The Reproductive Characteristics of *Garra tibetana*, an Endemic Labeonine Fish in the Lower Yarlung Tsangpo River, Tibet, China

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Abstract: The reproductive characteristics of an endemic labeonine fish, *Garra tibetana*, were investigated by examining 778 individuals collected monthly, from December 2015 to November 2016, in the lower Yarlung Tsangpo River, Tibet, China. Results showed that females predominated in the overall population with a sex ratio of 1:0.73, while the sex ratio for the mature individuals was 1:1. Standard length at first maturity was estimated as 82.4 mm for females and 55.4 mm for males based on logistic regression. Analyses based on the monthly variation of the gonad-somatic index, monthly proportion of gonad development, and frequency distribution of oocyte diameter demonstrated the spawning period of *G. tibetana* to be from February to April, with a peak in March. Absolute fecundity ranged from 113 to 440 oocytes, with a mean of 201.8 ± 58.7 oocytes. The relative fecundity ranged from 6 to 18 oocytes per gram, with a mean of 11.7 ± 2.6 oocytes per gram. Absolute fecundity showed positive correlations with standard length, body weight, and gonad weight, revealing that larger females produced more offspring. In conclusion, *G. tibetana* matures early and spawns synchronously from February to April, with low fecundity and large oocytes. These reproductive characteristics could explain why this species is dominant in its habitat and also provide valuable information for developing applicable management and conservation strategies.

Keywords: Cyprinidae; life-history trait; sex ratio; spawning season; fecundity

1. Introduction

Life-history traits (e.g., age, growth, mortality, and reproductive characteristics) are important indicators to assess the vulnerability of fish populations to extirpation as they determine how resilient a species is to disturbances such as habitat loss and invasion of alien species, which is crucial for fish population management and conservation [1,2]. Although niche conservatism theory has been emerging in recent years [3], as an important component of life-history traits, reproductive characteristics were still considered to be formed through natural selection during the course of evolution and reflected their local adaption of reproductive physiology and behavior to local environmental conditions to maximize population growth and development [4,5]. Studies on reproductive characteristics of fishes mainly include sex ratio, size at first maturity, spawning season, gonadal development, fecundity, and so on. A comprehensive understanding of these reproductive characteristics can aid in designing conservation strategies to prevent population decline, especially for some endangered and endemic fish species [6,7].

The Yarlung Tsangpo River, originating from the Angsi Glacier in southwestern Tibet and crossing south Tibet from west to east for 2057 km, is the largest river in Tibet and the steepest river in the world. In the lower reaches, the river rounds the Namjagbarwa Mountain in the eastern part of the Himalayas and turns south, forming the deepest and longest canyon in the world, the Yarlung Tsangpo Grand Canyon [8]. In the lower Yarlung Tsangpo River drainage, the unique canyon environment and humid climate harbor a
large number of endemic fish species, which are barely explored and affected by human activities. Specialized rheophilic species, such as labeonine fishes and glyptosternine fishes, are the core feature of this drainage, making it distinct from those in other drainages of the Tibet Plateau [9,10].

*Garra tibetana*, recently described by Gong et al. [11], is a small labeonine fish endemic to the lower Yarlung Tsangpo River drainage, which has been misidentified as *Garra kempi* for a long period. It mainly inhabits the mountain streams or small tributaries, where based on our survey data, *G. tibetana* is the most abundant species. To date, however, there are few published documentations of the fundamental life-history characteristics of *G. tibetana*, including its reproductive biology. Therefore, the objective of this study was to investigate the reproductive characteristics of *G. tibetana*, including (1) the sex ratio, (2) size at first maturity, (3) spawning season and pattern based on the analyses of the monthly variation in the gonadal somatic index (*GSI*), gonad development, and frequency distribution of oocyte diameter, (4) absolute fecundity, relative fecundity, and the relationships between absolute fecundity and several body parameters.

2. Materials and Methods

2.1. Fish Samples Collection

Individual fish were captured monthly from December 2015 to November 2016 by electro-fishing in 6 small tributaries of the lower Yarlung Tsangpo River, with an elevation range of 652–920 m (Figure 1). These sites allowed relatively convenient access for sampling, where *G. tibetana* primarily inhabited the fast-flowing and shallow waters. The fishing procedure was performed in an upstream direction for a standardized length of 50 m using back-carrying electro-fishing equipment in 6 sampling sites each month. For each individual, standard length (*SL*) was measured from the tip of the snout to the end of the caudal-fin base with an accuracy of 1 mm. Body weight (*BW*) and gutted-body weight (removing the visceral weight) were measured with an accuracy of 0.1 g, and gonad weight with an accuracy of 0.01 g.

![Figure 1](image-url)

*Figure 1.* Map showing the sampling sites of *G. tibetana* in the lower Yarlung Tsangpo River.
The relationship between body weight and standard length (length–weight relationship) was fitted using the following formula:

\[ BW = a \times SL^b \]

where \(a\) and \(b\) are constants. The difference in the \(BW\)-\(SL\) relationship between the sexes was examined based on covariance analysis. Examination of the significant difference in standard-length frequency distribution between sexes was determined by the Kolmogorov–Smirnov test.

2.2. Gonads Development Examination

After euthanasia (anesthetic: 250 mg/L MS-222), individuals were directly dissected, and gonads were assigned a gonadal developmental stage by examination macroscopically or under stereomicroscope if necessary. For each individual, the developmental stage of the gonads was determined as (I) immature; (II) developing; (III) spawning capable; (IV) regressing; and (V) regenerating. Determinations of gonadal development stages were based on gonadal morphologic features and the relative size and the presence of vitellogenic oocytes in females or milt in males, following the definition and terminology of Brown–Peterson et al. [12]. The ripe ovaries (at spawning-capable and regressing stages) were fixed in 8% formaldehyde solution to estimate the monthly frequency distribution of oocyte diameter and fecundity.

2.3. Size at First Maturity and Sex Ratio

Size at first maturity (\(SL_{50\%}\)), which refers to the standard length at which 50% of the individuals attain sexual maturity, was estimated to describe the macroscopical maturity for each sex [13]. The individuals assigned to developing or higher stages were considered sexually mature [14]. The following logistic equation fitted to the proportion of mature individuals was applied to calculate \(SL_{50\%}\):

\[ P = \frac{1}{\left(1 + e^{(k \times (SL_{mid} - SL_{50\%})})\right)} \]

where \(P\) is the proportion of mature individuals in each 10 mm standard-length interval, \(SL_{mid}\) is the midpoint of each standard-length interval, and \(k\) is a constant [15].

The sex ratio of the overall population (female: male) and the sex ratio by month based on mature individuals (defined as those whose standard length attained \(SL_{50\%}\)) were determined. The deviations from the null hypothesis of 1:1 ratio were determined using the chi-square (\(\chi^2\)) test following Yin [16].

2.4. Spawning Season

The spawning season of \(G.\ tibetana\) was established by analyzing the monthly variation of GSI, monthly gonad development, and the frequency distribution of oocyte diameter. The GSI based on mature individuals was calculated using the formula:

\[ GSI = 100 \times \frac{W_g}{W_{gt}} \]

where \(W_g\) and \(W_{gt}\) are values for gonad weight and gutted-body weight. The homogeneity of the GSI among months was examined by a Kruskal–Wallis nonparametric test.

To determine the water temperature at which spawning occurred, water temperature was measured using a multi-probe logger during the sampling day (10:00 am and 14:00 pm). The mean values for each month were applied in subsequent analysis.

2.5. Fecundity and Oocytes Diameters Estimation

The ovaries at matured and spawning stages were subsampled and weighed based on a gravimetric method for fecundity assessment [17]. The absolute fecundity was applied to describe the potential offspring number in spawning season, which generally focused on
only females possessing uterine eggs without macroscopic embryonic development [18], and calculated using the formula:

$$\text{absolute fecundity} = n \times \frac{W}{w}$$

where $n$ is the number of subsampled oocytes, $W$ is the weight of the ovary, and $w$ is the weight of the subsampled ovary.

Relative fecundity was used to compare the fecundity between different species or different populations and computed as the number of oocytes counted per gram of body weight [5]. Generalized additive model (GAM) was used to illustrate the relationships between $SL$, $BW$, $W_g$, and absolute fecundity of $G. tibetana$ [19]. Oocytes were photographed with a stereoscope (Nikon SM 2T45T), and their diameters measured with an accuracy of 0.1 mm using the software FishBC 3.0. To make the data comparable, we chose one ovary from the individuals whose standard lengths were 110–120 mm in each month to illustrate the monthly variation of oocyte diameter. For each ovary, no fewer than 100 oocytes were measured. ANOVA followed by Tukey’s post hoc test was adopted to examine if there were significant differences in the frequency distribution of oocyte diameter between months [20].

2.6. Statistical Analyses

The statistical data were expressed as means ± standard deviation. The adopted threshold for significance level was $p < 0.05$ or 0.01. Data processing and analyses were performed in SPSS version 18.0 (Armonk, NY, USA) and R version 4.0 (Vienna, Austria). Images were processed using Origin version 9.1 and Adobe Photoshop CS6 (San Jose, CA, USA).

3. Results

3.1. Size Distribution and Length–Weight Relationships

A total of 778 individuals were collected in the sampling period, including 34 individuals whose gender could not be identified. Of the 744 individuals for which sex could be determined, 426 were female with 41–145 mm $SL$, and 318 were male with 44–111 mm $SL$. The sample size for each month is shown in Table 1. The standard-length frequency distribution between females and males was significantly different (Kolmogorov–Smirnov $Z = 4.327$, $p < 0.01$). The proportion of males below 90 mm $SL$ was higher than the number of females, while there were almost no males above 110 mm $SL$ (Figure 2).

Table 1. Sample size and the monthly sex ratio of the mature group of $G. tibetana$ in the lower Yarlung Tsangpo River from December 2015 to November 2016.

| Month | Sample Size | Mature Group |
|-------|-------------|--------------|
|       | Female vs. Male | Female vs. Male | Sex Ratio | $\chi^2$ Value |
| 2015  | 12   | 17 vs. 12 | 12 vs. 11 | 1: 0.92 | 0.04 |
|       | 1    | 35 vs. 13 | 21 vs. 13 | 1: 0.62 | 1.88 |
|       | 2    | 64 vs. 56 | 38 vs. 50 | 1: 1.32 | 1.64 |
|       | 3    | 46 vs. 53 | 34 vs. 53 | 1: 1.56 | 4.42 * |
|       | 4    | 33 vs. 43 | 21 vs. 42 | 1: 2.00 | 7.00 * |
|       | 5    | 38 vs. 19 | 20 vs. 17 | 1: 0.85 | 0.24 |
|       | 6    | 30 vs. 25 | 24 vs. 21 | 1: 0.88 | 0.20 *
|       | 7    | 34 vs. 21 | 23 vs. 20 | 1: 0.87 | 0.21 |
|       | 8    | 33 vs. 18 | 23 vs. 18 | 1: 0.78 | 0.61 |
|       | 9    | 37 vs. 23 | 27 vs. 23 | 1: 0.85 | 0.32 |
|       | 10   | 33 vs. 21 | 19 vs. 21 | 1: 1.11 | 0.10 |
|       | 11   | 26 vs. 14 | 13 vs. 14 | 1: 1.08 | 0.04 |
| 2016  | 426 vs. 318 | 275 vs. 303 | 1: 1.10 | 1.36 |

* indicating the significant deviation from 1:1 ($p < 0.05$).
The length–weight relationships showed no significant difference between sexes ($p > 0.05$) and could be estimated as:

- For females: $BW = 7 \times 10^{-5}SL^{2.66}$ ($R^2 = 0.97$; $N = 426$; $p < 0.05$)
- For males: $BW = 7 \times 10^{-5}SL^{2.69}$ ($R^2 = 0.95$; $N = 318$; $p < 0.05$)
- For both sexes: $BW = 7 \times 10^{-5}SL^{2.67}$ ($R^2 = 0.96$; $N = 744$; $p < 0.05$)

Figure 2. Frequency distribution of standard length ($SL$) of $G. tibetana$ in the lower Yarlung Tsangpo River.

Figure 3. Relationship between body weight ($BW$) and standard length ($SL$) of $G. tibetana$ in the lower Yarlung Tsangpo River.
3.2. Size at First Sexual Maturity

The smallest mature individuals of *G. tibetana* were 58 mm and 53 mm SL for females and males, respectively. Standard length at first maturity showed visible differences between sexes, which was calculated to be 82.4 mm for females and 55.4 mm for males (Figure 4). The logistic functions were expressed as follows:

\[ P = \frac{1}{1 + e^{-0.16 \times (L_{mid} - 82.4)}} \]

for females;

\[ P = \frac{1}{1 + e^{-0.25 \times (L_{mid} - 55.4)}} \]

for males.

3.3. Sex Ratio

A total of 744 individuals were included in the sex ratio analysis, of which 426 were female, and 318 were male. The overall sex ratio was 1:0.73, showing a significant difference from the expected 1:1 (\( \chi^2 = 15.68, p < 0.05 \)). However, considering only the mature individuals (i.e., females SL > 82.4 mm and males SL > 55.4 mm), the result indicated that it had a balanced overall sex ratio. An analysis of monthly sex ratios of the mature group showed identical results except for March and April, when the preponderance of males over females was significant (Table 1).

3.4. Monthly Variation of GSI

GSI values based on mature individuals varied from 0.17 to 32.86 for females and from 0.34 to 13.21 for males throughout the sampling period. Significant differences in GSI among months were tested for both sexes (\( p < 0.05 \)). The monthly variation regularly showed that mean GSI values of females were significantly higher from February to April and reached a peak in March (18.07 ± 9.58). For males, the overall annual variation was similar to that of females, although the fluctuation was relatively narrow and GSI was uniformly high from October to April. For both sexes, the GSI values declined sharply from April to May and reached the bottom in July (1.68 ± 0.26 for females, 1.48 ± 1.62 for males). The standard deviations of GSI for each month were mostly greater in females than in males, evidently from December to June. Coinciding with the rapid growth of GSI, water
temperature gradually increased from January (10.6 °C) to April (15.3 °C), while the lower value and the peak occurred in January and August, respectively (Figure 5).

![Figure 5. Monthly variations of GSI of G. tibetana and mean water temperature in the lower Yarlung Tsangpo River from December 2015 to November 2016. Error bars represent the standard deviations.](image)

**3.5. Monthly Gonadal Development**

The monthly proportion of gonadal development stages for both sexes based on the mature individuals is illustrated in Figure 6. Development of most of the developing and spawning-capable ovaries began in December. From December to April, the proportion of spawning-capable plus regressing ovaries exceeded 50%, and the proportion of spawning-capable plus regressing testes exceeded 70%. For both sexes, the peak of spawning-capable and regressing gonads appeared in March, and regenerating gonads mainly occurred in May and June. Over 60% of the developing and spawning-capable gonads in the resting phase for females and males occurred from July to November.

![Figure 6. Cont.](image)
This distribution pattern indicated that a majority of oocytes matured (Figure 7, ANOVA, Tukey’s post hoc, p < 0.05), which approximately coincided with the monthly variation of the GSI and gonad development.

### 3.6. Frequency Distribution of Oocyte Diameter

A total of 89 mature ovaries from samples collected from December 2015 to April 2016 were subsampled for oocyte-diameter distribution analysis. During the spawning season of *G. tibetana*, the oocyte-diameter distribution always had one peak ranging from 1.7 mm to 3.5 mm. This distribution pattern indicated that a majority of oocytes matured and were spawned synchronously. The median oocyte diameter increased continually from 2.5 mm in November to 2.9 mm in April with significant inter-monthly differences (Figure 7, ANOVA, Tukey’s post hoc, p < 0.05), which approximately coincided with the monthly variation of the GSI and gonad development.
3.7. Fecundity

The ovaries from 89 individuals (81–145 mm SL) were used to estimate absolute and relative fecundity. Results showed absolute fecundity of *G. tibetana* varied from 113 (82 mm SL) to 440 (123 mm SL) oocytes with a mean of 201.8 ± 58.7 oocytes per fish. Relative fecundity varied from 6 to 18 oocytes per gram, with a mean of 11.7 ± 2.6 oocytes per gram.

Significant linear relationships between absolute fecundity and SL, BW, Wg explained by GAM are shown in Figure 8. Results indicated that SL, BW, and fecundity were distinctly positive correlated, although the curves were flat in certain intervals (*F* = 11.41, *p* < 0.01; *F* = 16.36, *p* < 0.01). The relationship between Wg and absolute fecundity was approximately linear, implying the absolute fecundity rose steadily with increasing ovary weight (*F* = 27.23, *p* < 0.01).

![Figure 8](image-url)

**Figure 8.** Generalized additive model (GAM) explaining the relationships between standard length (SL), body weight (BW), gonad weight (Wg), and absolute fecundity of *Garra tibetana*. Solid lines represent the estimated smooth function, and the dotted lines represent the 95% confidence interval.

4. Discussion

4.1. Size at First Sexual Maturity

Standard length at first sexual maturity of fishes varies greatly from one species to another as well as within the same species from different regions [21,22]. The former reflects the distinctive life-history strategies adopted by different fishes, while the latter reflects the plasticity of life-history traits of fishes under different environmental conditions [23–25]. In this study, the calculated SL50% corresponded to 3 years old for females and 1–2 years old for males, respectively (unpublished data). Earlier maturity of males at a relatively smaller size compared to females is a common phenomenon among many cyprinids, and devotion of more energy by females for reproduction could be related to the longer time taken by them to mature [26,27]. Sui et al. [28] concluded that earlier maturity was also an adaptive feature of fishes when they faced variable and unstable environmental conditions. As an early-maturation fish, *G. tibetana*, could be utilizing this reproductive strategy to shorten its
generation span to adapt to the unstable swift-flowing habitat and maintain its population in the lower Yarlung Tsangpo River.

4.2. Sex Ratio

Results indicated that females were more abundant than males in the overall population. Nikolsky [29] indicated that female predominance was beneficial to both population maintenance and density increase. This biased sex ratio could be caused by some biotic or abiotic factors, such as sex differences in longevity and habitat conditions favoring one sex over the other [14,30]. The sampling method is another important abiotic factor, such as different gears, different sampling times, and locations [31]. In this study, the sex ratio bias toward females is assumed to result from the earlier maturation and death of males. In addition, our results revealed that the mature group of *G. tibetana* had a balanced sex ratio during each month except March and April, the spawning season of *G. tibetana*, when males were more abundant. Yin [16] pointed out that some species of milters had a clump distribution pattern in the spawning season and were easily captured, which might explain why males dominated in March and April.

4.3. Spawning Season

Ovarian development in most teleosts has been classified as synchronous or asynchronous according to the growth pattern of the oocytes in the ovary at any one time [32]. In this study, the oocyte-diameter distribution was unimodal (Figure 7), demonstrating that *G. tibetana* is a synchronous spawner, which was similar to its congeners, such as *G. ghorensis*, *G. ceylonensis*, and *G. tana* [2,33,34]. Spawning activities mainly occurred from February to April. The spawning initiation of *G. tibetana* accompanies the rising water temperature and emerging flood caused by heavy rainfall and melted ice in the monsoon season of the lower Yarlung Tsangpo River. Some other cyprinids distributed in the middle reaches of this drainage (Schizothorax o’connori, Schizopygopsis younghusbandi younghusbandi, and Oxygymnocypris stewartii, for instance) have similar spawning seasons [6,27,35]. This spring-spawning strategy presumably provides suitable environmental conditions for the early embryos and larva development.

4.4. Fecundity

Information on fecundity is of great importance for understanding the reproductive potential and strategies adopted by different fishes. Here, we found that both absolute fecundity and relative fecundity of *G. tibetana* were at a low level, similar to several other small-sized labeonine fishes, such as *Crossocheilus bamaensis* [36], *Discogobio yunnanensis* [37], and *Placocheilus cryptonemus* [38], and also lower than some medium to large-sized labeonine fishes, such as *Pseudogyrinocheilus prochilus* [39], *Ptychidio jordani* [40], and *Semilabeo notabilis* [41] (Table 2). Although the fecundity is low, the oocyte size of *G. tibetana* is relatively large (median 2.9 mm in April), suggesting a larger-sized larva, which is beneficial to survival in its early life history. The absolute fecundity of *G. tibetana* increased significantly with standard length, body weight, and gonad weight, which revealed that larger individuals devoted more energy to reproductive activities than smaller ones. The regressions of correlations were characterized by significant coefficients of GLM test, indicating that these three body parameters were effective predictors of potential fecundity.
Table 2. Comparison of the fecundity and oocyte diameter characteristics of several labeonine fishes from different river drainages in China.

| Species                  | River Drainage       | Max Standard Length (mm) | Oocyte Diameter (mm) | Absolute Fecundity (Eggs) | Relative Fecundity (Eggs/g) |
|--------------------------|----------------------|--------------------------|----------------------|---------------------------|-----------------------------|
| Crossocheilus bamaensis  | Pearl River          | 152                      | 0.7–0.9              | 360–2035                  | 26–43                       |
| Discogobio yunnanensis   | Yangtze River        | 123                      | 2.0–2.2              | 126–718                   | 9–66                        |
| Garra tibetana           | Yarlung Tsangpo River | 145                      | 2.4–3.4              | 113–440                   | 6–18                        |
| Plocomocheilus cryptonemus | Nujiang River       | 125                      | 2.0–2.7              | 22–313                    | 2–21                        |
| Pseudogyrinocheilus prochilus | Pearl River    | 222                      | 1.5–1.9              | 1026–6674                 | 10–49                       |
| Ptychidio jordani        | Pearl River          | 296                      | 1.0–1.3              | 7878–48880                | 37–48                       |
| Semilabeo notabilis      | Pearl River          | 540                      | 2.5–2.8              | 17575                     | 2350                        |

5. Conclusions

*Garra tibetana* matures early and spawns synchronously from February to April, which implies short generation time and strong population regeneration power of this species. It has low fecundity with large-sized oocytes, indicating the tradeoff strategy in energy allocation between increasing the number of offspring and improving the survival of hatched larva. These characteristics could reflect its adaptations to local environmental conditions and also explains why *Garra tibetana* could dominate in its habitat. Nevertheless, as an endemic species, *Garra tibetana* is restricted to the small tributaries of the lower Yarlung Tsangpo River and might be especially vulnerable to habitat loss and invasion of alien species. For the conservation of this endemic species, it is suggested that its natural habitat should be preserved, and human-induced disturbances should be avoided, especially from February to April, its spawning season. Meanwhile, considering its low reproductive potential, the prevention of invasive species should also be taken into account to protect it against unpredictable population decline.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the animal ethics committee of the Institute of Hydrobiology, Chinese Academy of Sciences (IHB/LL/20220402 and April 2022).

Data Availability Statement: The raw data which support this study are available from the corresponding author at reasonable request.

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