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Reproductive biology of the European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) in the Turkish Aegean Sea

by

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Abstract

The present study investigated the reproductive biology of European anchovies obtained seasonally from fishermen in İzmir Bay (Aegean Sea). The total length range of the investigated individuals was 9.0 – 15.7 cm. Of the 750 samples in which sex was determined, 34% were males, and 66% were females, resulting in a female-to-male ratio of 1.91:1. The first maturation length was determined to be 10.47 cm for females and 9.95 cm for males. Based on the total values of the gonadosomatic index, it was concluded that the reproductive period of the species falls in the summer season. The batch fecundity of the species ranged from 2.123 to 6.951 oocytes, and the fecundity–length relationship was expressed as F = 1134.4TL – 10034 (R^2 = 0.84). Histological examination of the female ovaries revealed the presence of oocytes at different stages of development, indicating multiple spawning during the reproductive season.

Key words: European anchovy, *Engraulis encrasicolus*, batch fecundity, first length maturity, İzmir Bay, Aegean Sea

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1. Introduction

The family Engraulidae, which belongs to the order Clupeiformes, comprises 17 genera and 155 species occurring worldwide (Froese & Pauly 2023). Turkish waters are home to three species from three genera, including *Encrasicholina punctifer* and *Stolephorus insularis*, which are both alien species recently observed along the Mediterranean coasts of Türkiye. *Engraulis encrasicolus*, on the other hand, is a species of Atlanto-Mediterranean origin found in all seas surrounding Türkiye (Karataş et al. 2021). Its distribution extends from Scandinavia to West Africa along the eastern Atlantic coasts, encompassing the Mediterranean Sea, the Black Sea, and the Sea of Azov (Whitehead et al. 1988). Its main distribution areas within the Mediterranean Sea include the Catalan Sea (Gulf of Lion), the Adriatic Sea, and the northern Aegean Sea (Somarakis et al. 2004). Anchovy, a species that forms large schools in oceans and seas, prefers coastal areas for spawning, particularly lagoons and estuaries. It tends to migrate north and occupy surface waters during the summer months, while retreating to deeper regions in winter (Froese & Pauly 2023).

Like other fish species belonging to the order Clupeiformes, such as sardines and sprats, the European anchovy is a batch spawner, engaging in external fertilization without parental care (Alheit 1989). It exhibits significantly high fecundity, with the development of gametes depending on accumulated reserves and food availability during the spawning period (Hunter & Goldberg 1980). The European anchovy is characterized by rapid growth and a short life cycle. The attainment of sexual maturity is affected by ecological conditions and usually occurs at the end of the first year of life, although it may vary depending on food availability and temperature (Lisovenko & Andrianov 1996; Motos 1996; Nikolsky 1963; Blaxter 1969).

The European anchovy represents a crucial fishery resource for Mediterranean countries, with an average catch of 595,527 tons per year in 2022 (FAO 2022). It accounts for over 50% of the annual average production in Turkish waters, which can vary from year to year (TUIK 2023). Therefore, it is of paramount importance to identify and ensure the sustainability of its stocks. Previous studies on the reproduction of the European anchovy have been conducted in the Bay of Biscay, the Southern California Bight, the Black Sea, the Adriatic Sea, and the Atlantic coast, focusing on the reproductive period, gonad histology, spawning frequency, and other reproductive parameters investigated individually (Andréu 1950; Sanz & Uriarte 1989; Vega et al. 1990; Hunter & Goldberg 1980; Hunter & Macewicz 1985; Lisovenko & Andrianov 1996; Motos 1996; Uriarte et al. 1996; Millán 1999; Sinovčić & Zorica 2006; El Qendouci et al. 2020). However, no studies have been conducted on the reproductive biology of this species in the Eastern Mediterranean basin, including Turkish waters.

Reproductive parameters serve as the basis for studies using the Daily Egg Production Method (DEPM), which in recent years has become a widely used and reliable method for fish stock assessment (e.g. Somarakis et al. 2004; Somarakis 2005). This research aims to determine all qualitative and quantitative reproductive parameters of the European anchovy distributed in the Aegean Sea of Türkiye. These parameters include size and sex composition, size at maturity, spawning period, type and number of spawning events, batch fecundity, and histological features of female ovaries during the reproductive period. The preliminary findings from this research will provide the basis for future stock assessment studies related to this species.

2. Materials and methods

2.1. Sample collection

Fish samples were collected seasonally from commercial fishermen operating in Izmir Bay in the Aegean Sea throughout 2020. Data were collected from the decks of medium-sized fishery vessels (17–22 m long, with 240–400 HP) using European anchovy purse seine nets (net length 255–550 m, net height 50–140 m). Following their collection, fish samples were swiftly transported to the laboratory.

2.2. Gonadosomatic index and first maturity length

Samples brought to the laboratory were measured for total length (TL, cm), body weight (BW, g), and gonad weight (GW, g). Measurements were taken using a ruler with a 0.1 cm interval and a digital scale with an accuracy of 0.1 g. The specimens were then dissected to determine their sex, and the maturity stages of the gonads were assessed macroscopically following the criteria established by Holden and Raitt (1974; Table 1).

The gonadosomatic index (GSI) value was calculated using the formula:

$$GSI = \frac{Gw}{Bw} \times 100$$

where Gw is the gonad weight and Bw is the body weight (Bagenal & Tesch 1978). The initial maturity length for both sexes was determined using the formula:

$$P = \frac{1}{1 + exp[-rm(L - Lm50)]} \quad \text{(King 1995).}$$

2.3. Fecundity

Subsamples (anterior, median, and posterior) accounting for 2–5% of the ovarian weight were taken from 50 ovaries to determine fecundity (Murua et al. 2003). Hydrated oocytes were counted using the gravimetric method (NOAA 1985). Total fecundity was calculated using the formula:

$$F = \frac{n \times G}{g} \quad \text{(Valladolid \& Przybylski 2008),}$$

and the relationship between fecundity and total length was determined. In each subsample, 100 eggs were randomly selected and their diameters were measured. The minimum, maximum and mean diameter values were determined.

2.4. Histology

Ovaries identified as mature during macroscopic examination were used to obtain sections for histological analysis. The samples were processed through a series of alcohol and paraffin embedding. Sections with a thickness of 5 – 7 μm were stained with hematoxylin-eosin (Correia et al. 2009). Diameters of approximately 100 developing, mature, and hydrated oocytes in the ovaries were measured under a microscope. In addition, post-ovulatory follicles (POF) were identified. The age of POFs was determined based on their morphological characteristics and regression over time (Parrish et al. 1986; Hunter & Macewicz 1985).

3. Results

3.1. Sex ratio

A total of 773 individuals were examined during the study, and 23 (3%) were of indeterminate sex. Among the 750 individuals with an identified sex, the range of total length was 9.0–15.7 cm (mean ± SD: 11.42 ± 1.28 cm), and the range of weight was 3.26–26.73 g (mean ± SD: 9.19 ± 3.62 g). The female-to-male ratio was 1.91:1. The chi-square test assessed the statistical difference between female and male individuals, revealing a significant difference ($\chi^2_{calc\ 19.73} > \chi^2_{table\ 3.841}$, $p \leq 0.05$).

3.2. Length at first maturity

The total length at first maturity was determined to be 10.47 cm for females and 9.95 cm for males (Fig. 1).

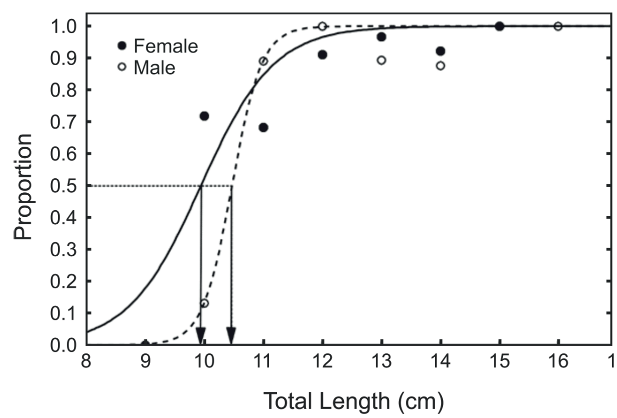

Figure 1

Logistic curve for estimating the size at first maturity (L_{50}) for both sexes

3.3. Gonadosomatic index

The highest values of the gonadosomatic index (GSI) were determined in the summer season in both

Table 1

Five-stage maturity scale for partial spawners (Holden & Raitt 1974)

Stage	State	Description
I	Immature	Ovary and testis approx. 1/3 of the total length of body cavity; ovary pinkish and translucent, testis whitish.
II	Maturing virgin and recovering spent	Ovary and testis approx. 1/2 of the total length of body cavity; ovary pinkish and translucent, testis whitish and more or less symmetrical.
III	Ripening	Ovary and testis are approx. 2/3 of the total length of body cavity; ovary pinkish-yellow with granular appearance, testis whitish-cream. No transparent or semi-transparent eggs visible.
IV	Ripe	Ovary and testis from approx. 2/3 of body cavity to the entire body cavity. Ovary orange-pink with large transparent, mature eggs visible; testes whitish-cream and soft.
V	Spent	Ovary and testis shrunken to approx. 1/2 of body cavity. Ovary and testis walls are lax. Ovary may appear darker or translucent and contain egg remnants. Testes are sagging.

females (GSI: 4.83; length: 11.38 cm) and males (GSI: 3.76; length: 10.51 cm). The lowest GSI values were found in the autumn season in both sexes (female GSI: 0.24; length: 11.90 cm; male GSI: 0.17; length: 11.08 cm; Fig. 2).

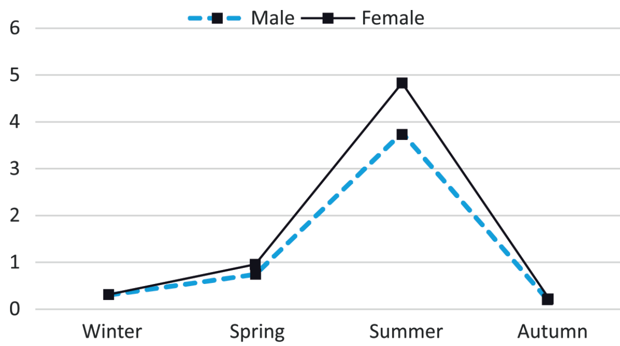

Figure 2

Seasonal values of the gonadosomatic index (GSI) for males and females of *Engraulis encrasicolus*

3.4. Maturity stages

As indicated by the gonadosomatic index, mature gonads (Stages 3–4) predominated in females and males during the reproductive season (summer). In the winter season, individuals with immature gonads (Stage 1) predominated (Fig. 3).

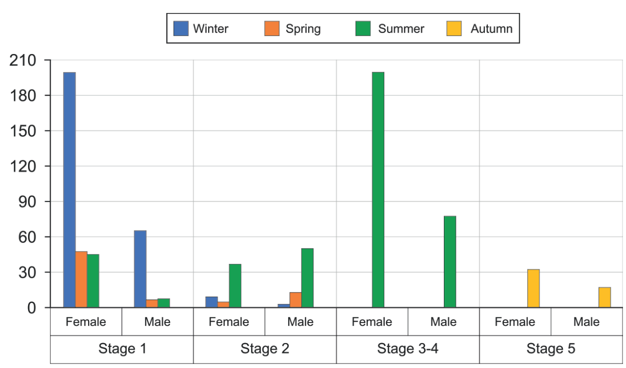

Figure 3

Sexual maturity stages of males and females of *Engraulis encrasicolus*

3.5. Fecundity

To determine the batch fecundity of the species, we examined 50 ovaries collected during the summer months. This analysis determined the minimum, maximum, and mean fecundity values as 2.123 (length: 10.6 cm) to 6.951 (length: 14.7 cm) oocytes, with an average of 4.383 ± 407. A strong linear relationship was found between total length and fecundity, expressed as F = 1134.4TL – 10034 (R^2 = 0.84; Fig. 4).

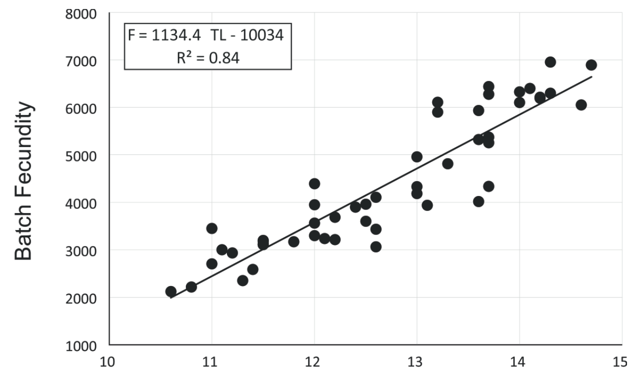

Figure 4

Relationship between mean fecundity and total length (TL) in *Engraulis encrasicolus*

Similarly, a positive linear relationship between fish weight and fecundity was observed as F = 348.26W – 393.12 (R^2 = 0.82; Fig. 5).

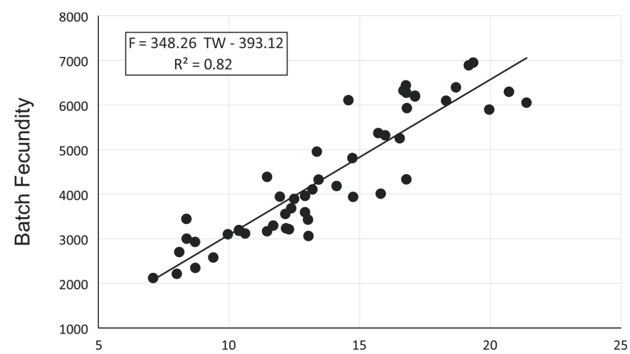

Figure 5

Relationship between mean fecundity and total weight (TW) in *Engraulis encrasicolus*

For each of the 50 ovaries used to determine fecundity, we randomly selected 50 hydrated oocytes and measured their diameters. The short-axis diameter ranged from 0.25 to 0.60 mm, while the long-axis diameter ranged from 1.05 to 1.26 mm (Fig. 6).

3.6. Histology

During the summer reproductive season, corresponding to the gonadosomatic index, we histologically examined a total of 93 ovaries in the mature stage from 157 female individuals (length: 9.0 – 15.7 cm). In addition to oocytes with completed vitellogenesis, we observed oocytes in

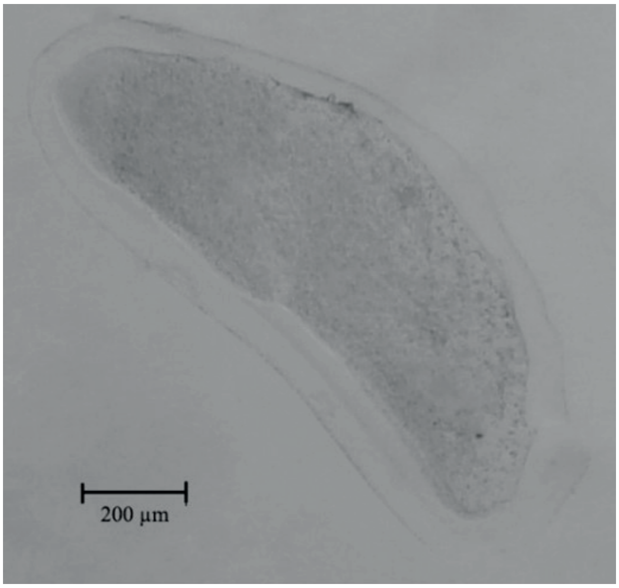

Figure 6

Hydrated oocyte in the ovary of *Engraulis encrasicolus*

the early vitellogenesis stage and inactive oocytes in reproduction. Furthermore, post-ovulatory follicles (POF) were detected in 43 ovaries, providing evidence of recent reproduction. Perinuclear oocytes (PO) with no yolk were polygonal in shape with a centrally located nucleus. The size of the oocytes was further increased due to the increase in the size of vitellogenic substances (Fig. 7c). Figure 7d shows the follicles after ovulation. Since anchovy is a batch spawner, it is possible to encounter oocytes at every stage in the ovary (Fig. 7). Analysis of the post-ovulatory follicles revealed that all follicles across all sections were one year old, indicating an interval equal to or longer than 24 h, but less than 48 h since ovulation. It is worth noting that the 50 ovaries used to determine fecundity were selected from ovaries without POF.

4. Discussion

Regarding the sex ratio, our study found that females predominated (F:M; 1.91:1), which is consistent with previous studies by Ünsal (1989), Özdamar et al. (1991), Mutlu (1996), Kayalı (1998), Mutlu (2000), Uçkun et al. (2005), Samsun et al. (2006), and Erdoğan Sağlam & Sağlam (2013). The dominance of female anchovies in these studies can be attributed to several factors, including the reduced ability of hydrated females to escape from the net, the vertical separation of females based on water column depth, and the preferential capture of female individuals with hydrated oocytes in purse seine nets. Furthermore, females may exhibit different horizontal distribution patterns depending on the area, with individuals carrying hydrated oocytes showing distinct distribution compared to those without (Alheit et al. 1984).

Based on the gonadosomatic index values, our study determined the summer season as the spawning season of the species. This finding is consistent with previous studies and the observation of eggs and larvae of the species in ichthyoplankton studies, which are more prominent in spring and summer, when water temperatures are higher (Table 2; Mater & Çoker 2002).

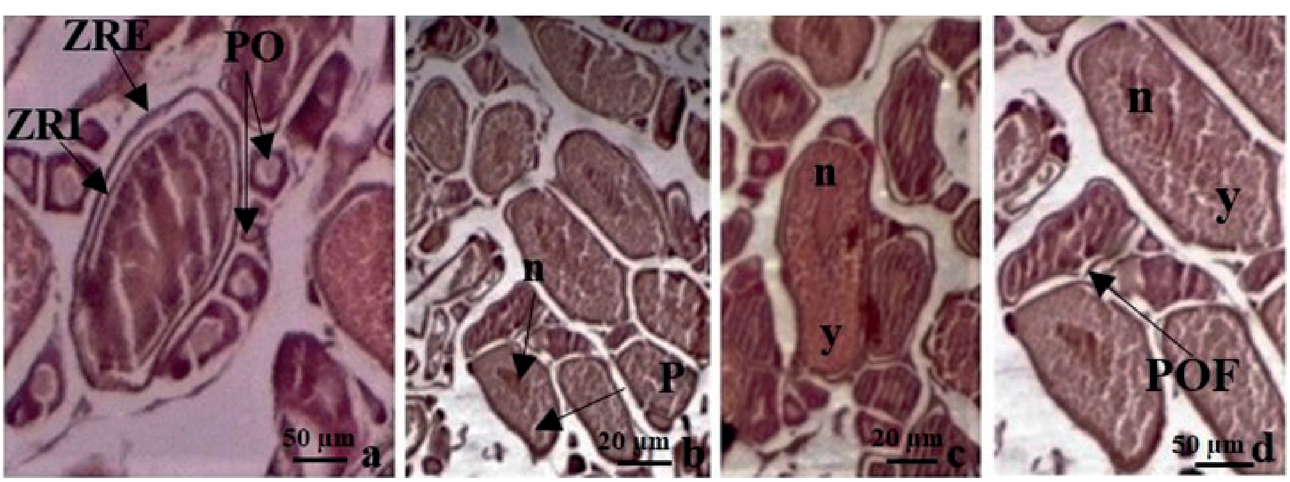

Figure 7

Anchovy ovary showing day-1 POFs and oocytes at the nuclear migration stage at the same time (PO – perinuclear oocytes with no yolk; P – partially yolked; n – nucleus; y – yolk globules; POF – postovulatory follicle; ZRE – zona radiata externa; ZRI – zona radiata interna)

Table 2

Comparison of the spawning season with other studies

Author	Area	Spawning season
Whitehead 1984	Italy	May–October
Whitehead 1984	Northern Europe and Mediterranean Sea	April–August
Santiago & Sanz 1992	Bay of Biscay	April–July
Esseen 1992	Italy	October–November
Lisovenko & Andrianov 1996	Black Sea, northwestern coast	May–August
Motos 1996	Bay of Biscay	April–June
Motos et al. 1996	Spain	March–August
Ré 1996	Portugal	March–May
Ribeiro et al. 1996	Portugal	May–September
Uriarte et al. 1996	France	March–August
Millán 1999	Bay of Cadiz	March–November
Muus & Nielsen 1999	Denmark	June–August
Veldre 2003	southeastern part of North Sea	June–August
Sinovčić & Zorica 2006	Croatia	April–September
ICES 2009	Italy	April–September
ICES 2010	Spain	March–October

As for the first maturation size, our study found that it was 10.47 cm for females and 9.95 cm for males. When comparing these values with previous studies, the first maturation size in European anchovy populations ranged from 8.2 cm to 14.3 cm, which is consistent with our findings (Table 3). The first maturation size is important for management and conservation of fish populations (Soares et al. 2020).

The batch fecundity of the European anchovy in our study ranged from 2.123 to 6.951 oocytes. Previous studies have reported considerable variation in batch fecundity (Table 4). Fecundity can be affected by various factors, such as fish size, weight, age, and environmental conditions, including food availability, population density, and temperature (Ali & Wootton 1999).

Table 3

Comparison of the first maturity length with previous studies

Author	Area	First maturity length ($Lm_{50,}$ cm)
Beverton 1963	Mediterranean	10.5
Beverton 1963	Bay of Biscay	11.0
Beverton 1963	Mediterranean	11.5
Beverton 1963	North Sea	13.5
Motos 1996	Bay of Biscay	14.3
Uriarte et al. 1996	Bay of Biscay	13.7
Anonymous 2001	Bay of Biscay	11.5
Sinovčić & Zorica 2006	Zrmanja estuary	8.2
ICES 2009	Strait of Sicily	11.2
ICES 2010	Bay of Biscay	11.5
El Qendouci et al. 2020	from Safi to Agadir	10.5

We also measured the diameter of the eggs used to determine fecundity. Given the elliptical shape of the eggs, we measured both the short- and long-axis diameters. The short-axis diameter ranged from 0.25 to 0.53 mm, while the long-axis diameter ranged from 0.60 to 1.26 mm. It is worth noting that the diameters of fertilized eggs in ichthyoplankton studies are generally larger than those of unfertilized eggs (Table 5). In addition, the differences in egg diameters can be attributed to differences in salinity levels between the seas where the eggs were found (Huret et al. 2016).

Histological analysis of the ovaries revealed that the spawning season of the European anchovy is characterized by the simultaneous occurrence of all stages of oocyte development. This indicates that the species follows asynchronous oocyte development and exhibits batch spawning, similar to *Engraulis mordax* (Hunter & Macewicz, 1985) and *Engraulis japonicus* (Funamoto et al. 2004), which are also members of the same family. The presence of post-ovulatory follicles (POFs) in the ovaries confirms the occurrence of spawning. The age of POFs is used to determine the spawning frequency. However, the age of POFs can only be determined up to 50 h after spawning, as after this period, atretic eggs can be mistaken for POFs (Hunter & Macewicz 1985). In our study, all histological samples showed post-ovulatory follicles at age 1 (POF-1), indicating that all female samples had spawned the previous night. These findings are important parameters in studies involving biomass estimation using the Daily Egg Production Method. Our study contributes important findings to the Daily Egg Production Method (DEPM), widely used and considered the most reliable method for assessing stocks of pelagic fish species.

Table 4

Comparison of batch fecundity with other studies

Author	Area	Batch fecundity
Sanz & Uriarte 1989	Spain (Bay of Biscay)	201 000–503 000
Motos 1996	Spain (Atlantic Ocean)	110 000–350 000
Motos 1996	Spain (Atlantic Ocean)	20 000–175 000
Muus & Nielsen 1999	Denmark (North Sea and English Channel)	13 000–20 000
Veldre 2003	North Sea	13 000–23 000
El Qendouci et al. 2020	Morocco (Saharan Upwelling)	7323–21 230
This study	Aegean Sea (Türkiye)	2.123–6.951

Table 5

Comparison of egg diameters in European anchovy with previous studies

Author	Area	Short-axis diameter (mm)	Long-axis diameter (mm)
Vodyanitsky 1954	Black Sea	0.80–1.20	1.50–1.90
Mater 1981	Aegean Sea	0.37–0.63	1.00–1.55
Yüksek 1993	Marmara Sea	0.65–0.80	1.05–1.55
Ak 2004	Mediterranean Sea	0.49–0.67	1.13–1.57
This study	Aegean Sea (Türkiye)	0.25–0.60	1.05–1.26

5. Conclusion

In conclusion, the sustainability of European anchovy stocks, which are of great importance to our country's fisheries, is of utmost importance. A comprehensive understanding of the species' reproductive biology is essential to ensure their continuity. Determining the first maturation size, establishing minimum catchable size regulations, and adhering to fishing bans are crucial for preserving the species. In addition, the reproductive parameters identified in our study will serve as a basis for future stock assessments.

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