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### FECUNDITY AND REPRODUCTIVE STRATEGY OF *PTYCHOBARBUS DIPOGON* POPULATIONS FROM THE MIDDLE REACHES OF THE YARLUNG ZANGBO RIVER

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Abstract: The Ptychobarbus dipogon of the Schizothoracinae subfamily, is an endemic economic fish species in Tibet Autonomous Region, China. Due to environment deterioration and invasion of alien species the population size of this species is decreasing; a study is urgently required to determine fecundity and reproductive strategy in order to conserve this natural resource. In this study, to investigate the fecundity and its relationship of body length, weight and age, we captured 1030 individuals in the middle Yarlung Zangbo River during two separate periods, one from February to March in 2013 and the other from February to June in 2014. The results showed that the standard length (SL) of males is concentrated in the 325—400 mm range, and the SL of females is above 375 mm. Sixty five females were at stages IV and V of sexual maturity, with SL 320—500 mm, weight 411.6—1328.0 g. Using the SL 50% method, the first female sexual maturity age was estimated to be 13.0 years and about 360.90 mm, while the first male maturity age was estimated to be 13.5 years and length 354.53 mm. The distributions of egg sizes, gonadal developmental stages and gonadosomatic indexes show that this species follows a synchronous spawning pattern concentrated in the period February to March, with an absolute fecundity at 3487 eggs, with a fecundity to SL ratio of 7.2 eggs/mm and fecundity to weight of 4.3 eggs/g. The absolute fecundity is positively correlated to the SL and the weight, but not significantly correlated to age. The overall male-female ratio was 1.23:1. Suggestions for its conservation have been made based on its low fecundity, late maturity and short breeding period.

Key words: Tibet Autonomous Region;Ptychobarbus dipogon;Fecundity;Reproductive strategyCLC number: S937Document code: AArticle ID: 1000-3207(2018)06-1169-11

The *Ptychobarbus dipogon* Regan is a member of the genus *Ptychobarbus* in the subfamily Schizothoracinae. It is a fish species with specialized adaptation for the middle reaches of the Yarlung Zangbo River<sup>[1]</sup>. Many sources have reported on its fecundity, including general-purpose surveys of local fish fau

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na<sup>[2]</sup>, with some specialized studies on wide-ranging topics, such as the species nutritional value<sup>[3]</sup>, feeding organs and behaviors<sup>[4]</sup>, reproductive strategies<sup>[5]</sup>, chromosome diversity<sup>[6]</sup>, age, growth and population dynamics<sup>[7]</sup>, correlation between age, growth and mortality<sup>[8]</sup>, age determination<sup>[9]</sup>, chromosome count<sup>[10]</sup>, length-weight relationship<sup>[11]</sup>, and mitochondrial sequencing<sup>[12]</sup>.

Presently, the most in-depth works on P. dipogon tend to focus on the evolution of Schizothoracine in the context of the rise of the Tibetan Plateau and subsequent climate changes. Cao, et al. offered a systematic postulation that the evolution of Schizothoracines based on fish trait variations is connected to environmental changes<sup>[13]</sup>. Through molecular phylogenetics and biogeography of the specialized Schizothoracine fishes, He, et al. demonstrated that their origin is intrinsically connected to the stepwise rise of the Tibetan Plateau; their evolution and distribution are a direct result of adaptation to those changing environments<sup>[14]</sup>. Wang investigated the molecular phylogenetics of East Asian Cyprinds and found that their earliest branching event may be concurrent with the development of the East Asian Monsoon climate caused by the plateau rise<sup>[15]</sup>. Yang, *et al.*<sup>[16]</sup> analyzed the mitochondrial DNA of Schizothoracines, and concluded their evolution was likely connected to the plateau rise. Estimated historical altitudes using fish fossils suggest a correlation between the rising height of the plateau and the evolution of Schizothoracines<sup>[17]</sup>. Transitional Cyprinid fossils of the Tertiary period, found in northern Tibet Autonomous Region, proved the eventual emergence of Schizothoracinaes to be an adaptation to the cooling climate<sup>[18]</sup>. The current Tibetan Schizothoracines are still in a process of speciation, with numerous taxonomic traits yet to be stabilized, suggesting that rapid environmental changes are still occurring in the plateau ecosystems<sup>[19]</sup>.

In recent years, the mainstream and branches of the Yarlung Zangbo River have suffered ecological problems including reduced wildlife populations, decrease of individual fish size, invasive alien species<sup>[2]</sup> and habitat deterioration<sup>[1]</sup>. Due to the low temperatures of highland lakes and rivers, Tibetan fish species naturally grow<sup>[20]</sup>, sexually mature and reproduce at a slower rate compared to fish elsewhere<sup>[19,21]</sup>. Currently, the deteriorating aquatic ecosystems endanger the habitats, feeding and breeding of Tibetan Schizothoracine fish<sup>[22]</sup>, also, the dam projects may obstruct their migratory passages and further effect their activities of foraging, overwintering and spawning<sup>[23]</sup>. The

continued existence of P. dipogon is under direct threat, and any such disturbance in the system may damage its population in ways that may be impossible to recover, or take a long time to recover from<sup>[24]</sup>.

To rationally conserve and manage the wild resources of *P. dipogon*, it is imperative to study the biology of reproduction and reproductive strategies of this species. In this paper, we studied the sex ratio, fecundity, reproductive period, age and size at first sexual maturity, and reproductive strategies. Our findings provide valuable information for the rational conservation and exploitation of P. dipogon.

#### 1 **Materials and Methods**

#### 1.1 Fish sample collection

We captured 19 female individuals of *P. dipo*gon at stages IV and V from February to March in 2013 in the Xigaze segment of the Yarlung Zangbo River in Xaitongmoin County (altitude ~4000 m, denoted by A1), and collected more individuals from February to June in 2014 in the upper reaches of the Lhasa River, a tributary of the Yarlung Zangbo River (altitude ~3800 m, A2), of which 33 individuals were caught in June, 37 in February, and more than 130 in each of the remaining months. In total, 1030 individuals were collected. Specimens were sampled using floating gillnets (mesh size 7.5 cm) and bottom gillnets (mesh size 6.5 cm). All fish were transported by live fish transport vehicles to the Tibetan Fish Reproduction & Nursery Base at the School of Tibet Agriculture and Animal Husbandry University for research. Their standard length, weight, gutted weight, ovary weight and egg size were measured with standard length (SL) accurate to within 1 mm, and weight to within 0.1 g. Six vertebrae were taken from each fish as materials to determine fish age.

#### Preparation and observation of vertebral columns 1.2

For each individual, its vertebral column was separated from muscular attachments, briefly immersed in boiling water and then the remaining muscle and other tissue cleaned by brush. After drying, scissors were used to carefully remove all ribs and protrusions around the column. The column was then numbered and preserved. A stereo microscope was used to observe the column and determine  $age^{[25]}$ . 1.3 Sex ratio

Individuals were divided into groups by standard length at intervals of 50 mm for study of the sex ratios of *P. dipogon* at different growth stages<sup>[26]</sup>. The chi-squared test is used to determine if the sex ratio conforms to 1:1.

### 1.4 Breeding season

The breeding season of *P. dipogon* was determined by comparing the distribution of gonadal developmental stages and egg sizes, and variation of the gonadosomatic index (*GSI*) among different months. Specifically, the breeding season is characterized by the appearance of stage IV and V gonads in substantial numbers.

Fat (*K*) and *GSI* were calculated using the following functions:

$$K = W/L^3 \times 100$$
  
$$GSI = W_1/W_0 \times 100$$

Where: W is body weight,  $W_0$  is gutted weight,  $W_1$  is gonad weight, K is fat, and GSI is the gonadosomatic index.

### **1.5** Egg sizes and distribution

The six-stage gonadal rank criteria developed by the Yellow Sea Fisheries Research Institute was used to determine the developmental stage of gonad via visual inspection<sup>[27]</sup>. The gonadal ranks were denoted with Roman numerals from I to VI, egg sizes were measured by diameter using matured or near-matured ovaries at stages IV and V<sup>[28]</sup>. The distributions of egg sizes in different months were used to determine the spawning pattern.

### **1.6** Size and age at first sexual maturity

Male and female individuals were respectively divided into intervals of 10 mm, and the  $SL_{50\%}$  (the length at which an individual has a 50% probability to be sexually mature) method was used to determine sample SL and age at first sexual maturity.  $P=1/\{1+\exp[-k(SL_{Tmid}-SL_{50})]\}^{[26]}$ . Where: P: percentage of mature individuals with length in the interval SL. k: the slope.  $SL_{Tmid}$ : median of the length interval.  $SL_{50}$ : average standard length at first sexual maturity. The age at first sexual maturity  $(A_{50})$  was appraised by fitting the logistic function to the proportion (P) of mature fish:  $P=1/\{1+\exp[-k(A-A_{50})]\}^{[26]}$ . where: A: the age of the individuals,  $A_{50}$ : the age at first sexual maturity.

### 1.7 Fecundity

A number of female fish at stages IV and V were randomly chosen to measure their total ovary weight and weight of sampled eggs (accurate to 0.1 g). The sampled eggs were preserved in a 10% Formalin solution. The number of sampled eggs was counted, and the absolute and relative fecundities were calculated as follows:

Absolute fecundity = (number of sampled eggs/weight of sampled eggs) × ovary weight

Fecundity relative to weight = absolute fecundity/gutted weight (g)

Fecundity relative to length = absolute fecundity/ length (mm)

### 1.8 Data analysis

Excel 2007 and SPSS 21 were used for data processing and regression analysis of fecundity and distribution of egg sizes. One-way analysis of variance (ANOVA) and Tukey test were used to detect significant differences in *GSI* and *K* per month. A difference was regarded as significant at P<0.05.

### **2** Results and Analyses

### 2.1 Secondary sexual characteristics

In non-breeding seasons, female and male *P. dipogon* are indistinguishable in appearance. During the breeding seasons, patches of lighter colors can be seen on the back, caudal peduncle, and fin rays of the mature male fish, especially near the anal fin; these patches are coarse to the touch. The mature female fish has no such patches, but its abdomen becomes swollen and soft, while its cloaca protrudes and turns red.

### 2.2 Sex ratio

A total of 1030 individuals were acquired. The sex of 68 individuals could not be determined. 530 males and 432 females were identifiable, and the male-female ratio was 1.23:1, which significantly differed from 1:1 by Chi-squared test ( $\chi^2$ =4.99, *P*<0.05). The standard length of individuals were in the range 155—550 mm, with weight in the range 46.5—1704.5 g. The *SL* of most males was concentrated in the range 325—400 mm, and the *SL* of most females was above 375 mm (Fig. 1). Analysis shows 350 mm to be a dividing point. The male-to-female ratio increases with length below 350 mm, and decreases with length above. No male specimens longer than 500 mm were caught (Fig. 2).

# **2.3** Distribution of gonadal developmental stages over months

Tab. 1 illustrates features of *P. dipogon* at different gonadal stages. Females in stage II were seen throughout February to June; stage III appeared in February, March and May; stage IV appeared from February to May and were most prominent in February (45%); stage V were seen from February to April, and reached to a peak of 36.43% in May; stage VI appeared from April to June (Fig. 3A). Male individuals in stage IV were more prominent from February to June, and stage V appeared from February to April (Fig. 3B). It can be seen that the breeding season of the species lasts from February to April, with March as the height of breeding activities.

# **2.4** Variations of gonadosomatic index and fatness in different seasons

The females caught from the Xaitongmoin area had remarkably higher *GSI* values than the females from the upper Lhasa River in the same months of 2014. Among the 2014 specimens, the *GSI* is highest

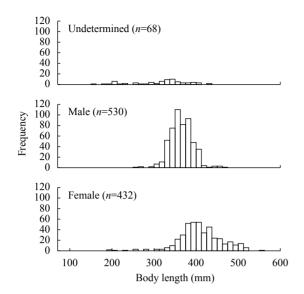


Fig. 1 Body length distribution of P. dipogon

in March, and not very different from April to June. There is a significant difference between the GSI of female specimens from Xaitongmoin in March and all female specimens from the Lhasa River (P < 0.05); the

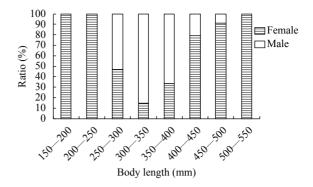


Fig. 2 Sex ratios of P. dipogon in different SL groups

difference is insignificant between the months from February to May in the Lhasa River specimens, but the female *GSI* from these months are significantly different from June (P<0.05) (Tab. 2).

The females show higher average fatness than the males. Among females from the upper Lhasa River, there was significant difference between their fatness in June and that in the other months (P<0.05); significant differences in fatness also exist in males

Tab. 1 Characteristics of gonadal development in P. dipogon

Gonadal developmental stages	Ovaries	Testes
I	Gonads are undeveloped, appearing as translucent wires on the abdomen close to the swim bladder. Sex cannot be determined visually. No visible eggs. No blood vessels on the surface	Similar to the stage I ovaries
Π	Development has started. Each ovary is generally a flattened, semi-translucent stripe, with a slightyellow hue under light. No visible eggs. Sex can be determined visually. Small amounts ofvessels, not highly visible	Development has started. Each testis is generally a thin, semi-translucent stripe, with a white hue under light. Sex can be determined visually
Ш	Gonads are maturing. Each ovary is cylindrical in the middle, appearing as thick, flattened stripes in both ends, light yellow in color. White or light yellow eggs with yolk are visible. When dissected, the eggs are neither large nor round, and difficult to remove from the ovariolar sheath	Gonads are maturing. Each testis is a gray-white thick and flattened stripe in the back end, and rod-like in the middle and front end. Its surface is divided into lobules. No semen leaks out when the abdomenis lightly depressed
IV	Gonads nearing maturity. The ovaries take up a thirdof the body cavity. Eggs are highly visible as orange- yellow spheres. When dissected, eggs are easy to remove from the sheath. Eggs are separate from each other	Gonads nearing maturity. The testes take up a third of the body cavity, and are milky in color. No semen leaks out when the abdomen is lightly depressed
V	Gonads fully matured. The ovaries take up 1/3—1/2 of the body cavity. Eggs are highly visible and orange- yellow. When dissected, eggs are round and full, already detached from the sheath and have free movement in the ovariolar cavity. Eggs will leak out from the cloaca if the fish is lifted by its head, and the abdomen is lightly depressed	Gonads fully matured. The testes are milky and filled with semen, taking up $1/3$ — $1/2$ of the body cavity. Their surfaces have highly visible vessels. Semen will leak out from the cloaca if the fish is lifted by its head, and the abdomen is lightly depressed
VI	Post-spawning. The ovaries are dark red and shrunken. The ovariolar sheath is loose and thickened. When dissected, most eggs are white and small, with only small amounts of matured eggs remaining. When the abdomen is lightly depressed, only few eggs and yellow sticky matter will leak out. Eggs are round and slightly flattened	Post-spermiation. The testes are shrunken. When the abdomen is lightly depressed, only translucent mucous leaks out, rather than white semen

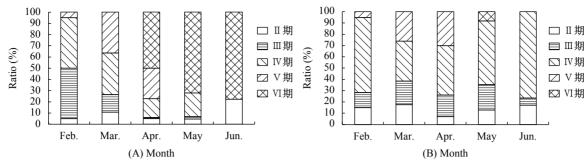


Fig. 3 Breakdown of gonadal developmental stages for female (A) and male (B) P. dipogon

Tab. 2 Female *GSI* values of *P. dipogon* between sampling areas and periods

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Sampling area and period	N	GSI (%)
A1-Feb. 2013	12	$7.862 \pm 3.047^{ab}$
A1-Mar. 2013	6	$8.313 \pm 2.687^{a}$
A2-Feb. 2014	49	$5.162 \pm 4.517^{bc}$
A2-Mar. 2014	62	$5.269 \pm 4.970^{bc}$
A2-Apr. 2014	205	$2.574{\pm}1.758^{cd}$
A2-May 2014	38	$2.66{\pm}1.747^{cd}$
A2-June 2014	9	$1.985 \pm 1.043^{d}$

Note: *N* denotes sample size. Within the same column, items with different superscript letters have significant differences (P < 0.05)

Tab. 3 Variations of male/female fatness for *P. dipogon* for different months

Month	Female fatness (K)		Male fatness (K)	
	N	Mean±S.D.	N	Mean±S.D.
Feb.	58	1.038±0.100 <sup>b</sup>	57	1.019±0.129 <sup>c</sup>
Mar.	89	1.095±0.122 <sup>b</sup>	144	1.110±0.113 <sup>b</sup>
Apr.	218	1.120±0.193 <sup>b</sup>	190	$1.101 \pm 0.118^{b}$
May	43	1.101±0.231 <sup>b</sup>	85	$1.095 \pm 0.131^{b}$
June	9	1.258±0.375 <sup>a</sup>	47	1.162±0.100 <sup>a</sup>

Note: *N* denotes sample size. Within the same column, items with different superscript letters have significant differences (P < 0.05)

between February, June, and the months in-between (P<0.05). This is an indication of the continual maturation of gonads from February to April (Tab. 3).

### 2.5 Fecundity

The eggs from 60 female individuals at stages IV and V were counted, with *SL* in the 320—500 mm range, and weight in the 507.0—1566.0 g range. The absolute fecundity ranged from 1078 to 9590 (3487± 1731), the relative fecundity to *SL* from 3.2 to 13.9 (7.2±2.5) eggs/mm, and relative fecundity to weight from 1.6 to 7.6 (4.3±1.4) eggs/g. For absolute fecundity, another 106 individuals were added to the calculation (Tab. 4).

The data displays a general trend of absolute fecundity increase with *SL* (Fig. 4A), with the best fit equation: F = 11.4SL - 1717.3,  $R^2 = 0.139$ , n = 60

(P<0.01); absolute fecundity also tends towards increase with weight (Fig. 4B), with the best fit equation: F= 4.5W-360.7,  $R^2$  = 0.313, n=166 (P<0.01). There is no significant correlation between absolute fecundity with age (P>0.05) (Fig. 4C).

### 2.6 Egg sizes

In total, 7749 eggs at stages IV and V were measured, obtaining an average size of (3.63±0.25) mm. Female specimens caught in both the Xaitongmoin and Lhasa River areas displayed an egg size peak around 3.6 mm in both February and March. For females in the Lhasa River, the April peak was around 4.2 mm, while some eggs were as small as 2.3 mm, indicating they were approaching the end of breeding season in April. A2-March has the highest peak. Except for A2-April, most plots are single-peaked (Fig. 5).

# **2.7** Size at first sexual maturity and smallest sexually mature individual

Logistic regressions to the proportion (*P*) of mature fish were performed on the size ( $SL_{50}$ ) and age ( $A_{50}$ ) at 50% maturity of females and males as follows:

Size at 50 % maturity:

Female:  $P = 1/\{1 + \exp[-0.042 (SL-360.900)]\},\ R^2 = 0.937$ 

Male:  $P = 1/\{1 + \exp[-0.030 (SL-354.530)]\},\$  $R^2 = 0.780$ 

Age at 50 % maturity:

Female:  $P = 1/\{1 + \exp[-0.252 (A-13)]\}, R^2 = 0.829$ 

Male:  $P = 1/\{1 + \exp[-0.099 (A-13.5)]\}, R^2 = 0.461$ 

At first sexual maturity, the female *P. dipogon* has a *SL* of 360.90 mm, 13.0 years old, the male has a *SL* of 354.53 mm, 13.5 years old by fitting the logistic regressions. Among the 1030 captured individuals, the smallest female individual has a *SL* of 338.0 mm, weighs of 429.5 g, 18 years old; the smallest male has a *SL* of 310.0 mm, weighs of 327.0 g, 9 years old.

### 2.8 Living environment and reproductive habits

The caught specimens show that *P. dipogon* tends to inhabit the middle reaches of the Yarlung Zangbo River at an altitude of 3580—4000 m. Larger

Tab. 4Fecundity of P. dipogon			
Fecundity	Mean±SD	No. of individuals	Range
Absolute fecundity (eggs)	3487.3±1730.5	166	1078.4—9589.6
Relative fecundity to SL (eggs/mm)	7.2±2.5	60	3.2—13.9
Relative fecundity to weight (eggs/g)	4.3±1.4	166	1.6—7.6
Standard length (mm)	413.4±36.9	60	320-500
Weight (g)	853.5±214.6	166	507.0—1566.0

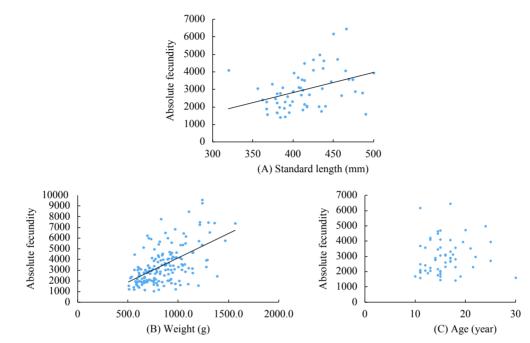


Fig. 4 Correlations between absolute fecundity and SL, weight and age in P. dipogon

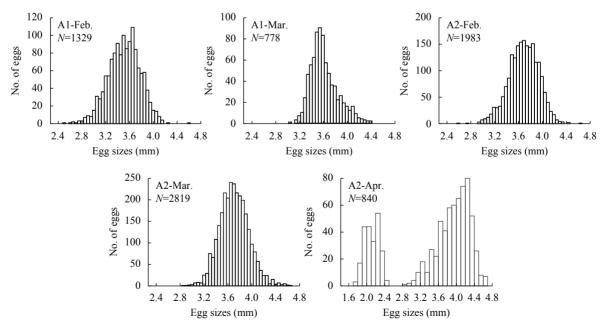


Fig. 5 Egg size distribution of P. dipogon in different months

individuals tend to inhabit medium to deep layers of the river, in areas with strong currents, deep water, and riverbed of large rocks. Smaller individuals tend to live in shallower areas with wide river surfaces, slow water flow, and gravel riverbed. The species largely consume benthic animals and phytoplanktons. From mid-February to early April, the species migrate to shallow areas with wide and calm surfaces, abundant plankton with more gravel and less mud in the riverbed, suitable for breeding purposes.

### **3** Discussion

### 3.1 First sexual maturity

In recent years, many studies<sup>[29–37]</sup> paid attention to the reproductive biology of Schizothoracine fishes.  $Wu^{[22]}$  noted that Schizothoracines from different regions exhibit different spawning patterns related to their climate and environment, also the sex ratios of their reproductive populations vary with time, *SL* group, and habitat<sup>[38]</sup>.

The age of first sexual maturity is a determining factor for specie reproductive potential via the influences of the duration of its fertility and the size of reproductive populations<sup>[39]</sup>. Fish population age and size at sexual maturity are the combined result of its bionomic and genetic features and environmental factors; fishing pressure, abundance of predators and feed and composition of the population are some examples of biological and non-biological factors<sup>[40]</sup>. Food supply and other factors that affect growth rate all have an impact on first sexual maturity<sup>[29]</sup>. The majority of Schizothoracines become sexually mature in the third to sixth year, and the males tend to mature earlier than the females<sup>[22, 30, 33, 35, 37]</sup>; but there are also species that require ten years or longer to mature<sup>[32]</sup>.

Among the Schizothoracines of Tibet Autonomous Region (Tab. 5), the first sexual maturity of *P*. *dipogon* comes the second latest, only earlier than *Schizothorax oconnori*. The first maturity age in our study is consistent with Li's<sup>[5]</sup> finding. Compared to overall age, there is a stronger correlation between first sexual maturity age and *SL*. For female and male *P. dipogon*, the *SL* at first sexual maturity are 360.90 mm and 354.53 mm respectively, at ages of respectively 13.0 and 13.5. Their slow growth rate and late maturity should be directly connected to the scarcity of food and low temperature in the harsh highland environment. This conclusion is similar to that proposed for *Schizothorax waltoni*<sup>[29]</sup>, *Oxygymnocypris stewartii*<sup>[31]</sup>, *Schizothorax oconnori*<sup>[32]</sup>, *Gymnocypris przewalskii*<sup>[35]</sup> and *Schizopygopsis malacanthus baoxingensis*<sup>[37]</sup>. The *SL*<sub>50</sub> and first sexual maturity ages of *P. dipogon* are a good basis to devise measures for its monitoring and management.

### 3.2 Individual fecundity and reproductive strategy

**Fecundity** Fecundity is an important characteristic in showing how a species or population reacts to environmental change, and its understanding can help predict population changes. The fecundity of fish is influenced by a multitude of factors including environment, such as water quality and other fish populations in the habitat, and the population itself. We find individuals of similar age carry different numbers of eggs, which may suggest differences in individual nutrition intake caused by the scarcity of food<sup>[32]</sup>. Different groups in the same area also have different breeding periods and significantly different fecundities<sup>[28]</sup>.

Most Schizothoracines have yellow demersal eggs of 1.54—4.0 mm diameter. Their absolute fecundities ranged from 2300 to 16000, and their average relative fecundities ranged from 10 to 45 eggs per gram<sup>[35, 37]</sup>. Generally speaking, Schizothoracine absolute fecundity increases with length and weight<sup>[28]</sup>.

The absolute fecundity of *P. dipogon* is positively correlated to length and weight, and is not significantly related to age, this is similar to that reported for *Schizothorax oconnori*<sup>[32]</sup>, and *Oxygymnocypris stewartii*<sup>[31]</sup>, *Schizopygopsis malacanthus baoxingensis*<sup>[37]</sup>.

Compared to the absolute fecundity of other cold-water Schizothoracine (Tab. 6), the *P. dipogon* is less fecund, yet has larger eggs. This strategy ensures

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Species	Female first sexual maturity age (year)	Male first sexual maturity age (year)	Source
Schizothorax yunnanensis Norman	3	2	Xu, et al. <sup>[41]</sup>
Schizothorax davidi Sauvage	5	3	Peng, <i>et al</i> . <sup>[42]</sup>
Schizothorax waltoni Regan	13.5	10.2	Zhou <sup>[29]</sup>
Schizothorax biddulphi Günther	4+	3+	Nie, <i>et al</i> . <sup>[43]</sup>
Schizothorax sineusis Herzenstein	5	4	Leng <sup>[36]</sup>
Schizothorax oconnori Llord	16.2	12.5	Ma <sup>[32]</sup>
Ptychobarbus dipogon Regan	13	13	Li, <i>et al</i> . <sup>[5]</sup>
Ptychobarbus dipogon Regan	13.0	13.5	This study
Gymnodiptychus dybowskii Kessler	3.4	2.5	Niu <sup>[44]</sup>
<i>Gymnocypris chilianensis</i> Li et Chang	5	5	Wang <sup>[45]</sup>
Gymnocypris selincuoensis	9	8	Chen, <i>et al</i> . <sup>[46]</sup>
Oxygymnocypris stewartii Lloyd	7.3	5.1	Huo <sup>[31]</sup>

Tab. 5 Comparison of first sexual maturity ages among cold-water Schizothoracine fishes

Species	Absolute fecundity (No. of eggs)	) Sampling Location	Source
Schizothorax yunnanensis Norman	10980	Miju River	Xu, <i>et al</i> . <sup>[41]</sup>
Schizothorax waltoni Regan	21693	Yarlung Zangbo River	Zhou <sup>[29]</sup>
Schizothorax biddulphi Günther	1983—11894	Weigan River	Nie, <i>et al</i> . <sup>[43]</sup>
Schizothorax lissolabiatus Tsao	4049	Pearl River system, Beipan River	Xiao and Dai <sup>[47]</sup>
Schizothorax sineusis Herzenstein	7563	Jialing River system, upper	Leng <sup>[36]</sup>
Schizothorax prenanti Tchang	25600	Sichuan Yaan Fish Breeding Co.	Zhou, <i>et al</i> . <sup>[48]</sup>
Schizothorax grahana Regan	10000—15000	Wu & Chishui Rivers, upper	Zhan, <i>et al</i> . <sup>[49]</sup>
Schizothorax oconnori Llord	21190	Yarlung Zangbo River	Ma <sup>[32]</sup>
Schizothorax kozlovi Nikolsky	8681	Wu River, upper	Chen, et al. <sup>[33]</sup>
Ptychobarbus dipogon Regan	4597	Lhasa River	Li, <i>et al</i> . <sup>[5]</sup>
Ptychobarbus dipogon Regan	3487	Lhasa River	This study
Gymnodiptychus pachycheilus Herzenstein	n 3043—42158	Yellow River, upper	Lou, <i>et al</i> . <sup>[50]</sup>
Gymnodiptychus dybowskii Kessler	3087	Ili River, tributary of	Niu <sup>[44]</sup>
Gymnocypris przewalskii Kessler	4338	Qinghai Lake	Zhang, et al. <sup>[51]</sup>
Gymnocypris chilianensis Li et Chang	4236	Qilianxue Nursery	Wang <sup>[45]</sup>
Gymnocypris waddellii Regan	4446	Yamdrok Lake	Yang <sup>[52]</sup>
Gymnocypris selincuoensis	12607	Siling Lake	Chen, <i>et al</i> . <sup>[46]</sup>
Oxygymnocypris stewartii Lloyd	34211	Yarlung Zangbo River	Huo <sup>[32]</sup>
Schizopygopsis chengi Fang	2659	Zhuosijia River	Hu <sup>[53]</sup>
Schizopygopsis stoliczkae Steindachner	19380	Sengge River	Wan <sup>[34]</sup>
Platypharodon extremus Herzenstein	12630	Yellow River, upper, segment in Maqu Cour	nty Zhang, et al. <sup>[54]</sup>

Tab. 6 Comparison of absolute fecundities of cold-water Schizothoracine fish

relatively high hatchability and survivability by giving eggs and larvae a good endogenous nutritional supply, and makes them more likely to achieve their first feeding as fry. This is consistent with the conclusion from a prior study of *Schizothorax oconnori*<sup>[32]</sup>.

With an absolute fecundity of 3330.6, the *P*. *dipogon* ranks fairly low among currently studied Tibetan Schizothoracines. Comparison to Li's<sup>[5]</sup> result shows a decline in the fecundity of *P*. *dipogon* in the same area, suggesting an increasing threat to its propagation in the future. The difference may be attributed to the long-term overfishing of the species.

**Reproductive strategies** Each fish population has evolved its own reproductive strategy to ensure the survival of its offspring, which can include the season and duration of breeding activities<sup>[40]</sup>. The spawning of fish is dictated by a combination of internal reproductive cycle and external signals (e.g. temperature, daylight duration and water flow)<sup>[28]</sup>, of which water temperature and daylight duration may be the most crucial<sup>[55, 56]</sup>. The *P. dipogon* has a short breeding season that concentrates breeding activities in March, and ends in early April. Because eggs take longer to hatch in the cooler waters of the plateau, an early spawning date may ensure a better environment for the early development of the larvae and fry<sup>[37]</sup>. The fatness of a fish indicates its nutritional and environmental condition<sup>[28]</sup>. Females of the *P. dipo-gon* have higher fatness than males. Their fatness shows a slight increase in the early part of the breeding season from the end of winter to early spring; it decreases after breeding due to the energy expended by spawning or spermiation; the fatness slightly increases again for a while after breeding.

The *P. dipogon* is a cold-water fish native to the Tibetan environment, with low fecundity, late sexual maturity, and short breeding period. These characteristics make them highly sensitive to environmental damage and, once reduced, their repopulation would be difficult even over a long period. This fact promotes the importance of protecting their habitat and regulation of fishing.

### 3.3 Spawning pattern

Knowledge of spawn pattern is essential to the estimation of fish species fecundity, and for tracing its repopulation and bionomic strategy. Based on the developmental morphology of oocytes in ovaries, Shi<sup>[57]</sup> divided spawning patterns into three categories: completely synchronized, batch-synchronized, and batch-unsynchronized.

Spawning patterns can be determined using histological observation of gonads, cyclical variation of *GSI*, and frequency distribution of egg sizes<sup>[37]</sup>. The *P. dipogon* only exhibits one egg size peak in March, and two peaks in April, which proves the existence of a completely synchronized pattern, similar to *Oxygymnocypris stewartii*, <sup>[31]</sup> *Schizothorax waltoni*<sup>[29]</sup>, *Schizothorax oconnori*<sup>[32]</sup>, and *Schizopygopsis stoliczkae*<sup>[34]</sup>.

### **3.4** Suggestions on conservation

The construction of new hydropower dams<sup>[58]</sup>, reservoirs<sup>[59]</sup>, mines, quarries<sup>[60]</sup>, and other projects has increasing impacted the habitats of the *P. dipo-gon*, including spawning grounds, feeding, overwintering and migration. In light of its low fecundity, late sexual maturity and short breeding season, we have the following suggestions for policymakers regarding its preservation:

(1) Stricter regulation and control should be rigorously enforced on overfishing, unlicensed quarrying of stone and sand, and industrial wastewater and solid wastes in order to protect the species habitat.

(2) Measures should be taken to address the impact on fish migration routes of closed-off water bodies, such as reservoirs.

(3) The breeding season of *P. dipogon* could be designated a closed season, to protect its reproductive population, eggs and larvae.

(4) Release of alien fish species should be prohibited, as they tend to out-compete local species. Regulation should be imposed on the Buddhist animal release rituals popular among tourists, also outreach efforts should be made to inform the populace of the necessity to preserve local ecosystems.

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## 雅鲁藏布江中游双须叶须鱼群体繁殖力与繁殖策略研究

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**摘要:** 双须叶须鱼*Ptychobarbus dipogon*隶属裂腹鱼亚科,叶须鱼属,是西藏特有经济鱼类,研究通过对其繁殖 力和繁殖策略的研究,旨在为双须叶须鱼的科学保护和合理开发提供理论依据。于2013年2月至3月以及 2014年2月中旬至6月中旬,在雅鲁藏布江中游区段采集到1030尾双须叶须鱼,雄鱼体长主要集中在325— 400 mm,雌鱼体长主要集中在375 mm以上,其中IV期、V期的雌鱼65尾,体长为320—500 mm,体重为 411.6—1328.0 g。采用*SL5*0%的方法,雌鱼初次性成熟体长为360.90 mm,初次性成熟年龄为13.0龄;雄鱼初次 性成熟体长为354.53 mm,初次性成熟年龄为13.5龄。从不同月份不同性腺发育期所占比例,不同月份的性体 指数变化以及不同月份卵径分布图,可以看出双须叶须鱼属于同步产卵类型,集中在2—4月份。双须叶须鱼 平均绝对繁殖力为3487粒,平均相对体长繁殖力为7.2粒/mm,平均相对体重繁殖力为4.3粒/g,绝对繁殖力与体 长、体重呈正相关,与年龄无显著相关性。群体性比为1.23:1。综上所述,双须叶须鱼是繁殖力较低、性成 熟较晚和繁殖期较短的鱼类,我们针对其繁殖特性提出了一些保护性建议。

关键词:西藏自治区;双须叶须鱼;繁殖力;繁殖策略