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SEXUAL DIMORPHISM IN SEXUALLY MATURE *GLYPTOSTERNUM MACULATUM* IN TIBET AUTONOMOUS REGION, CHINA

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Abstract: Sexual dimorphism refers to the morphological differentiation between males and females in the same population, such as sizes, morphological features, and colors. Cold water fish *Glyptosternum maculatum*, is an endemic teleost species of notable economic importance and with high potential for controlled rearing of the species in Tibet Autonomous Region. In this study, morphological measurements and related analysis were conducted on sexually mature *G. maculatum* to investigate their morphological differences in Tibet Autonomous Region. Results indicated the sexually mature populations indeed display really displayed significant sexual dimorphism: males exhibited significantly greater body length, weight, and males also exhibited in addition to greater tail length while females exhibited significantly greater eye diameter, body depth, trunk length, and lengths of dorsal, pectoral, pelvic, anal, and caudal fins at a given body length. Principal components analysis on 16 morphological parameters showed that the differences were largely due to features of the body shape (including lengths of body parts and fins) and the head, and differences in the above features differences contributed 76.7%. The *t*-statistic test on the allometry index *b* in the total length-weight function showed that during the breeding season, the total lengths and weights of *G. maculatum* females developed proportionally, while the male specimens were allometrical.

Key words: *Glyptosternum maculatum*; Morphological features; Sexual dimorphism

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Sexual dimorphism refers to the morphological differences between male and female members of the same animal population, such as sizes, colors, and physical structures^[1,2]. Sexual selection, fecundity selection, and niche differentiation may all have contributed to its occurrence^[2,3]. According to Darwin's theory of sexual selection, as members of one particular sex (usually male) compete for the right to mate with the opposite sex, features more attractive to the opposite sex are selected and developed, e.g. the crests of cocks, the trains of peacocks, and the manes of male lions^[4-6]. The fecundity hypothesis argues that reproductive pressure selects females for features that help them produce more offspring, e.g. the capacity to store and produce more egg cells^[7]. The niche

differentiation hypothesis posits that the two sexes of the same population evolve by moving into different niches, therefore increased ecological pressure may accelerate their divergence, while intensifying interspecies competition may suppress it^[8-11]. Additionally, food and nutrition intake during the reproductive period may have a strong effect on the reproductive potential of females, and female sizes may be influenced by the distribution of energy between growth and reproduction^[12].

The *Glyptosternum maculatum* (Regan) is a cold-water fish species adapted to plateau environments, belonging to the Sisoridae family within the Siluriformes order^[13]. Consisting of only three recognized species, *Glyptosternum* is the most primitive

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genus among sisorid catfishes^[14]. *G. maculatum* species is uniquely endemic to the Yarlung Zangbo River (Brahmaputra) river system and surrounding waters in China and India. With the rapid dwindling of its populations due to overfishing and niche disruption in recent years, it is considered a critically endangered (CR) species by the Red List of China's Vertebrates, 2015, the only one among China's 77 known sisorid species to be given this ranking. Early research on *G. maculatum* focused on its taxonomy, geographical distribution, phylogenetics, and evolution^[15–18]; recent studies by Chinese investigators had begun to examine its idiobiology (including age determination, growth characteristics, reproductive strategies, food habits and digestive organs), physiology, population ecology, and genetic diversity^[19–27], but no work has been done on the morphological features of its sexually mature populations. Since there is a visually apparent size difference between female and male members of *G. maculatum*, an empirical study of the difference, and examination of its causes are warranted. This paper is to investigate these questions in order to 1) identify morphological difference between male and female *G. maculatum*, 2) suggest possible mechanisms for the rise of observed differences, and 3) better understand this species, and for conservation purposes.

1 Materials and Methods

1.1 Specimens

Specimens of *G. maculatum* were obtained from the Xietongmen (3900 m above sea level), Lhaze

(4100 m), Namring (4300 m), and Saga (4500 m) Counties of Xigaze Municipality in the Tibet Autonomous Region. The specimens contained 171 females, 98 males, and 11 of indeterminate sex.

1.2 Standard biological measurements

Standard measurements were made on specimens. Total lengths and body lengths were measured by rulers, accurate to 0.1 mm. Vernier calipers were used to measure head length, trunk length, tail length, body width, body depth, head depth, snout length, eye diameter, postorbital head length, interorbital width, dorsal fin length, pectoral fin length, pelvic fin length, anal fin length, and caudal fin length for all specimens, accurate to 0.01 mm (Tab. 1). Body weights were measured by electronic scales, accurate to 0.01 g. Sexes were determined and recorded by the following criterion: males have a sharp genital papilla with a slight depression at its end, females have a blunt, round papilla, which is sometimes swollen with hyperemia, and some females have swollen abdomens containing eggs that can be felt by hand. Female and male *G. maculatum* have obvious differences in secondary sexual characteristic after their sexual maturation, but are difficult to distinguish by appearance before their sexual maturation^[15]. For this reason, smaller specimens which sex could not be visually determined were not included in the tally.

1.3 Data processing

In order to remove the influence of individual specimen dimensions, the following were chosen as the parameters: the dimorphism of head morphology would be represented by the ratios of head depth,

Tab. 1 Measurement methods for morphological parameters of *G. maculatum*

Morphological parameters	Measured by
Total length (<i>L</i>)	Horizontal rectilinear distance (Hor. RD) between the end of the snout & the end of the anal fin
Body length (<i>BL</i>)	Hor. RD between the end of the snout & the end of the caudal vertebrae
Head length (<i>HL</i>)	Hor. RD between the end of the snout & the back edge of an operculum
Trunk length (<i>TL1</i>)	Hor. RD between the back edges of an operculum & the back edge of the anus
Tail length (<i>TL2</i>)	Hor. RD between the back edge of the anus & the back edge of the last caudal vertebra (base of caudal fin)
Body width (<i>BW</i>)	Hor. RD at the widest part of the body
Body depth (<i>BD</i>)	Vertical (Ver.) RD between the highest point & ventral side of the body
Head depth (<i>HD</i>)	Ver. RD between the highest point & ventral side of the head
Snout length (<i>SL</i>)	Hor. RD between the end of the snout & the front edge of an orbit
Eye diameter (<i>ED</i>)	Hor. RD between the back edge & front edge of an orbit
Postorbital head length (<i>PHL</i>)	Hor. RD between the back edge of an orbit & the back edge of an operculum
Interorbital width (<i>IW</i>)	Hor. RD between the orbits
Dorsal fin length (<i>DL</i>)	RD between the starting point & end of the dorsal fin at its base
Pectoral fin length (<i>PL</i>)	RD between the starting point & end of the pectoral fin at its base
Pelvic fin length (<i>PFL</i>)	RD between the starting point & end of the pelvic fin at its base
Anal fin length (<i>AL</i>)	RD between the starting point & end of the anal fin at its base
Caudal fin length (<i>CL</i>)	RD between the starting point & end of the caudal fin at its base

snout length, eye diameter, postorbital lead length, and interorbital width to head length; the dimorphism of body morphology was estimated by calculating ratios of other measured values to body length. Since the comparison is made between two independent samples (females, and males), the independent samples *t*-test was used to process the data. The descriptive statistics were represented as (average \pm standard deviation), with significance level set to $P=0.05$. The morphological data were evaluated with principal component analysis (PCA). The relation between total length and body weight was described by the power equation $W=aL^b$ (where W is weight, L is total length, a , b are parameters to be estimated and b is the allometry index). Analysis of covariance (ANCOVA) was used to determine whether growth differences exist between the two sexes^[28]; Pauly's *t*-statistic method^[29] was used to determine the relation between the allometry index b and 3 via the equation $t = \frac{SD(L)}{SD(W)} \times \frac{|b-3|}{\sqrt{1-r^2}} \times \sqrt{n-2}$ where $SD(L)$ is the standard deviation of the logarithm of the body length, $SD(W)$ is the standard deviation of the logarithm of the weight, n is the number of specimens, and r is the correlation coefficient. All data were computed and analyzed by the Excel 2007 and SPSS 19 software.

2 Results

2.1 Comparison of morphological parameters between sexually mature males and females of *G. maculatum*

The independent samples *t*-test showed that the BL , $TL2/BL$, and W/BL for *G. maculatum* females were significantly lower than males ($P<0.05$), while ED/HL , BD/BL , $TL1/BL$, DL/BL , PL/BL , PFL/BL , AL/BL , and CL/BL were significantly greater than males ($P<0.05$). Differences in other parameters were insignificant (Tab. 2).

2.2 Principal components analysis of morphological parameters for sexually mature *G. maculatum*

Principal components analysis was performed on 16 morphological parameters of 75 specimens. Tab. 3 shows the factor loadings of each main component. The first five main components have a cumulative contribution rate of 76.7%. The 1st main component has a contribution rate of 41.7%, with greater loadings for morphological features of the body including BL , HL/BL , $TL1/BL$, $TL2/BL$, BD/BL , DL/BL , PL/BL , PFL/BL , AL/BL , and CL/BL ; the 2nd, 3rd and 4th main components have a total contribution of 28.7%, with greater loadings for morphological features of the head including HD/HL , SL/HL , IW/HL , ED/HL , and PHL/HL ; the 5th main component has a 6.3% contribution, with greater loading for BW/BL , which may represent features related to the feeding beha-

Tab. 2 Differences between morphological parameters for sexually mature males and females of *G. maculatum* in Tibet Autonomous Region

Morphological parameter	Female number	Male number	Female (F)	Male (M)	Independent samples <i>t</i> -test result
TL (mm)	72	97	177.96 \pm 22.93 ^b (135.0—236.0)	221.67 \pm 55.67 ^a (127.0—344.0)	F<M
HD/HL	39	36	0.4925 \pm 0.0439	0.4793 \pm 0.0403	
SL/HL	39	36	0.4385 \pm 0.0236	0.4465 \pm 0.0294	
PHL/HL	39	36	0.5148 \pm 0.0261	0.5141 \pm 0.030	
ED/HL	39	36	0.0585 \pm 0.0064 ^a	0.0494 \pm 0.0068 ^b	F>M
IW/HL	39	36	0.2849 \pm 0.0158	0.2930 \pm 0.0246	
HL/BL	39	36	0.2618 \pm 0.0118	0.2539 \pm 0.0233	
$TL1/BL$	39	36	0.4734 \pm 0.0183 ^a	0.4374 \pm 0.0355 ^b	F>M
$TL2/BL$	39	36	0.2648 \pm 0.0172 ^b	0.3087 \pm 0.0551 ^a	F<M
BD/BL	39	36	0.1507 \pm 0.0207 ^a	0.130 \pm 0.0160 ^b	F>M
BW/BL	72	97	0.2273 \pm 0.0162	0.2249 \pm 0.0222	
DL/BL	39	36	0.1769 \pm 0.0107 ^a	0.1610 \pm 0.0171 ^b	F>M
PL/BL	39	36	0.2179 \pm 0.0119 ^a	0.1972 \pm 0.0247 ^b	F>M
PFL/BL	39	36	0.1532 \pm 0.0091 ^a	0.1364 \pm 0.0141 ^b	F>M
AL/BL	39	36	0.1570 \pm 0.0069 ^a	0.1455 \pm 0.0176 ^b	F>M
CL/BL	69	92	0.1323 \pm 0.0304 ^a	0.1224 \pm 0.0275 ^b	F>M
W/BL	72	96	0.4753 \pm 0.1299 ^b	0.6793 \pm 0.3135 ^a	F<M

Note: Different superscript letters indicate significant differences between groups ($P<0.05$); significant results are shown as either F<M or F>M; W: weight. Other abbreviations are as shown in Tab. 1

vivors of *G. maculatum* (Tab. 3).

2.3 Development of sexually mature *G. maculatum*

Distribution of total lengths Sixty-nine female total length specimens were acquired, which ranged between 147.0 and 259.0 mm, for an average of (200.9±26.6) mm; ninety-two male total length specimens were acquired, ranging between 141.0 and 377.0 mm, with an average of (245.3±61.9) mm.

The majority of female total lengths fall in the 150 to 250 mm range; the majority of male total lengths lie in the 150 to 300 mm range.

Distribution of weights One hundred and seventy-one female weight specimens were acquired, ranging between 30.3 and 303.5 g, with an average of (99.2±46.9) g; ninety-eight male weight specimens were acquired, ranging between 28.6 and 502.6 g, with an average of (166.0±110.9) g.

The majority of female weights are in the 50 to 150 g range, and the majority of male weights are in the 50 to 200 g range.

Relationship between total length and weight

Scatter plot analysis was applied to the total lengths and weights of female and male specimens (Fig. 1, Fig. 2). Through fitting, power function rela-

tionships were obtained between weight *W* and total length *L* ($n_{\text{♀}}=69, n_{\text{♂}}=92$): $W(\text{♀})=2 \times 10^{-5} L^{2.8647}$ ($r^2=0.9030$), $W(\text{♂})=5 \times 10^{-5} L^{2.7073}$ ($r^2=0.9424$). The r^2 describes how much of the variability in the data is explained by the model. ANCOVA showed significant differences in allometry between the sexes (Sig.=0.117>0.05). Pauly's *t*-statistic results were $t=1.18$ for females and $t=4.20$ for males. Compared to $t_{0.05}=1.96$, it can be seen that during the breeding season, the total lengths and weights of *G. maculatum* females developed proportionally, while the male specimens did not.

2.4 Summary morphological data

Significant sexual dimorphism was found in the sexually mature specimens of *G. maculatum* by comparing normalized morphological parameters. Primary dimorphic features included: 1) males were significantly larger than females in both total length and weight; 2) at given body length, females had significantly larger eye diameters; 3) at given body length, females had significantly greater body depths, and trunk lengths; and 4) at given body length, females

Tab. 3 Factor loadings for morphological parameters for sexually mature *G. maculatum* in Tibet Autonomous Region

Morphological parameter	Factor loading				
	PC1	PC2	PC3	PC4	PC5
HD/HL	0.145	0.667	0.397	-0.391	0.011
SL/HL	-0.91	0.664	-0.330	0.269	0.023
PHL/HL	0.124	0.519	-0.078	0.565	0.248
ED/HL	0.573	0.343	-0.543	-0.326	-0.105
IW/HL	-0.352	0.550	0.323	-0.096	-0.078
TL	-0.787	-0.105	0.391	0.314	0.053
HL/BL	0.707	-0.443	0.355	0.115	-0.094
TL1/BL	0.834	0.105	0.029	0.285	-0.006
TL2/BL	-0.894	0.104	-0.166	-0.254	0.042
BD/BL	0.683	0.180	0.385	-0.332	-0.128
BW/BL	0.178	-0.139	0.018	-0.262	0.916
DL/BL	0.839	-0.053	-0.001	-0.017	-0.024
PL/BL	0.854	0.001	-0.035	0.069	-0.142
PFL/BL	0.910	-0.004	-0.046	-0.082	0.076
AL/BL	0.807	-0.049	-0.230	0.132	0.124
CL/BL	0.664	0.265	0.396	0.158	0.125
Contribution rate (%)	41.734	12.075	9.382	7.198	6.283
Cumulative Contribution rate (%)	41.734	53.809	63.191	70.389	76.671

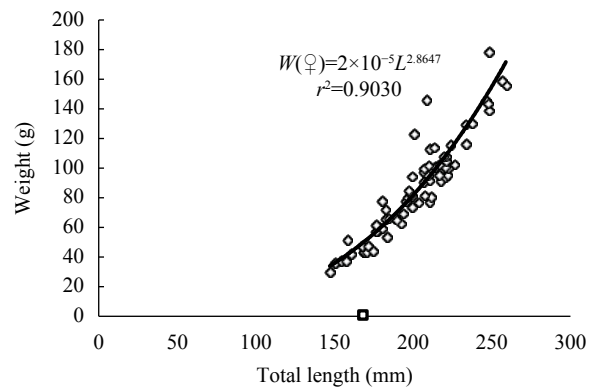


Fig. 1 Relationship between total length and body weight of *G. maculatum* females in Tibet Autonomous Region

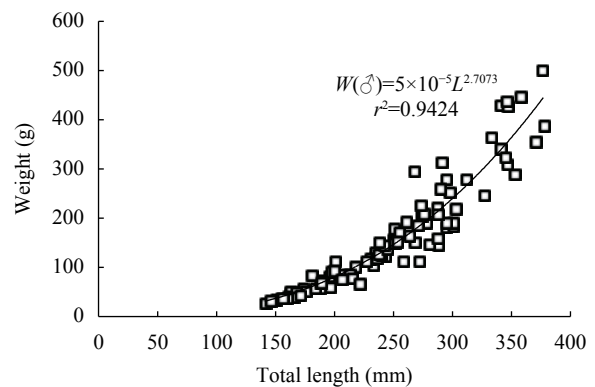


Fig. 2 Relationship between total length and body weight of *G. maculatum* males in Tibet Autonomous Region

had significantly longer dorsal, pectoral, pelvic, anal, and caudal fins. PCA on 16 parameters suggests that the differences were caused by morphological characteristics of the body (including the lengths of body parts and fins) and the head. Through t-statistic test on the allometry index b from the total length-weight function, it is found that during the breeding season, the lengths and weights of females developed at the same rate, which is not the case for males.

3 Discussion

3.1 Sexual dimorphism in sizes of sexually mature *G. maculatum*

Size tends to be one of the most prominent ways in which sexual dimorphism is expressed, and fish species are no exception. Fish generally fall into three categories: females larger than males, e.g. *Gambusia affinis*^[30], and *Paracanthobrama guichenoti*^[31]; similarly sized females and males, e.g. *Silurus asotus*^[32], and *Acrossocheilus wenchowensis*^[33]; and males larger than females, e.g. *Clarias fuscus*^[32], *Odontobutis obscurus*^[12], *Abbottina rivularis*^[34], and *Pseudoba-grus fulvidraco*^[35]. The results of this study have shown that sexually mature males of *G. maculatum* tend to be larger than females, which might be explained by the sexual selection theory: reproductive competition between males, as larger males are more likely to win out in the reproductive competition between males, and produce offspring. The fish populations of the Yarlung Zangbo River are relatively undiversified; as an example, the only fishes living with *G. maculatum* in the Xietongmen river segment are four species of Triplophysa (e.g. *T. tibetana*) and six species of Schizopygopsis (e.g. *S. younghusbandi*)^[27]. This relative lack of interspecies competition for living space and food may have accentuated sexual dimorphism in *G. maculatum*. Research on its behavior had found *G. maculatum* to generally live as small groups in areas abundant in rock and gravel, and only move within a small range^[27]. This behavior may have reinforced their intra-species competition, thus affecting the size differences. In addition, the sizes of females may be influenced by the distribution of energy between growth and reproduction^[12]. During breeding seasons, the total length and weight of females *G. maculatum* growing proportionally, which likely means they have allotted more energy towards reproduction-related activities, leaving less to their own growth, while the males' total length and weight develop at different rates, which probably indicates more energy has been devoted to growing their own bodies probably because of dedicating more energy to growing themselves. This suggests maybe energy

distribution as another possible is also a factor.

3.2 Sexual dimorphism in features

In order to carry the offspring, female fishes tend to need greater abdominal cavities to contain more eggs. This difference in evolutionary pressure may be why *G. maculatum* females have greater trunk lengths and body depths.

Fish species depend on fins for locomotion and maintaining balance. The body of *G. maculatum* is flattened horizontally in the front half, and flattened vertically in the back half; this, along with the round caudal fin gives it relatively weak locomotive capabilities. Instead, its pectoral and pelvic fins both spread out horizontally, and have small folds on their surfaces, which increases their friction, and helps it anchor itself on the riverbed. At given length, a female *G. maculatum* have significantly longer dorsal, pectoral, pelvic, anal, and caudal fins than a male, which may be the result of niche differentiation and environmental factors, as the stronger fins can help the comparatively smaller females better anchor themselves to the bottom in strong currents, and give them better locomotion capabilities to find food and meet the need for nutrition in breeding seasons.

The *G. maculatum* has small, simple eyes on the top of its head, while the barbels on its top jaw are highly developed, with broad films connecting them to the snout skins and the lips. Its mouth faces the bottom, and has a large, wide opening and strong teeth^[27]. Researchers found the species As a benthic predator, *G. maculatum* feed by actively predated on the *Triplophysa* and juvenile *Schizopygopsis*^[27]. It has small, simple eyes on the top of its head, while the barbels on its top jaw are highly developed, with broad films connecting them to the snout skins and the lips. Its mouth faces the bottom, and has a large, wide opening and strong teeth^[27]. In this study, at a given length, sexually mature *G. maculatum* females were shown to have significantly larger eyes than males. The cause of this dimorphism warrants further investigation, as more work is needed to determine whether the eye size is related to the species' visual capabilities, or beneficial to their predatory behavior. It can be reasoned that the significantly larger eyes of females may also be related to selective pressure from the need for nutrition in breeding season.

In summary, *G. maculatum* exhibits sexual dimorphism in both sizes and local physical features. Examining its related selective pressure and adaptive

mechanism should help us better understand and protect this rare fish species.

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西藏黑斑原鲮繁殖群体两性异形研究

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摘要: 两性异形是指在同一种群内, 雌雄间的形态特征产生分化, 如在个体大小、局部形态构造、体色等方面呈现出差异的现象。为了解西藏黑斑原鲮 (*Glyptosternum maculatum*) 雌雄间是否也存在两性异形, 在其繁殖期对性成熟个体进行了形态学测量及相关分析。结果表明: 西藏黑斑原鲮繁殖群体间存在显著的雌雄异形, 一是雄性个体的体长、体重及特定体长下的尾长显著大于雌性个体; 二是在特定体长下, 雌性个体眼径、体高、躯干长以及背鳍、胸鳍、腹鳍、臀鳍、尾鳍长度均显著大于雄性。16个形态参数的主成分分析表明, 雌雄间的差异主要是由体型特征(包括身体各部分长度、各鳍长度等)及头部形态特征引起的, 贡献率达76.7%。对全长-体重方程中异速生长指数 b 的 t 检验表明, 繁殖期黑斑原鲮雌性个体呈等速生长而雄性个体呈异速生长。

关键词: 黑斑原鲮; 形态特征; 两性异性