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SEASONAL VARIATIONS IN THE REPRODUCTION OF TWOSPOT CATFISH *(Mystus nigriceps)* IN THE CICINGGULING RIVER, KEBUMEN, CENTRAL JAVA

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ABSTRACT

The Cicingguling River in Kebumen Regency is a freshwater ecosystem that serves as a critical habitat for native fish species, including *Mystus nigriceps*, which holds high ecological and economic value. This study aims to examine the reproductive characteristics of *M. nigriceps* over a full annual cycle (November 2022 - October 2023) using a survey method with purposive sampling. Field and laboratory sampling were conducted monthly to evaluate sex ratio, gonadal maturity stage (GMS), gonadosomatic index (GSI), fecundity, and egg diameter. The results showed an unbalanced sex ratio, with the number of female fish far exceeding that of males throughout the year. The GMS III and IV were higher during the rainy season, with GMS III at 295 individuals (female) and 170 individuals (male), and GMS IV at 259 individuals (female) and 144 individuals (male). The GSI and fecundity increased from December to February, coinciding with increased rainfall. The highest GSI and fecundity were recorded in January at 12.98% (females) and 6.63% (males), with fecundity reaching 17,927 eggs, accompanied by high rainfall (403 mm). Egg diameter during the rainy season showed a single peak (0.41–0.50 mm). In conclusion, *M. nigriceps* exhibits a seasonal reproductive pattern, indicating that the development of aquaculture and population dynamics depend on season-based management strategies. These findings provide a biological basis for data-driven conservation and support the sustainable management of freshwater fish resources in Central Java.

Keywords: Cicingguling; Gonad; *M. nigriceps*; Reproduction; Seasonal

INTRODUCTION

The Cicingguling River is one of the rivers that flows through Kebumen Regency, Central Java. The upper reaches of the Cicingguling River originate from Sempor Reservoir, while its lower reaches flow into the Indian Ocean near Suwuk Beach, Kebumen (BPDAS Serayu Opak Progo, 2015). The Cicingguling River is 49 km long, with Sempor Reservoir serving as its primary water source. The water in Sempor Reservoir comes from the Kedungwringin, Sempor, and Mampang Rivers. Traditional fishing activities, such as angling and net fishing, are frequently conducted along the Cicingguling River. One of the fish species inhabiting the Cicingguling River ecosystem is *Mystus nigriceps*.

Twospot catfish, or *M. nigriceps*, is a member of the Bagridae family and is found in abundance in the rivers of Sumatra, Java, and Kalimantan. This fish has not yet received much attention, despite its high ecological and economic value (Ahmadi, 2018). Kebumen Regency is known for using *M. nigriceps* as a food fish, processing it into fish chips and successfully marketing them both within and outside the region. In contrast, its production waste is used as livestock feed. Due to high demand for this fish, its wild population is declining, leading to overfishing. To address this, it is necessary to mitigate fish resource scarcity by understanding the reproductive biology of *M. nigriceps* (Syafrialdi *et al*., 2024).

The sustainability of fish populations and the balance of freshwater ecosystems depend on reproduction. Reproductive studies provide critical information for the management and conservation of economically and ecologically important freshwater fish species. For efficient fisheries management and conservation, reproductive parameters such as sex ratio, gonadal maturity stage (GMS), gonadosomatic index (GSI), fecundity, and egg diameter are important to understand (Nur *et al*., 2017; Olowo *et al*., 2023).

Seasonal variations strongly influence these reproductive parameters through fluctuations in temperature, rainfall, and food availability, which are regulated by seasonal cycles that govern fish spawning and reproductive patterns. As one of Indonesia's important indigenous fish species to conserve, *M. nigriceps* requires an understanding of its reproductive patterns, which are crucial to maintaining its population's sustainability. However, research on seasonal variations in the reproduction of M. nigriceps remains limited, particularly in Indonesian rivers, which exhibit diverse fish habitat dynamics. Additionally, previous studies on the reproduction of *M. nigriceps* in the Kampar Kiri River, Riau (Walidaini & Elvyra, 2022), and the Citarum River, West Java (Makri *et al*., 2020) have primarily focused on specific parameters, such as gonadal maturity. In contrast, this study examines their relationship to seasonal variations.

Seasonal variations, particularly in rainfall and temperature, influence environmental factors such as nutrient availability and water flow in tropical freshwater systems. For many fish species, these factors are closely linked to the reproductive cycle (Adekoya *et al*., 2019). Research on seasonal variations in the reproduction of *M.nigriceps* in the Cicingguling River is essential for determining policies regarding population management, fishing, and domestication. By understanding fish reproductive aspects, appropriate conservation approaches can

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be implemented, particularly for populations classified as Least Concern (LC) and those at risk, to ensure the sustainability of fish resources. The objective of this study is to analyze sex ratio, GMS, GSI, fecundity, and egg diameter of *M. nigriceps* in the Cicingguling River.

RESEARCH METHOD

Research Time and Location

The research was conducted on the Cicingguling River over 1 year, from November 2022 to October 2023. Sampling was conducted 12 times a year (once a month). The method used in this study was a purposive sampling survey. Research stations at the study site were selected purposively based on the three river flow zones: upstream, middle, and downstream. After the station locations were established, fish sampling at each station was conducted randomly, ensuring that every individual fish had an equal chance of being caught and resulting in more representative data. The minimum target catch was set at 15 or more fish per month. The sampling locations are shown in Figure 1, with the coordinate points presented in Table 1.

Table 1. Research Station Coordinates

Zone	Station	Coordinates
Upstream	1	7°34'52.3"LS, 109°29'40.9"BT
	2	7°35'22.5"LS, 109°29'50.0"BT
	3	7°36'34.6"LS, 109°29'19.8"BT
Middle Part	4	7°38'31.7"LS, 109°29'29.9"BT
	5	7°39'31.0"LS, 109°29'28.7"BT
	6	7°40'43.3"LS, 109°29'23.1"BT
Downstream	7	7°41'48.0"LS, 109°29'10.0"BT
	8	7°42'42.4"LS, 109°28'56.9"BT
	9	7°43'02.8"LS, 109°28'56.2"BT

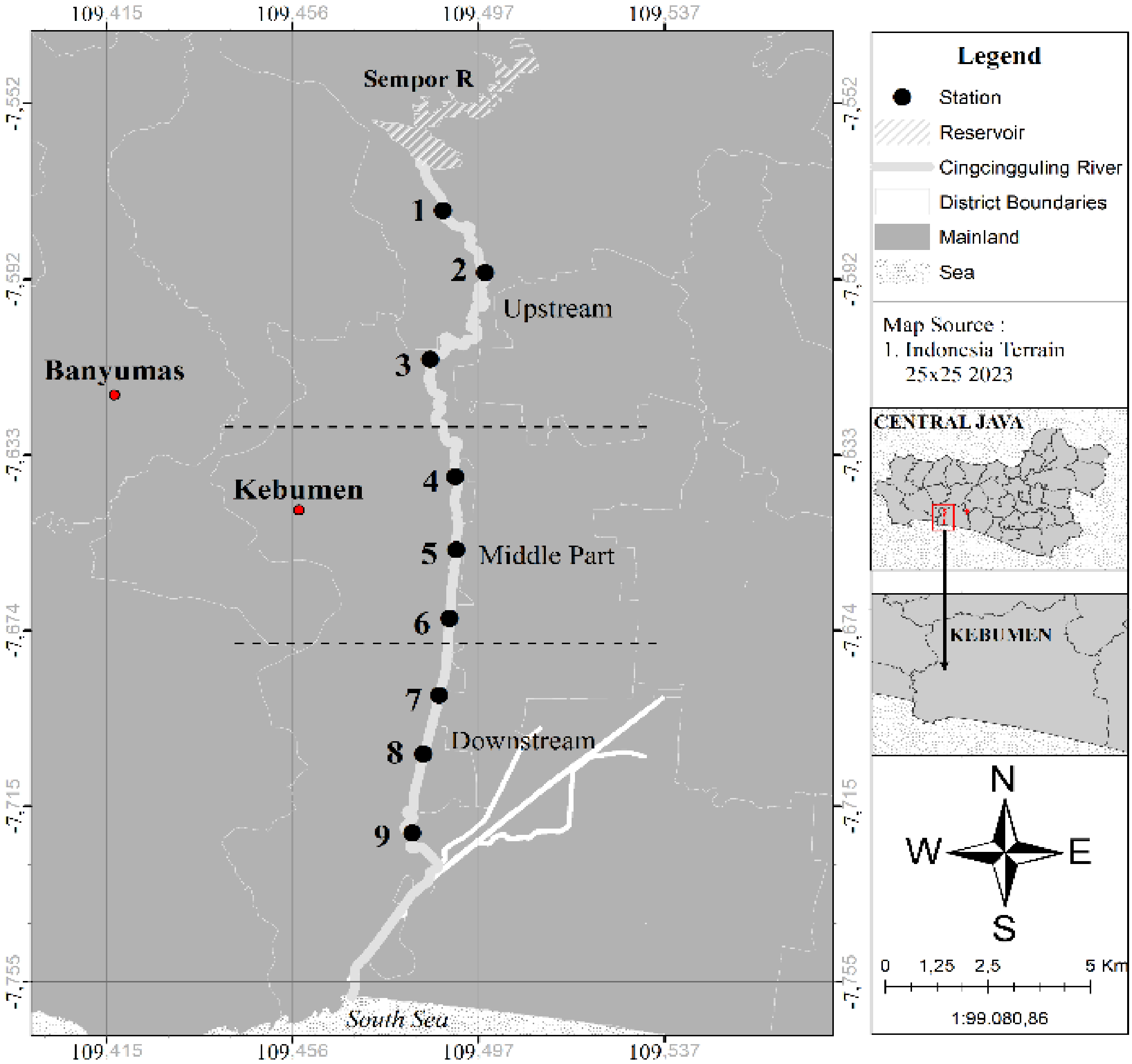

Figure 1. Map of The Study Area in the Cicingguling River, Kebumen (Source: Digitized RBI)

Equipment and Research Materials

The equipment used included a boat, cast nets and gillnets (mesh sizes ½ and 1"), vertical and horizontal traps, tweezers, an ice box, trays, laminating blocks, a camera, digital scales (0.1 g and 0.01 g), surgical instruments, a basin, a lamp, a microscope equipped with a micrometer, microscope slides,

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and a counter. The materials used in this study were *Mystus nigriceps*, Gilson's solution (to release ovarian connective tissue), 4% and 10% formalin, 70% alcohol (for preserving fish), ice cubes, tissues, and latex gloves.

Measurement of Length, Weight, and Dissection

The data used consists of primary data on *M. nigriceps*. Fish samples were rapidly euthanized in ice water; the fish were then measured for length and weighed, and then dissected. Dissection began at the anus along the medioventral line of the body toward the front until near the pectoral fin, and the upper muscle flap was opened with forceps. Dissection continued from the anus toward the dorsal portion of the body.

Gonadal Observation and Determination of Gonadal Maturity Stage.

The gonads were visually examined for color and shape, then compared with the gonadal maturity stage table (Sjafei *et al*., 2008), and the gonadal maturity stage of *M. nigriceps* was determined.

Determination of GSI and Fecundity

The fish gonads were removed using forceps and then weighed. Next, approximately 10% of the total gonad weight was sampled from three sections—anterior, middle, and posterior—to be weighed, and the number of eggs was counted; this value represents the partial egg count.

Measurement of Egg Diameter

Some of the egg samples were placed in sample bottles, and Gilson's solution was added until the gonads were submerged. Before measuring the egg diameter, the egg samples were gently shaken until all eggs were separated from the ovarian tissue. After that, the egg diameter was measured using a microscope equipped with a micrometer. The egg diameter measurements included both vertical and horizontal dimensions, and the results obtained were averaged.

Seasonal Determination on the Cicingguling River, Kebumen, Central Java

Sampling was conducted over one year, from November 2022 to October 2023. According to BMKG (2023), the seasonal pattern in the Kebumen region indicates that March through August is the dry season (peaking in August), while October through February is the rainy season. This seasonal timeline serves as the basis for analyzing variations in the reproductive patterns of *M. nigriceps*.

Data Analysis

The analysis of *M. nigriceps* reproductive data based on this study includes sex ratio, gonadal maturity stage (GMS), gonadosomatic index (GSI), fecundity, and egg diameter.

Sex Ratio

The collected *M. nigriceps* specimens were counted to determine the number of male and female individuals. The results were then analyzed using a Chi-Square test based on the formula by Sokal & Jamesrohlf (1987).

$$\chi^2 = \sum_{i=1}^{n} \frac{oi-ei}{ei} \quad \text{.......(1)}$$

where: χ^2 = Chi-Square; ei = Expected frequencies of male and female fish in cell 1; oi = The frequency of male or female fish observed.

Gonadal Maturity Stage (GMS)

The identification of gonadal maturity stages in *M. nigriceps* is based on the reference (Sjafei *et al*., 2008), specifically the gonadal maturity stages of fish in the Order: Siluriformes, Species: *M. montanus*, as shown in Table 2.

Table 2. Gonad Maturity Stage (GMS) Criteria

Stages	Testes	Ovaries
Pre-Spawning I (Immature)	The testicles are very smooth, colorless, elongated, and thread-like; the left one is slightly longer than the right.	The right and left ovaries are roughly the same length and size; the eggs are colorless to whitish, very small, and distinguishable only under a microscope.
Pre-Spawning II (Maturing Development)	The testicles are reddish or creamy white. The left one is slightly longer than the right.	The ovaries are much larger; white or yellowish-white eggs in the process of maturing are visible under the microscope through the wall, and the left ovary is longer than the right.
Pre-spawning III (Mature)	The testes in stages II and III cannot be clearly distinguished; the two stages are more or less similar.	The ovary is yellowish-white; shorter than a fully mature one; differing from a fully mature one in color (fairly yellow in the case of a fully mature ovary).
Spawning IV (Ripe)	The testicles are elongated, swollen, and dark red to yellowish or creamy white, with red spots.	The ovaries are yellowish to yellowish-white; they are visible through the translucent abdominal wall; the genital organs turn dark red.
Post-spawning V (Spent/ Resting)	The testicles are white; red spots are still visible; the left testicle is longer than the right; the testicles appear flat dorsoventrally.	The ovary is slightly shorter than when fully mature; several immature oocytes and some mature oocytes remain in the nearly empty sac; the sac is transparent, and the yolk is visible from the outside.

Gonadosomatic Index (GSI)

The gonadosomatic index (GSI) is calculated using the equation from Devlaming *et al*. (1982).

$$\text{GSI} = \frac{\text{gonadal weight}}{\text{body weight}} \times 100\% \quad \text{.......(2)}$$

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Fecundity

Fecundity was calculated using the equation from Holden & Rait (1975).

$$F = \frac{G \times X}{Q} \quad (3)$$

where: F = fecundity (eggs); G = total gonad weight (g); X = number of eggs in a portion (eggs); and Q = weight of a portion of the gonad (g).

Egg Diameter

Egg diameter is calculated using the equation from Bonisławska *et al*. (2001).

$$ED = \left(\frac{D_V + D_H}{2}\right) \times C \quad (4)$$

where: ED = egg diameter (mm); DV = vertical diameter of the egg (mm); DH = horizontal diameter of the egg (mm); and C = calibration factor.

Statistical Analysis

The statistical analysis used in this study was Pearson's correlation analysis to establish a relationship between rainfall and the gonadosomatic index (GSI).

RESULTS AND DISCUSSION

Sex Ratio

The number of female and male *M. nigriceps* collected over 12 months is presented in Table 2. Table 2 shows that more female *M. nigriceps* were collected each month than males. Therefore, a Chi-square test was conducted to determine whether there was a significant difference in the sex ratio of *M. nigriceps* in the Cicingguling River. The results of the Chi-square test are presented in Table 3.

The results of the Chi-Square test indicate that there is a highly significant difference between the number of male and female fish in the Cicingguling River. The unbalanced sex ratio (1:1) indicates that female fish far outnumber male fish throughout the year. This study assumes that the use of fishing gear does not affect the sex ratio of *M. nigriceps* in the Cicingguling River because the same fishing gear was used throughout the study: cast nets (mesh sizes ½ and 1"), vertical and horizontal traps in the upstream section, and cast nets and gillnets (mesh sizes ½ and 1") in the middle and downstream zones. The dominance of females in the Cicingguling River is due to their spawning migration, which often occurs in large groups in specific areas (such as the middle or lower zones) to seek optimal spawning sites or environments with abundant food.

In addition, the dominance of females in a fish population within a habitat can be influenced by various biological or environmental factors, such as migration patterns, selection of fishing gear, or habitat conditions that favor one sex over the other. This was observed in the species *Hemibagrus nemurus* in the Upang River, Bangka Belitung, where a higher female-to-male sex ratio was found due to the influence of the behavioral patterns of the fish population, where individuals of the same sex tend to school together, thereby affecting catch results (Wulandari *et al*., 2020). Fish behavioral factors can also influence the sex ratio. This was observed in the species *M. nigriceps* in the Batang River, South Kalimantan, where a higher proportion of females was recorded compared to males because female *M. nigriceps* generally have larger body sizes than males and move more slowly in the water, making them easier for fishermen to catch (Ahmadi, 2018).

Table 2. Number of Female and Male *M. nigriceps* Over 12 Months

Month	Female	Male
November	30	13
December	33	7
January	15	1
February	75	16
March	76	18
April	203	43
May	118	45
June	249	55
July	180	46
August	173	21
September	420	29
October	307	60

Table 3. Chi-Square Test Results for Sex Ratio

Gender	Observation (O)	Expected (E)	(O−E)²/E
Female	1,879	1,116.5	520.74
Male	354	1,116.5	520.74
Total	2233	2233	1,041.48

$\chi^2_{calculate} = 1{,}041.48$.
Degrees of freedom (*df*) = k – 1 = 2 – 1 = 1.
$\alpha = 0.05$, so we obtain $\chi^2_{table} = 3.841$.
$\chi^2_{calculate} = 1{,}041.48 > \chi^2_{table} = 3.841$, so H0 is rejected

Gonad Maturity Stage (GMS)

Observations of the gonadal maturity stage (GMS) of *M. nigriceps* in the Cicingguling River showed variations from month to month and across both seasons. The results of the observations are presented in Figure 2.

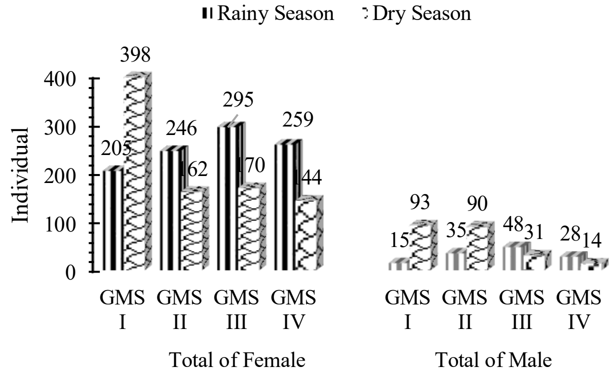

Figure 2. Gonad Maturity Stages

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Gonadal maturity stages (GMS) I–IV vary between male and female fish. In female fish, GMM I is highest during the dry season and GMS II–IV during the rainy season; in male fish, GMS I and II are highest during the dry season, and GMS III and IV are highest during the rainy season. The highest gonadal maturity stage (GMS) III and IV in both female and male fish were observed during the rainy season, indicating that spawning occurs at this time. This is also consistent with findings in the species *M. nigriceps* in the Kampar River, Riau, where the highest GMS III and IV levels occur during the transition to the rainy season (Syafrialdi *et al*., 2024), and in *M. tengara* in the Ganges River, India, where the highest gonadal maturity was observed in June the early peak of the rainy season (Mitu, 2017) due to environmental conditions that support the reproductive process. Increased river flow from rainfall expands the habitat and provides safe, nutrient-rich spawning sites for larvae. Additionally, more suitable water temperatures and increased dissolved oxygen levels during the rainy season stimulate hormonal activity that triggers vitellogenesis and oocyte maturation (Mitu, 2017; Syafrialdi et al., 2024; Vo et al., 2023).

Gonadosomatic Index (GSI)

The gonadosomatic index of *M. nigriceps* in the Ijo River varies with season. This is based on rainfall data from November 2022 to October 2023, as shown in Figure 3, and on gonadosomatic index (GSI) calculations for female and *male M. nigriceps* in the river, as presented in Figure 4.

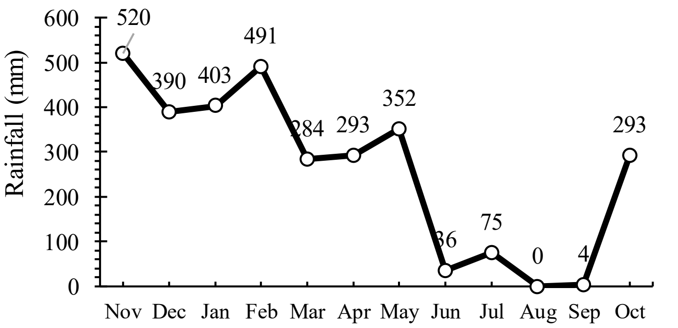

Figure 3. Rainfall in Kebumen Regency, Central Java

The gonadosomatic index (GSI) of *M. nigriceps* in the Cicingguling River indicates that the GSI of female fish is higher than that of male fish. The highest GSI values were recorded at the end of the rainy season, in January, at 12.98% for female fish and 6.63% for male fish. The highest recorded gonadosomatic index (GSI) values indicate that both female and male fish are approaching reproductive maturity. This aligns with the BMKG (2023) rainfall graph (Figure 3), which shows an increase in rainfall from December through February. River catfish spawn during the rainy season, suggesting that increased rainfall can trigger gonadal maturation. These conditions result in abundant food availability, allowing the fish to allocate significant energy toward gonadal development (Vo *et al*., 2023).

The same pattern was observed in *M. cavasius* in the Brahmaputra River, India, where the highest gonadosomatic index (GSI) was recorded in June, as this month coincides with the rainy season and the phase leading up to the peak spawning season (Rajbongshi *et al*., 2025), and for the species *M. gulio* in the Bengal River, India, the highest GSI was recorded in female fish in August, coinciding with the monsoon season (Chakraborty *et al*., 2024). An increase in GSI reflects intensive gonadal growth and maturation in preparation for reproduction. Seasonal factors strongly influence this phenomenon; specifically, June marks the onset of the rainy season in Assam, India, which triggers environmental conditions such as increased river flow and the availability of natural food two factors that ecologically support fish reproductive activity. Thus, seasonal patterns play a crucial role in determining the annual reproductive cycle (Chakraborty *et al*., 2024; Rajbongshi *et al*., 2025).

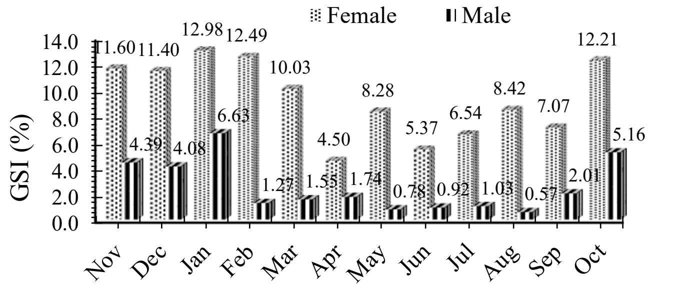

Figure 4. GSI of *M. nigriceps* in the Cicingguling River

Fecundity

Fecundity data for *M. nigriceps* over 1 year show results that are relatively consistent with the gonadosomatic index (GSI) shown in Figure 5. The fecundity of *M. nigriceps* in the Cicingguling River ranged from 5,425 to 17,927 eggs. The highest fecundity, coinciding with the peak GSI, was recorded at the start of the rainy season in January, amounting to 17,927 eggs. To estimate fish fecundity, various factors must be considered, including the peak gonadosomatic index (GSI), spawning season, and fish size (Hasan *et al*., 2020; Hossain *et al*., 2010).

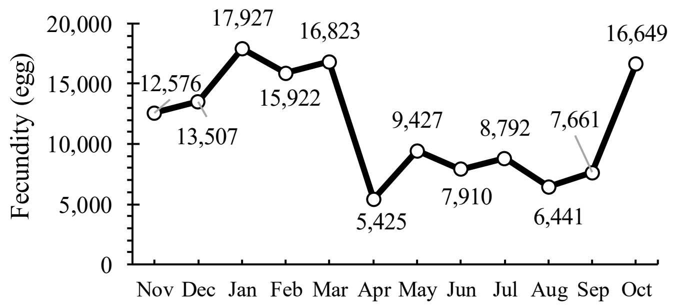

Figure 5. Fecundity of *Mystus nigriceps* in the Cicingguling River

Similar results were obtained for *M. vittatus* in the Ichamati River, India, where the highest fecundity of *M. vittatus* was recorded at the start of the monsoon season, specifically in June in Kolkata, India (Basu, 2015). The same was found in the species *M. bleekeri* in the Surma River, Bangladesh, where the highest fecundity was recorded at the start of the monsoon season, specifically in June, at 14,790 eggs (Sultana *et al*., 2022). Increased rainfall during this season serves as a strong environmental signal that regulates the gonadal cycle and stimulates spawning in various Mystus species. Better water flow conditions, more spawning substrates, and greater food availability during the rainy season encourage fish to spawn, leading to higher egg numbers and greater gonadal maturity (Basu, 2015; Sultana et al., 2022).

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Egg Diameter

The results of egg diameter measurements for *M. nigriceps* in the Cicingguling River are presented in Figure 6. The range of egg diameters for *M. nigriceps* in the Cicingguling River was 0.1-0.61 mm. The distribution pattern of egg diameters varied by season. The rainy season shows a unimodal pattern of egg diameter at 0.41-0.50 mm, with 8,294 eggs, and the highest number occurring in January. The dry season exhibits an early-stage asynchronous pattern, meaning egg development within the ovary is uneven, with small eggs coexisting alongside larger ones. This indicates that the early-stage asynchronous development of *M. nigriceps* eggs in the Cicingguling River during the dry season will shift toward full synchrony, as all eggs are stimulated to mature simultaneously by environmental conditions when rainfall is suitable for egg development. The diameter of *M. nigriceps* eggs ready to spawn ranges from 0.40-0.75 mm, indicating that *M. nigriceps* spawns once during the rainy season or is a total spawner (Sari *et al*., 2016; Walidaini & Elvyra, 2022).

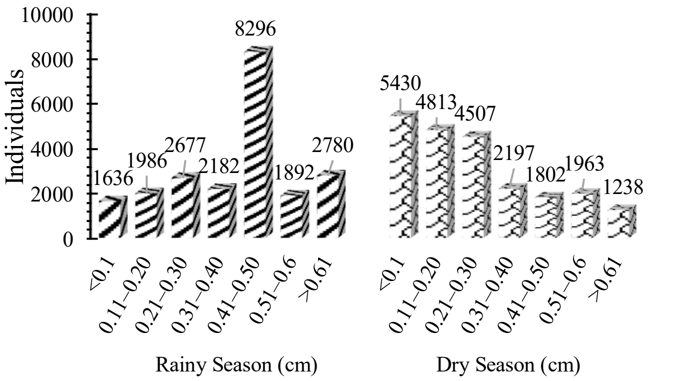

Figure 6. Egg Diameter of *M. nigriceps* in the Cicingguling River

Similar results were also obtained for the species *M. mysticetus* in the Mekong River, Vietnam, where the highest peak in egg diameter occurred during the rainy season, ranging from 0.63-0.64 mm (Vo *et al*., 2023), and for *M. tengara* in the Ganges River, India, the egg diameter distribution reached a single peak during the rainy season, ranging from 0.60-0.85 mm (Mitu, 2017). The increase in egg diameter during the peak of the rainy season indicates that spawning occurs during this period. This is attributed to higher water levels, improved flow velocity, and a broader river habitat resulting from rainfall, which creates favorable conditions for gonadal maturation, reproductive behavior, and the survival of eggs and larvae (Alam *et al*., 2018; Mitu, 2017; Vo *et al*., 2023).

CONCLUSION

This study concludes that *M. nigriceps* in the Cicingguling River exhibits a sex ratio imbalance, with females significantly outnumbering males over 12 months. Additionally, *M. nigriceps* exhibits a reproductive pattern influenced by the season, particularly the rainy season, which triggers gonadal maturation (TKG and GSI), fecundity, and egg diameter. Females reach gonadal maturity faster than males. The peak of reproductive activity occurs during the rainy season, marked by high GSI values, fecundity, and egg diameter, indicating total spawning (total spawner). Environmental factors, such as increased water flow, optimal temperatures, and nutrient availability during the rainy season, play a crucial role in supporting the annual reproductive cycle of this species.

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