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Reproductive Biology of One-Stripe Spiny Eel, *Macrognathus aral* (Bloch and Schneider, 1801)

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ABSTRACT

A comprehensive analysis of the reproductive biology of Macrognathus aral is presented in this work. The study was conducted on 256 M. aral specimens, consisting of 134 males and 122 females, obtained from six distinct locations along the Kangsabati river. Comparative analysis of the reproductive biology of the one-stripe freshwater spiny eel was conducted, focussing on sexual dimorphism, sex ratio, length at first sexual maturity, gonadal maturation, gonadosomatic index, and fecundity. During the breeding season, both sexes show sexual differentiation in body color; males have dorsally brown and ventrally whitish-yellow, and females have dorsally yellowish-brown and ventrally yellowish body colors, allowing sexual dimorphism. An analysis using the Chi-square test (ϱ2) confirmed that the male-to-female sex ratio was 1:0.72 ($p < 0.05$). Approximately half of males and females reached their initial sexual maturity at body lengths ranging from 17.5 to 19.4 cm and 19.2 to 22.4 cm, respectively. In July, the mean gonadosomatic index values for males and females were 1.95 ± 0.19 and 14.51 ± 0.81, respectively. July featured the greatest abundance of stage IV gonads, indicating the onset of spawning. The absolute fecundity varied between 3312 ± 34.23 to 12321 ± 299.85, the mean value being 6822.56. Fecundity exhibited a substantial and statistically significant association value of 0.9297 with total length, 0.8539 with body weight, and 0.9640 with gonad weight ($p < 0.05$). This research aims to enhance comprehension of species population dynamics and conservation mechanisms. Investigations into its reproductive behaviors can unveil its life cycle, patterns of movement, and preferences for specific habitats. Fisheries management can use this knowledge to enhance breeding practices, make informed decisions on catch limits, and implement conservation interventions.

Keywords:
Fecundity; GSI; Macrognathus aral; sex ratio; sexual dimorphism.

1. Introduction

India is one of the world's mega-biodiverse countries, boasting a rich variety of wildlife. The nation hosts a diverse fish population totaling about 2,500 species. Among these, 930 species have habitats in freshwater, while 1,570 are from marine environments (Kar, 2003). Within the biodiversity, India proudly showcases around 374 indigenous freshwater ornamental fish species and approximately 700 indigenous marine ornamental fishes (Raja et al., 2019). The majority of fish production and capture find their way to local markets across India (Bhanja et al., 2023). The one-stripe spiny eel, *Macrognathus aral* (Bloch and J.G. Schneider, 1801) is one of the important fish species under the family Mastacembelidae, mainly inhabit freshwater and brackish water, both water bodies can be running and stagnant (Talwar and

Jhingran, 1991). They prefer to live in slow- moving streams and shallow waters of plains and estuaries and are also found in canals, beels (seasonal large water-logged area), ponds, and inundated fields (Abujam and Biswas, 2011; Dutta and Banerjee, 2014). This species is widely distributed to India and other nations, including Nepal, Myanmar, Bangladesh, Pakistan, and Sri Lanka (Talwar and Jhingran, 1991). In the international market, *M. aral* is popularly known as 'peacock eel' and is in high demand for excellent ornamental value. According to the 'Conservation Assessment and Management Plan' (CAMP) report (1998), *M. aral* is included under the 'Lower Risk Near Threatened' (LRNT) category in India. The IUCN has considered the species to be of "Least Concern" (Vishwanath, 2010).

In most organisms, basic survival behaviors are adapted to biological rhythms through daily, monthly, or annual physiological changes (Ripperger, 2007). The marked reproductive seasonality in fish and most vertebrates ensures that reproduction and offspring development are coordinated with optimal environmental and nutritional conditions. The reproductive biology of fish is essential for assessing the stock's commercial potential, life history, cultural practices, and fisheries management (Schaefer, 1998). Also, reproductive biology is a prerequisite for evaluating fish's conservation and management possibilities (Jakobsen et al., 2009; Hliwa et al., 2017; Cardoso et al., 2019). Studies on the reproductive biology of fish include sex ratio, stages of gonadal development, duration of the breeding season, GSI (Gonado Somatic Index), and measurement of the reproductive capacity (Maurua et al., 2003; De Carvalho et al., 2009; Fontoura et al., 2009; Cardoso et al., 2019). Evaluating the annual breeding cycle of the cultivable fish is very important for the success of fish culture. Thus, it is possible to know the peak breeding season of the fish. GSI has been considered an indicator of fish reproductive activity, which has been used to determine the fish breeding season (Sley et al., 2015; Almukhtar et al., 2016). Studies on fecundity have the potential to gain knowledge not only about the potential stock of a species but also about fisheries management, cultural practices, and the species' life cycle (Lagler, 1956; Doha and Hye, 1970).

Chakraborty and Goswami (2016b), Dutta and Banerjee (2016a), Borah et al. (2017), Deka and Barman (2020), Das et al. (2023) studied the length-weight relationship and also the condition factors of *M. aral*. Abujam et al. (2013) and Dutta and Banerjee (2014) worked on the food and feeding habits of *M. aral.* Very minute work has been done on the reproductive biology of *Macrognathus* species. However, there are no reports on the reproductive biology of *M. aral* in West Bengal. The sexual dimorphism of *M. pancalus* was studied by Swarup et al. (1972). Karim and Hossain (1972) studied the sexual maturity and fecundity of *M. pancalus*. Sharma and Lavanya (2002) have worked on the biology of *M. aral* from the Krishna irrigation system. Faridi et al. (2020) worked on the reproductive biology of the *M. aculeatus* from the river Ganga, Uttar Pradesh. Abujam and Biswas (2011), Dutta and Banerjee (2016, b), and Chakraborty and Goswami (2016a) studied the reproductive biology of *M. aral*. Pathak et al. (2012) studied the reproductive traits of *M. pancalus* from the both lentic and lotic ecosystems of the Gangetic basin. Therefore, the present study focused on reproductive biology and the sex ratio, length at first sexual maturity, GSI, and fecundity of the *M. aral* from Kangsabati River, West Midnapore.

2. Materials and Methods

2.1 Study area and period

Six different sampling sites of the Kangsabati River provided sources of *M. aral* specimen sampling every month between May 2021 to April 2022. The six sampling sites along the Kangsabati River lie between 22°29'18.1"N 87°33'19.2"E and 22°28'56.6"N 87°05'28.4"E. Fish species were captured through a combination of cast net, bag net, gill net, and manual handpicking methods, with the assistance of local fishermen at the six designated sampling sites of Kangsabati River (Table 1).

Table 1. *M. aral* sampling sites of Kangsabati River

Study Site	Area Name	Latitude and longitude of the sampling site
Site-I	Shal dahari	22°29'18.1"N 87°33'19.2"E
Site-II	Gopinathpur	22°26'06.9"N 87°31'00.8"E
Site-III	Pathra	22°24'22.5"N 87°25'06.5"E
Site-IV	Harishpur	22°24'04.2"N 87°21'47.3"E
Site-V	Kankabati	22°24'58.9"N 87°14'54.9"E
Site-VI	Dherua	22°28'56.6"N 87°05'28.4"E

2.2 Sampling

A total of 256 *M. aral* specimens were collected, of which 134 were males and 122 were females. Out of 122 female species, 23 species were examined separately for the study of fecundity. At first, the specimens were separated by observing the sexual dimorphism characters.

2.3 Morphological measurements

Then, their total lengths (in centimeters) were measured by using a digital vernier caliper (nearest to 0.01 cm), and total weights (in grams) were measured by using the MH- 200 series scale (200gm/ 0.01gm). Male and female fish were distinguished by examining morphological characters and internal anatomy study. The maturation stages of *M. aral* were assessed by examining both gonadal morphology and histology.

2.4 Data analysis

Following Fisher's (1970) approach, the Chi-square test ($\chi 2$) was used to evaluate the significance of the comparison in the sex ratio of the species. Length at the first sexual maturity of the species ($Lm50$ or Length at the first sexual maturity) was calculated, following Hossain et al. (2010). The gonado somatic index (GSI) of both sexes was calculated by using the following method developed by June (1953), GSI = 100 × Weight of the gonad (g)/ Weight of the body. The gravimetric method, one of the important methods described by Lagler (1956) for fish fecundity, is the most effective and provides reasonably precise results. Mustafa et al. (1980) and Blay (1981) also applied this technique. About 20 specimens were painstakingly removed during the breeding season. After washing and weighing, the ovaries were preserved in a 4% formalin concentration. Three sub-samples of each ovary of known weight were weighed. After that, a drop of distilled water was added to each sub-sample placed in the Petri dish to count the number of eggs. The fecundity was obtained by the following formula: $F1$ =

(number of eggs in the sub-sample × weight of gonad)/weight of the sub-sample of gonad. Then, the average number of eggs obtained from the three sub-samples (F1, F2, and F3) was added, and also the following equation was used to calculate each bird's fecundity, i.e., Fecundity (F) =(F1+F2+F3)/3. Relative fecundity = absolute fecundity/ body weight (g).

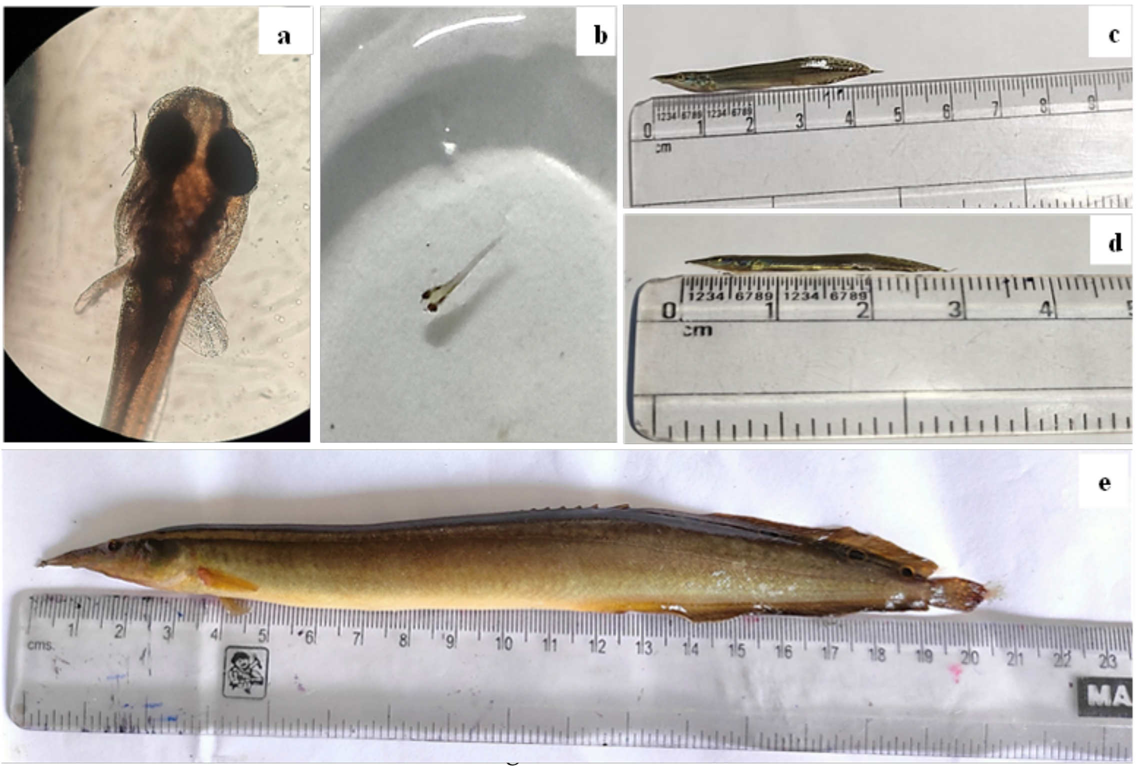
2.5 Statistical analysis

Microsoft Excel 2019 tool pack was utilized for the statistical analysis of the witnessed data and the data analysis itself.

3. Results and Discussion

3.1 Sexual dimorphism

In general, identifying the sexual dimorphism of *M. aral* based on outward characteristics is challenging. However, during the breeding season, the sexes become externally distinguishable, primarily attributed to variations in body color. The body color of the male species is dorsally brown and ventrally pale yellow, whereas the female species is dorsally brownish-yellow and ventrally yellow. (Plate 1)



3.2 Sex ratio

A total number of 233 *M. aral* specimens in lotic environments were able to be sexed, of which 134 were males and 99 were females. Male and female species of *M. aral* represented 58.20% and 41.78%, respectively, of the total number (Table 2). The sex ratio (*M: F*) was 1: 0.72 ($p<0.05$, verified by the Chi-square test).

Table 2. Monthly sex ratio variation of *M. aral* collected from Kangsabati River

Month	Sample Size	Male (Observed value)		Female (Observed value)		Sex Ratio	
		Number	Percentage	Number	Percentage	Male	Female
January	13	08	61.53	05	38.46	1	0.62
February	19	11	57.89	08	42.10	1	0.72
March	23	12	52.17	11	47.82	1	0.91
April	27	15	55.55	12	44.44	1	0.80
May	21	12	57.14	09	42.85	1	0.75
June	23	13	56.52	10	43.47	1	0.76
July	20	11	55.00	09	45.00	1	0.81
August	23	12	52.17	11	47.82	1	0.91
September	13	08	61.53	05	38.46	1	0.62
October	20	12	60.00	08	40.00	1	0.66
November	17	11	64.70	06	35.29	1	0.54
December	14	09	64.28	05	35.71	1	0.55
Total	233	134		99			

3.3 Length at first sexual maturity

Fifty percent of male and female *M. aral* specimens reached their first stage of maturity in the length range of 17.5-19.4 cm and 19.5-22.4 cm, respectively.

3.4 Maturation stages of male and female species:

3.4.1. Stage I (immature)

Testicles are narrow, thread-like, whitish colored, and translucent. Small and thin ovaries are observed. Ovaries are reddish-pinkish colored and thread-like semi-transparent (Plate 2a, Plate 3a).

3.4.2. Stage II (maturing and recovering spent)

Testes are slightly elongated and indistinct vasa differentia. Ovaries are elongated, pink in color with small ova, and visible to the bared eyes.

3.4.3. Stage III (ripening)

Testes are swollen, and creamy white and vasa differentia are distinct. Ovaries contain the ova. Ova is visible to the naked eye. Yellow color ovaries contain approximately 2/3rd of the gut (Plate 2b, Plate 3b).

3.4.4. Stage IV (ripe)

The testicles are large and creamy white in color, and white milt discharges at gentle pressure. Ovaries are bright green, occupying about the 3/4th of the abdomen (Plate 2c, Plate 3c).

3.4.5. Stage V (spent)

Testes are shrunken, the weight of the testis decreases, and the milt is not removed with gentle pressure. Ovaries are flabby and shrunken with some residual undeveloped ova (Plate 2d, Plate 3d).

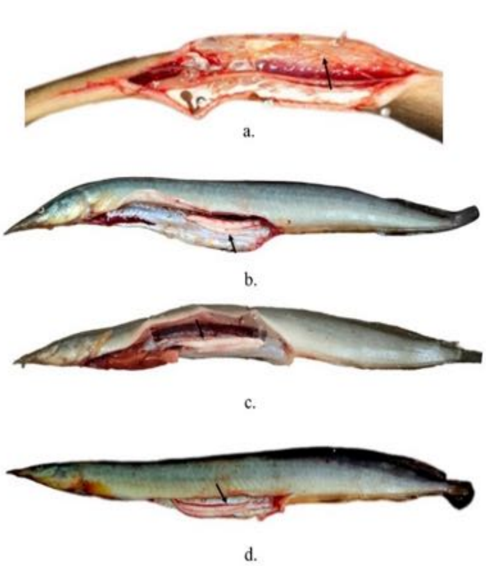


Plate 2. Different maturation stages of gonads of male *M. aral*: a. Immature stage, b. Ripening stage, c. Ripe stage, d. Spent stage.

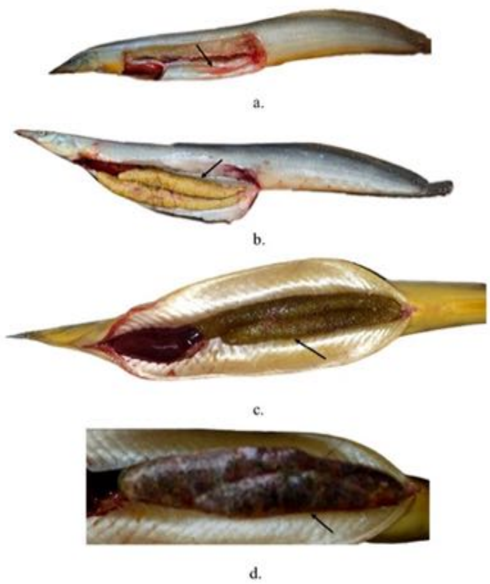


Plate 3. Different maturation stages of gonads of female *M. aral*: a. Immature stage, b. Ripening stage, c. Ripe stage, d. Spent stage.

3.5. Gonado-somatic index of the species

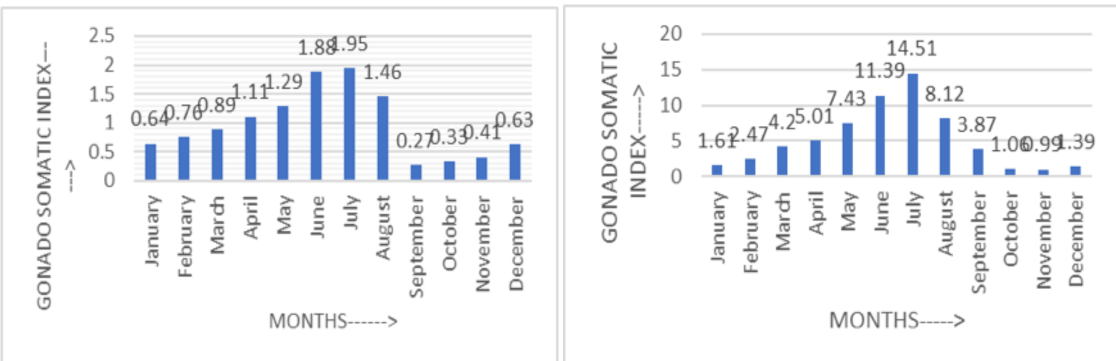


Fig 1. Graphical representation of monthly GSI variation of *M. aral* species (a= Male, b= Female)

In *M. aral*, the cycle of gonad maturation and depletion occurs only once a year and is synchronous in both sexes. The present study observed that both males and females of the *M. aral* species have higher GSI values from June to August, indicating that the fish has only one spawning season. The male species' peak value of the GSI is highest during July, which was 1.95 ± 0.19, while the value is lowest during September, which was 0.27 ± 0.01. Similarly, the peak value of the GSI for the female species is highest during July, 14.51 ± 0.81, and lowest during November, which was 0.99 ± 0.06 (Fig 1).

3.6. Fecundity

The fecundity of 23 different species of *M. aral* was assessed using the sample and direct counting method. During the study period from April to August, female species of *M. aral* with different body weights between 32.68 gm to 152.00 gm and lengths between 20.5 cm to 31.9 cm had a mean absolute fecundity that varied from 3312.31 ± 34.23 to 12321.53 ± 299.85, with an average value of 6822.56, while the relative fecundity ranged from 80.55 to 145.84, with an average value of 111.36 ± 19.23 of the same samples. The correlation coefficient (r) was highly significant ($p < 0.05$) for *M. aral* between fecundity-total length (0.9297), fecundity-body weight (0.8539), and fecundity-over weight (0.9640), represented in Table 5. It has been found that there is a linear relationship between fecundity and each of these traits (Fig 2).

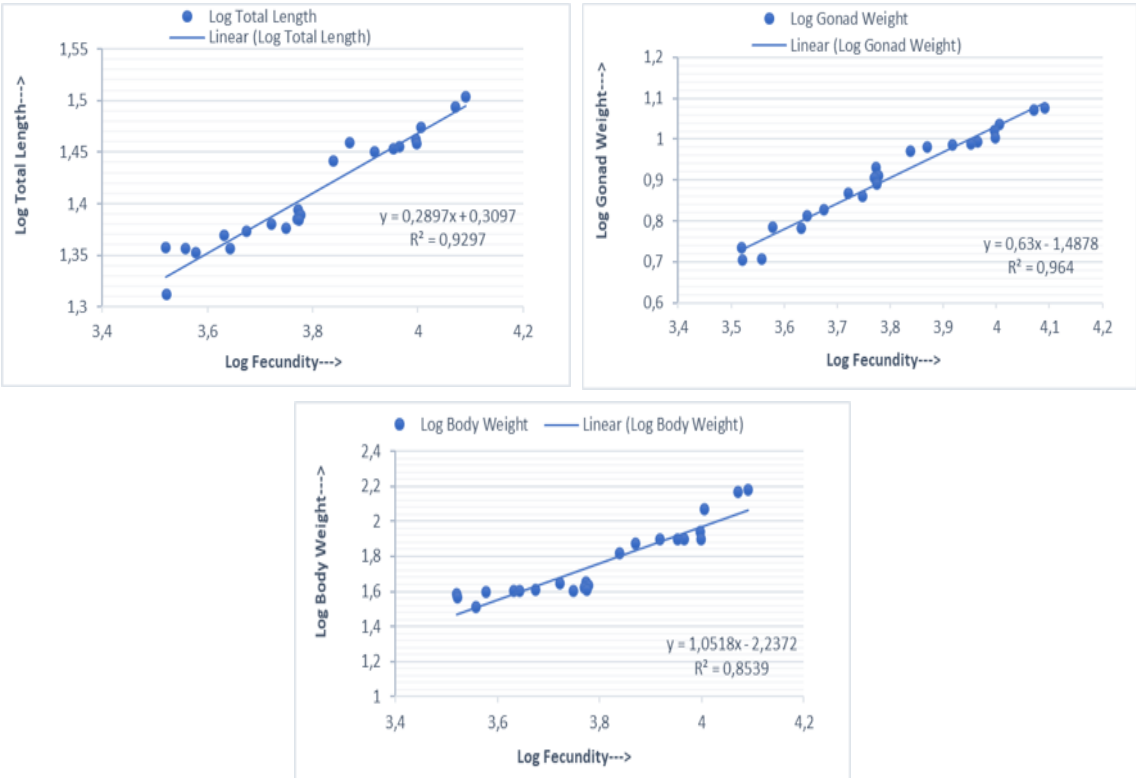


Fig 2. Graphical representation of Mean absolute fecundity relationship of *M. aral* (Log value).

4. Discussion

The sexes of the species are easily differentiated internally by dissection and the examination of the gonads. Ovary color ranged from reddish-pinkish in the immature ovaries to bright green in the mature ovaries. In contrast, the testes' color ranged from white in the immature testes to creamy white in the mature testes. A similar result of sexual dimorphism was reported by Das et al. (2023). In the study period, it was observed that the gonad of this fish is 'bi-lobed unequal', and both the right ovary and testes were somewhat smaller than the left. Mian et al. (2020) observed a similar result in *Channa punctatus*.

Researchers led by Swarup et al. (1972) observed that the sex ratio of bisexual species is nearly 1:1, and this finding was corroborated in a study conducted on *Tor tor*; *Schizothorax plagiostomus* by (Magloo et al., 2024); *Oreochromis niloticus* (Abdalla et al., 2024) and *Cirhinus mrigala* by Pathani (1978) and Jhingran and Khan (1979), respectively. In the present study, the sex ratio (M: F) was 1: 0.72 ($p<0.05$, verified by the Chi-square test), which did not so much variation from the speculative sex proportion of 1:1 and it indicates males are more dominant than the female species of *M. aral*. The maximum sex ratio (M: F) was observed for March and August (1:0.91), while the minimum sex ratio was recorded for November month (1:0.54). Abujam and Biswas (2011) have reported the male dominance of the same species in the population of both lotic and lentic ecosystems of the two districts in Dibrugarh and Tinsukhia from Upper Assam, reasons proposed for the predominance of females in lotic and males in lentic environments in this study may be the segregation of both sexes at different times of the year, differences in size, gear selection associated with sex differences in the morphology and the physiological activity, and differences in the natural or artificial mortality. Variations in different environmental stresses and food availability during critical developmental stages can lead to imbalances in primary sex ratios within certain species (Geffroy & Wedekind, 2020; Abdollahpour et al., 2022). Sharma and Lavanya (2002) reported similar results. Chakraborty and Goswami (2016a) reported an average male-to-female ratio of 1:0.862 for the natural environment of wild stock and a male-to-female ratio of 1:1.25 for the captive environment. Dutta and Banerjee (2016, b) reported that the overall sex ratio in *M. aral* was 1:1.65 (M: F), so females are more dominant ($p< 0.01$) than males. However, some workers reported male dominance over the female fish, such as Rangaswami (1974) in *Mugil cephalus* and Gowda and Shanbhogue (1988) in *Valamugil seheli*, Hoda and Qureshi (1989) in *Liza klunzingeri*. On the other hand, female dominance was also reported by several workers such as Suresh et al. (2006) in *Macrognathus pancalus*, Rahman et al. (2012) in *Macrognathus aculeatus*, Qayyum and Quasim (1964) in *Ophiocephalus punctatus*, Effendi and Sjapal (1976) in *Mugil dussumieri*, Fatima and Khan (1993) in *Rhinomugil corsula*, Cardoso et al. (2019) in *Prochilodus lacustris,* Mandal and Mandal (2022) in *Lepidocephalichthys guntea*, Ribolli et al. (2023) in *Pimelodus maculatus.*

Sharma and Lavanya (2002) have reported the early maturation of males at the length of 16 cm and females at 17.5 cm for *M. aral*, while Abujam and Biswas (2011) stated that 12.05 cm in males and 16.05 cm in females for the same species. Dutta and Banerjee (2016b) and Chakraborty and Goswami (2016a) also get similar results. Abujam and Biswas (2011) reported that higher GSI values for males and females were observed in Upper Assam from February to August (peak in May) and March to September (peak in August), respectively. Changes in hydrology, climate, the availability of food, the health of the fish, etc, may bring on this geographic difference in breeding frequency. The study found that June, July, and August had higher GSI values than the other months. So, based on these results, it can be clearly said that the three months of June to August were the species' breeding season. From the current study, it is also observed that the male species of *M. aral* mature earlier than a similar- length female species. In this research work, most stage IV (ripe) gonads were recorded from June to August, spawning peaking during the core monsoon. An increase in the GSI value indicates progress in maturity. High GSI values in both sexes of the species during the June-August period indicated full development of the gonads. Recovering stage II, gonads remained dormant from October to January as temperature and photoperiod remained minimal. In February-March, the development of the gonads intensified with rising temperatures. The maximum development of the gonads was recorded in July when temperature and photoperiod reached their maximum.

Assessing the fecundity of fish is a prerequisite for successful breeding programs, as a species' fecundity indicates a species' reproductive potential. Bagenal & Braum (1978) and Mignien & Stoll (2024) found that the fecundity of fish species was characteristically different between individuals of the same size and age. Fagade et al. (1984) and Lombo et al. (2024) proposed that the variability in fertility might be due to different amounts of food. So, the one stripe spiny eel a moderately fecund. The fecundity of the species was highest during April to August. During the study period, the fecundity of the species was not seen from October to February. Abujam and Biswas (2011) studied from Upper Assam and have reported that the fecundity of *M. aral* ranges from 250 (21.6cm / 27.4gm) to 5,220.1 (27.2cm / 66.3gm) while relative fecundity ranges from 39.63±18.61 to 88.76±28.31, and on the other hand, from the lower Krishna irrigation ponds, Sharma and Lavanya (2002) have documented that the fecundity of *M. aral* ranges between 1,065 to 4,961 from the ponds in lower Krishna irrigation system. Also, the fecundity range of 622.49 ± 226.96 to 4511.73 ± 498.59 has been reported by Dutta and Banerjee (2016b). According to Narejo et al. (2002), spiny eels' overall length and body weight were the key determinants of their fertility. Dutta and Banerjee (2016b) stated that the correlation coefficient between fecundity-TL, BW, and GW were 0.49, 0.45, and 0.95, respectively ($p < 0.01$).

5. Conclusion

To better understand the biological mechanisms that could be in charge of preserving the underlying stock structure and to ensure adequate conservation and management of *M. aral* in its native environment, the study provides vital information on the reproductive pattern of this species. The findings from the present study on the reproductive biology of *M. aral* have revealed that males are more dominant than the female species and mature earlier than females. The gonad of this fish is 'bi-lobed unequal'. June to August was the species' breeding season, so it can be clearly said that the species breeds only once a year. The total length, body weight, and gonad weight of the fish in *M. aral* were significantly correlated with fecundity. This study will facilitate comprehension of fish species' life cycle and population dynamics. An indispensable contribution to sustainable fishing techniques, this study offers vital insights into fish stock recruitment, spawning patterns, and genetic variability. A comprehensive knowledge of reproductive biology enables managers to enhance fishing techniques, therefore mitigating the potential for overfishing and the subsequent reduction of fish populations.

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