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Reproductive Characteristics of *Pseudecheneis sulcatus* (Siluriforms: Sisoridae) in the Lower Yarlung Zangbo River, Tibet

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Abstract: The present research offers novel understandings of the reproductive biology of *Pseudecheneis sulcatus* (McClelland, 1842) found in the Yarlung Zangbo Grand Canyon, a biodiversity hotspot. Reproductive characteristics of *Pseudecheneis sulcatus*, including their maturation age, fertility, spawning season, and maximum body size, constitute important aspects of its life-history traits. We collected a total of 310 specimens from the Yarlung Zangbo River's Motuo reach between March and November 2016 and found the male-to-female ratio to be 0.73:1, markedly different from the anticipated 1:1. The total length of individuals ranged from 72 to 207 mm, while overall weight ranged from 2.5 to 65.0 g. Their length at first maturity was estimated as 131 mm in males and 132 mm in females. Based on the adult proportion and fluctuation in the monthly gonadosomatic index (GSI) and size distribution of oocytes, spawning was determined to occur from March to June. The absolute fecundity ranged between 247 and 2886 eggs, while the fluctuation in average relative fertility ranged between 16.8 and 77.1 eggs/g of fish. The fecundity of *P. sulcatus* was linearly correlated to the overall weight and length of the fish along with the ovarian weight. In conclusion, *P. sulcatus* spawned synchronously in spring and early summer, exhibiting low fecundity and large oocytes. Our results provided basic information in understanding how this species adapted well to the unique canyon environment, which may be useful for developing a sustainable conservation plan for *P. sulcatus* at the lower reach of the Yarlung Zangbo River.

Keywords: reproductive biology; first maturity; spawning season; fecundity; environmental adaptation

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1. Introduction

Today, nearly a third of freshwater fish species are threatened with extinction due to anthropogenic events, such as habitat destruction, dam construction, and overfishing [1,2]. An increasing number of studies have realized that reproductive biology is an essential component of conservation biology required to preserve fish diversity [3]. Moreover, reproductive parameters, including sexual maturity, fertility, and spawning season, all considered important components of life-history traits, provide vital information for understanding the ability of fish species to adapt to rapid changes occurring in the surrounding environment [4]. Acquiring knowledge of fish reproduction areas is a necessary prerequisite for establishing biological techniques for captive breeding and artificial propagation and sustainable management of the natural stocks, especially some endangered and endemic fish species [5].

Yarlung Zangbo River, the largest river (around 2,057 km in length) in the Tibetan Plateau, originates from Gyima Yangzoin Glacier at an altitude of 5200 m above sea level in the northwestern part of Tibet–Himalayas. This river flows in a west–east direction across the southern Tibetan Plateau, making a sharp turn near the Namjagbarwa Mountain in its lower reaches to form the Yarlung Zangbo Grand Canyon (YZGC), the deepest and longest canyon found globally [6]. Due to the unique canyon environment and humid climate, YZGC harbors thousands of plants and animals, making it a globally recognized biodiversity hotspot. Recently, novel plant and animal species are being continually discovered in the canyon [7]. Typical species of fish assemblages in YZGC include the oriental fauna species (e.g., *Neolissochilus hexagonolepis*, *Psilorhynchus homaloptera*, *Exostoma tenuicaudata*, *Garra tibetana*, and *Pseudecheneis sulcatus*) and Tibetan plateau species (e.g., *Schizothorax molesworthi* and *Schizothorax curilabiatus*) [8].

Pseudecheneis sulcatus (McClelland, 1842), commonly known as sucker throat catfish, is the only species of *Pseudecheneis* occurring in the basin of the Yarlung Zangbo River [9]. *P. sulcatus* prefers deep riffles and is well adapted to lotic environments. The fish swims over gravel and cobble substrates and feeds on aquatic invertebrates and algae [10]. Meanwhile, this species has a great economic importance for local livelihoods. As it is renowned for the tender meat, delectable flavor, and fewer spines, *P. sulcatus* is an important food resource in this remote mountainous area where hunting is prohibited. It can also provide a relatively cheap source of protein, vitamins, and other essential nutrients in areas where other nutritional sources are unavailable or prohibitively expensive. Thus, *P. sulcatus*, a dominant species in the lower Yarlung Zangbo River, plays an important role in food supply and nutritional security in this mountain area. Nevertheless, *P*. *sulcatus* have been rare in catches recently, because of regional ecological change (including the Sedongpu landslide in 2018) and increasing human activities. Therefore, the conservation of this species has become a focus of research in the lower-reach areas of this river.

Despite extensive exploration of the biological traits of Siluriformes species, *P. sulcatus* has seldom been researched in general. Studies on this species have mainly involved the description of morphology and adhesive organ, and the discussion of genetic diversity and phylogenetic position [11,12]. Recent studies have described the length–weight correlations of *P. sulcatus* [13–15]. Although *P. sulcatus* is important and has suffered due to increasing environmental pressure, no studies have focused on its reproductive biology to develop effective stock management of this species. Therefore, in this study, we selected the Motuo reach of the Yarlung Zangbo River to study the reproductive biology of this species. The purpose of this study was to assess several reproductive traits, including the size at maturity, species fertility, and spawning season, which may facilitate species preservation and management programs along with captive breeding.

2. Materials and Methods

2.1. Study Area and Fish Samplings

Sampling sites (29.17°–29.59° N, 94.98–95.37° E) were located at Motuo County, where the Yarlung Zangbo River breaks through YZGC and flows across the southern part of Tibet (Figure 1). The features of rivers and streams in this area include lotic flow and high gradient. The area experiences humid and warm air currents from the Indian Ocean's southeast Bay of Bengal, which penetrates the Tibetan Plateau and the Himalayas. Thus, the investigated sites possess a humid climate, with a yearly air temperature of 16 °C and precipitation above 2200 mm/a. The predominant substrate components are cobbles and boulders, with little gravel and sand [16].

Overall, 310 *P*. *sulcatus* specimens were sampled in the lower reach and tributaries of the Yarlung Zangbo River using backpack electro-fishing gear between March and November 2016 (Figure 2). After sampling and euthanasia (250 mg/L anesthetic MS-222), the total length (*TL*) and total weight (*TW*) of individual fish were measured and then dissected for biological materials. For total length, the precision for slide caliper-based measurement was 1 mm, and for total weight and gonadal weight (W_G), the precision for digital balance-based measurement was 0.1 g. Sex was determined by observing external morphological differences (the presence or absence of the genital process, i.e., gonapophysis) and the appearance of the gonads, macroscopically. In situ water temperature at each sampling site was determined using a thermometer.

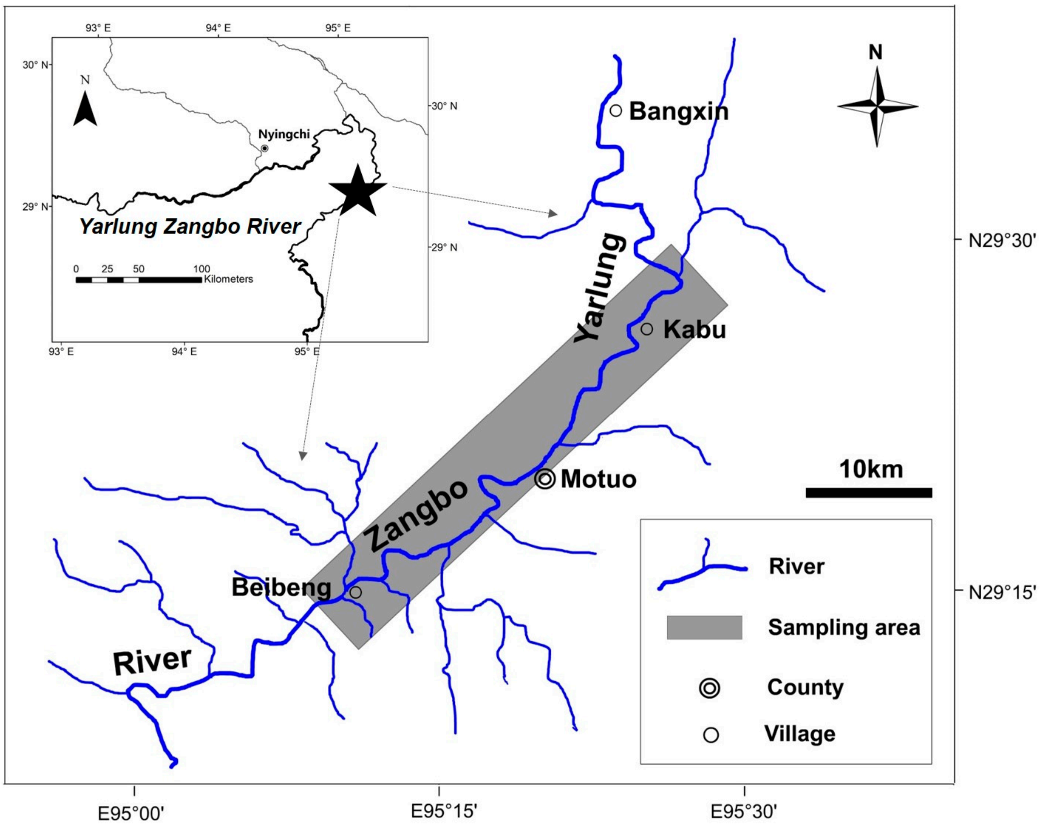

Figure 1. Location of the sampling area of *Pseudecheneis sulcatus* in the lower Yarlung Zangbo River from March to November 2016.

Figure 2. *Pseudecheneis sulcatus* (184 mm in TL and 54.1 g in TW) collected from the lower Yarlung Zangbo River.

2.2. Size at Sexual Maturity

The fish length at sexual maturity (stage of maturation and thereafter) was defined as the length at which half of the population matured sexually (TL_{t50}) [17]. Based on macroscopic features (such as morphology, color, distension degree, and occupation of relative space in the body cavity) in males and females, the gonad development status in each fish was grouped into the following five stages: (I) immature; (II) developing;

(III) maturing; (IV) mature; (V) spent, which followed the definition and terminology used by Ding et al. [18] and Brown–Peterson et al. [19].

The proportion (P) of mature individuals was estimated using the following logistic formula, which was set at the length of 10 mm size intervals [20]:

$$P = (1 + e^{-k(TL\text{mid}-TL50)})^{-1}, \quad (1)$$

where TL_{mid} signified the midpoint for the TL class, TL_{50} represented the average TL at sexual maturity, k referred to the slope, and P denoted the mature fish proportion.

2.3. Spawning Season

The spawning season of *P. sulcatus* was assessed by comprehensive analysis and judgment of monthly variations in macroscopic maturity phases, gonadosomatic index (*GSI*), and oocyte size–frequency distributions. The *GSI* of the mature fish was computed as follows [21,22]:

$$GSI = W_G/TW \times 100, \quad (2)$$

We subsampled the ovaries of *P. sulcatus* from 34 mature female adults in total from March to August and preserved them in formalin (7%) to assess the monthly distribution of oocyte size. The diameter of the egg was estimated by capturing the egg images with a Nikon SMZ 745T, with a precision of 0.001 mm in Image Pro Plus 6.0. ANOVA and subsequent Tukey's post hoc test were used to examine significant inter-month *GSI* differences [23,24].

2.4. Fecundity

To study fecundity, we preserved the ovaries of 52 females in 7% formalin and treated them gravimetrically [25]. Ovarian subsampling was accomplished at the anterior, middle, and posterior sites. Fecundity (F) was computed using the equation [26]:

$$F = n \times TW/w, \quad (3)$$

where n and w separately denoted the oocyte counts in the subsamples and subsample weights, while W referred to the whole ovarian weight. During the absolute fecundity evaluation, the entire oocytes that already began vitellogenesis were considered potentially mature eggs. Additionally, the relative fecundity was estimated as the quantity of ova per gram of total weight [27]. The correlations between absolute fecundity, TL, TW, and ovary weight (W_o) of *P. sulcatus* were assessed using regression analysis.

2.5. Statistical Analysis

We used Microsoft Excel 2016 and Origin Pro 2016 for data processing and graph creation. Statistical analyses were performed in SPSS 16.0 at a significance level of $\alpha = 0.05$.

3. Results

3.1. Distribution of Length Frequency and Sex Ratio

Samples ranged from 72–207 mm TL while TW ranged from 2.5–60.0 g. Males ($n = 131$) ranged from 91 to 205 mm TL while females ($n = 179$) ranged from 72 to 207 mm TL. Length frequency distributions were significantly different between males and females (Kolmogorov–Smirnov $Z = 1.341$, $p < 0.05$). The modal length intervals were the 120–140 mm TL group, which included 41% of all specimens (Figure 3).

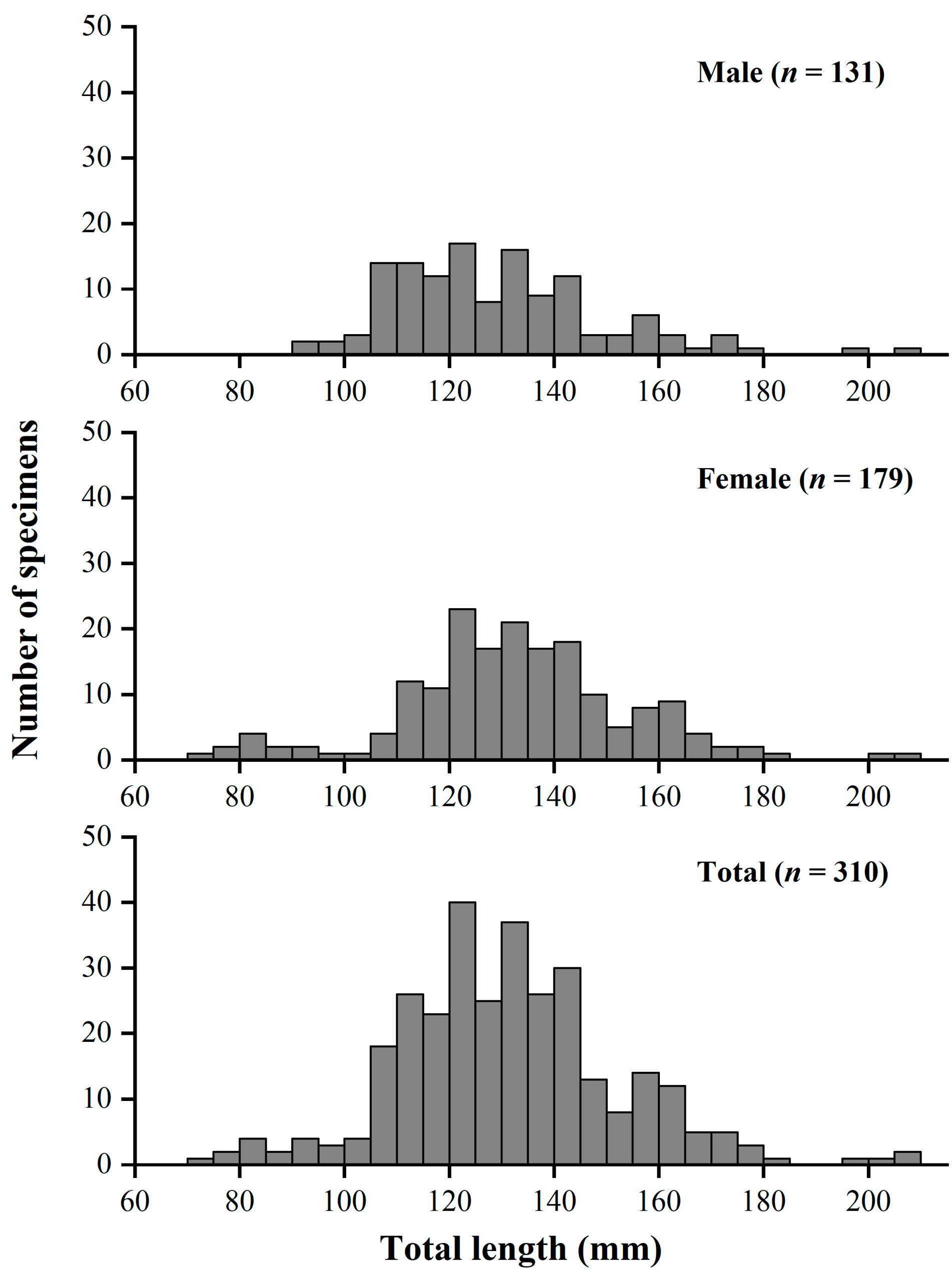

Figure 3. Size frequency distribution of males, females and all samples combined of *Pseudecheneis sulcatus* captured in the lower Yarlung Zangbo River.

The male-to-female ratio was 0.73:1, revealing a markedly different value from the anticipated 1:1 (χ^2 = 7.432, $p < 0.05$). Between March and June, the proportion of females was much higher than that of males, whereas the proportion of males and females was balanced between August and November (Figure 4).

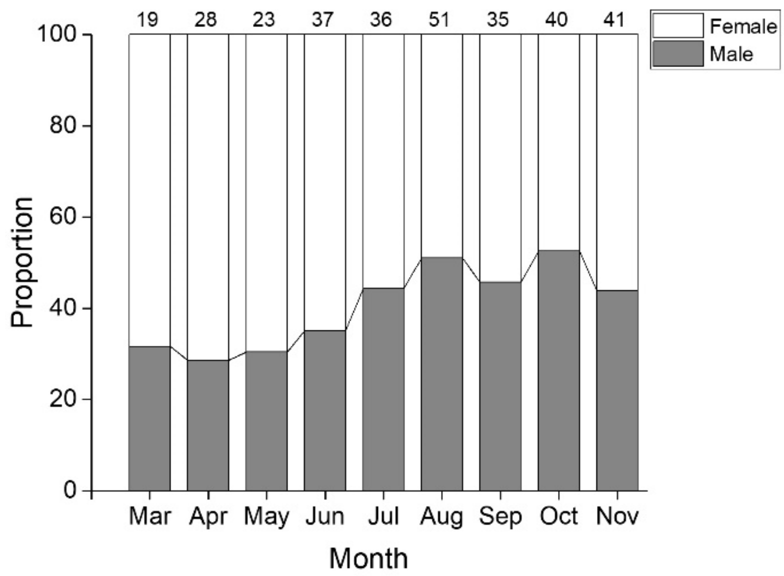

Figure 4. Monthly distribution of the male: female sex ratio in *Pseudecheneis sulcatus* during the period from March to November 2016. Numbers on bars represent sample size.

3.2. Length at Sexual Maturity

Only a slight gender disparity was noted in length at first maturity (Figure 5). The logistical model computed the TL_{50} values as 131 mm in males and 132 mm in females and resulted in the following logistic equations: $P = (1 + e^{-0.035(TL\mathrm{mid}-130.7)})^{-1}$ (n = 131, R^2 = 0.831) in males and $P = (1 + e^{-0.046(TL\mathrm{mid}-131.7)})^{-1}$ (n = 179, R^2 = 0.856) in females.

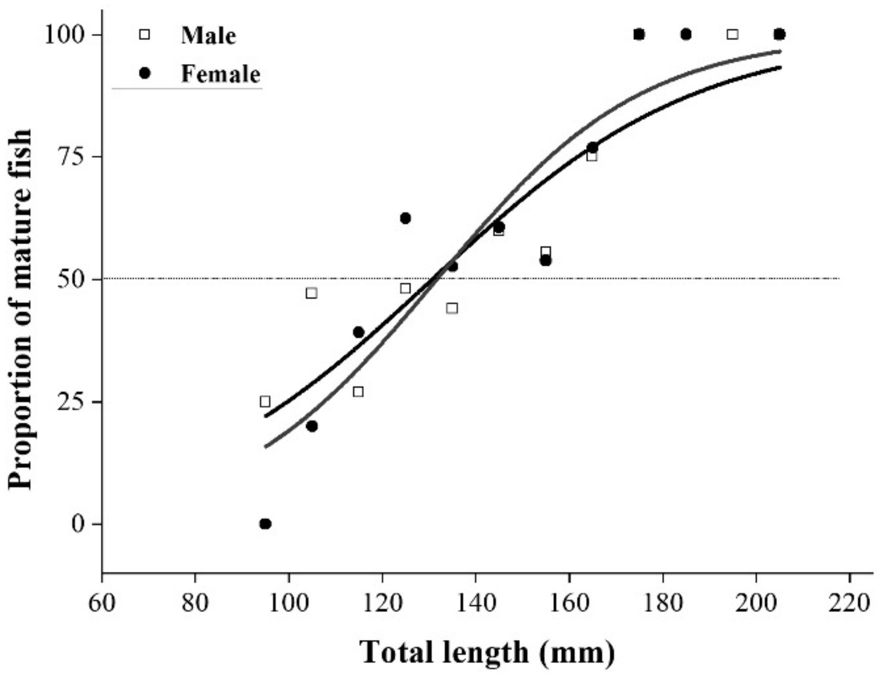

Figure 5. Logistic functions for *Pseudecheneis sulcatus* fitted to cumulative percentage of sexual maturity in samples in relation to size.

3.3. Spawning Season

The statistics for monthly variations in the gonadal maturity phases of ovaries and testes are illustrated in Figure 6. The monthly testicular and ovarian trends were found to be similar. Proportions of mature testes exceeded 90% between May and June (Figure 6a).

Highest numbers of mature and spent ovaries were noted from March to June, exceeding 80% in May, indicating that spawning ovaries appeared between May and June (Figure 6b).

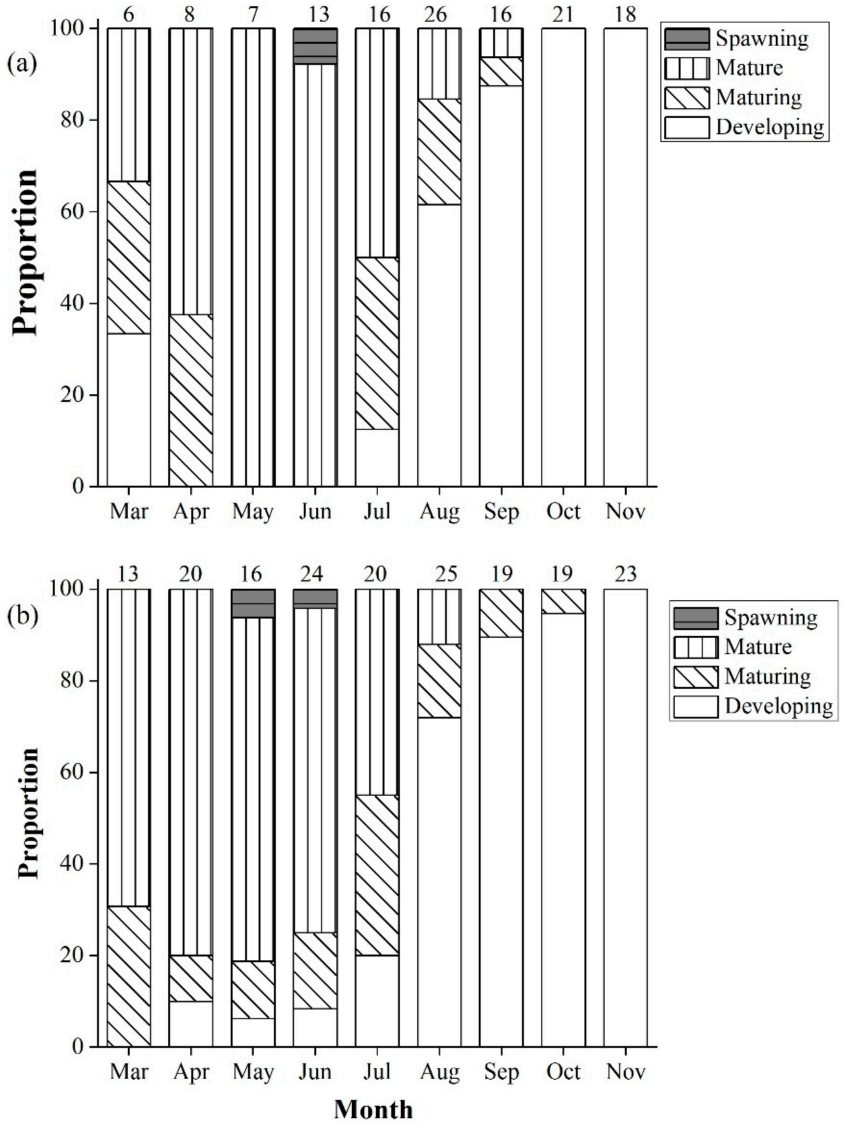

Figure 6. Monthly distribution of macroscopic gonad maturity stages in *Pseudecheneis sulcatus* male fish (**a**) and female fish (**b**) during the period from March to November 2016. Numbers on bars represent sample size.

Female *GSI* was highest March–May, declining through July before levelling off August–November. Male *GSI* was even throughout the sampling period (Figure 7).

The oocyte diameter was determined from mature ovaries between March and August of 2016 (Figure 8). The mean egg diameter was found to be significantly higher in May (2.39 ± 0.21 mm) compared to the eggs in other months (ANOVA, Tukey's post hoc, $p < 0.05$) (Figure 8).

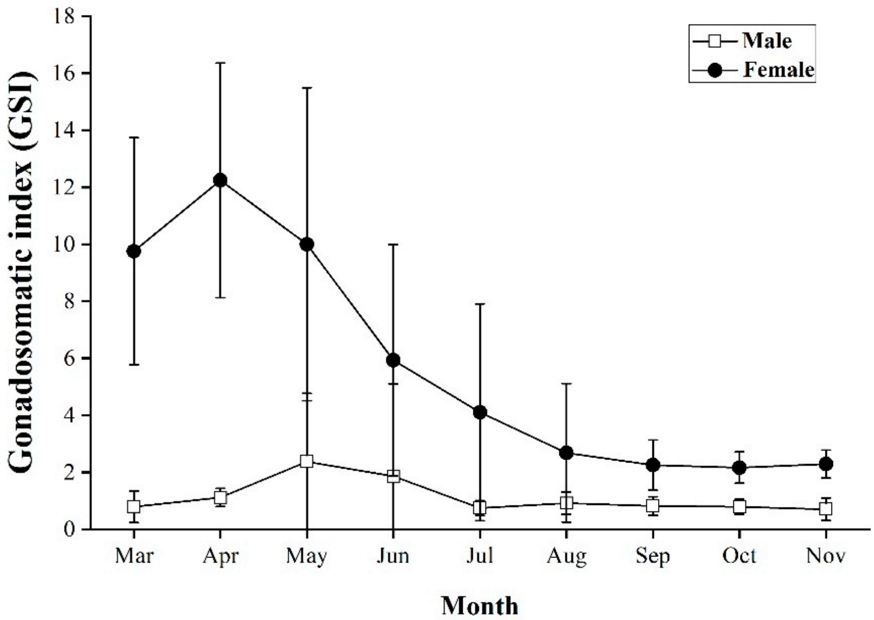

Figure 7. Monthly gonadosomatic index (*GSI*) of *Pseudecheneis sulcatus* males and females in the lower Yarlung Zangbo River between March and October 2016. Values are expressed as means ± SD.

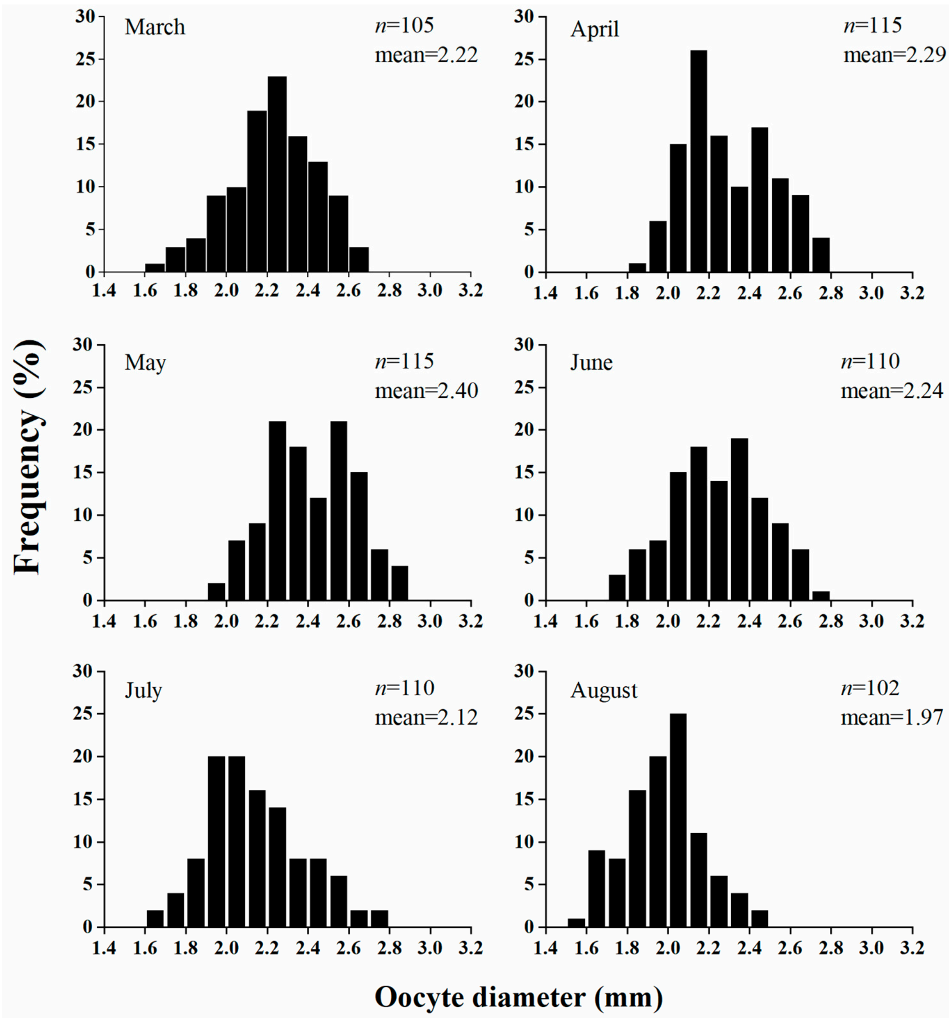

Figure 8. Monthly oocyte diameter distribution in *Pseudecheneis sulcatus* from March to August 2016. Values in the figure are the number of oocytes measured (n) and the mean diameter of oocytes (mean).

The monthly variations in the maturity stages, mean *GSI* values, and mean oocyte diameter showed that *P. sulcatus* spawned between March and June, with a peak in the month of May. The final maturation of gonads and ovulation were found to be well synchronized with spawning temperature, with spawning occurring at water temperatures above 15 °C (Figure 9).

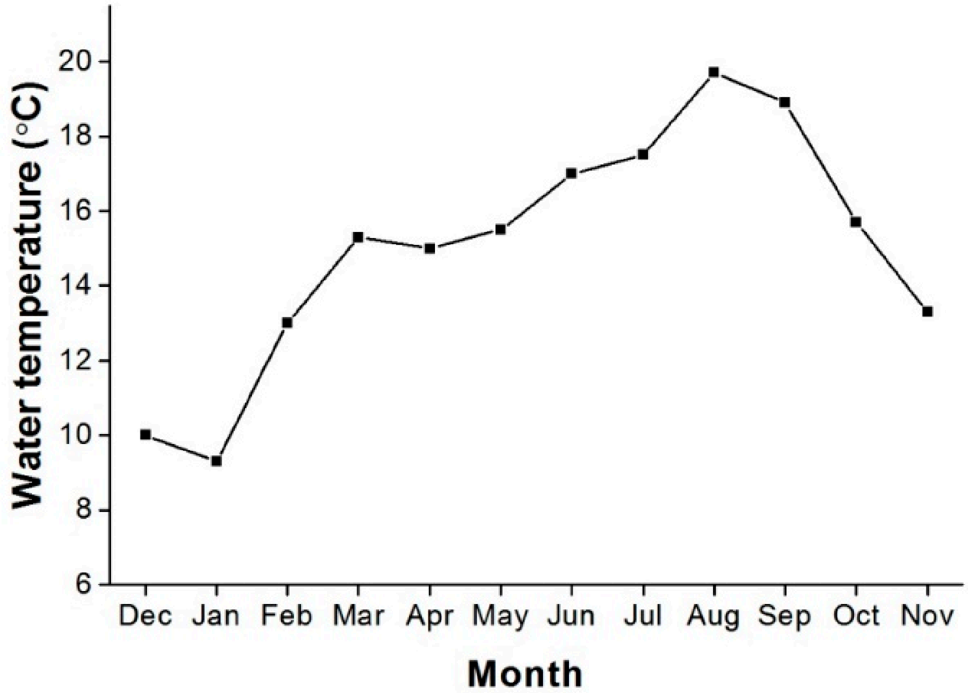

Figure 9. Monthly variation in water temperature at Motuo, the lower reaches of the Yarlung Zangbo River between December 2015 and November 2016.

3.4. Fecundity

The absolute fecundity of *P. sulcatus* ranged from 247 to 2886 oocytes, with an average of 911 ± 544 oocytes per fish and *TL* varying between 114 and 200 mm. The estimated relative fecundity was 40.4 ± 13.3 oocytes per gram, exhibiting a range of 16.8–77.1 oocytes per gram.

The absolute fecundity of *P. sulcatus* showed a linear relationship with its total length and weight along with its ovary weight, which is displayed in Figure 10. The regression equations were fitted as shown below:

Total length: $F = -2031.966 + 21.210TL$, $n = 52$, $R^2 = 0.519$;

Total weight: $F = -60.103 + 43.932TW$, $n = 52$, $R^2 = 0.602$;

Ovary weight: $F = 180.746 + 354.336W_0$, $n = 52$, $R^2 = 0.607$.

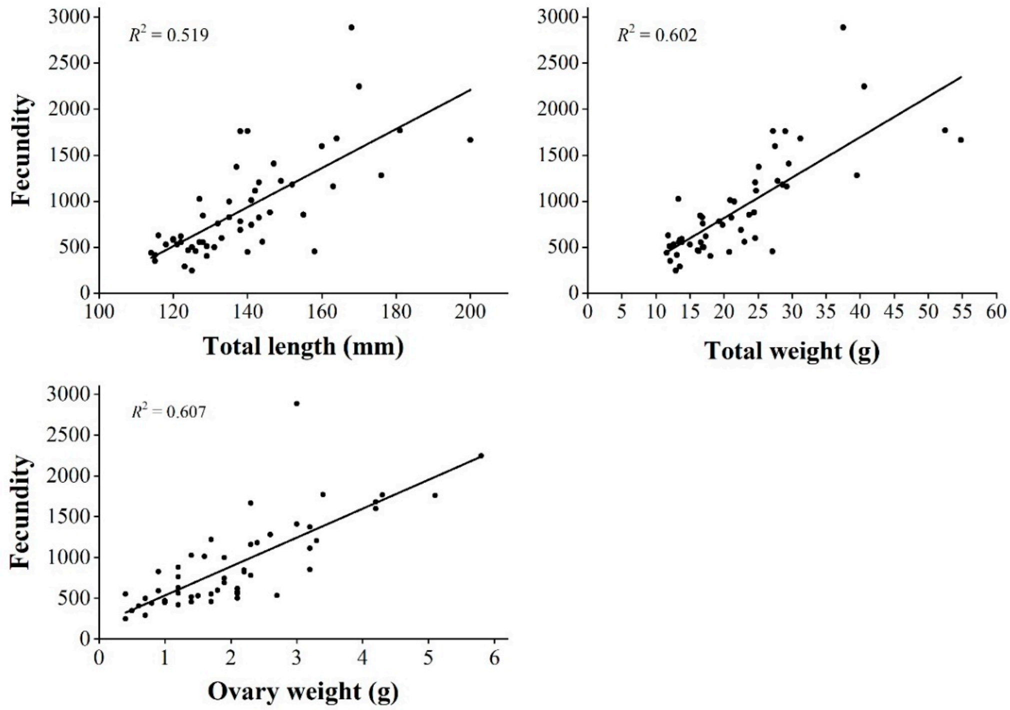

Figure 10. Relationships among the total length, total weight, ovary weight, and fecundity of *Pseudecheneis sulcatus*.

4. Discussion

4.1. Sex Ratio and Size at First Maturity

In evolutionary and population ecology, sexual size dimorphism (SSD) and sex ratio have persistently remained a concern. Theoretically, the sex ratio of most species should be close to 1:1 because that differential investment in gametes would be promoted by natural selection to ensure population persistence over evolutionary time [28]. In contrast, several studies have usually revealed skewed sex ratios among teleost species. In our *P. sulcatus* study, females were found to be the prevailing sex. Similar results were also observed in *G. tibetana* [26] and *P. homaloptera* [29] in the same region, and some Schizothoracinae fishes [24,30] in the middle Yarlung Zangbo River along with other catfish *Glyptosternum maculatum* [18] and *Euchiloglanis kishinouyei* [31]. In *P. sulcatus*, the female-biased sex ratio could be due to biotic or abiotic factors, including local interspecific and intraspecific competitions, male-female differential mortalities, habitat types, and selective fishing [4,32].

Moreover, SSD represents one of the most well-acknowledged and common forms of intraspecific trait alteration in the wild. According to the life-history theory, both sexual and natural selection can be the causes of SSD. Such alterations resulting in successful reproduction may lead to gender disparity in the optimal body size [4]. Length at first maturity is an important index that quantitatively describes SSD, which helps in understanding the reproductive dynamics of specific fish species. In our study, the length at first maturity in males was slightly smaller than that of females, indicating that female-biased sexual size dimorphism existed in the *P. sulcatus* population. This phenomenon was consistent with many other fish species in the same region, including *P. homaloptera* [29] and *Schizothorax curvilabiatus* [33], whose males were more precocious and matured at a smaller length compared to females. This can be attributed to the faster growth of females compared to males or the high mortality of adult males. Meanwhile, SSD alteration was also seemingly caused by sex-specific energy allocation measures [34]. The requirement of more energy for reproduction may prolong the maturity time in females, which could benefit population maintenance while facing variable and unstable fast-flowing habitats in the lower Yarlung Zangbo River [26].

4.2. Gonadosomatic Index (GSI) and Spawning Season

GSI, the ratio between gonad weight and body weight, is an important parameter in fish reproductive biology. Monthly changes in *GSI* values can be used to determine the fish reproductive season based on the gonadal size and developmental stage over time [4]. In the present study, *GSI* values peaked in March–May and then declined from June through July, indicating that the spawning season of *P. sulcatus* in the lower Yarlung Zangbo River extended from March to June. This result indicates that *P. sulcatus* is a spring/early summer spawner. Similar spawning seasons of other species in the Yarlung Zangbo basin, such as *Schizothorax o'connori*, *Oxygymnocypris stewartia*, *G. tibetana*, and *G. maculatum*, can be found in previous studies [18,23,24,29]. These are consistent with the general pattern of most fishes' spawn in spring and summer months in temperate areas where seasonality is distinct [4]. In the fast-flowing river environment of the lower Yarlung Zangbo River, the spring/early summer-spawning strategy would ensure suitable environmental conditions for the larval growth and the accessibility of suitable habitat patches when the water temperature rises. Moreover, the mean females GSI in *P. sulcatus* was much higher than that of males between March and June which suggested that females allocated much more body reserves towards gonads than males. These results provided baseline information on the maturity phases and reproductive periodicity of *P. sulcatus* in its wild habitat.

Methodologically, the *GSI*-based macroscopic method and histology analyses are both widely used to assess maturity in fish. Though histology analysis is the most accurate diagnostic method for assessing the developmental stage of ovaries and testes through sectioning, staining, and microscopically examining the individual histological or cellular structures, the *GSI*-based method exhibits great potential in determining maturity stage due to inexpensive cost and simple calculations [4,35]. Moreover, Flores et al. (2019) showed that the *GSI*-based method to estimate the maturity ogive agreed well with the microscopic method using histology [35]. Thus, the *GSI*-based method has considerable practical advantages for estimating the gonadal maturity stage in such cases where histology staging is lacking or experimental conditions in the field are poor.

4.3. Reproductive Effort and Fecundity

The reproductive effort can be considered as an investment toward producing viable offspring to sustain and enlarge the existing gene counts of the organism in context. Ovary weight per female and the product of oocyte diameter and fecundity can be used as evaluation indicators [36]. Knowledge of fish fecundity is of great importance in understanding the reproductive potential and strategies adopted by different fishes. Relative fecundity provides a better comparison of reproductive effort and avoids the variation in absolute fecundity with fish age and size [4]. Teletchea et al. [37] compiled the reproductive traits of 65 freshwater fish species and revealed that relative fecundity varied considerably among species, exhibiting a range between 0.7 and 2650 eggs/g. In this study, the relative fecundity of *P. sulcatus* was found to be at a lower level compared to other fishes in the nearby waters, for example, the relative fecundity of *Labeo goniu* found in the Brahmaputra was 198 eggs/g [38] while it was 275 in *Barilius bendelisis* found in Gaula River, central Himalayas [39], and 692 in *Hemiculter leucisculus* in Iran wetlands [40]. Similar results were also found in other Sisoridae fish, including *Glyptosternon maculatum* (14.7 eggs/g) [18] in the middle Yarlung Zangbo River along with *Euchiloglanis kishinouyei* (6.6 eggs/g) [31], *Glyptothorax madraspatanum* (128 eggs/g) [41], *Glyptothorax silviae* (105 eggs/g) [42], and *Bagarius yarrelli* (35.6 eggs/g) [43] (Table 1). Hence, in the event of riverine environment degradation, the Sisoridae fishes in the Yarlung Zangbo River may be susceptible to endangerment because of their low relative fecundity.

Another crucial determinant of the reproductive effort of fish was its egg diameter. Coburn [44] studied the oocyte diameters of 71 fish species and found that they varied from 0.7 mm in *Hybognathus hankinson* and *Hybopsis aestivalis* to 2.0 mm in *Campostoma anomalum*. Since most developed oocytes of *P. sulcatus* were over 2.1 mm in diameter, the reproductive traits of *P. sulcatus* were characterized by large eggs and relatively low fecundity. Large eggs are more likely to produce relatively larger and well-developed offspring [45]. This yields several vital benefits: (1) larvae from larger eggs have better behavioral and physiological capabilities than less developed larvae from smaller eggs in hill streams; (2) they are more resistant to starvation conditions because of larger yolk reserves [46]. Therefore, the trade-off between relatively low fecundity and large eggs can increase the offspring fitness or larvae survival ability. These characteristics are well described as the equilibrium strategy guild, which principally agrees with the *K*-selectors that define species with low resources accommodating in their habitats [47].

Table 1. Comparison of the reproductive characteristics of several Sisoridae fishes.

Species	River Drainage	Max Standard Length (mm)	Oocyte Diameter (mm)	Absolute Fecundity (Eggs)	Relative Fecundity (Eggs/g)
Pseudecheneis sulcatus	Yarlung Zangbo River	177	2.39	911	40.4
Glyptothorax madraspatanum	Western Nayar River	152	1.62	3213	128
Glyptosternon maculaturn	Lhasa River	243	2.83	1244	14.7
Glyptothorax silviae	Maroon River	96	1.29	1129	105
Euchiloglanis kishinouyei	Dadu River	168	4.38	248	6.6
Bagarius yarrelli	Lancang River	800	1.20	640,200	35.6

4.4. Reproductive Characteristics and Protection Measures

The distribution of Sisorid catfishes is restricted primarily to the rivers of the Tibetan Plateau and the Himalayas [9,11]. Apart from *P. sulcatus*, there are several Sisorid catfishes inhabiting in the lower Yarlung Zangbo River, such as *Glyptothorax annandalei*, *Pareuchiloglanis kamengensis* and *P. hodgarti* [8]. Due to the relatively wild and remote riverine environment, this particular fauna is vulnerable to anthropogenic events. Although that quality and quantity of essential spawning habitat decreased by anthropogenic events such as land use or water flow alterations, and these influences may have resulted in selecting for smaller size at maturity, potentially reducing reproductive potential [33]. Hence, it is crucial to understand the reproductive traits of this species to preserve their stock. The monthly variations in macroscopic maturity stages, GSI estimates, and distribution of *P. sulcatus* egg diameters suggest their low fecundity along with large-sized oocytes spawning synchronously between March and June. These characteristics are likely due to the tradeoffs between the investment in larger and higher-quality larvae instead of the increased larvae quantity to accommodate the rigorous environmental conditions. The above traits could also explain why *P. sulcatus* is dominant in its habitat. However, this species is vulnerable to threats due to the drastic changes in the riverine environment and frequent geological disasters.

Thus, to achieve fish stock sustainment for future generations, a few rational preservation strategies are recommended. Firstly, human-induced disturbances should be avoided, especially between March and June, in its spawning season. Secondly, a nature reserve should be established to protect the spawning ground with an urgent need to develop the captive breeding of *P. sulcatus*. Moreover, further studies are needed to understand in-depth life history traits and stock conservation of this species in its natural habitats.

5. Conclusions

In the present study, we provided basic information on the reproductive biology of *P. sulcatus* in the lower Yarlung Zangbo River, where the conflict between environmental conservation and economic development exists. *P. sulcatus* matures in spring and early summer and spawning occurs from March to June. Low fecundity and large oocytes are the main reproductive characteristics of this species, which may increase the offspring fitness. These results could help us better understand how fish adapt to extreme riverine environments. Meanwhile, our findings may aid in developing effective population conservation and management measures of *P. sulcatus*.

Author Contributions: Conceptualization, P.L. and X.G.; methodology, formal analysis, software, and writing—original draft preparation, P.L. and H.H.; investigation, resources and data curation, P.L., H.H., Z.G. and J.W.; writing—review and editing, visualization, supervision and project administration, X.G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the ethics committee of the Institute of Hydrobiology, Chinese Academy of Sciences (IHB/LL/20220402). The effects were conducted to minimize the potential suffering for fish.

Data Availability Statement: The datasets that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: All authors declare no conflict of interest.

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