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Original Article

# Assessment of reproductive biology of *Garra langlungensis* (Teleostei: Cyprinidae) from the Langlung River, Nagaland, India

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**Abstract**
The stone sucker, *Garra langlungensis* Ezung, Shaingningam & Pankaj, 2021, is confined to the Langlung River, near Zutovi village in Dimapur district of Nagaland, India. This endemic fish is a common food item among the local people. There is scarce information available on this species. Despite its rising exploitation, the lack of data could contribute to its decline and potential loss. The present study involved analysis of 213 specimens from the Langlung River, collected monthly over a period of one year, to determine the sex ratio, gonadosomatic Index (GSI), and fecundity. The sex ratio of males to females was 1:0.43 (deviation from the expected 1:1 ratio), showing a male-dominated population. The monthly assessment of GSI indicated that *G. langlungensis* breed once a year, with maximum GSI values of 8.1 (male) and 11.5 (female) in April and a short breeding season extending from February to May. Fecundity, based on data from 16 mature fish specimens, was the highest (844 eggs) in 77.9 mm TL (0.56 g) and the lowest (319 eggs) in 55.2 mm TL specimen (0.23 g). A significant correlation between fecundity and ovary weight ($r$ = 0.915) was recorded. This study provides insights into the sex ratio, GSI, and fecundity of *G. langlungensis*, which facilitate the identification of fish stocks in their natural habitats and the development of appropriate conservation measures.

**Keywords:** endemic fish; fecundity; GSI; linear relationship; male-dominated population; spawning season

## 1 | INTRODUCTION

*Garra langlungensis* Ezung, Shaingningam & Pankaj, 2021 is a small-sized, bottom-dwelling freshwater fish species that is classified as a proboscis species group based on its snout morphology amongst the *Garra* species. It belongs to the labeonine genus *Garra* Hamilton, 1822, which typically inhabits mountain torrents and rapidly flowing rivers and streams (Ezung *et al.* 2021). These fish exhibit unique adaptations to their surroundings, such as adhering to the substratum and manoeuvring against swift currents using their oral suctorial disc and the horizontally placed paired fins, particularly the pectorals (Menon 1964). *Garra langlungensis*, known locally as “pathor mass” in Nagaland (in Nagamese ‘pathor’ = stone, and ‘mass’ = fish), particularly as this fish inhabits a river with mainly rocks and boulders as its substratum (Ezung *et al.* 2021). This fish is a popular food item among locals of Nagaland, and currently found exclusively in the Langlung River, Nagaland. Apart from being recognized as a food delicacy, this fish fetches a higher price in the local market (between ₹500 - ₹1000 per kg; 1 US$ = 86.58₹), and owing to its high market value, it also contributes to the local economy. This, on the other hand, is due to increased exploitation and overfishing, which is having an adverse impact on the natural stock (Pham *et al.* 2023).

Over time, anthropogenic activities such as boulder excavation, sand mining, and the exploitation of fish using destructive fishing tactics have degraded the fish's habitat, potentially contributing to a decline in fish populations in Nagaland (Kechu and Pankaj 2023). Understanding the reproductive biology of fish is critical for successful aquaculture practices and scientifically informed fishery management strategies in various aquatic environments (Jacob 2013). Therefore, it is necessary to generate basic information on the reproductive biology of *G. langlungensis*. Furthermore, the reproductive behaviour of fish species is essential for unraveling fundamental aspects of fish biology and their effective management and conservation (Borthakur 2018).

*Garra langlungensis* is an endemic species in Nagaland and is highly esteemed for its culinary appeal. However, despite its local significance, there is a lack of scientific research on the reproductive biology of this fish species. Therefore, the present study aims to fill this knowledge gap by providing comprehensive information on the reproductive biology of *G. langlungensis*, with a specific focus on the sex ratio, gonadosomatic index, and fecundity. This research endeavor will contribute to a better understanding of the fish stock within its natural habitat and assist in its effective management and conservation.

## 2 | METHODOLOGY

### 2.1 Study site

Fish specimens were collected from Langlung River (25°42′55.98″N 93°39′51.07″E), a tributary of Dhansiri River near Zutovi village in Dimapur District of Nagaland, India. It originates near New Jalukie, Peren District, flows through Zutovi Village, Dimapur, and joins the Dhansiri River, finally confluencing into the Brahmaputra. Water parameters recorded during the study included water temperature (24.75 – 31.66°C), pH (7.0 – 8.0), and water flow velocity (0.74 – 3.89 $ms^{-1}$).

### 2.2 Collection of fish samples and laboratory analysis

A total of 213 specimens of *G. langlungensis* were collected from different sites along the Langlung River using standardized fishing gear, including cast nets, gill nets, and scoop nets. The cast nets used had a diameter of 3 meters, a mesh size of 2 cm, and were made from durable nylon material. Gill nets of varying mesh sizes, ranging from 1.5 cm to 4 cm, were employed, with the nets typically measuring 10 meters in length and 1.5 meters in height. Scoop nets, with a mesh size of 2.5 cm and a handle length of 1.5 meters, were also used to collect specimens from shallow areas. These nets were all made of nylon to ensure consistency and durability in sample collection.

Sampling was carried out at five designated sites along the stretch of Zutovi village, with distances between sites ranging from 400 to 800 meters. The sampling occurred at the end of each month, from March 2017 to February 2018, between 9:00 AM and 12:00 PM. The coordinates for the sampling sites were as follows: Site 1 (25°42′57.45″N, 93°39′50.85″E), Site 2 (25°43′9.84″N, 93°39′46.68″E), Site 3 (25°43′20.36″N, 93°39′53.28″E), Site 4 (25°43′31.43″N, 93°39′45.10″E), and Site 5 (25°43′50.90″N, 93°39′40.10″E). The collected fish specimens were preserved in a 10% formalin solution for further laboratory analysis. The total length of each specimen was measured using a digital Vernier caliper (INSIZE, India), with a precision of 0.1 mm, and the whole body weight and gonad weight were determined using an electronic weighing scale with a precision of 0.1 g. Dissection was performed to determine the sex of each specimen, and the gonads were carefully excised and examined. Standardization of the sample techniques was maintained by employing comparable equipment at all sites and conducting sampling at regular intervals to ensure the validity and comparability of the study's findings.

### 2.3 Sex ratio

The sex ratio was calculated as the percentage of males to females for each month. To assess the homogeneity of the sex ratio, a Chi-square test (Snedecor and Cochran 1967) was employed to determine whether the observed ratio deviated significantly from the ideal 1:1 ratio of males and females.

### 2.4 Gonadosomatic index (GSI)

The GSI was estimated on a monthly basis by applying the formula by Nikolsky (1963) as follows: GSI = (weight of the gonad / weight of the fish) × 100.

### 2.5 Fecundity

Fecundity was calculated based on 16 ripe females with sizes ranging from 55.2 mm to 78.7 mm TL. The ovaries of each individual were dissected, and three sub-samples representing the anterior, middle, and posterior regions were weighed separately. The number of ova in each sub-sample was determined by visual examination under a Huvitz stereo zoom microscope (HSZ-ILST6, South Korea). Fecundity was calculated using the gravimetric method (Hunter *et al.* 1992) with the following formula:

> Fecundity = (weight of the ovary × average number of eggs per sub-sample) / (average weight of the sub-sample)

Regression analysis was performed to explore the relationship between computed fecundity and various parameters such as total length, body weight, and ovary weight following log10 transformation of the respective X and Y values (Joshi and Khanna 1980).

### 2.6 Data analysis

Data collected on gonadosomatic index and fecundity were analyzed using Microsoft Office Excel. The sex ratio was analyzed using the Chi-square test with the null hypothesis that the sex ratio is 1:1. The analysis was carried out separately for males and females. The data were presented using descriptive statistics, including mean, standard deviation, and percentage. Variables that significantly deviated from normality ($p < 0.05$) and exhibited skewness were subjected to logarithmic transformation (log10) to normalize their distribution and satisfy the assumptions of parametric tests such as regression analysis. The transformed data were then re-assessed for normality before further analysis. The Shapiro-Wilk test indicated that total length and body weight data met the normality assumptions, and therefore, required no data transformation. However, ovary weight and fecundity showed significant positive skewness and were normalized using log10 transformation. These adjustments enabled valid parametric analyses to assess relationships between fecundity and biometric parameters.

**TABLE 1** Normality test and Skewness analysis for biometric and fecundity data of *Garra langlungensis*.

| Variable | Shapiro-Wilk *W* | *p*-value | Skewness | Normality conclusion | Transformation applied |
|---|---|---|---|---|---|
| Total Length (mm) | 0.934 | 0.325 | -0.142 | Normally distributed | No |
| Body Weight (g) | 0.911 | 0.162 | 0.136 | Normally distributed | No |
| Ovary Weight (g) | 0.821 | 0.016* | 0.872 | Data is not normal ($p < 0.05$) | Log10 |
| Fecundity | 0.803 | 0.010* | 1.043 | Data is not normal ($p < 0.05$) | Log10 |

*Significant deviation from normality.

## 3 | RESULTS

### 3.1 Sex ratio

Out of the 213 specimens examined, 149 were males and 64 were females. The percentage occurrence of males was found to be 69.95, while that of females was 30.04. The month-wise distribution of males and females of *G. langlungensis* shows that males outnumbered females in all months (Table 2). The mean sex ratio of males to females was observed to be 1:0.43, which deviated from the ideal 1:1 ratio. The chi-square value of 33.92 indicated that the variation in the sex ratio of *G. langlungensis* was significant ($p < 0.05$).

### 3.2 Gonadosomatic index (GSI)

The GSI ranged between 0.3 and 8.1 in males, and between 0.5 and 11.5 in females. The GSI was found to be higher from February to May and lower from September to November in both male and female populations. The peak in GSI was observed in March and April among the male population, while among females, the peak was observed in April. A declining trend was observed right after the peak point in both the male and female populations (Figure 1). The values of the GSI indicate that the breeding season of *G. langlungensis* extends from February to May.

### 3.3 Fecundity

The fecundity of *G. langlungensis* ranged from 319 – 844 in fishes of 55.2 – 78.7 mm TL. The highest fecundity (844) was observed in individual weighing 5.31 g whereas; the lowest fecundity (319) was recorded in individual of 2.19 g body weight. The average absolute fecundity was worked out to be 565.

Regression analysis was conducted to examine the relationship between fecundity and total length, body weight, and ovary weight, as illustrated in Figures 2 to 4. The scatter diagram revealed a linear relationship between fecundity and body parameters. The value of the correlation coefficient between fecundity and body parameters showed that fecundity is strongly correlated with ovary weight.

**TABLE 2** Monthly sex ratio of *Garra langlungensis* collected from Langlung River, Nagaland.

| Months | Total | Male | % of male | Female | % of female | Ratio of M:F | $\chi^2$ | *df* | *p*-values |
|---|---|---|---|---|---|---|---|---|---|
| February | 22 | 15 | 68.18 | 7 | 31.81 | 1:0.47 | 1.92 | 1 | > 0.05 |
| March | 21 | 12 | 57.14 | 9 | 42.85 | 1:0.75 | 2.9 | 1 | > 0.05 |
| April | 44 | 30 | 68.18 | 14 | 31.81 | 1:0.47 | 0.42 | 1 | > 0.05 |
| May | 29 | 22 | 75.86 | 7 | 24.13 | 1:0.32 | 5.82 | 1 | **<0.05** |
| June | 17 | 12 | 70.58 | 5 | 29.411 | 1:0.42 | 7.76 | 1 | **<0.05** |
| July | 8 | 6 | 75 | 2 | 25 | 1:0.33 | 2.88 | 1 | > 0.05 |
| August | 11 | 8 | 72.72 | 3 | 27.27 | 1:0.38 | 2 | 1 | > 0.05 |
| September | 9 | 7 | 77.77 | 2 | 22.22 | 1:0.29 | 2.28 | 1 | > 0.05 |
| October | 14 | 10 | 71.42 | 4 | 28.57 | 1:0.40 | 2.78 | 1 | > 0.05 |
| November | 13 | 9 | 69.23 | 4 | 30.76 | 1:0.44 | 2.58 | 1 | > 0.05 |
| December | 12 | 9 | 75 | 3 | 25 | 1:0.33 | 1.92 | 1 | > 0.05 |
| January | 13 | 9 | 69.23 | 4 | 30.76 | 1:0.44 | 3 | 1 | > 0.05 |
| Total | 213 | 149 | 69.95 | 64 | 30.04 | 1:0.43 | 33.92 | 11 | **<0.05** |

Boldface *p*-values indicate statistically significant differences.

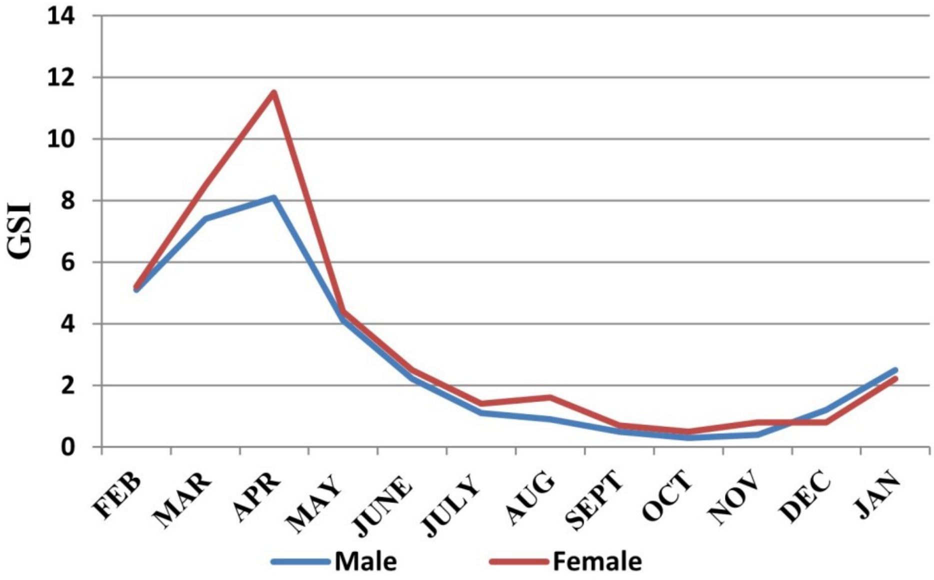


**FIGURE 1** Monthly variation in Gonadosomatic index (GSI) values of male and female *Garra langlungensis* from Langlung River of Nagaland, India.

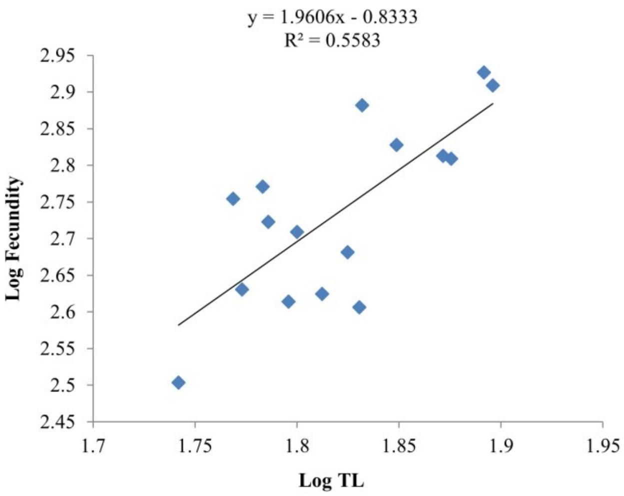


**FIGURE 2** Relationship between fecundity and total length (TL) of *Garra langlungensis*.

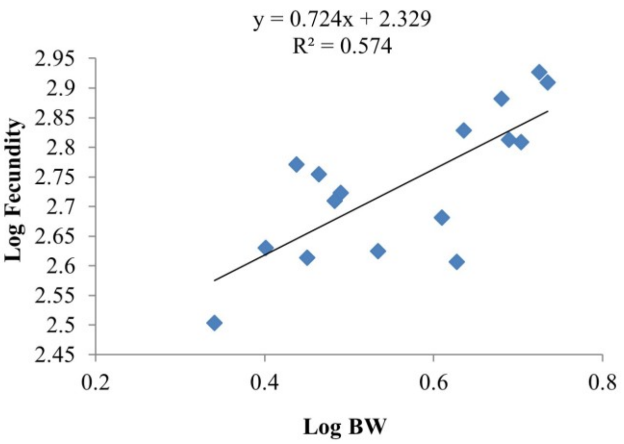


**FIGURE 3** Relationship between fecundity and body weight (BW) of *Garra langlungensis*.

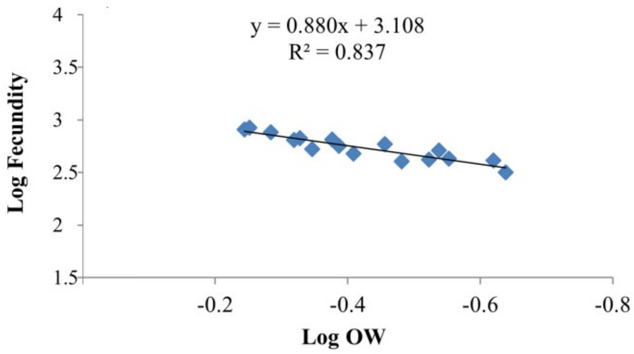


**FIGURE 4** Relationship between fecundity and ovary weight (OW) of *Garra langlungensis*.

**4 | DISCUSSION**

Knowledge of sex ratio estimation in a fish population is essential for understanding the abundance of the sexes in a natural habitat during different seasons or specific times. Ideal sex ratio in a wild population is close to 1:1; however, drastic deviations from this optimum can occur due to various factors (Nikolskii 1969). In the present investigation, the mean sex ratio of males to females was 1:0.43 ($\chi^2$ = 33.92), indicating that males dominated females in all months during the study period. However, considerable variations were observed during April and May, which is the peak spawning season for this fish.

A significant variation in sex ratio, with males outnumbering females, has been reported in *Garra surendranathanii*, with a sex ratio of 1:0.34, as noted by Thampy (2009). A sex ratio of 1:0.8 was reported by Bindu and Padmakumar (2014) in *Etroplus suratensis*. Ajibare and Loto (2023) also reported a sex ratio of 3.21:1 in *Sarotherodon melanotheron*. Indarjo *et al.* (2021) reported that a higher proportion of males compared to females within a population suggests overexploitation of the species' natural stocks and indicates a fishing preference for one sex, posing challenges for sustainable fishery management. According to Nikolsky (1999), a sex ratio greater than one, favoring males over females in fish populations, may indicate an increased likelihood of fertilization. However, the present study observed a significant deviation from the ideal 1:1 sex ratio, with male dominance in *G. langlungensis*, suggesting a reduced potential for successful fertilization in this species. Therefore, prior to establishing and implementing management approaches, further investigations must be carried out to determine what factors might exclude more females in the study area.

The GSI is a valuable measure for determining reproductive periodicity in fishes. The maximum GSI value indicates the breeding season of the fish in its natural habitat (Hamza 1980; Sharma 1987; Rao 1993). The highest GSI value for this fish was recorded in March and April for males, and in April for females. The lowest value was recorded in October for both males and females. The GSI typically increases with fish maturation, reaching a maximum during the period of peak maturity and then declining abruptly thereafter (Borthakur 2018). It was observed that the mean GSI value gradually increased from November, rising higher from February, reaching its peak in April, and subsequently declining to its lowest point in October.

The GSI differs by species; however, similar findings with one peak have been reported by Patimar *et al.* (2010) in *Garra rufa*; Kanwal (2017) in *Garra lamta*; Wani *et al.* (2022) in *Schizothorax niger*; Poudel *et al.* (2023) in *Glyptothorax telchitta*. The present study shows that *G. langlungensis* breed once a year, indicating its peak breeding period in April, with a short duration extending from February to May.

Knowledge of fecundity is essential for assessing the productive potential, life history, and commercial potentialities of a fish stock, as well as for efficient fish culture (Lagler 1956; Mian and Dewan 1984). Fecundity in *G. langlungensis* ranged from 319 to 844, with an average value of 565. The highest fecundity value (844) was obtained, with a total length of 77.9 mm, a body weight of 5.31 g, and an ovary weight of 0.56 g. The lowest fecundity value (319) with a total length of 55.2 mm, 2.19 g body weight, and 0.23 g ovary weight. In teleosts, fecundity ranges from a few hundred to millions, such as *Cirrhinus reba* with 19549 – 265042 (Jewel *et al.* 2019) and *Cyprinus carpio* with 18280 – 390600 (Bakht *et al.* 2020). The range of fecundity observed in the present study was low compared to other high-fecund fish, which produce thousands to several lakhs of ova. Similar findings with low fecundity were reported in *G. surendranathanii* (Thampy 2009); *G. rufa* (Abedi *et al.* 2011); *Garra regressus* and *Garra tana* (Geremew *et al.* 2015); *Xenontedon cancila* (Borthakur 2018); and *Puntius ticto* (Bahuguna *et al.* 2021). The findings of this study also show that the number of ova varies within the same size group of this species.

The statistical analysis reveals a linear and positive relationship between fecundity and total length, body weight, and ovary weight. Similar observations were reported by Rahman and Miah (2009) in *Mastacembelus pancalus*; Angami (2012) in *Danio dangila* and *Puntius chola*; Kant *et al.* (2016) in *Puntius sophore*; Borthakur (2018) in *X. cancila*; and Bahuguna *et al.* (2021) in *P. ticto*. The correlation coefficient value showed that fecundity is significantly correlated with total length ($r = 0.747$), body weight ($r = 0.758$), and ovary weight ($r = 0.915$). However, the highest correlation was observed between fecundity and ovary weight. This finding is in agreement with observations reported in *Gerres abbreviates* (Sivashanthini *et al.* 2008); *G. rufa* (Abedi *et al.* 2011); and *Garra lamta* (Kanwal 2017). Ezung and Pankaj (2022) reported on the length-weight relationship and relative condition factor of the ornamental fish population *G. langlungensis*, concluding that the length-weight relationship deviated from the cube law and exhibited negative allometric growth. Fecundity in *G. langlungensis* is thus directly proportional to the weight of the ovary in the present study.

## 5 | CONCLUSIONS

The present study provides valuable knowledge on specific aspects of the reproductive biology of *G. langlungensis*. This study reveals a male-dominated population in its native habitat, with females having a lower fecundity compared to other fish species. The study on gonadal maturity indicates that it breeds once a year, with a limited breeding season. Based on the current findings, an effective initiative could be planned to establish a conservation strategy for restocking the exploited stock, as well as raising awareness among the local population against the exploitation of fish stocks. Suggested measures include restricting the catch of undersized fish to conserve resources, limiting fishing of females during breeding seasons, and reducing the use of harmful fishing equipment. The limited sample size in this study restricted statistical comparisons of fecundity across sexes or groups. Although GSI values were calculated year-round for both sexes, only descriptive analysis was performed to identify reproductive trends, without inferential testing. The sex ratio shows significant deviation from the expected 1:1 ratio. Future studies should use larger, balanced samples to enable inferential analyses (e.g. *t*-test and ANOVA) for more comprehensive insights.

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**CONFLICT OF INTEREST**

The author declares no conflict of interest.

**AUTHORS' CONTRIBUTION**

SE: Data collection, data analysis, manuscript writing. PPP: Research design, supervision, data analysis, Critical review and editing of the manuscript.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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