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Reproductive Biology of *Lepidocephalichthys guntea* (Hamilton, 1822) from Kangsabati River, West Bengal

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ABSTRACT

The purpose of this research was to evaluate the sexual dimorphism, sex ratio, gonadosomatic index, first maturity length, and fecundity of *Lepidocephalichthys guntea* in the waters of the Kangsabati river. Pectoral fin changes are evident in males of the species, with the seventh and eighth rays of the pectoral fin joining together to produce a thicker ray known as the laminar circuli, which allows for sexual differentiation between males and females of the species. During mating season, females have a pinkish bilobed ovary, while males' testes are white. As a whole, the male to female ratio was 1: 1.61 (M: F). It was calculated that at 50% maturity, the length would be 6.46 centimeters. June is the month with the highest GSI values for both sexes. Females of the genus *L. guntea* with a mean body weight of 2.01 gm to 9.73 gm had a mean absolute fecundity that varied from 2832.19 ± 40.88 to a high of 9951.28 ± 142.92. The GSI and fecundity results indicated that the months of June and July are optimal for the species' reproductive success. The research results will help grow this species commercially and make it easier to breed it.

Key words: *Lepidocephalichthys guntia*, Gonado-somatic index, Fecundity, Sexual dimorphism, Sex ratio

The term "aquaculture" refers to a wide-open field that is included in the scope of the fishing industry. This field provides the human population with an inexpensive supply of protein. In addition to this, it affords the possibility of employment to millions upon millions of people all over the world. Because of aquaculture, farmers who want to produce animals can always count on having a continual supply of seed that is of good quality and has not been contaminated. It is impossible to gather eggs or seeds from the river for the purpose of using them in the growing process. There is no likelihood of success. Therefore, a different way of manufacturing seeds of the highest possible quality ought to be possible as an alternative. The "induced breeding approach" is the name given to this alternate methodology. One needs to have some knowledge of the mating seasons and the reproductive biology of the species they are trying to breed in order to successfully produce fish in an area that has a restricted amount of space. Research on fecundity has the ability to yield knowledge not only about a species' potential stock but also about fisheries management, practical culture, and the life cycle of the species [1-2]. When it comes to reproductive biology, having a precise understanding of the fecundity of a species is essential to establishing an efficient breeding program for that species. This is because fecundity is directly related to the number of offspring that a species can produce. The term "fecundity" refers to the number of eggs that a female of a species is able to produce throughout the duration of the breeding season. The study of fecundity is being carried out in order to ascertain whether or not there is a connection between the size, age, and age of a fish and the number of offspring it may produce [3-5]. This endeavor's goal is to compile a report on the current status of the populations and stocks of a variety of fish species; specifically, the focus will be on density as a determining factor [6-7]. In quantitative terms, fecundity can be thought of as the relationship that emerges between egg production and recruitment [8-9]. Estimates of the fecundity of a wide variety of species have been the subject of a great number of studies that have been carried out by a limited group of researchers. The evaluation of fecundity on *Puntius stigma* [10-11] *Channa gachua* [12-14] called *Amblypharyngodon mola* are some instances of these kinds of studies. *Colisa fasciata* [15]. There has been some use of *Sarotherodcin nilotica* [16]. *Oreochromis nilotica* [17-18] and *Xenentodon cancila* [19]. One of the species that is considered to be in the least amount of danger is the *Lepidocephalichthys guntia*, which can be found on the IUCN Red List. The Guntea loach, also referred to as *L. guntea*, is an important indigenous fish in India and belongs to the family Cobitidae. This species has been spotted not only in moving streams but also in still pools of clear water [20], and it has also been observed in beels and marshes. In addition to this, it has a significant market presence in a number of other nations, like Bangladesh, Pakistan, and Nepal [21]. They are able to live in water that is either still or moving when they are in their natural environment. As fish that dwell on the bottom, they frequently

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find their source of nutrition in the larvae of other insects as well as in a variety of forms of detritus. It has previously been classified as a threatened species because of the alterations that humans have brought about in the ecosystems of aquatic environments; the disappearance of naturally occurring breeding and nursery grounds; and the overexploitation of fisheries [22]. This fish has great flavor and is a good source of many different nutrients. The changes that humans have brought about in the aquatic ecology; the disappearance of natural breeding and nursery grounds; and the overexploitation of fisheries are all contributing factors in the progressive decline of the species' abundance. The most important purpose of this investigation was to give up-to-date information on a number of the biological properties of this native species. The ratio of men to females, sexual dimorphism, the gonadosomatic index, and fecundity were these features.

MATERIALS AND METHODS

The Kangasabati River and all of its tributaries provided the location for the collection of *Lepidocephalichthys guntia* specimens on a monthly basis. During the time period beginning in January 2020 and ending in December 2020, a total of 1065 specimens were collected, including 408 males, 657 females. First, the specimens were separated from one another by noting the various sexual dimorphism characteristics, and then their total lengths (in centimeters) and total weights (in grams) were measured. The male to female ratio was calculated each month using the percentage of males to females (M: F). We used the Chi-Square test with a significance level of 0.05 to figure out if the ratio of men to women is significantly different from 1:1. Based on the scale that [23] made, the stages of maturation were figured out.

The gonado-somatic index (GSI) was calculated and characterized using a formula developed by [24-25]. The formula is as follows:

$$GSI = 100 \times G / W$$

Where (G) denotes the weight of the gonad and (W) denotes the weight of the body. Lm stands for "length at first sexual maturity."

Using a model that is based on GSI, researchers were able to determine the length of time at which fifty percent of *L. guntea* reach their sexual maturity (Lm = TL vs. GSI [26]). There are different ways to estimate the fecundity of fish. The gravimetric method, which is one of the methods described by [1], is regarded as being the most effective and provides results that are reasonably precise. This technique was also applied by [27-29], with the same favorable results. Fecundity During the breeding season, approximately twenty specimens were painstakingly removed (May to July, 2021). After being washed and weighed to the nearest 0.01 gm, the ovaries were then stored in formalin at a concentration of 4%. The weight of each of the three subsamples that were obtained from each ovary was recorded to the nearest 0.01g. Each subsample was put in a Petri dish with one drop of distilled water and then looked at with a binocular microscope to count the number of eggs. This was accomplished using the following formula:

$$F_1 = \text{Number of eggs in sub-sample multiplied by gonad weight / Sub-sample weight}$$

After that, the mean number of eggs from three subsamples (F_1, F_2, and F_3) were added together, and the following formula was used to figure out each bird's fecundity:

$$\text{Fecundity} = F_1 + F_2 + E_3 / 3$$

Relative fecundity was determined by the successive equations.

$$\text{Relative fecundity} = \text{Absolute fecundity / body weight (g)}$$

We looked into the relationship between fecundity and total body length, body weight, and gonad weight. We then put this relationship in logarithmic form (Log Y = log a + b log X).

Statistical analysis

All of the reproductive factors that were mentioned above, including sex ratio, GSI, fecundity, and relative fecundity, were examined. Utilizing Microsoft Excel, we were able to determine the linear association as well as the correlation coefficient (r) between overall length and fecundity, as well as gonad weight and fecundity. For the statistical data analysis, the computer program SPSS version 15.0 (Statistical Package for Social Science) was used to get the job done.

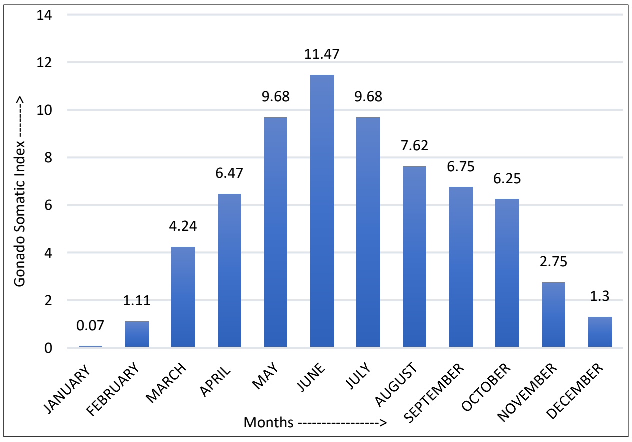


Fig 1 Monthly variation of Gonado Somatic Index of female species

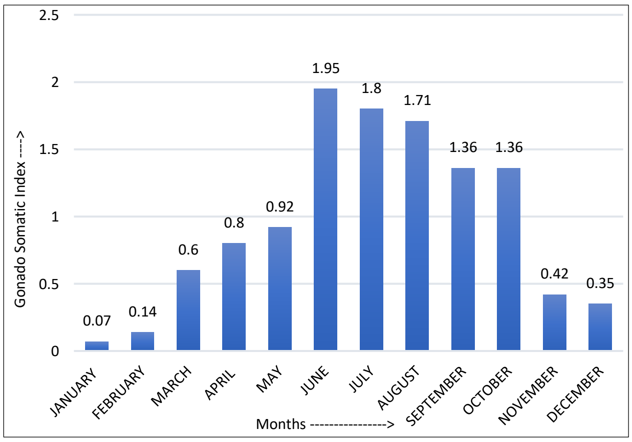


Fig 2 Monthly variation of Gonado Somatic Index of male species

RESULTS AND DISCUSSION

Sexual dimorphism

For the sake of breeding, it was necessary to accurately and quickly distinguish between male and female by using some exterior characteristics. Females of this species are often larger than males of this same species, both in terms of their overall size and the depth of their bodies. Adults were classified as males if they possessed modified pectoral fins, whilst females were classified if their pectoral fins were unmodified. Pectoral fin alterations are present in males of a species where the 7^{th} and 8^{th} rays of the pectoral fin join together to form a thicker ray known as the laminar circuli (Fig 6). In females of this species, the rays do not connect together in this way. At the time of year when they are reproducing, the females of this species have a rosy tint to their bodies. During the dissection, it was discovered that males have gonads that are white in color, while females have gonads that are pink in color and contain eggs (prominent during the breeding season) (Fig 7a) and male possess a whitish testis (Fig 7b).

Sex ratio

With a total of 1065 *L. guntea* specimens that were able to be sexed, there were 408 males, which represented 38.03% of the total number, and 657 females, which represented 61.95% of the total number, for an overall sex ratio of 61.95 :38.03 (1 males: 1.61 females). The results of the study revealed that for the entirety of the research period, the proportion of female *L. guntea* specimens to male specimens was consistently higher than the reverse. The data was collected from January through December of 2020 (Table 1). The month of January had the largest percentage of males (41.17%), while August had the lowest percentage (34.21%) of males. On the other hand, the month of August had the largest percentage of females (65.78%), while the month of November saw the lowest value (56.36%). During the month of November, when the running stage was recorded for both sexes, the percentages of male and female runners were relatively similar. Chi-Square showed that there was a statistically significant and large difference between the two genders ($P < 0.05$).

Table 1 Monthly variation of sex ratio of *Lepidocephalichthys guntea* from kangsabati river

Month	Sample size	Male (Observed value)		Female (Observed value)		Ratio	
		Number	Percentage	Number	Percentage	Male	Female
January	85	35	41.17	50	58.82	1	1.42
February	90	31	34.44	59	65.55	1	1.90
March	74	28	37.83	46	62.16	1	1.64
April	92	37	40.21	55	59.78	1	1.48
May	69	24	34.78	45	65.21	1	1.87
June	98	37	37.75	61	62.24	1	1.64
July	84	29	34.52	55	65.47	1	1.89
August	76	26	34.21	50	65.78	1	1.92
September	104	41	39.42	63	60.57	1	1.53
October	81	31	38.27	50	61.72	1	1.61
November	110	48	43.63	62	56.36	1	1.29
December	102	41	40.19	61	59.80	1	1.48

Table 2 Size at first sexual maturity of *Lepidocephalichthys guntea*

Species	Number of samples	Minimum length (cm)	Maximum length (cm)	Size at 1st sexual maturity (Lm, In cm)
Lepidocephalichthys guntea	126	5.4	10.71	6.46

Size at the age of sexual maturity

Fifty percent of all female specimens will have reached their first stage of maturity at a length of 5.4 to 10.71 centimeters. It has been found that most females with mature ovaries are longer than 5.5 centimeters in June and July, which is when they spawn (Table 2).

Gonad of female and male species

Immature: the testicles are milky white and opaque, the gonads are thread-like and slender, and the ovaries are pinkish and translucent.

Maturing and recovering: During this stage, the gonads grow larger, eventually taking up between one third and half of the body cavity. Ova is quite small and can be seen with a trace of yolk.

Nearly ripe: at this stage, the gonads take up approximately two-thirds of the whole length of the body cavity. When they get to the ovary, the eggs get bigger, yellower, and easier to push out when you apply pressure. Testicles are colorless and their wall thickness decreases as they mature.

Ripe stage: the body cavity is completely occupied by the gonads. The testes of males consist of milky secretions that emerge from the vent (Fig 8b), whereas the eggs of females are produced in response to pressure from the abdomen (Fig 8a).

Running: Ovaries are small, ruddy organs that are spherical, have a wide front edge, and are vascularized with blood vessels. The testicles become smaller and more flaccid and floppier as the condition progresses.

Spent: Gonads are flaccid. A dark crimson tint can be seen throughout the ovary, and there are only a few eggs left behind (Fig 9a). The testicles have a grayish brown tint, and there may be some milt that has not been removed completely (Fig 9b).

Table 3 Monthly variation of Gonado Somatic Index of female species

Month (Jan.-Dec.2020)	Gonado-Somatic Index (Average)	SD
January	0.07	± 0.05
February	1.11	± 0.44
March	4.24	± 0.33
April	6.47	± 0.79
May	9.68	± 0.12
June	11.47	± 2.63
July	9.68	± 0.42
August	7.62	± 0.34
September	6.75	± 3.81
October	6.25	± 3.15
November	2.75	± 1.17
December	1.3	± 0.42

Gonadosomatic index of the species

The Gonado-somatic index (GSI) of fishes is a factor that can be used to estimate the timing of the breeding season. During the investigation of GSI, a total of 240 different species were considered and taken into account. During the course of the research that was conducted between January 2020 and December 2020, the average value of *L. guntea* GSI was calculated. During the course of the research, the month of June had a GSI mean value of female species that was 11.47 ± 2.63 and the month of January had a GSI mean value that was 0.07 ± 0.05 (Table 3, Fig 1). Similarly, the peck value of GSI for males of the species is highest in the month of June, coming in at 1.95 ± 0.35, while it is at its lowest in the month of January, coming in at 0.07 ± 0.05 (Table 4, Fig 2). The highest GSI mean value was found in June. According to the findings of the study, the months of May, June, and July had a higher GSI value when compared to the other months. Based on these results, the mating season for the species occurred during the months of May to July. During the breeding season, a fully grown egg was between 116.242 and 118.452 micrometers across.

Table 4 Monthly variation of Gonado Somatic Index of male species

Month (Jan.-Dec.2020)	Gonado-Somatic Index (Average)	SD
January	0.07	± 0.05
February	0.14	± 0.02
March	0.6	± 0.05
April	0.8	± 0.1
May	0.92	± 0.16
June	1.95	± 0.35
July	1.8	± 0.23
August	1.71	± 0.17
September	1.36	± 1
October	1.36	± 1.21
November	0.42	± 0.2
December	0.35	± 0.06

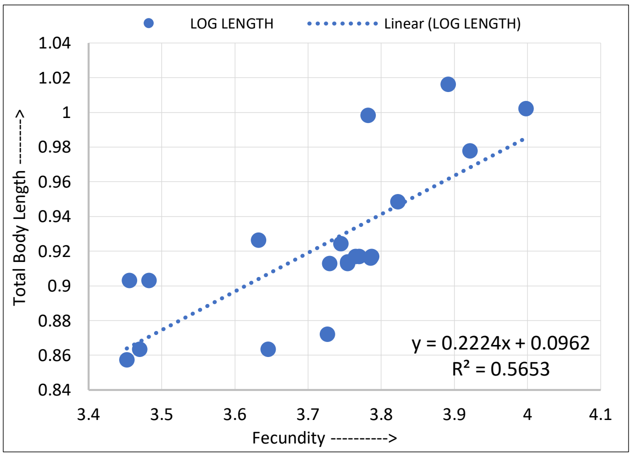


Fig 3 The mean values of absolute fecundity per length (cm) of *Lepidocephalichthys guntea*

Fecundity

The number of eggs that are thought to be contained within the ovary of a mature female specimen is referred to as the fecundity. The approach of sampling and direct counting was used to determine the fecundity of 20 different species. For females of *L. guntea* with an average body weight ranging from 2.01 gm to 9.73 gm, the mean fecundity ranged from a low of 2832.19 ± 40.88 to a high of 9951.28 ± 142.92 throughout the study period, which corresponded to the breeding season (Table 5). The relative fecundity of the species with respect to the body weight varies from 854.75 to 1538.16 (Mean value 1312.17 ± 191.1825), with respect to total length, ranges from 356.85 to 990.17 (Mean value 646.77 ± 162.47). The scatter diagram showed that there is a linear relationship between the total weight of the species and the fecundity of the species (Fig 3), and the coefficient of correlation was statistically significant ($P < 0.05$). (Table 6) has a log equation that expresses the link between fecundity, denoted by the letter "F," and the total weight of the species, denoted by the letter "TL." The scatter diagram demonstrated that there is a linear link between the total weight of the species and the fecundity of the species (Fig 4), and the coefficient of correlation was statistically significant ($P < 0.05$). A log equation (Table 6) shows the relationship between fecundity, which is shown by the letter "F," and the total weight of the species, which is shown by the letter "TL." (Fig 5)'s scatter plot revealed a linear link between the species' fecundity and the overall weight of its gonads, with a statistically significant coefficient of correlation ($P < 0.05$). In (Table 6), a log equation shows the relationship between fecundity (shown by the letter "F") and the total weight of the gonad (shown by the letter "TL").

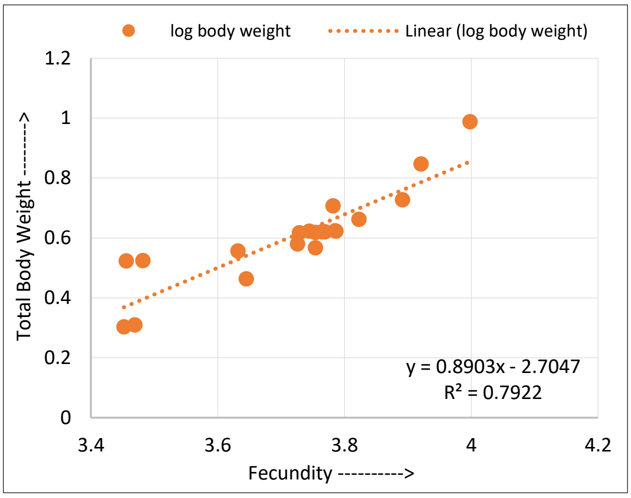


Fig 4 The mean values of absolute fecundity per body weight (gm) of *Lepidocephalichthys guntea*

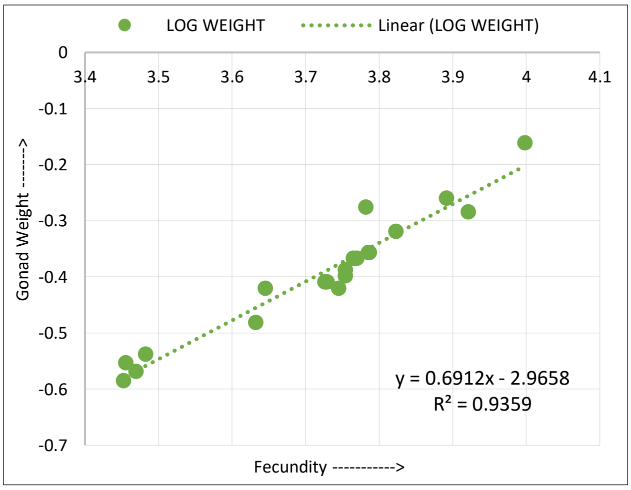


Fig 5 The mean values of absolute fecundity per gonad weight (gm) of *Lepidocephalichthys guntea*

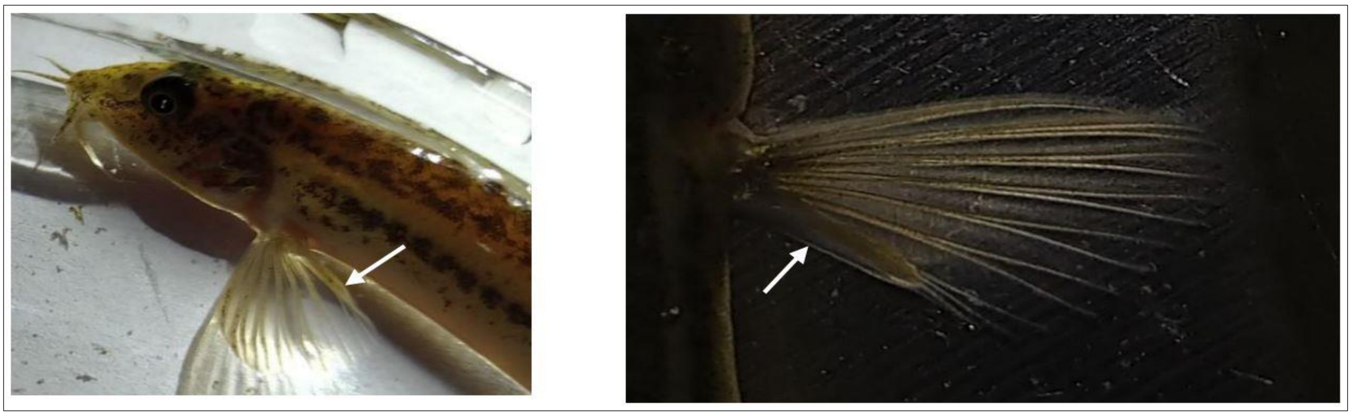
There is a significant need for research on the processes of maturation and spawning in fish. When it comes to the reproduction of a species' stock, the fertility of a fish is an extremely important factor to consider. The management of brood stock in aquaculture operations is based on information regarding reproduction and the behavior associated with reproduction. In order to ensure the development of high-quality seeds for culture, efficient management of brood stock depends on an accurate forecast of ripening tissue. This allows for the most effective management of brood stock. A comprehensive understanding of the maturation, ovulation, and spawning processes of the candidate species is necessary for the reliable collection of gametes of high quality. In order to improve the effectiveness of brood stock management and larval rearing programs, it is important to have accurate estimations of fecundity [30]. From the study, the most prominent sexual dimorphism characters were observed in male species. Pectoral fin alterations are present in males of a species where the 7th and 8th rays of the pectoral fin join together to form a thicker ray, known as Lamina circularis. This Laminar circuli was absent in the female species. A similar observation was also reported by [31]. Researching the gender distribution of a fish in its natural population is of the utmost significance. The male-female ordination pattern can be seen in a body of water through the examination of the monthly sex ratio. According to the findings of this study, females are more common than males at all times of the year. The results of the analysis showed that the total sex ratio was 1:1.61, which suggested that the collected male and female distribution of *L. guntea* in the natural water of the Kangasbati River was determined to be female dominated and statistically significant (P <0.05) from the parity test. It was discovered that the female was larger than the male [32-35]. It is not quite clear why females outnumber males in this fish population; nonetheless, it is possible that this is a process of population regulation that was described by [36] in the past. On the other hand, [37] reached the conclusion that the metabolic trait of spawning was generally greater in older men than in older females. This observation translates to greater mortality among males than females, specifically during the time of spawning in the population that was studied. It is possible that the earlier maturation of male Mystus species compared to females accounts for the increased strain experienced by males. Many researchers [39-39] have observed the same tendency of early maturity of males in comparison to females for various fish species, which lends support to the findings of the current study [40].

Table 5 Fecundity of *Lepidocephalichthys guntea* during breeding season

S. No.	Total body length (cm)	Body weight (gm)	Gonad weight (gm)	Mean absolute fecundity	SD
1	8.26	4.18	0.43	5819.8	± 21.16
2	8.18	3.69	0.4	5675.82	± 15.12
3	7.2	2.01	0.26	2832.19	± 40.88
4	7.3	2.91	0.38	4417.71	± 12.23
5	8	3.34	0.28	2854.85	± 7.1
6	8.4	4.2	0.38	5554.41	± 25.82
7	10.05	9.73	0.69	9951.28	± 142.92
8	9.5	7.04	0.52	8334.33	± 57.46
9	8.26	4.18	0.43	5881.3	± 231.12
10	8.44	3.6	0.33	4286.5	± 110.08
11	8	3.35	0.29	3035.01	± 104.7
12	7.3	2.04	0.27	2947.77	± 191.65
13	8.2	4.16	0.41	5675.11	± 111.77
14	8.18	4.15	0.39	5359.16	± 138.25
15	8.26	4.2	0.44	6119.68	± 250.57
16	8.24	4.21	0.44	6100.91	± 232.67
17	7.45	3.8	0.39	5323.03	± 157.47
18	8.88	4.6	0.48	6648.98	± 109.67
19	9.96	5.1	0.53	6051.77	± 79.45
20	10.38	5.34	0.55	7789.73	± 35.09

Table 6 Regression and logarithm equation of fecundity

S. No.	Relationship Characters	Regression	Log Equation
1	Fecundity and Total body Length	0.56	Log F = 0.0962 + 0.2224 Log TL
2	Fecundity and Total body weight	0.89	Log F = - 2.7047 + 0.8903 Log BL
3	Fecundity and Total gonad weight	0.93	Log F = - 2.9658 + 0.6912 Log GW



(6.a) (6.b)

Fig 6 Distinct sexual dimorphism character "laminar circuli" of male species shown in figure

(7.a) (7.b)

Fig 7 Sexually mature female ovary (7.a) and male testis (7.b) in body cavity

(8.a) (8.b)

Fig 8 Fully mature female ovary (8.a) and male testis (8.b)

(9.a) (9.b)

Fig 9 Spent female ovary (9.a) and male testis (9.b)

According to some authors [41], the selection of permitted capture size at first maturity is widely employed and also used as a key instrument in fisheries management in open waters. In this study, it was found that the fish reached sexual maturity at an average length of 6.46 cm. According to [42], *L. guntea* matures at a length of 6.03 cm. According to a number of variables, including feeding rate, sex and gonadal development, behavior, season, water velocity, population density, water temperature, and food, the Lm of fish specimens may vary [43-44] of females are double-lobed, tiny, and have a pinkish hue; they are dorso-laterally connected to the gut. Both of the ovaries were lengthy, with the middle being significantly larger than the extremities, and they were almost exactly the same size and form. The long insertion line is located in the center of the ovary. Ovaries, when fully formed, ova are visible to the naked eye in the gonad. The adult testis has a whitish color, but the juvenile testis has a slightly pinkish color and a translucent appearance. According to (Fig 2), the maximum mean GSI value was calculated to be 11.47 ± 2.63 in June and the lowest mean GSI value was 0.07 ± 0.05 in January. According to the study, the months of May, June, and July had higher GSI values than other months [45-46]. Between May and July, when GSI corresponds to enhanced gonadal development, there is a progressive decline in value, which is similar to [47]. The GSI revealed a single peak from April to July, indicating that a higher proportion of fish were reaching maturity during this time. The abrupt decrease in GSI during the month of August strongly suggested the start of spawning activity in this fish, demonstrating the existence of a distinct breeding season for this species of fish each year. The mean fecundity for females of *L. guntea* with typical body weights of 2.01 g to 9.73 g ranged from 2832.19 ± 40.88 to a maximum of 9951.28 ± 142.92. In this study, June and July saw an increase in the mean female fecundity compared to other months. From September to January, fecundity was not seen during the research period. According to the study, the *L. guntea* species' reproductive season lasted from May to June. The research conducted by [48-49] supports this fact. The fluctuation in relative fecundity in relation to body weight is brought on by size variance, study site, and study length. In different locations, the same species' fertility was reported to vary greatly. This can arise as a result of the availability of food in both the wild and in captivity [50]. The species' fecundity correlates positively with total body length, total body weight, and gonad weight, with gonad weight showing the strongest correlation. The linear link between the regression equations was significant ($P<0.05$) [51].

CONCLUSION

This research aims to provide light on the sexual dimorphism, sex ratio, gonadosomatic index, onset of sexual maturity, and fertility of *L. guntea* in the Kangsabati River. The preceding criteria were deemed vital to the management plan for this species. Sex dominance in the *L. guntea* population, as well as fundamental data needed for reproduction and assessing stock size, are the focus of the study on sex ratio. Finding out how often and when *L. guntea* reproduces can help in planning recruitment efforts.

Acknowledgments

We appreciate Vidyasagar University's administration for facilitating our research by providing a fully functional fishing laboratory. In addition, we are indebted to the financial support of the Department of Science and Technology (Govt. of India).

Conflicts of interest

We declare that we have no conflict of interest.

Ethics approval

We declare that all applicable international, national and/or institutional guidelines for sampling, care and experimental use of fishes for the study have been followed, and that all necessary approvals have been obtained.

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