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Gonad histology and some reproductive parameters of the big mouth Lotak, *Cyprinion macrostomum* (Cyprinidae) in the Little Zab River, Tigris River drainage, Iran

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Abstract

The reproductive characteristics of *Cyprinion macrostomum* in the Little Zab River, Iran, were investigated by monthly collections from November 2021 to September 2022. A total of 175 specimens were collected and transferred to the laboratory, where biometric measurements were performed. For the histological studies, the gonads were fixed in 10% buffered formalin, hydrated, and embedded in paraffin. They were then sectioned transversely at a thickness of 5-7 µm and stained with Hematoxylin–Eosin. Based on the results, the sex ratio was 1M:1.16F. The age groups for males and females were 0-4 years. The maximum length (TL) and weight of males were 16.02 cm and 53.7 g, and those of females were 16.24 cm and 60.1 g. The average condition factor was 1.23 and 1.21 for males and females, respectively. The minimum and maximum absolute fecundity were 1250 and 21857 (9611±6539). The mean of the gonadosomatic index (GSI) was 1.46±1.36 in males and 1.60±1.18 in females. The GSI showed significant differences in different months in males, but there was no significant difference in females. The maximum value of GSI was observed in July for females and in March for males. According to histological studies, five stages of sexual maturity were observed, including immature, maturing, ripening, ripe, and spent. Based on GSI values, egg diameter, and histological studies, this species has a relatively long spawning period from late March to early July. Our results indicated that the ovary in *C. macrostomum* is group Asynchronous.

Keywords: Histology, Egg diameter, Fecundity, GSI, Tigris

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Introduction

Studies on the reproductive biology of fish are crucial and a basic requirement for planning better conservation and management strategies for fishery resources (Grandcourt *et al*., 2009; Poorbagher *et al*., 2025), examining basic life history, and evaluating the impacts of environmental factors on the dynamics of fish populations (Schlosser, 1990). Studies on reproduction biology have been a popular topic in the last decades, providing valuable information for selecting fish candidates for diversification in the aquaculture industry (Muchlisin, 2013). Important aspects of fish reproductive biology that must be determined for effective management strategies include the follicle development process, reproductive cycle, and timing of spawning. The usefulness and importance of histological techniques in reproductive studies have been widely illustrated for fish species (West, 1990). Histology offers a powerful tool for reproductive studies and is routinely used for sex verification, assessing reproductive phases, or quantifying atresia (Blazer, 2002).

There are approximately 292 fish species in the inland waters of Iran, belonging to 36 families, of which 102 species are endemic, 29 species exotic, and others native (Eagderi *et al*., 2022). Cyprinidae has the most diverse family of Iranian freshwater fishes, with 74 species (25.34%) (Mouludi-Saleh *et al*., 2024). Among them is the genus *Cyprinion* (Cyprinidae), comprising nine valid species, of which five are reported in Iranian inland waters. Three species (*C. kais*, *C. macrostomum*, and *C. tenuiradius*) are distributed in the Iranian part of the Persian Gulf basin. *Cyprinion kais* and *C. macrostomum*are are distributed in the Tigris River drainage in Iran, Iraq, Turkey, and Syria. *Cyprinion macrostomus*, known as Lotak-e Dahan Bozorg (Big Mouth Lotak) in Iran, is an economically valuable species for sport fishing and is also consumed by locals as a protein source (Nasri *et al*., 2015; Ghafouri *et al*., 2025a).

Based on the backgrounds mentioned above, this study aimed to examine some reproductive characteristics of *C. macrostomum*, collected from the Little Zab River, a tributary of the Tigris River drainage, including condition factor, sex ratio, gonadosomatic and hepatosomatic indices, ovum diameter, absolute fecundity, and relative fecundity, as well as the gonad histology. The results of this study will provide the basic data for the management and conservation of *C. macrostomumas*, as well as for its artificial breeding, a candidate for aquaculture.

Material and methods

Study area and sampling

The Little Zab River originates in northwest Iran and joins the Tigris River in the Kurdistan region of Iraq. It is approximately 400 km long and drains an area of about 22,000 km^2. For this study, fish were collected monthly from November 2021 to October 2022 using an electrofishing device (Samus Mp

750) from the Iranian part of the Little Zab River, a tributary of the Tigris River drainage, West Azerbaijan Province, Iran (Ghafouri *et al*., 2025b). The total body weight (W) of the fresh specimens was measured using an electronic balance to the nearest 0.001 g, and their total length (TL) was measured using a calliper to the nearest 0.1 cm. Then, the specimens were preserved in 10% buffered formalin after anaesthesia using a clove oil solution. After transferring the specimens to the laboratory, they were then put in 70% ethanol.

Calculating reproductive parameters

The chi-square test was used to assess deviation from a 50:50 sex ratio (Robards *et al*., 1999). The condition factor (K) was calculated monthly using the formula $K = (W/L^3) \times 100$, where W and L are the total weight and total length, respectively (Biswas, 1993). The fish were dissected laterally, and sex was ascertained macroscopically. To examine the monthly changes in the gonads for estimating the spawning season, the gonadosomatic index (GSI) was used, calculated by the formula GSI = (weight of gonads/weight of fish) × 100 (Nikolsky, 1963). The hepatosomatic index was calculated as HIS = (HW/TW) × 100, where HW is the HP weight and TW is the total weight of the fish. The ovaries were fixed in a 10% formalin solution to determine the ovum diameter. The diameters of ova from all of the subsamples were measured using a stereomicroscope that was fitted with an ocular micrometer. The fecundity was defined as the number of ripe and maturing oocytes in the ovaries prior to spawning, and the absolute fecundity (F) was measured. The relative fecundity (number of ova per unit of body weight) was also estimated based on Bagenal (1967). Maturity stages were determined following Brown-Peterson *et al*. (2011). Reproductive seasonality was determined by examination of the monthly changes in the gonadosomatic index. One-way ANOVA was used to analyze differences in means of GSI% and egg diameter of fish at a 95% probability level, followed by the Duncan post hoc test in SPSS (version 19).

Histological examination

Subsamples were taken from the anterior, middle, and posterior regions of the ovary for histological examination. Then, the samples of ovaries and testes were processed using standard histological techniques (Humason, 1979; Eagderi *et al*., 2013). All tissue blocks were sectioned at 5-6 µm and stained with hematoxylin and eosin. Histological slides were examined under a light microscope, and their images were captured. The monthly alternation of the ovaries was analyzed based on Eagderi *et al*. (2013), using the occupied areas for each developmental stage of the oocytes.

Results

The length and weight of females ranged from 4.81 to 16.24 cm (11.92±1.98) and 1.20 to 60.1 (22.78±11.49) g, and those of males from 4.13 to 16.02 cm

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(10.96±3.07) and 0.8 to 53.7 (20.39±13.97) g, respectively (Table 1). Based on the results, the 12-16 cm length group was the most frequent in both sexes (Fig. 1).

Table 1: The range and average of the total length and total weight of *Cyprinion macrostomum* collected from the Little Zab River.

Sex	Number	Total length (cm)		Total weight (g))	
		Range	Average (±SD)	Range	Average (±SD)
Female	94	4.81-16.24	11.92±1.98	1.2-60.1	22.78±11.49
Male	81	4.13-16.02	10.96±3.07	0.8-53.7	20.39±13.97
Total	175	4.13-16.24	11.48-2.58	0.8-60.1	21.68-12.72

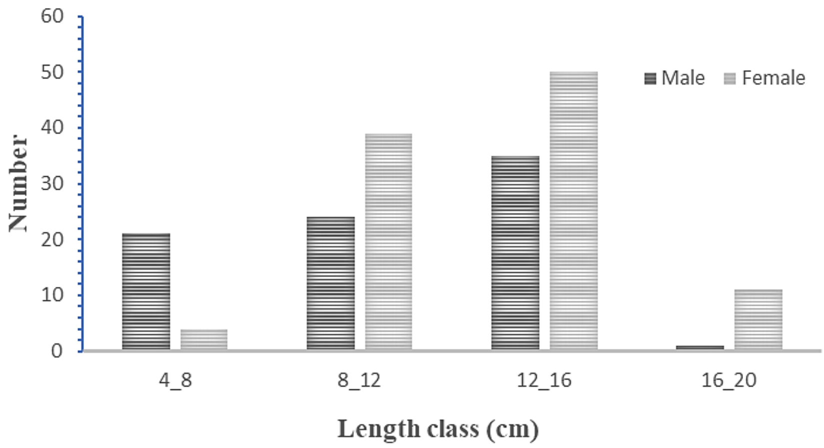

Figure 1: Length frequencies of *Cyprinion macrostomum* collected from the Little Zab River.

The average condition factor was 1.23±0.17 and 1.21±0.15 for males and females, respectively. The monthly changes of the condition factor showed no significant difference in males between the months (P>0.05) (Fig. 2A). There was a significant difference in females between the months of the year (p<0.05) (Fig. 2B).

The sex ratio of male to female was 1M:1.16F which was no significant difference from the expected 1:1 ratio (p>0.05). The oldest males and females were 4 years old, representing the dominant age group 1 in both sexes (Fig. 3).

Significant differences were found in the males' GSI (p<0.05) across different months, but there was no significant difference between the females in different months (p>0.05). GSI in females started to increase from April to July and decreased in August (Fig. 4A). The GSI value for males had a peak in March and it began to decline in June (Fig. 4B). Thus, the reproductive period for this species in the Little Zab River was estimated from March to July when GSI is considerably higher. The lowest hepatosomatic index was recorded in October, and the highest in February, with a negative correlation with GSI (Fig. 5).

The egg diameter ranged between

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0.05-1.20 (0.62±0.30) mm and was significantly different during the months of the year ($p<0.05$). The egg diameter started to increase in April and reached its highest value in June. The highest mean egg diameter (0.59±0.25 mm) was observed in June, and the lowest value in August (0.09±0.03 mm) (Fig. 6). The highest egg diameter in July was 1.20 mm.

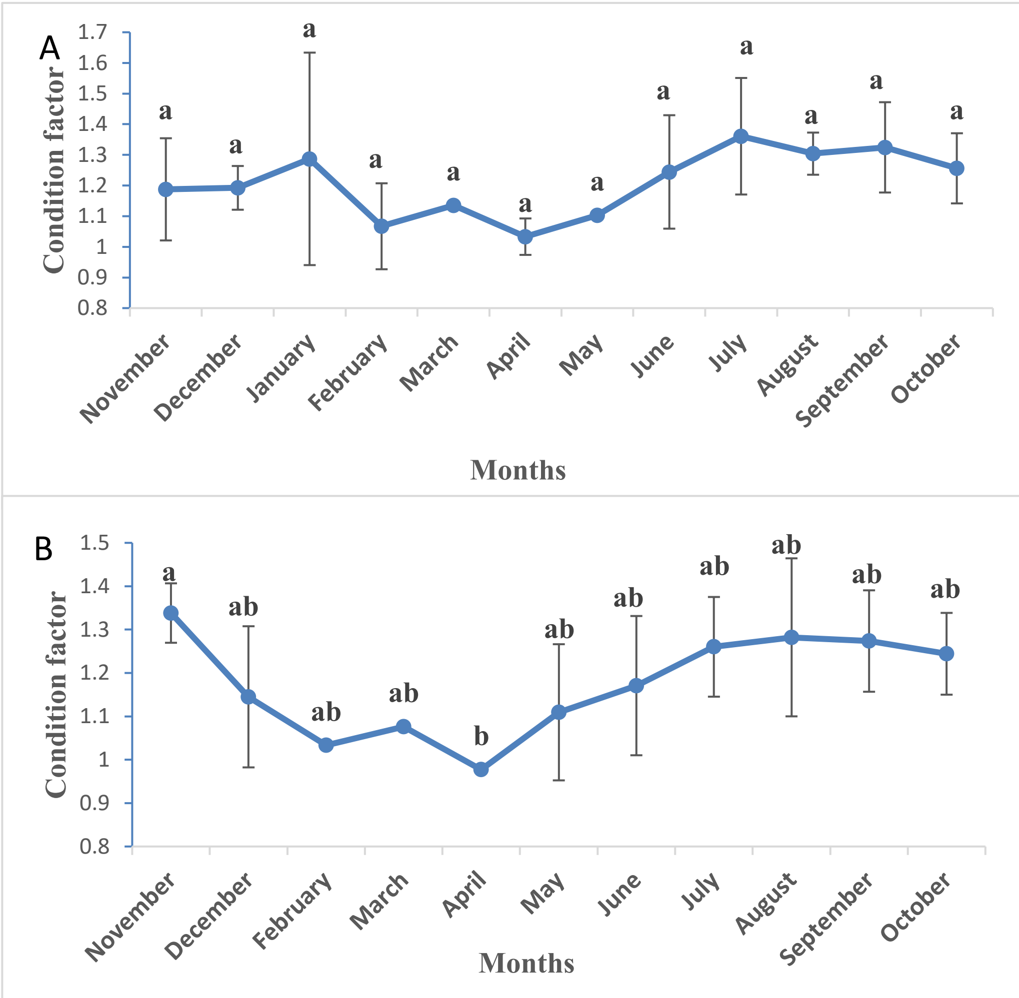

Figure 2: Mean condition factor and standard error in different months for (A) males and (B) females of *Cyprinion macrostomum* in the Little Zab River (Different letters show significantly different at $p \leq 0.05$).

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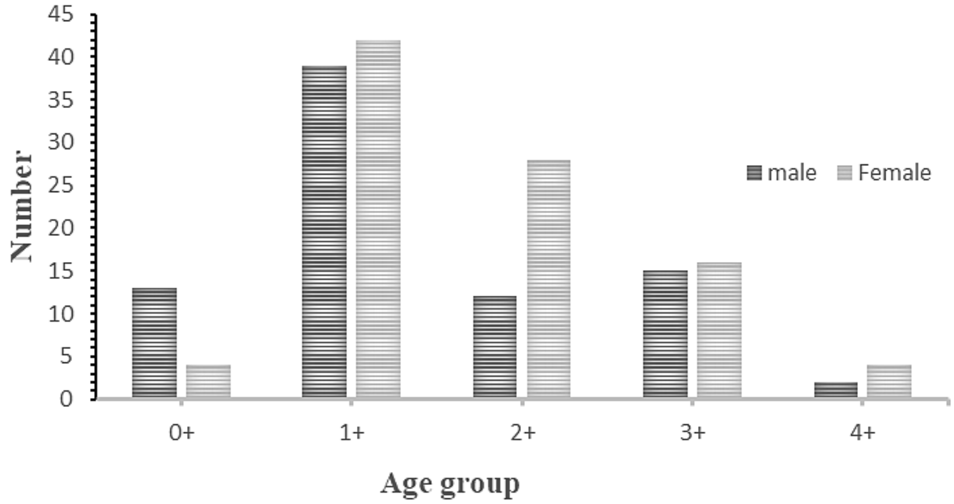

Figure 3: Age frequencies of *Cyprinion macrostomum* in the Little Zab River.

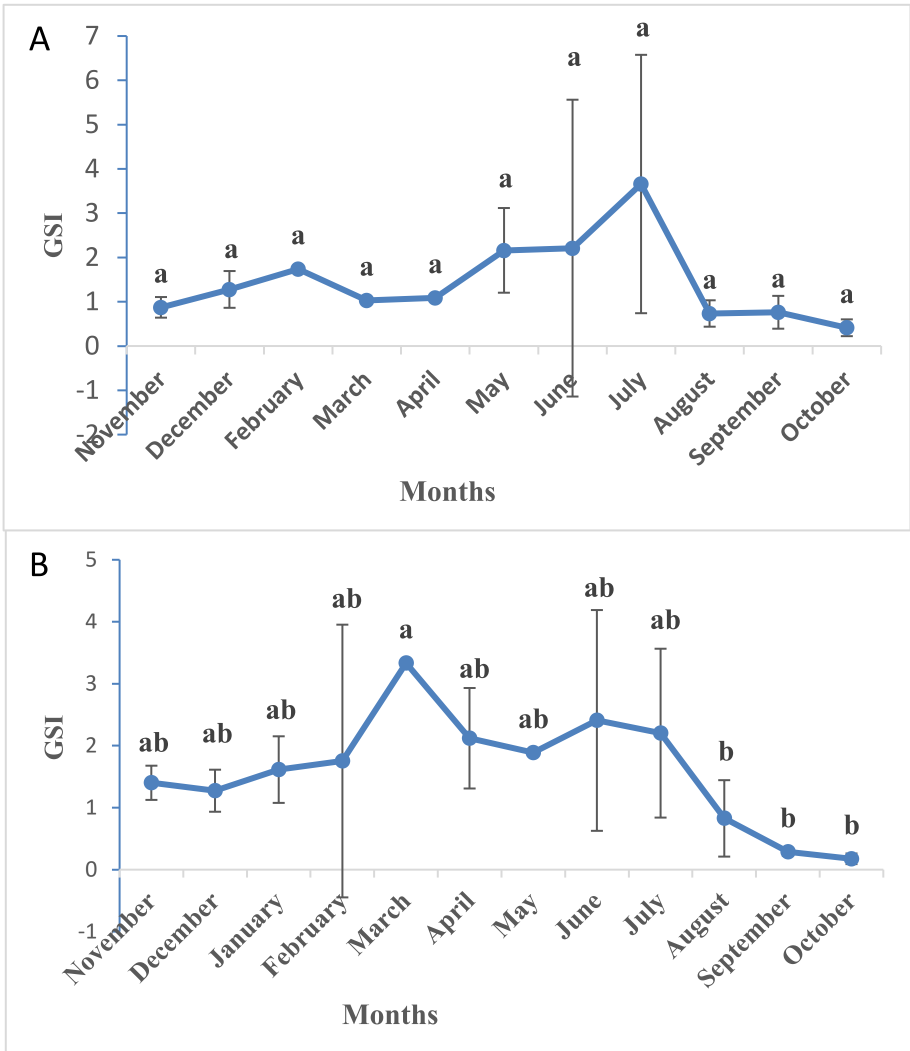

Figure 4: Variation of mean gonadosomatic index (GSI) of (A) female and (B) male *Cyprinion macrostomum* in the Little Zab River in different months.

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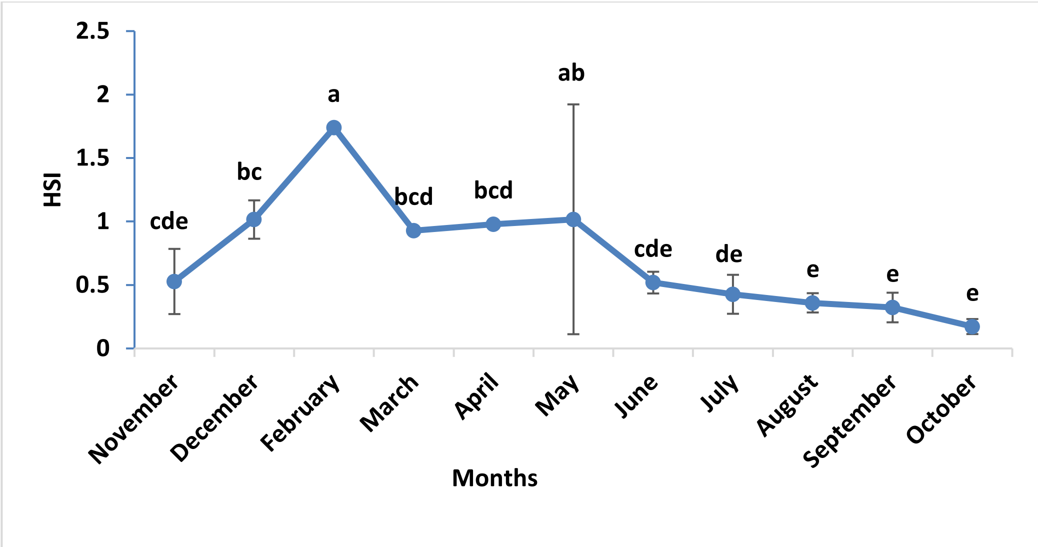

Figure 5: Variation of the mean hepatosomatic index (HSI) of the female *Cyprinion macrostomum* from the Little Zab River in different months.

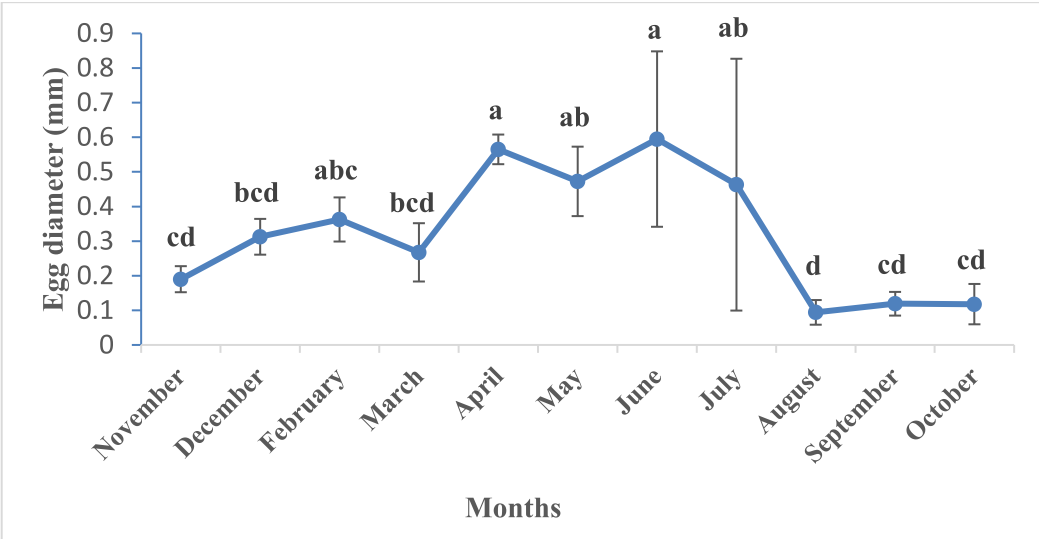

Figure 6: Variation of mean ova diameter (mm) in the female *Cyprinion macrostomum* from the Little Zab River in different months.

The absolute fecundity during the spawning period was 1250-21857 (6611±6539) eggs, and relative fecundity was 84-967 (329±277) eggs/g of body weight. The relationships between the fecundity-ovary weight, length, and body weight were as: F=2086.4X0.08(r^2=0.0031), F=.007L5.48 (r^2= 0.21), F= 6.847W2.32 (r^2=0.46), respectively (Figs. 7 to 9).

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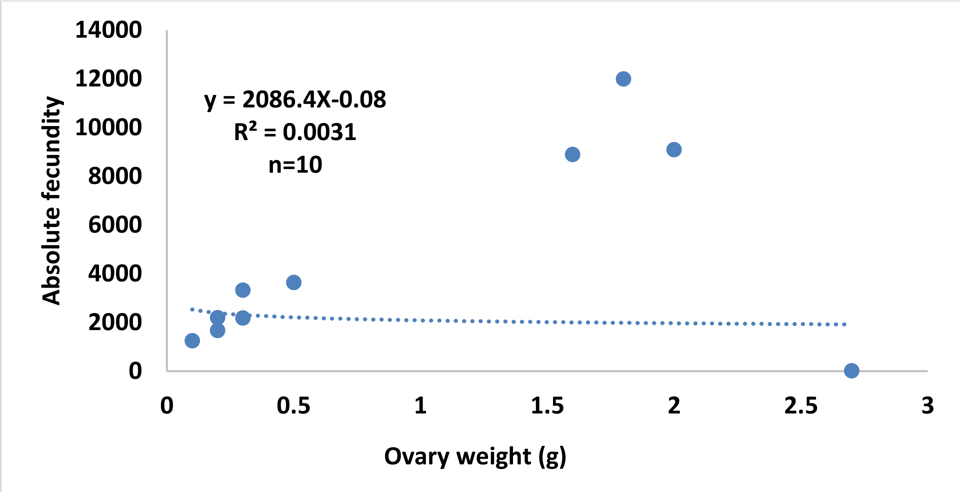

Figure 7*:* Absolute fecundity-ovary weight relationships in *Cyprinion macrostomum* in the little Zab River.

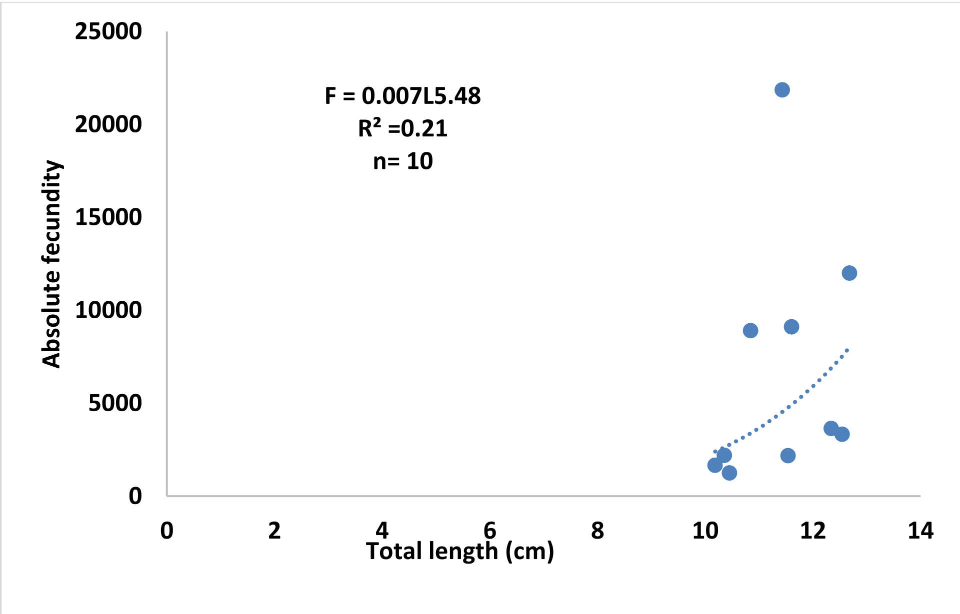

Figure 8*:* Absolute fecundity-length relationships in *Cyprinion macrostomum* in the Little Zab River.

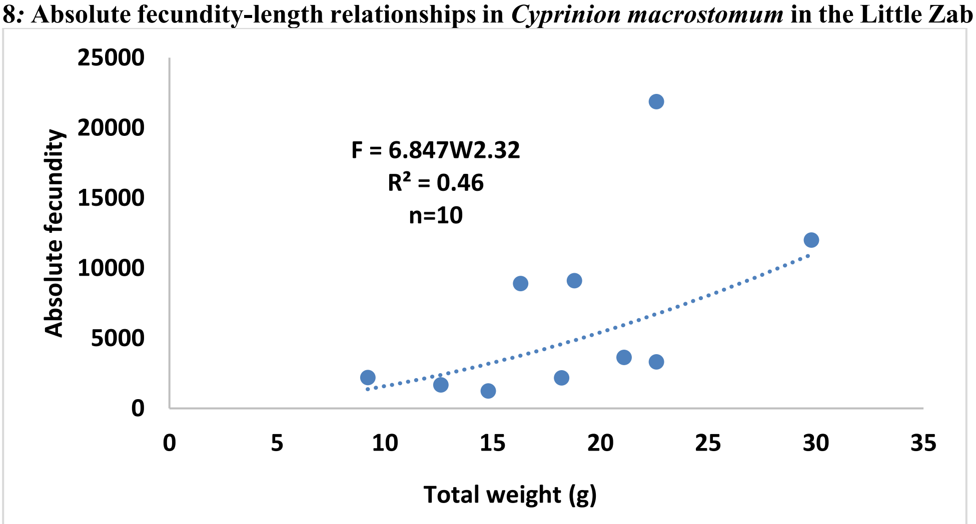

Figure 9*:* Absolute fecundity-weight relationships in *Cyprinion macrostomum* in the Little Zab River.

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According to morphological and histological examinations, five stages of sexual maturity were observed, including immature, maturing, ripening, ripe, and spent. Based on the histological results, the oocyte development of this fish species was divided into five stages, including:

(1) *Oogonia*: In this stage, small oocytes are characterized by a relatively large nucleus. The cytoplasm is stained with hematoxylin, and the follicles are visible in the form of batches (Fig. 10A). (2) *Pre-yolk oogenesis stage*: Developing oocytes have a large nucleus and cytoplasm stained with hematoxylin. Some lateral nuclei are observed in the oocyte (Fig. 10B). (3) *vitellogenesis*: In this stage, developing oocytes are visible in the ovary. Yolk vesicles expand in the cytoplasm, forming one or more layers and occupying a large part of the cytoplasm. The nucleus of the yolk has become larger and occupies a greater volume than the cytoplasm. At this stage, the follicles are visible in hematoxylin stain (Fig. 10C). (4) *Ovulating*: The mature follicles are visible before ovulation. Yolk vesicles are observed scattered in the cytoplasm (Fig. 10D). (5) *Ovulation*: In this stage, the ovulated oocytes can be seen. A large number of lateral vesicles and yolk can be seen on the internal surface of the oocyte (Fig. 10E).

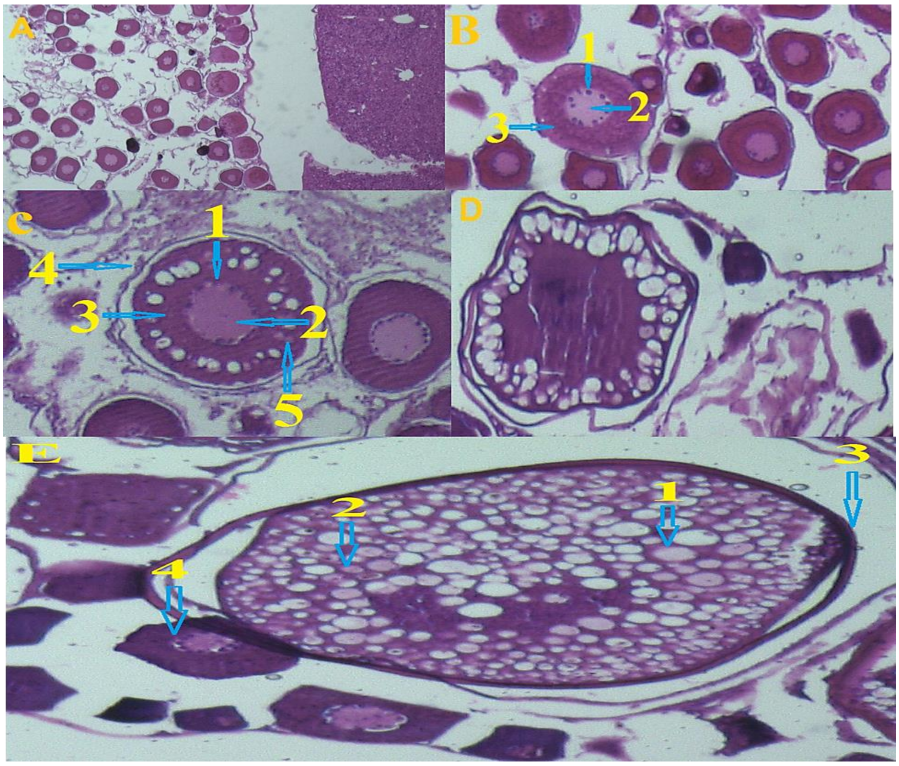

Figure 10: Stages of oocyte maturation A: A view of the cross-section of the ovary of the *Cyprinion macrostomum* in stage I of development maturation B: Stage II of development maturation 1- Nucleolus, 2- Nucleus 3- Cytoplasm C: Stage III 1- Nucleolus, 2- Nucleus, 3- Cytoplasm, 4- oocyte membrane, 5- Oocyte membrane vesicles in the preparation phase, nuclei are observed near the nuclear membrane D: stage IV of development maturation E: 1- mature oocyte stage 5 2- yolk vacuoles 3- follicular layer 4- stage I immature oocyte and II maturation.

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The monthly alternation of oocyte composition (Stages I, II, III, IV, and V) in November, December, February, April, May, June, July, August, September, and October is presented in Figure 11 and Table 2. Stage I was the most frequent in August and September. Most ovaries exhibited stage II maturation in various months. Stages III and IV were the most frequent from April to July, and stage V was the most frequent in June and July.

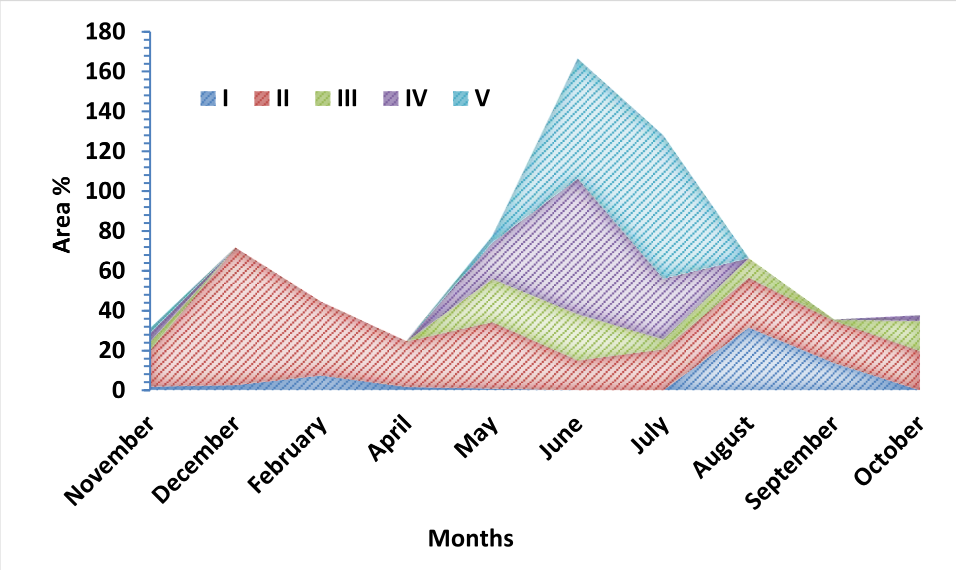

Figure 11: Monthly values of occupied areas for each developmental stage of the oocyte (II = Stage II, III = Stage III, IV = Stage IV, V = Stage V, and VI= Ovulation Stage VI).

Table 2: The composition changes of ovaries during one-year sampling (Oocyte maturation stages (I–V), present (+), and not present (-).

Months	Stages				
	I	II	III	IV	V
November	+	+	+	+	+
December	+	+	-	-	-
February	+	+	-	-	-
April	+	+	-	-	-
May	+	+	+	+	+
June	-	+	+	+	+
July	-	+	+	+	+
August	+	+	+	-	-
September	+	+	+	-	-
October	-	+	+	+	-

The results of the histological examinations revealed that the reproductive period of *C. macrostomum* is relatively long, lasting from May to July, which is consistent with the results of the gonadosomatic index and monthly changes in egg diameter. The results also showed that in most months of the year, and even during the reproductive season, stage I and II immature oocytes were present in the ovary, indicating that the reproductive period of this species is prolonged and its spawning pattern is asynchronous.

Discussion

Although males outnumbered females, the results of the current work revealed no significant differences in the sex ratio. The ratio of males to females was 1M:1.16F. This finding was in agreement with the findings of Uckun and Gokce (2015) in Karakaya Dam

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Lake. The sex ratio of *C. macrostomus* was 0.75:1 in the Dalaki River (Sedaghat and Hoseini, 2012), and it was 0.80:1 in the Murat River (Aydın *et al*., 2008). The sex ratio of fish populations varied based on the spawning season, life stage of the fish, spawning ground, and migration (Nikolsky, 1963; Bartulovic *et al*., 2004). The total length of *C. macrostomus* specimens ranged from 4.13 to 16.24 cm; in general, females had longer total lengths. In this study, the age distributions were between age groups 0^+ and IV, and the majority of specimens belonged to age group I for both sexes, indicating that the fish populations were young. The oldest specimens of *C. macrostomus* reported were 6 years old in the Murat River (Aydın *et al*., 2008).

Gonad development, as determined by the gonadosomatic index (GSI%), was at maximum level for females (3.65±2.91) in July and males (3.33±0.0) in March. The GSI reached its maximum value in July and gradually declined in early August. Based on GSI, the reproductive activity of this species in Karakaya Dam Lake was between June and August (Uckun and Gokce, 2015). The examined gonad indices in males and females fluctuated and reached a peak in May, with reproduction lasting until August, when the water temperature ranged from 16 to 24°C (Faghani Langroudi and Mousavi Sabet, 2018). In fish species that spawn in late spring and summer, these indices remain low in winter and then rise sharply just before spawning (Wootton, 1979). Al-Rudainy (2008) reported the spawning season for *C. macrostomum* of May and June in Iraq on gravel beds in shallow water with fast currents. Additionally, Ünlü (2006) observed the spawning season of *C. kais* in the Turkish Tigris River, which occurs over sand, stones, and gravel, in May and June. The GSI and HSI are used as biological indicators to determine the fish spawning season (Hunter and Macewicz, 2001). The HSI index for females is more important because vitellogenesis, which is essential for egg production, occurs in the liver. Typically, in many fish species, the GSI reaches its highest level at the peak of spawning, while the HSI is at its lowest (Keivany and Mohammadiyani, 2021). Based on the results of the current study, the lowest value of HSI was observed in females in October, while the highest value was observed in February. Variation in the HSI might contribute to the vitellogenesis in the liver. The monthly changes in the HSI index were lower in males than in females.

The study of egg diameter is essential for understanding the nature of reproduction during the spawning season, including whether the fish spawns multiple times a year, to determine spawning frequency, and to determine the duration of the spawning season. Ripe eggs in *C. macrostomum* in the present study varied in mean diameter between 0.05 and 1.2 mm. The monthly changes in egg diameter showed that it was the highest in June and the lowest in August. The oocyte size recorded in the present study was 0.2 to 1.7 mm in diameter (Faghani

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Langroudi and Mousavi Sabet, 2018). The maximum egg diameters reported for *C. macrostomum* and *C. watsoni* are 1.4 mm and 1.2 mm, respectively (Coad, 2016). Also, Esmaeili and Gholamifard (2012) recorded oocyte diameters from 0.1 to 1.3 mm in *C. tenuiradius* from the Rudbal River, southern Iran. The size of eggs can vary from one population to another or even from year to year, depending on factors such as temperature and food availability. The absolute fecundity of 10 females determined during the spawning period was 1250-21857 (6611±6539) eggs, and relative fecundity was 84-967 (329±277) eggs/g of body weight. Coad (2016) reported up to 150 eggs from the Genow hot spring in *C. watsoni*. Faghani Langroudi and Mousavi Sabet (2018) reported that the averages of absolute and relative fecundity (relative to body weight) were 3642.51 eggs (SD±1219.92) and 55.04 (SD±14.12) per gram of body weight, respectively. The differences in fecundity found among species may reflect the varying environmental conditions of their habitats, food availability, female size and age, and life history strategies (Wootton, 1990).

Histological studies provide the most reliable and objective data in gonad staging, and they are also significant in determining the maturation cycle. The histological analysis of the ovary in *C. macrostomum* has provided the seasonal gonad differentiation of this species. Based on the results, in most cases, the resting, previtellogenic, and vitellogenic stages were visible from the spawning season to the following March. Additionally, in most specimens collected in May, June, and July, the main phase of nuclear migration and hydration was observed, and in some instances, wrinkled follicles appeared. However, the maximum weight of the gonads and GSI, as well as the maximum diameter of the eggs, were observed in the same month. The hydration stage in fish is known as an indicator of readiness for spawning (Kiani *et al*., 2021). During the rest period, cells had a large, single nucleus with a high nucleus-to-cytoplasm ratio. The nucleus contained one to four nucleoli, and the cytoplasm was strongly basophilic. The number and size of the nucleolus increased in the perinucleolar stage and migrated to the nuclear margin.

Based on ovarian follicle formation, the first stage of oocyte growth occurs during the peripheral nucleolus stage, coinciding with the covering of oocytes by follicular cells. Hepatic yolk uptake and storage of the oocyte cytoplasm mark the beginning of vitellogenesis. The main phase of oocyte growth occurs during vitellogenesis (Thome *et al*., 2012). As yolk begins to accumulate, vesicles are visible in the marginal cytoplasm, and toward the end of vitellogenesis, they increase in number and size. The present study indicates that this species has a relatively long reproductive period in the Zab River, and histological data support other indices. Our findings suggest that the ovary in *C. macrostomum* is group asynchronous.

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