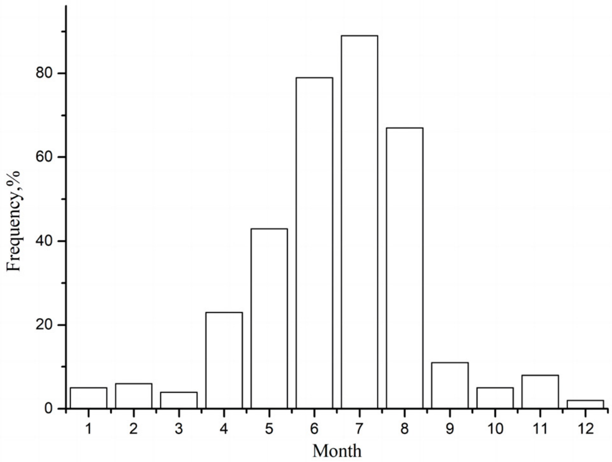

Fig. 7. Monthly size frequency of oocytes for *T. yarkandensis*.

Spawning type

From the 940 samples, we successfully collected 342 ovarian samples. Figure 7 shows that the ovaries begin to develop in April, and the deposition of yolk continues to increase. From June to August, a mean egg diameter of 0.60 mm is dominant. From September to March, the vertical mean egg diameter is 0.30 mm. The frequency distribution of oocyte diameter presented a unimodal pattern, indicating synchronous spawning in *T. yarkandensis* (Fig. 7). The egg diameter of the fish was 0.38 ± 0.15 mm, and the eggs were sticky.

Fig. 8. Habitat about the feeding ground, wintering ground and spawning ground.

Spawning location

Observations from the sampling point in the Alar section of the Tarim River (Fig. 8) indicated that *T. yarkandensis* can live in the sandy bottom of rapid and deep waters and swim upstream in spring and autumn. With the arrival of spring floods from March to May each year, the water level rises and volume increases, and *T. yarkandensis* at full maturity swim upstream in search of a suitable location for spawning, which is also referred to as reproductive migration. According to the local fishermen, more fish are available in rapid waters. However, as the flood ends, the riverbed expands and the water volume decreases, and *T. yarkandensis* tends to swim into the alkali channels, leading to the mainstream or shallow waters where they are easier to catch and overfishing is intensified. In August and September of each year, the Tianshan snow water flood, which is the largest flood of the year, occurs, and because of the abundance of food, parent fish and larvae return to the main stream, which includes too much sediment and causes a high death rate of larvae. When the flood ends in September and the water volume decreases, *T. yarkandensis* search for a new location to overwinter. Certain individuals return to the slow waters, which are approximately 3.00-4.00 m deep, whereas others overwinter in the mainstream, which gradually narrows.

Fecundity

In the 342 ovarian samples of 88 females in fecundity stages IV or V, the TL ranged from 30-195 mm and TW ranged from 3.59-114.04 g, the absolute fecundity was 1101-56320 (9944 ± 5487) tablets, and the relative fecundity was 824-1140 (982 ± 158) eggs/g. The population fecundity (Fp) of *T. yarkandensis* was 403.46 eggs.

The relationship between TL and fecundity was determined by the formula $Y = 88.83X^{2.0641}$ ($R^2 = 0.8666$), and the relationship between TW and fecundity was determined by the formula $Y = 1370.675X^{0.7106}$ ($R^2 = 0.8160$). That suggested the absolute fecundity of *T. yarkandensis* in the Alar section of the Tarim River increased as the TL increased, whereas it increased slowly as the TW increased.

DISCUSSION

In this study, the sex ratio of *T. yarkandensis* deviated from the normal 1:1. Studies have suggested that in fish, the sex ratio may change based on a series of factors, such as mortality and population growth rate (Vazzoler, 1996). These factors may affect the two genders differently (Oliveira *et al.*, 2017). Behavioral differences between sexes, differences in growth rates between the sexes, and differences in morphology and physiological activity can also cause gender deviations (Barzotto *et al.*, 2017). The higher percentage of males found in the present study may be related to a combination of several factors stated above. Overall, more males were captured in the intermediate size classes. In short, it can be stated that the *T. yarkandensis* collected from the Tarim River exhibited a skewed sex ratio favoring males during a prolonged period.

The relationship between the body length and body weight of fish is a biological index with interspecific differences that is closely related to biological processes such as population growth and reproduction. The main influencing factors include temperature, food sources and fishing pressure (Froese *et al.*, 2014). The variables a and b in the equation $W=a L^b$ for fish body length and body weight were used to assess *T. yarkandensis.* Fish growth status is represented by two factors-a, the growth factor, and b, the conditional factor, or allometric index. Allometric growth, which is connected to habitat adaptability, shows relative preference for body length growth (Moutopoulos *et al.*, 2002; Sani *et al.*, 2010). In this study, male and female *T. yarkandensis* are allometric growth.

The growth advantage and speed of female individuals were worse than those of male individuals, so females were thinner than males. Some studies suggested that sex, growth and development of fish and living environment can affect the a and b values, and the difference in the a and b values of male and female *T. yarkandensis* may reflect the influence of the above factors. The male and female populations in Hotan River were higher than those in Tarim River (males: b=2.9283; females: b=2.8768), which was indicative of less human activity there (Wang, 2022). Therefore, in a specific ecosystem, it is important to determine the relationship between fish body length and body weight to guide fish conservation and management (Nallathamb *et al*., 2020).

Seasonal changes in GSI reflect the different proportions of energy input to the reproductive biology of fish (Mohan *et al*., 2018; Fouche and Venter, 2011). From March to May in the Alar section of the Tarim River, the rising water temperature and abundant illumination and food are favorable for the development of embryos and growth of larvae, and this period represents the best season for reproduction. The male reproductive period lasts from March to August, which explains why more and larger males were caught in July and August in this study. According to fish behavioral research, suitable spawning times and locations are selected for larvae to exit the membrane, survive, and grow in favorable environments (Zhu, 1989; Abujam and Biswas, 2011). Based on the size and frequency of oocyte diameters, *T. yarkandensis* is a synchronous spawner, which is consistent with the results of the ovarian histological section analysis. In addition, the results for the spawning pattern of *T. yarkandensis* are similar to those of other studies (Zhu, 1989; Zeng and Tang, 2010).

The *GSI* values for *T. yarkandensis* were high from February to July, and the values were higher for females than for males; however, from July to September, males presented higher values than females and showed two peaks. Moreover, during the reproductive season, in addition to spawning upstream, *T. yarkandensis* competes for food and even dies from over-eating (Chen and Yao, 2008). However, these behaviors are also reasonable when considering that *T. yarkandensis* must accumulate the necessary energy to overwinter and develop ovaries. Previous studies have shown that with the same energy input in reproduction, individual fecundity could be improved at the cost of lowering the quality of the oocyte, i.e., oocyte diameter. Low oocyte quality has a negative impact on population recruitment by directly influencing the survival rate of fertilized eggs and larvae (Mohamed and Al-Absawy, 2010; Vinod and Basavaraja, 2010; Fouche and Venter, 2011; Liu *et al*., 2011). This is also one of the reasons why its resources have not been significantly recovered.

In fish reproduction, the age at maturity has a large impact on the reproductive duration and reproductive population size; therefore, this age is critical for reproduction and determines the reproductive potential of the entire population (Lashari *et al*., 2007). In this study, we found that the maturity of *T. yarkandensis* was earlier, which is closely related to the objective conditions of the Tarim River itself (such as insufficient feed abundance, high sand content, long and strong sunshine, etc.) (Chen, 2012).

The ecological habitat influences the adaptability of fish to their environment, and during reproduction, the habitat influences the ability of the larvae to exit the membrane and survive, which affects the survival rate of the offspring (Wootton, 1990). Gonadal hormones, water temperature, illumination, nutrition, water flow, and other factors influence gonadal maturity (Chen and Yao, 2008; Yin, 1995; Jobling, 1994). The gonadal development of *T. yarkandensis* starts in April when the water temperature of the Tarim River gradually rises to 20°C (the temperature is 19°C the rest of the year), volume increases, food becomes more abundant and water flows more rapidly; all of these factors promote the development of ovaries and result in the peak period of reproduction.

T. yarkandensis has a relatively larger oocyte diameter than most Cobitidae, although its fecundity presents the opposite characteristics. Bagenal (1967) described the empirical equation stating that the fecundity of most fish species changes with size, which was also the case with *T. yarkandensis*, the fecundity of which increased with increasing TW and TL, but the relationship between fecundity and age was insignificant. Research has shown that oocyte numbers and fecundities depending on food abundance and living environments, and fecundity improves with increased weight and other factors (Naeem *et al*., 2011; Guo *et al*., 2011). The population fecundity of the *T. yarkandensis* in Hotan River in this study was significantly higher than that of other *Triplophysa* in the genus, and there was a significant correlation between its absolute fecundity *F* and body length and body weight, which is consistent with the findings of the *Triplophysa bleekeri*, *Triplophysa tibetana*, and *Triplophysa brevicauda* (Wang *et al*., 2013; Wang, 2017; Hou *et al*., 2010; Gang *et al*., 2019; He *et al*., 1999; Liu *et al*., 2009). In recent years, the environmental management of the waters of the Hotan River has been quite effective, the effect of stocking and releasing is obvious, and the superior water environment is conducive to the flourishing of the *T. yarkandensis*.

CONCLUSION

In conclusion, *T. yarkandensis* is a euryhaline fish that lives in cold water, and it reaches maturity at a young age, features low fecundity and has a long reproductive season. According to Yin (1995) and Ye (2002), the spawning population is classified as Type II (i.e., P = K + D, K > D, where P represents the spawning population, K represents early maturing individuals, and D represents later maturing individuals). These characteristics are representations of selected behavioral adaptations to their living surroundings (Grossman *et al*., 2002). Therefore, it is imperative to formulate proper fishery management for the environment and conduct long-term monitoring for fish resource protection.

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IRB approval

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

Ethics statement

All experimental procedures were approved by the Animal Research Ethics Committee of Tarim University (TDDKYXF20160412).

Statement of conflict of interest

The authors have declared no conflict of interest.

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