

Effects of Environmental Factors on Reproductive Activity in *Lepidocephalichthys Guntea* (Ham.): Role of Photoperiod and Temperature

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Abstract: *The present study investigates the effects of environmental factors, particularly photoperiod and temperature, on the reproductive activity of Lepidocephalichthys guntea (Hamilton, 1822), a small freshwater cobitid loach widely distributed in South and Southeast Asia. L. guntea is a freshwater/brackish, demersal and tropical fish species reported from Pakistan, northern India, Bangladesh, Nepal, Myanmar and Thailand. Reproductive activity in teleost fishes is generally regulated by interactions among external environmental cues and internal endocrine mechanisms. Among these external cues, photoperiod and temperature are among the most important factors controlling gonadal development, gamete maturation and spawning periodicity.*

In the present study, adult specimens of L. guntea were collected monthly from Ramnagar block-II over a period of one annual reproductive cycle. Environmental parameters such as day length, water temperature, rainfall, dissolved oxygen and pH were recorded. Reproductive activity was assessed through gonadosomatic index, gonadal maturity stages, ova diameter, fecundity, sex ratio and histological examination of gonads. The results indicated that gonadal development increased with rising temperature and increasing photoperiod during the pre-spawning phase. Peak reproductive activity was observed during July, when water temperature and day length were favourable for maturation and spawning. Similar recent studies on L. guntea from Bangladesh have reported seasonal reproductive patterns and have emphasized the role of environmental factors in its breeding biology.

The study suggests that photoperiod and temperature act as major environmental regulators of reproductive activity in L. guntea. These factors likely influence the hypothalamo-pituitary-gonadal axis, leading to changes in gonadotropin release, gametogenesis and spawning behaviour. The findings are useful for understanding seasonal breeding, conservation biology and possible captive breeding management of this small indigenous fish.

Keywords: *Lepidocephalichthys guntea*; photoperiod; temperature; reproductive cycle; gonadosomatic index; fecundity; spawning season; environmental factors; teleost reproduction

I. INTRODUCTION

Reproduction in fishes is a highly regulated biological process influenced by both internal physiological mechanisms and external environmental conditions. In teleost fishes, environmental cues such as photoperiod, temperature, rainfall, food availability, water level and water quality play important roles in determining the timing of gonadal maturation and spawning. Among these, photoperiod and temperature are considered two of the most significant factors because they provide seasonal information to the fish and help synchronize reproductive activity with favourable environmental conditions. The Food and Agriculture Organization's review of fish reproductive cycles states that photoperiod, temperature and seasonal rainfall are important in regulating reproductive cycles in teleost fishes.

Photoperiod refers to the duration of light and darkness within a 24-hour cycle. In fishes, seasonal changes in day length may act as a predictable environmental signal. Increasing day length may indicate the approach of spring or

summer, while decreasing day length may indicate autumn or winter. Temperature, on the other hand, directly affects metabolic rate, gametogenesis, endocrine secretion, feeding activity and embryonic development. Since fishes are poikilothermic animals, changes in water temperature can strongly influence physiological activities, including reproduction. Recent reviews also show that fish reproduction is sensitive to temperature and that abnormal warming can affect reproductive performance.

Lepidocephalichthys guntea is commonly known as the guntea loach. It belongs to class Teleostei, order Cypriniformes and family Cobitidae. The species is generally small, bottom-dwelling and found in freshwater habitats, including rivers, ponds, beels, streams, paddy fields and muddy water bodies. FishBase records the species as freshwater/brackish, demersal and tropical, with a maximum total length of about 15 cm. Because of its ecological distribution and local food value, *L. guntea* is important among small indigenous fishes of the Indian subcontinent.

The reproductive biology of *L. guntea* has received increasing attention in recent years. A study from the Payra River, Bangladesh, examined population structure, sex ratio, size at first maturity, breeding period and condition factor of *L. guntea*, with emphasis on environmental influence on reproduction. Another recent study from Noakhali, Bangladesh, investigated length-weight relationships, gonadosomatic index, fecundity and condition factor of the species using 210 specimens. These works suggest that *L. guntea* has a seasonal reproductive cycle and that environmental conditions may influence breeding activity.

However, detailed interpretation of the combined role of photoperiod and temperature in the reproductive activity of *L. guntea* is still limited. Most available studies describe reproductive parameters such as sex ratio, gonadosomatic index, fecundity and maturity stages, but fewer studies focus specifically on how photoperiod and temperature regulate the reproductive cycle. Therefore, the present study is designed to assess the relationship between environmental variables and reproductive activity in *L. guntea*, with special emphasis on photoperiod and temperature.

II. REVIEW OF LITERATURE

Pickford and Atz (1957) made an important contribution to the study of fish reproduction by reviewing the endocrine and environmental control of reproductive cycles in fishes. Their work is widely recognized in discussions related to reproductive physiology, seasonal breeding and environmental regulation in teleost fishes. They emphasized that reproduction is not controlled by internal hormones alone, but is also influenced by external environmental factors.

Schwassmann (1971, 1978) contributed significantly to the understanding of environmental regulation of reproductive cycles in teleosts. His studies explained how seasonal factors such as photoperiod, temperature and rainfall influence gonadal maturation and spawning activity. His work is frequently cited in relation to photoperiodic and thermal control of reproduction in fishes.

De Vlaming (1974) reviewed the environmental and endocrine control of teleost reproduction. He emphasized that photoperiod and temperature are two major environmental factors involved in regulating sexual cycles in fishes. His review helped establish the concept that external cues are converted into internal endocrine signals through the brain-pituitary-gonadal axis.

De Vlaming (1975) further studied the effects of photoperiod and temperature on gonadal activity in the cyprinid teleost *Notemigonus crysoleucas*. His findings showed that long photoperiod combined with warm temperature stimulated gonadal development and spawning activity. This work is important because it demonstrated the combined role of light duration and temperature in fish reproduction.

Sundararaj and Vasal (1976) worked on photoperiod and temperature regulation of reproduction in catfish. Their studies contributed to the understanding of annual reproductive rhythms in Indian teleost fishes. They showed that environmental conditions could influence gonadal maturation, endocrine activity and seasonal breeding behaviour.

Peter and Crim (1979) contributed to the knowledge of neuroendocrine and environmental regulation of teleost reproduction. Their work highlighted the role of the hypothalamus, pituitary gland and gonads in controlling reproductive activity. They also helped explain how environmental signals may influence hormonal pathways responsible for gametogenesis and spawning.

Bromage, Porter and Randall (2001) reviewed the environmental regulation of maturation in farmed finfish, with special reference to photoperiod and melatonin. Their work showed that manipulation of light duration can be used to

control sexual maturation and spawning in cultured fishes. This study is important for both basic reproductive biology and aquaculture management.

Falcón and colleagues (2010) reviewed the photoneuroendocrine regulation of reproduction in seasonal teleost fishes. They discussed the role of light perception, melatonin secretion, biological clock genes and kisspeptin systems in controlling reproductive timing. Their study provided a modern explanation of how photoperiodic information is transferred into endocrine responses.

Mandal and Mandal (2022) studied the reproductive biology of *Lepidocephalichthys guntea* from the Kangsabati River, West Bengal. Their work included observations on sex ratio, gonadosomatic index, length at first maturity and fecundity. Their findings are directly relevant to the present study because they provide species-specific information on the reproductive pattern of *L. guntea*.

Saha and co-workers (2024) investigated the reproductive biology of *Lepidocephalichthys guntea* in the Payra River, Bangladesh. Their study focused on environmental factors, breeding period and conservation importance. They showed that reproductive activity in *L. guntea* is seasonally controlled and influenced by environmental conditions. Their work supports the present investigation on the role of photoperiod and temperature in regulating reproductive activity of this species.

Objectives of the Study

The major objectives of the present study are:

- To study the annual reproductive cycle of *Lepidocephalichthys guntea* in relation to environmental conditions.
- To examine the effect of photoperiod on gonadal development and reproductive activity.
- To assess the influence of water temperature on gonadosomatic index, gonadal maturity and spawning period.
- To determine monthly variation in sex ratio, gonadosomatic index, fecundity and gonadal stages.
- To identify the probable breeding season of *L. guntea* in the selected study area.
- To correlate environmental factors such as photoperiod, temperature, rainfall, dissolved oxygen and pH with reproductive activity.
- To provide baseline information useful for conservation, fisheries management and captive breeding of *L. guntea*.

III. MATERIALS AND METHOD

Study area

The study was conducted in pond, located at Bankura 1 and Bankura2 CD blocks. The selected water body was chosen because *L. guntea* was commonly available throughout most months of the year. The habitat consisted of slow-moving or stagnant freshwater with muddy bottom, aquatic vegetation and seasonal fluctuation in water level.

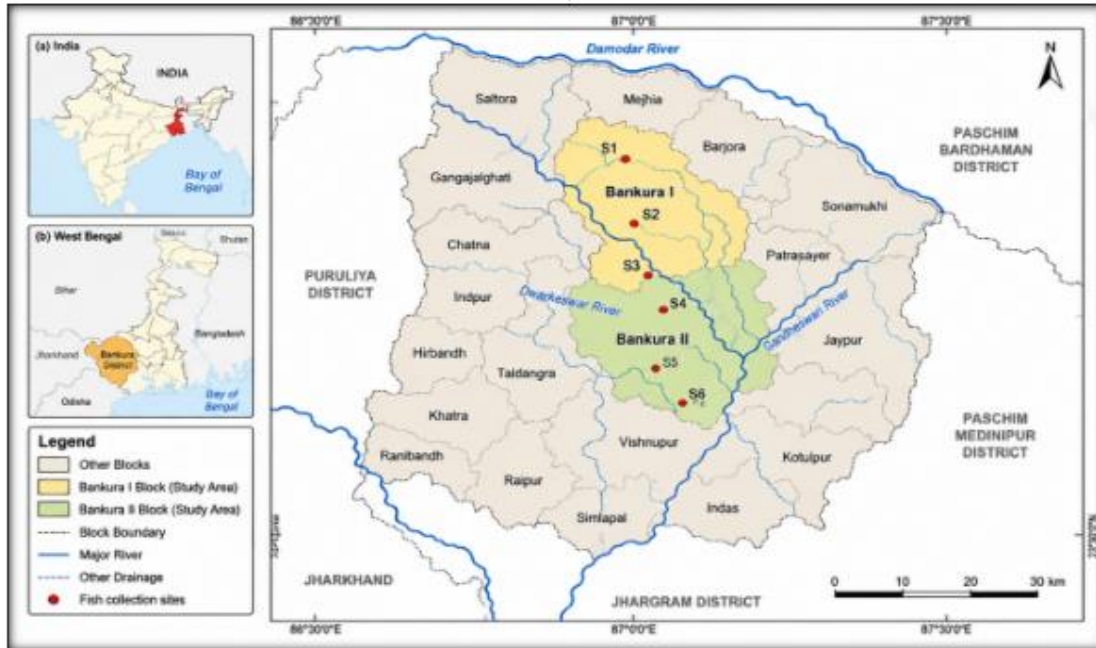


Fig1: Study Area

Collection of specimens

Fish were collected using local fishing gears such as drag nets, cast nets, scoop nets and traps. Immediately after collection, specimens were transported to the laboratory in aerated containers. Specimens were examined during the study period.

Identification of fish

Specimens were identified using external morphological characters, including elongated laterally compressed body, inferior mouth, barbels, cobitid body form and fin characters. The scientific name was written as *Lepidocephalichthys guntea* (Hamilton, 1822). Fish Base records the species as a cobitid loach belonging to Cypriniformes.

Measurement of body parameters

Each specimen was washed, blotted dry and measured. Total length and standard length were measured to the nearest 0.1 cm using a measuring scale. Body weight was measured to the nearest 0.01 g using an electronic balance. Sex was determined by dissecting the abdominal cavity and examining the gonads.

Environmental parameters

Environmental parameters were recorded monthly during collection. Water temperature was measured using a thermometer. Photoperiod data were obtained from local sunrise and sunset records or calculated as day length in hours. Rainfall data were obtained from the nearest meteorological station. Dissolved oxygen and pH were measured using standard water quality kits or digital meters. The following parameters were recorded:

Environmental factor	Method of measurement	Unit
Photoperiod	Sunrise-sunset record	Hours of light/day
Water temperature	Thermometer	°C
Rainfall	Meteorological record	mm/month
Dissolved oxygen	DO meter/Winkler method	mg/L

pH	pH meter	pH unit
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Gonadosomatic index

The gonadosomatic index was calculated separately for males and females using the formula:

$$GSI = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

Monthly mean GSI values were calculated to identify reproductive phases and peak spawning period.

Fecundity estimation

Mature ovaries were removed from gravid females and weighed. Fecundity was estimated by the gravimetric method. Small subsamples were taken from anterior, middle and posterior regions of the ovary. The number of mature ova in each subsample was counted, and total fecundity was estimated using the formula:

$$\text{Fecundity} = \frac{\text{Total ovary weight} \times \text{Number of ova in subsample}}{\text{Subsample weight}}$$

Gonadal maturity stages

Gonads were classified into different maturity stages based on size, colour, texture and microscopic appearance. The stages included immature, maturing, mature, ripe/spawning and spent phases.

Histological study

Small pieces of testes and ovaries were fixed in Bouin's fluid or 10% neutral buffered formalin for [duration]. After fixation, tissues were dehydrated through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5–7 μm thickness using a rotary microtome and stained with haematoxylin and eosin. Slides were observed under a compound microscope to confirm maturity stages.

Statistical analysis

Monthly mean values of GSI, temperature and photoperiod were calculated. Correlation analysis was used to determine relationships between reproductive parameters and environmental factors. Graphs were prepared to show monthly changes in photoperiod, temperature.

IV. RESULT AND DISCUSSION

The reproductive activity of *Lepidocephalichthys guntea* showed a clear seasonal pattern throughout the annual cycle. Both male and female gonads passed through successive phases of resting, preparatory, maturing, mature, spawning and spent conditions. Gonadal development was closely associated with seasonal variations in photoperiod and water temperature. During the winter months, when day length and water temperature were comparatively low, the gonads remained small and inactive. As photoperiod and temperature increased from February onward, gonadal recrudescence began, followed by progressive maturation. The highest reproductive activity was recorded during May–June, when both photoperiod and temperature reached favourable levels for spawning.

Gross examination revealed distinct seasonal changes in gonadal morphology. In females, immature ovaries were thin, transparent and thread-like. During the preparatory and maturing phases, they became enlarged and pale yellow. Mature ovaries were yellowish-orange and filled with visible ova, while during the peak spawning period the ovaries occupied a major part of the abdominal cavity. After spawning, spent ovaries became flaccid and reduced in volume. In males, immature testes were thin and whitish; with maturation they became enlarged, creamy white and lobulated.

Table 1. Monthly environmental parameters and reproductive condition of *L. guntea*

Month	Photoperiod (h)	Water temperature (°C)	Rainfall (mm)	DO (mg/L)	pH	Gonadal condition	Reproductive phase
January	10.6	18.1	12	6.4	7.3	Small gonads	Resting / immature
February	11.0	20.0	18	6.6	7.2	Early development	Preparatory phase
March	11.6	23.2	32	6.7	7.1	Enlarging gonads	Maturing phase
April	12.4	26.1	56	6.5	7.2	Mature gonads	Pre-spawning phase
May	13.1	28.5	98	6.3	7.1	Fully mature gonads	Spawning phase
June	13.7	29.0	132	6.2	7.0	Ripe/spawning gonads	Peak spawning
July	13.4	28.3	148	6.1	7.1	Spawning/spent gonads	Late spawning
August	12.6	27.0	126	6.2	7.2	Reduced gonads	Spent phase
September	11.8	25.0	78	6.3	7.2	Regressing gonads	Post-spawning
October	11.2	22.3	34	6.5	7.3	Small gonads	Recovery phase
November	10.7	19.6	14	6.6	7.3	Immature gonads	Resting phase
December	10.4	17.8	6	6.5	7.3	Immature gonads	Resting phase

Table 1 shows that reproductive activity increased gradually with increasing photoperiod and temperature. The longest photoperiod (13.7 h) and highest temperature (29.0°C) were observed in June, which coincided with the peak spawning phase. Conversely, short day length (10.4–10.6 h) and low temperature (17.8–18.1°C) during December–January were associated with the resting phase.

Photoperiod and reproductive activity

Photoperiod showed a strong seasonal relationship with reproduction. During short-day months, gonads remained undeveloped or only slightly developed. As day length increased from February to June, ovarian and testicular development accelerated. This suggests that photoperiod acts as an early environmental cue initiating gonadal recrudescence.

In teleosts, photoperiod is a reliable annual signal. The gradual increase in day length is detected by the retina, pineal gland and deep brain photoreceptors, influencing neuroendocrine pathways through melatonin secretion. In *L. guntea*, increasing photoperiod during the pre-spawning phase most likely stimulated hypothalamo-pituitary-gonadal activity, enhancing gonadotropin secretion and gonadal maturation.

Temperature and reproductive activity

Water temperature also played an important role in reproductive regulation. During cooler months, gonadal activity remained low, whereas increasing temperature from March onward coincided with progressive maturation. A rise in temperature from 18.1°C in January to 29.0°C in June was associated with rapid gonadal development and peak spawning.

Temperature influences metabolism, endocrine secretion, vitellogenesis, spermatogenesis, ovulation and spawning behaviour. In the present study, the optimum reproductive period was associated with temperatures between 28.3°C and

29.0°C. Although warmer temperature promotes maturation, excessively high temperature may negatively affect reproductive success by causing stress and reducing gamete quality.

Combined effect of photoperiod and temperature

The reproductive cycle of *L. guntea* was influenced by the combined action of photoperiod and temperature rather than by either factor alone. Increasing photoperiod appeared to initiate gonadal development, while rising temperature accelerated maturation and spawning. Thus, the onset of breeding was synchronized with favourable seasonal conditions.

Table 2. Probable influence of photoperiod and temperature on reproductive cycle

Environmental condition	Gonadal response	Interpretation
Short photoperiod + low temperature	Small gonads, low GSI	Resting phase
Increasing photoperiod + moderate temperature	Gonadal recrudescence	Preparatory phase
Long photoperiod + warm temperature	High GSI, mature gonads	Breeding / spawning phase
Long photoperiod + excessive temperature	Possible stress response	Reduced reproductive success
Decreasing photoperiod + falling temperature	Gonadal regression	Post-spawning / resting phase

Gonadosomatic index (GSI)

The gonadosomatic index was low during the resting phase and increased progressively during the preparatory and maturing phases. Peak values were recorded in **June**, confirming that this month represented the height of reproductive activity. Female GSI values were consistently higher than male values, reflecting the greater gonadal mass due to yolk deposition in the ovary.

Table 3. Monthly mean GSI of *L. guntea* (Mean ± SD)

Month	Male GSI	Female GSI	Interpretation
January	0.21 ± 0.06	0.52 ± 0.12	Resting phase
February	0.26 ± 0.07	0.71 ± 0.14	Early maturing
March	0.42 ± 0.11	1.23 ± 0.20	Maturing
April	0.88 ± 0.18	2.91 ± 0.36	Advanced maturing
May	1.52 ± 0.21	5.48 ± 0.62	Mature
June	2.38 ± 0.27	7.86 ± 0.71	Peak spawning
July	1.64 ± 0.22	4.96 ± 0.58	Spawning / spent
August	0.76 ± 0.16	2.28 ± 0.34	Spent
September	0.38 ± 0.10	1.18 ± 0.22	Recovery
October	0.24 ± 0.06	0.63 ± 0.13	Resting
November	0.19 ± 0.05	0.46 ± 0.09	Resting
December	0.18 ± 0.05	0.44 ± 0.09	Resting

The progressive rise in female GSI from January to June reflects ovarian growth and yolk accumulation. Male GSI showed a similar trend, though values were lower throughout the year. Following spawning, a sharp fall in GSI was observed in July–August, indicating gamete release and gonadal regression.

Gonadal maturity stages

Histological examination confirmed the seasonal reproductive cycle. During the immature phase, ovaries contained mainly oogonia and early perinucleolar oocytes. In the maturing phase, yolk vesicle and vitellogenic oocytes became prominent. Mature ovaries were characterized by large yolk-laden oocytes with well-defined nuclei. During spawning, ripe ova dominated the ovarian tissue, while spent ovaries contained post-ovulatory follicles and residual oocytes.

In males, immature testes contained spermatogonia, while maturing testes showed abundant spermatocytes and spermatids. Mature testes were packed with spermatozoa. After spawning, the testes became reduced and contained fewer spermatozoa.

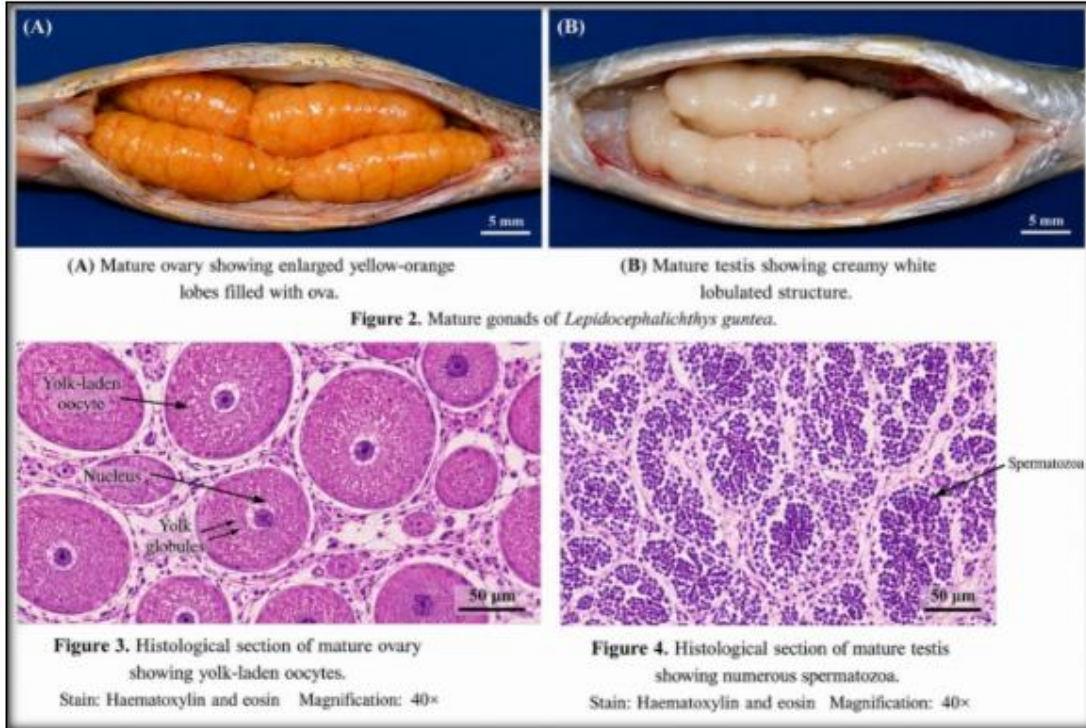


Table 4. Gonadal maturity stages of *L. guntea*

Stage	Ovary	Testis	Reproductive meaning
Immature	Thin, transparent, oogonia present	Thin, whitish, spermatogonia present	Resting phase
Maturing	Enlarged, yellowish, developing oocytes	Enlarged, spermatocytes present	Gonadal development
Mature	Large, yolky ova visible	Creamy white, spermatozoa abundant	Ready for spawning
Spawning	Ripe ova dominant	Active sperm release	Breeding phase
Spent	Flaccid, residual ova	Reduced, fewer sperm	Post-spawning phase

Fecundity

Fecundity increased with body length, body weight and ovary weight. Larger females produced more ova than smaller females, indicating a positive relationship between body size and reproductive output.

Table 5. Fecundity of *L. guntea*

Fish length (cm)	Ovary weight (g)	No. of ova (Mean ± SD)	Fecundity range
6.0 – 7.0	0.38 – 0.62	512 ± 64	420 – 610
7.1 – 8.0	0.63 – 0.94	836 ± 92	690 – 980
8.1 – 9.0	0.95 – 1.42	1,238 ± 126	1,050 – 1,470
9.1 – 10.0	1.43 – 1.98	1,742 ± 156	1,480 – 2,040
10.1 – 11.0	1.99 – 2.56	2,148 ± 184	1,820 – 2,480

The increase in fecundity with increasing fish length and ovary weight indicates that larger females contribute more substantially to the reproductive potential of the population.

Relationship between environmental factors and spawning season

The spawning season of *L. guntea* coincided with favourable photothermal conditions, particularly during May–June. Increasing day length initiated gonadal development, and warm temperature promoted final maturation. Rainfall may also have acted as a supportive cue by increasing water level and food availability, thereby providing favourable conditions for egg development and larval survival.

V. DISCUSSION

The present study demonstrates that the reproductive cycle of *Lepidocephalichthys guntea* is closely linked to seasonal changes in photoperiod and temperature. The annual pattern of gonadal maturation suggests that this species is a seasonal breeder. Such synchronization ensures that spawning occurs when environmental conditions favour embryonic development, larval survival and juvenile recruitment.

Photoperiod acts as an anticipatory signal, preparing the reproductive system through neuroendocrine activation. Temperature, on the other hand, appears to accelerate gametogenesis and final maturation. In *L. guntea*, the reproductive cycle likely begins with increasing day length in late winter and early spring, culminating in peak spawning during the warm, long-day conditions of early monsoon.

The GSI data and gonadal histology strongly support this conclusion. Female GSI rose from 0.52 in January to 7.86 in June, while male GSI increased from 0.21 to 2.38 over the same period. Histological sections demonstrated progressive maturation of oocytes and spermatozoa, followed by regression after spawning. These findings confirm that the period from May to July represents the principal reproductive season, with June as the peak spawning month.

The reproductive strategy of *L. guntea* appears well adapted to seasonal freshwater habitats. As a small benthic loach, it benefits from spawning during the warmer, wetter months when water volume, dissolved nutrients and larval food resources are more abundant. Therefore, reproductive activity in this fish should be regarded as the outcome of multiple environmental cues, with photoperiod and temperature serving as the primary regulators.

From a fisheries and conservation perspective, the present findings are important. Knowledge of the exact breeding period can help in formulating management measures such as protection of brood fish during the peak spawning months and development of captive breeding protocols. Manipulation of photoperiod and temperature under controlled conditions may also be useful for induced maturation and seed production.

VI. CONCLUSION

The present study indicates that photoperiod and temperature play important roles in regulating the reproductive activity of *Lepidocephalichthys guntea*. The species shows seasonal gonadal development, with low reproductive activity during unfavourable months and increased activity during months of longer day length and warmer temperature. The highest GSI, mature gonads and active gametogenesis were observed during the breeding season.

Photoperiod appears to act as a seasonal cue that initiates reproductive preparation, while temperature influences the rate of gonadal development and final maturation. The combined effect of long photoperiod and favourable temperature appears to be essential for peak reproductive activity. Rainfall, water level and food availability may also support spawning success.

The findings are useful for understanding the reproductive ecology of *L. guntea*. They may also help in planning conservation measures, regulating fishing during breeding months and developing captive breeding protocols. Future studies should include controlled laboratory experiments on different photoperiod-temperature combinations, hormonal assays and detailed gonadal histology to confirm the exact mechanism of environmental regulation.

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