



## Reproductive Pattern and Length at First Sexual Maturity of The Whiting (*Merlangius merlangus*, Nordmann, 1840) in The Black Sea

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**Abstract:** Samples of whiting (*M. merlangus*) (n = 1545) were collected using bottom trawl in the southern Black Sea during 2017–2018 and were subjected to reproductive biology assessments. These assessments included the gonadosomatic index, macroscopic and microscopic gonad phases, oocyte diameter distributions, batch fecundity, microscopic analysis of oocytes, and histology. The assessment results revealed a significant difference in sex ratio the samples (female: 877, male: 668). Analyses of the gonadosomatic index (GSI), along with macroscopic and microscopic phase evaluations, indicated that the most intense reproductive periods occurred in May and November, with two peaks observed during the studied year. Examination of oocyte structures and diameter distributions in histological sections taken from the ovaries each month showed that the whiting in the southern Black Sea exhibited multi-spawning behavior and had determinate fecundity. Additionally, the monthly batch fecundity (FB) and relative batch fecundity (FBR) of spawning whiting were calculated throughout the year. The average FB was  $17823 \pm 21,353$  hydrated oocytes, while the FBR was  $243 \pm 143$  hydrated eggs per gram. The length at first maturity for males and females were 12.80 cm and 13.72 cm, respectively. In light of the study's results, it is recommended to develop a new fisheries management strategy aimed at contributing to the sustainability of whiting stocks in the Black sea. This strategy should consider the most efficient spawning periods identified in this study, as well as other reproductive strategies, and focus on preserving larger individuals that can enhance egg production within the stocks.

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## 1. Introduction

Recent reports on the sustainability of global fisheries are concerning. As global fisheries production continues at maximum levels, the recovery of aquatic resources may soon become nearly impossible, with stocks at risk of collapse due to current fishing activity. International organizations emphasize the need to maintain fish stocks at optimal sustainable levels and ensure that depleted stocks are restored to exploitable levels. To achieve these global targets, the institutions and organizations responsible for fisheries management must establish and implement effective strategies and policies based on the current status of fish stocks (Mora et al., 2009; FAO, 2021).

Reproduction is a physiological process essential for the continuation of species across all organisms. As fecundity and reproductive strategy are two key topics in fish population dynamics and biological studies, understanding the reproductive strategy of a stock is crucial for effective fisheries management (Murua and Saborido-Rey, 2003).

Sustainable management of fish resources is directly linked to the reproductive potential of fish stocks. This potential is a critical factor for commercial demersal and semi-demersal species (Ferreri et al., 2009). Recent advancements in the understanding of reproductive biology and growth have led to a more precise definition of fecundity. Given the subjectivity and variability in the available information, it is essential to examine the developments occurring in the gonads of fish to evaluate fishery resources accurately. Microscopic studies are necessary to determine reproductive timing, maturation periods, spawning stock biomass, and the reproductive potential of stocks in various geographical and environmental contexts. In particular, the microscopic examination of partially spawning fish allows for accurate identification of spawning maturity stages (Ferreri et al., 2009).

The fishing activities and anthropogenic pollutants have adversely impacted the ecosystem of the Black Sea over time (Prodanov et al., 1997; Daskalov, 2002; Akoglu et al., 2014). This unfavorable situation has reduced biodiversity of this ecosystem and is gradually reducing the productivity of commercial species from this region. This situation has also threatened the sustainability of whiting stocks, one of the most important commercial species obtained from the Black Sea. Since 2000, the annual whiting production of the countries bordering the Black Sea has been fluctuating (FAO, 2019; TÜİK, 2021). In this regard, it becomes important to elucidate the reproductive biology of this species and relate it to fisheries to ensure the sustainability of whiting stocks in the Black sea.

In this context, the aim of the study is to determine the spawning peaks of whiting in the Black Sea, examine the changes in oocyte structures within the gonads, analyze monthly variations in oocyte diameters, assess fecundity parameters, and investigate seasonal changes in spawning frequency and the lengths at first sexual maturity.

## 2. Materials and Methods

The study was conducted in the southern Black Sea from March 2017 to February 2018 (Figure 1). A total of 1545 specimens of *Merlangius merlangus* were collected during monthly sampling operations with the Karadeniz Araştırma research vessel. Samples were taken from depths of 5–65 m using a bottom trawl net with a mesh size of 12 mm.

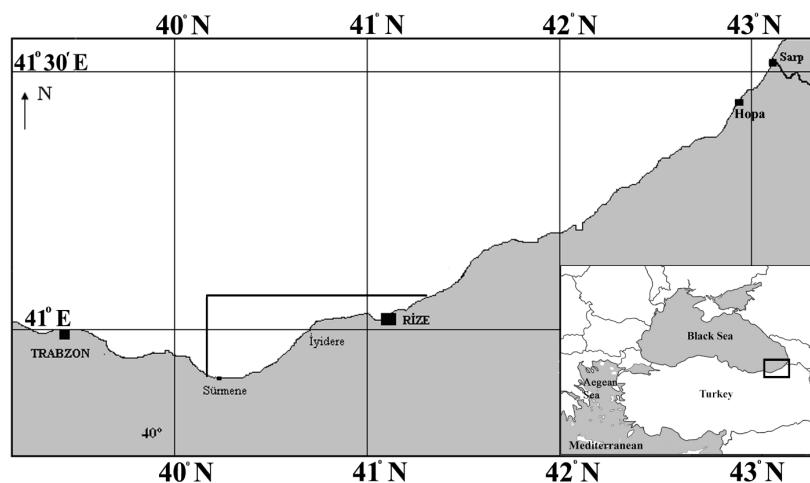


Figure 1. Study area.

The total length (TL) of each fish specimen was measured to the nearest millimeter (1 mm). Gonads were removed through dissection, and sex determination was performed. Each specimen's body and gonads were weighed with an accuracy of 0.01 g. The reproductive period throughout the year was assessed by calculating the gonadosomatic index (GSI), which was computed using the following formula (Craig et al., 2000; Nunes et al., 2011; Flores et al., 2015).

$$GSI = \left( \frac{GW}{TW - GW} \right) * 100 \quad (1)$$

Where GW represents the weight of the gonads and TW represents the total body weight of the fish specimen.

Gonads were examined both macroscopically and microscopically to detect changes in gonadal and oocyte structures in the ovaries during the reproductive period. These examinations were categorized into five phases: immature (I), developing (II), spawning (III), regressing (IV), and regenerating (V), as described by Brown-Peterson et al. (2011).

A total of 877 female ovarian specimens were examined macroscopically, while 307 of these were subjected to microscopic examination. For the microscopic analysis, ovarian samples intended for both histological examination and oocyte frequency distribution analysis were placed in 10% neutral buffered formalin for one day, followed by preservation in 70% alcohol. The histological samples were then washed under running water overnight and passed through an alcohol concentration gradient. After cleaning with xylene, the samples were embedded in paraffin blocks at 65 °C overnight. Using a microtome, the paraffin-embedded ovarian samples were sectioned into thin slices of 5–10 µm and stained with hematoxylin-eosin. (Hunter and Goldberg, 1980; Hunter et al., 1992; Murua and Motos, 2006). The stained samples were examined under a microscope to stage the oocyte structures.

Batch fecundity is defined as the number of hydrated oocytes in each ovary sample, estimated using the gravimetric method (Hunter et al., 1985; Hunter et al., 1992). Only ovaries containing hydrated oocytes were used to calculate partial fecundity, while those with post-ovulate follicles were excluded. A total of 61 ovaries were included in the determination of partial fecundity. Hydrated oocytes from the anterior, middle, and posterior regions of the gonad samples (0.1 g each) were counted under a binocular microscope. Batch fecundity (FB) was calculated using the following equation (Hunter et al., 1985; Murua et al., 2003; Macchi et al., 2005).

$$F_B = \sum_{i=1}^m \left( \frac{n_i * G_i}{g_i} \right) * \left( \frac{1}{m} \right) \quad (2)$$

Where Gi denotes the weight of the gonad sample from the ith female, gi denotes the subsample weight of the ith female, ni denotes the number of the hydrated oocytes in the subsample from the ith female, and m denotes the total number of samples used to estimating batch fecundity.

The relative batch fecundity (FBR) was calculated by dividing the number of hydrated oocytes in the batch fecundity by the body weight.

Annual fecundity (FA) was defined as the number of hydrated eggs released by a female in a year (Hunter et al., 1992; Murua et al., 2003; Murua and Saborido-Rey, 2003). The relationships of body weight with FB and length with FB were determined through regression analysis. The diameters of oocytes in mature gonads during the spawning season were measured using a binocular microscope and used to calculate oocyte frequency distributions. The length at first sexual maturity was calculated as the ratio of mature individuals to total individuals based on macroscopic examination of gonad samples collected at the peak of the reproduction period. The length at first maturity (Lm<sub>50</sub>) was calculated using the logistic function provided below (Saborido-Rey and Junquera, 1998; Flores et al., 2015).

$$P = 1/(1 + \exp(a + b * L)) \quad (3)$$

$$Lm_{50} = -(a/b) \quad (4)$$

Where P denotes the proportion of mature individuals in the length class, and L denotes total length; a: intercept, b: slope.

Statistical significance levels of the data (GSI, FB, FBR) were determined using ANOVA, Kruskal-Wallis test followed by Dunn test for non-parametric data, chi-square test and independent t-test using SigmaPlot 12.0 software.

### 3. Results

A total of 1545 whiting (*M. merlangus*) specimens were collected over a duration of 12 months. The length distribution of all specimens ranged from 7.5 to 25 cm; for females, the range was 7.5 to 25 cm, while for males, it was 7.5 to 19 cm. The sample set comprised 57% females (n = 877) and 43% males (n = 668). The chi-squared test performed to compare the sex ratios in the length classes (7.5 to 19) revealed a significant difference in the sex ratios in the length groups ( $\chi^2_{(df: 12)} = 169,127$ ;  $p < 0.001$ ). The proportion of males (43%) and females (57%) in the sample set also demonstrated a significant deviation from the standard 1:1 ratio ( $\chi^2_{(df: 1)} = 28,272$ ;  $p < 0.001$ ).

The monthly GSI values calculated for the whiting specimens, categorized by sex, revealed two peaks occurring in May and November during the year (Figure 2).

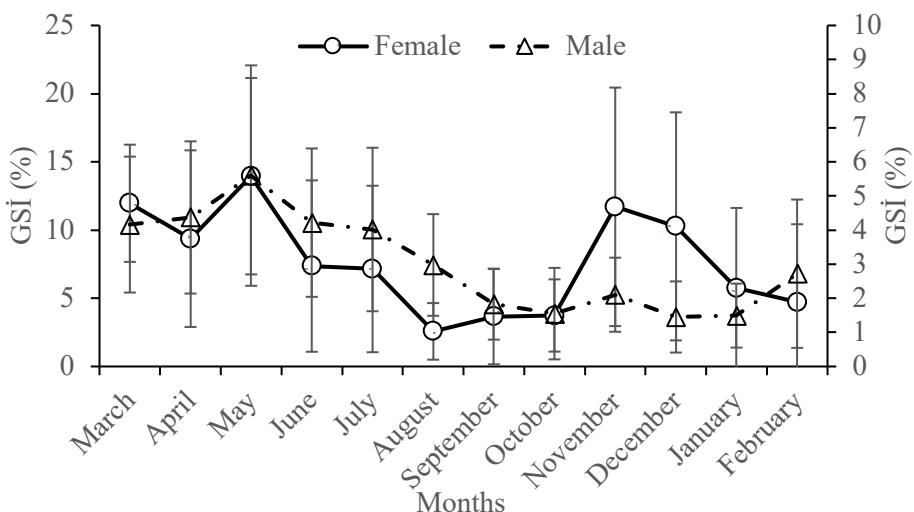


Figure 2. Monthly gonadosomatic indices for female and male *M. merlangus* specimens.

The highest GSI values were recorded in May, with females exhibiting a value of  $13.94 \pm 7.200$  and males showing  $5.59 \pm 3.231$ . Statistical analysis of the monthly GSI values for females revealed significant differences in peak months ( $F_{(df: 11)} = 29.93$ ;  $p < 0.001$ ), and similar results were obtained for males ( $F_{(df: 11)} = 20.36$ ;  $p < 0.001$ ). In order to determine the maturity phase of the ovaries, ovary samples from females were examined macroscopically every month to reveal the five phases (Figure 3). The examinations revealed the presence of all five phases in all months except for March.

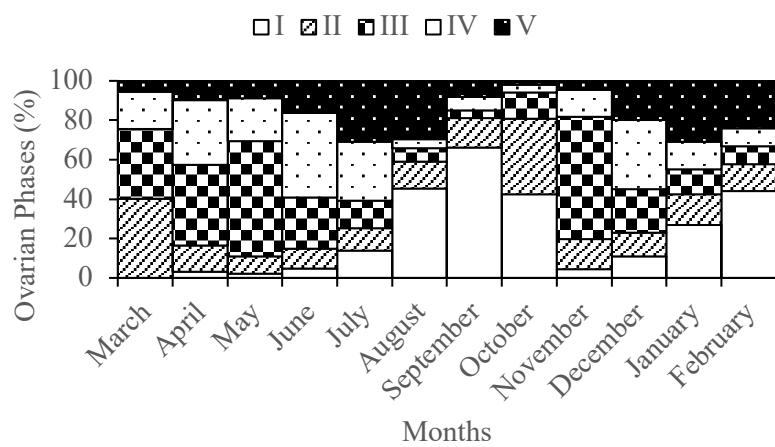


Figure 3. Monthly macroscopically determined gonad maturation phases in *M. merlangus*.

Histological sections were received from the ovaries taken every month to determine the maturity phases of the ovary. The sections were categorized as having one of five phases according to the changes that occurred in the oocyte structures within the ovary (Figure 4).

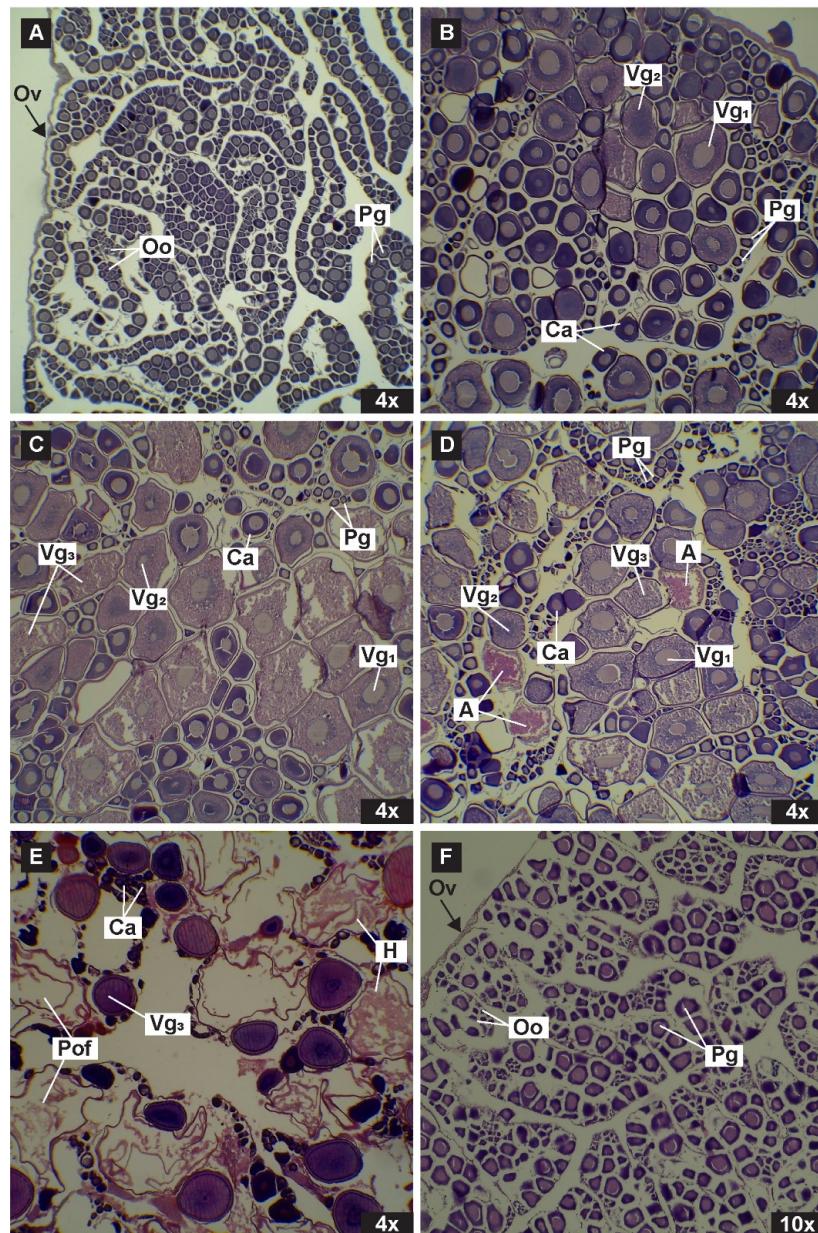


Figure 4. Histological sections showing developmental phases in ovary and oocyte structures: A, immature I; B, developing II; C-D, spawning III; E, regressing IV; F, regenerating V (Ov: ovarian wall; Oo: oogonia; Pg: primary growth oocyte; Ca: cortical alveolar oocyte, Vg1: primary vitellogenic oocyte; Vg2: secondary vitellogenic oocyte; Vg3: tertiary vitellogenic oocyte; h: hydrated oocyte; Pof: postovulatory follicle; A: atresia oocyte).

Phase I: This phase was characterized by a high presence of oogonia (Oo) and primary growth (Pg) oocytes. The oocyte nuclei were considerably large and occupy a significant portion of the oocyte cytoplasm. No atretic oocytes (A) are observed (Figure 4A).

Phase II: This phase was characterized by the presence of Pg, cortical alveolar (Ca), primary vitellogenic (Vg1), and secondary vitellogenic (Vg2) oocytes structures. In addition, the diameters of a few oocytes were enlarged while the nucleus diameters were decreased. The atresia structures were not observed in this phase (Figure 4B).

Phase III: The atresia and tertiary vitellogenic (Vg3) oocytes were the first ones to be identified in this phase. While Pg, Ca, Vg1, Vg2, and Vg3 were observed heterogeneously within the ovary, the Vg1, Vg2, and Vg3 oocytes were further intense (Figure 4C and 4D).

Phase IV: This phase was characterized mainly by hydrated oocytes (H) and postovulatory follicles (Pof) with a few Ca and atresia oocytes (A) (Figure 4E).

Phase V: In this phase, the reconstruction of the ovary had begun, the ovary walls had thickened, and the oogonia and Pg oocytes had been remodeled (Figure 4F). The monthly evaluation of the histological sections revealed the presence of all five phases in the ovaries of the fish specimens during all months.

The fecundity of 61 female specimens was estimated by evaluating a minimum of three samples each month (Phase III). Only hydrated oocytes were used to calculate batch fecundity. Average batch fecundity values (FB) and relative batch fecundity values (FBR) were calculated for all months (Table 1).

Table 1. Monthly mean batch fecundity (FB) and mean relative batch fecundity (FBR) along with the corresponding standard deviations

Months	N	Average (FB)	Average FBR
March	3	2217±874	67±3
April	5	9520±4660	328±162
May	3	33142±49792	311±152
June	3	2335±2134	80±58
July	7	9539±6021	222±124
August	3	3070±504	110±160
September	4	8751±5343	267±160
October	3	7090±3724	107±36
November	11	25859±17652	334±118
December	6	25412±22424	247±140
January	8	18293±8364	221±98
February	5	35726±39734	325±181
Total/Average	61	17823±21353	243±143

The Kruskal-Wallis test revealed a significant difference among the monthly mean batch fecundity values ( $H_{(df:11)} = 27.979$ ,  $p < 0.05$ ). Similarly, the difference among the monthly mean relative batch fecundity values was also significant ( $H_{(df:11)} = 25.098$ ,  $p < 0.05$ ). Among the evaluated samples, the smallest spawned individual measured 13.7 cm (24.11 g) and had a batch fecundity of 4542 hydrated oocytes, while the largest individual measured 32.6 cm (279.58 g) and had a batch fecundity of 108353 hydrated oocytes. The average batch fecundity was estimated at  $17823 \pm 21353$  hydrated oocytes, while the relative batch fecundity ranged from 40 to 589 hydrated oocytes  $g^{-1}$ , with an average of  $243 \pm 143$  hydrated oocytes  $g^{-1}$  (Table 1). Furthermore, regression analysis revealed that the batch fecundity of whiting was significantly and positively correlated with female body weight and length ( $p < 0.05$ ) (Figure 5A and 5B).

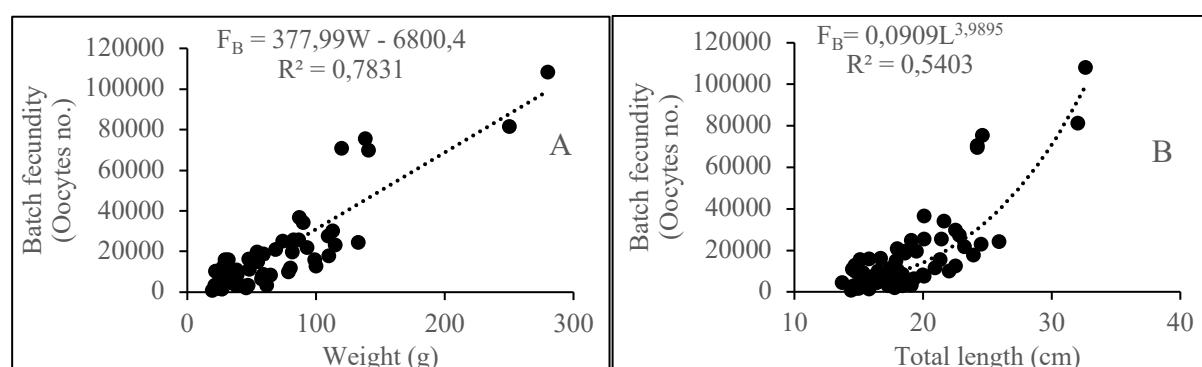


Figure 5. The relationship between the total weight and batch fecundity (A) and the total length and batch fecundity (B).

To determine the egg diameter of whiting fish, samples were collected monthly from mature gonads. Oocytes with diameters greater than 0.1 mm were measured. The oocyte diameters measured each month are presented in Table 2 and Figure 6.

Table 2. Monthly-measured average diameters of hydrated oocytes and total oocytes along with standard deviations

<b>Months</b>	<b>N</b>	<b>Hydrated oocyte diameter (mm)</b>	<b>N</b>	<b>Total (mm)</b>
<b>March</b>	630	1.01±0.195	1419	0.65±0.330
<b>April</b>	362	1.18±0.224	1277	0.68±0.349
<b>May</b>	432	1.27±0.131	1232	0.77±0.395
<b>June</b>	261	0.87±0.084	684	0.62±0.221
<b>July</b>	196	1.03±0.151	508	0.71±0.280
<b>August</b>	486	1.08±0.234	1113	0.78±0.322
<b>September</b>	165	1.12±0.126	994	0.57±0.269
<b>October</b>	115	1.39±0.099	237	0.91±0.479
<b>November</b>	798	1.34±0.217	2486	0.75±0.433
<b>December</b>	725	1.29±0.284	2637	0.72±0.401
<b>January</b>	690	1.49±0.167	2119	0.83±0.483
<b>February</b>	687	1.49±0.213	2178	0.84±0.473
<b>Total/Average</b>	5547	1.21±0.177	16874	0.74±0.098

The average diameter of the oocytes measured throughout the year was  $0.74 \pm 0.098$  mm, while the average diameter of the hydrated oocytes was  $1.21 \pm 0.177$  mm. The Kruskal-Wallis test revealed a significant difference among the monthly measured average diameters of the oocytes ( $H_{(df:11)} = 545.68$ ,  $p < 0.001$ ). Similarly, the difference among the monthly measured diameters of the hydrated oocytes was also significant  $H_{(df:11)} = 2575.57$ ,  $p < 0.001$ ). The monthly measured diameters of the hydrated oocytes ranged from 0.7 mm to 1.9 mm.

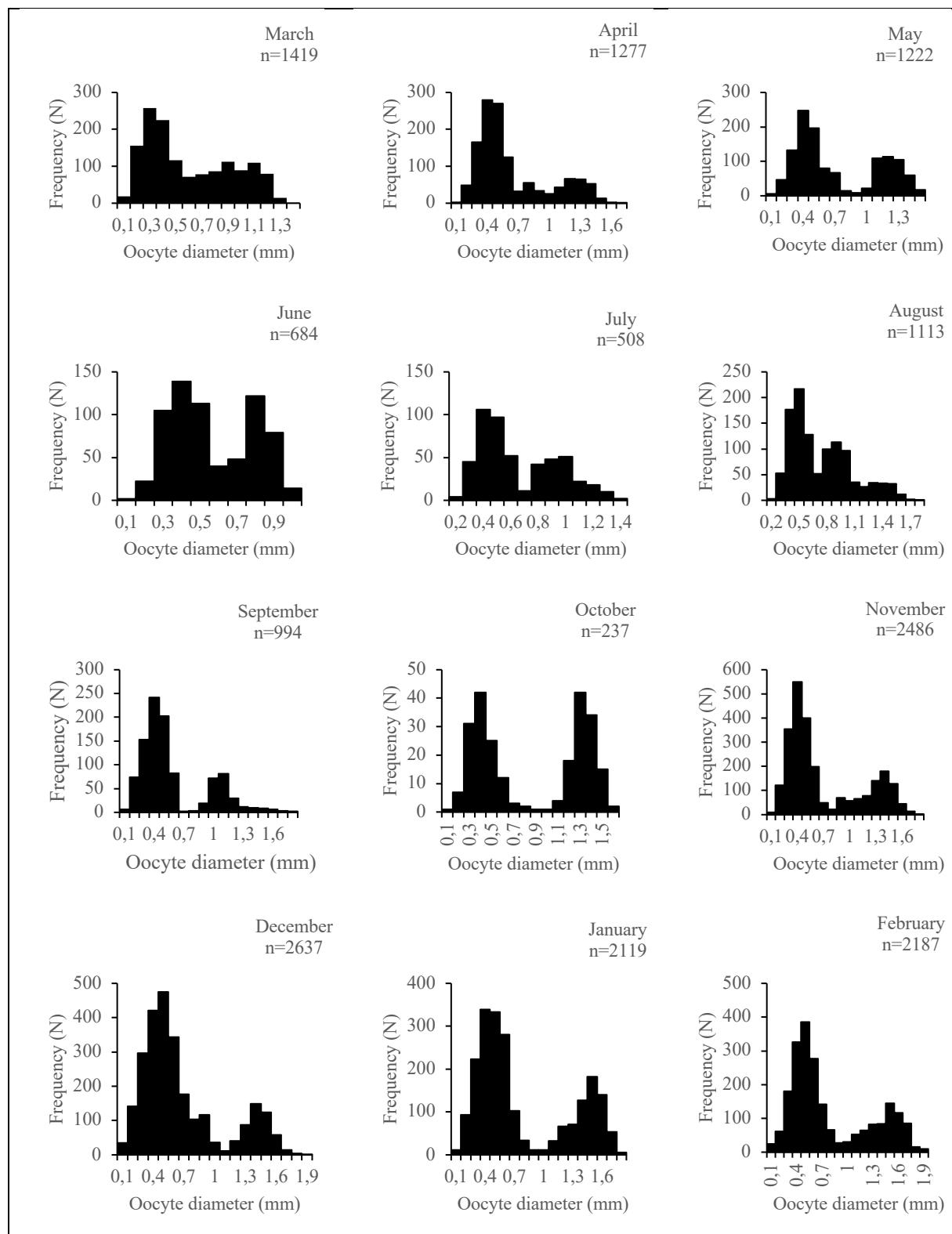


Figure 6. Monthly oocyte frequency distributions.

The length at first sexual maturity ( $L_{m50}$ ) was determined for both mature males and females (Figure 7), observed to be 12.80 cm and 13.72 cm, respectively.

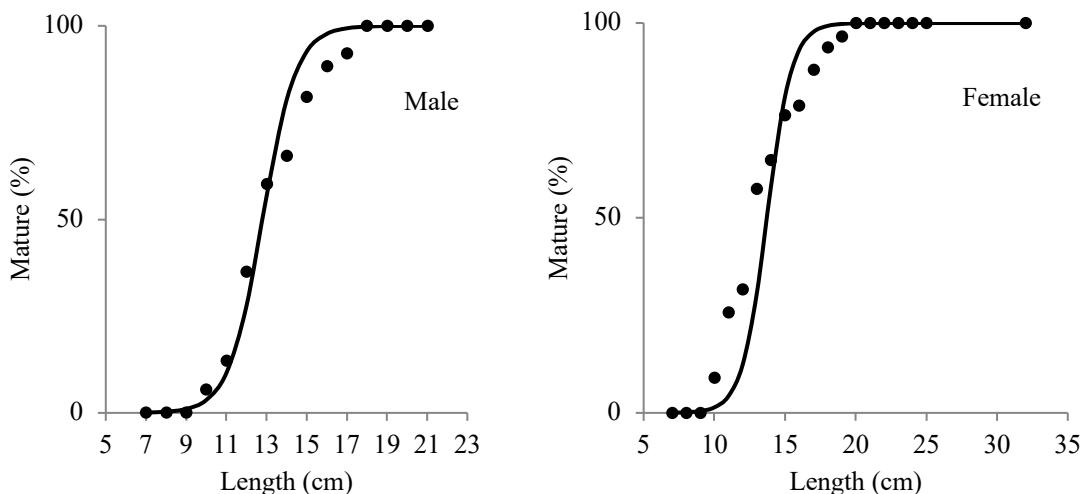


Figure 7. The length at first sexual maturity in *M. merlangus*.

#### 4. Discussion

Considering the biometric data obtained in this study, female whiting stocks from the Black Sea were found to be larger than their male counterparts and dominated the length classes and sex ratio significantly differed from the expected 1:1 ratio. Similar findings have been documented in previous studies on whiting stocks in the Black Sea (Çiloğlu et al., 2001; Bilgin et al., 2012; Sağlam and Sağlam, 2012; Mazlum and Bilgin, 2014; Yıldız and Karakulak, 2019).

The reproductive period and fecundity are two critical factors for fishery management. Additionally, the gonadosomatic index (GSI), allows for a quick and cost-effective determination of the reproductive period (De Vlaming et al., 1982; Somarakis et al., 2004; Stahl and Kruse, 2008; Nunes et al., 2011; Flores et al., 2015). The monthly GSI values for males and females throughout the year, as determined in the current study, peaked twice—first in May and then in November (Figure 2). Based on the GSI values obtained for females, it can be inferred that whiting spawned heavily during the months of May to August and November to February. Male gonads were predicted to be emptied during the months of May to September and November to January. In previous studies on the whiting population in the Southern Black Sea, reproductive periods were identified as occurring from spring to autumn (Genç et al., 1998) and extending into late summer, mid-autumn, and early winter (Bilgin et al., 2012). Other studies conducted in the Black Sea indicated that whiting fish reproduce at temperatures of 7–15 °C and spawn throughout the year (Genç et al., 1998; Çiloğlu et al., 2001; Samsun, 2005).

Reproductive timing in fish is crucial for successful spawning and recruitment (Tomkiewicz et al., 2003; Lowerre-Barbieri et al., 2011). Marine fish exhibit various reproductive timing characteristics, including annual, seasonal, and diel patterns (Lowerre-Barbieri et al., 2009). One of the approaches for determining the reproductive period and reproductive strategy in fish is the histological approach, which is further categorized into macroscopic and microscopic examinations. The macroscopic examination is a rapid and cost-effective way to assess the reproductive status of fish (Tomkiewicz et al., 2003). However, this method can be subject to margins of error in the visual staging of fish gonads. In contrast, microscopic analysis, while more expensive and time-consuming, offers valuable insights into the reproductive period. This method examines the oocyte structures within fish gonads and provides greater reliability in determining reproductive phases (Saborido-Rey and Junquera, 1998; Tomkiewicz et al., 2003; Vitale et al., 2006; Stahl and Kruse, 2008).

The present study attempted to determine the reproductive period by establishing the maturity phases of the whiting ovaries sampled using macroscopic and microscopic examinations. Macroscopic phasing was established based on the developments in the external appearance of the gonads considering the criteria specified in the method. In regard to phase III based on the macroscopic examination, which is the beginning of the egg-laying stage, it was observed that the reproductive periods occurred almost

during the periods determined based on the GSI, i.e., during May–August and November–February (Figure 2–3). In separate studies, Samsun (2005) and Bilgin et al. (2012) had observed the presence of almost all phases throughout the year in the macroscopic staging of the whiting ovaries in the southern Black Sea. In the present study as well, all phases were observed throughout the year, except for phase (I) based on macroscopic phasing, which occurred in March.

Since determining the reproductive strategies of fish is crucial for fisheries management, it becomes important to accurately evaluate ovarian development to determine the ovary maturation process, reproduction period, and oocyte recruitment (Murua and Saborido-Rey, 2003; Lowerre-Barbieri et al., 2011; Alonso-Fernandez et al., 2013). Subsequently, the results of GSI, macroscopic and microscopic methods were by being compared to evaluated. All maturity phases were observed in the ovarian sections sampled from March, the first month of sampling, through February, the last month. This indicates that whiting in the Southern Black Sea reproduce year-round. Additionally, based on the microscopic phasing results, it can be inferred that whiting in this region exhibit a multiple spawning pattern. Atretic oocytes were detected at lower rates throughout the year in the monthly ovarian sections. Histological examination revealed that the peak spawning periods for whiting coincided with those identified by the gonadosomatic index (GSI) and macroscopic methods, specifically in spring and autumn. Furthermore, regressing (phase IV) and regenerating (phase V) phases were rarely observed in microscopically examined ovarian samples from March to May. In contrast, these regressing and regenerating phases were observed at significantly higher rates during visual examinations of specimens from the same months (Figure 3). It was difficult to determine the gonad structures of the ovaries in the regenerating phase (V) and those in the immature phase (I) using the visual examination due to considerable similarity; in addition, there was a change in the ratio of the number of individuals in these phases. Stahl and Kruse (2008) and Brown-Peterson et al. (2011) also reported difficulties and confusion in visually staging the regeneration and immature phases of gonads. Additionally, inaccurate visual phasing of individuals that have reached sexual maturity but skipped the breeding period without spawning (Rideout et al., 2005; Rideout and Tokievicz, 2011) complicates the assessment of the actual number of productive females in the stock, negatively impacting the determination of spawning stock biomass. In this context, it can be said that microscopic staging is a more accurate method for determining spawning stock biomass.

The estimation of spawning biomass in multiple-spawning fish depends on the number of batches spawned and the number of eggs in each batch (Hunter and Goldberg, 1980). Determining fecundity is crucial for predicting spawning biomass (Hunter et al., 1985; Murua et al., 2003; Rogers et al., 2019). In this context, estimating fecundity, which is influenced by environmental and other biological factors, helps us understand not only the basic mechanisms that regulate annual variability in fecundity but also variability in recruitment (Murua and Saborido-Rey, 2003). In the present study, the fecundity status of whiting specimens was evaluated. It was found that the values of relative batch fecundity (FBR) and batch fecundity (FB) determined from the monthly samples exhibited variability, peaking during the intensive spawning periods in May and February (Table 1).

Statistical analysis revealed that the differences between the monthly values of both FB and FBR were not significant ( $p > 0.05$ ). However, the months with the most intense whiting spawning were identified as May and February. The consistent detection of FB throughout the year further indicates that whiting spawn year-round in the Black Sea. In studies on the egg fecundity of whiting in the Black Sea, annual fecundity has been determined by counting all oocytes without considering batch fecundity. İşmen (1995) reported annual fecundity values of 313 000, 314 000, and 263 000 eggs for whiting in the Black Sea in the years 1991, 1992, and 1993, respectively. Ak (2009) reported an annual fecundity of 179 672 eggs in the southern Black Sea, while Samsun (2005) found it to be 140 838 eggs in the western Black Sea. When the fecundity value was statistically compared with those from previous studies in the Black Sea, the difference was found to be insignificant ( $p > 0.05$ ). In developing a management plan for multiple spawning stocks, it is crucial to consider partial fecundity as a unit of measurement to more accurately assess egg productivity, spawning stock, and individual recruitment. The most effective measure for determining egg productivity in multiple spawning fish is batch fecundity (Hunter et al., 1985; Lowerre-Barbieri et al., 1996).

In the present study, a positive relationship was observed between batch fecundity and somatic weight and also between batch fecundity and total length (Figure 5A-5B). As the somatic weight of whiting in the Southern Black Sea increases, batch fecundity also increases linearly. In contrast, as total

length increases, batch fecundity rises exponentially. Thus, it can be concluded that batch fecundity is a function of both total length and somatic weight. Similar findings have been reported for whiting in the Black Sea (İşmen, 1995; Samsun, 2005). Hislop (1974) found in his study of *Merlangius merlangus* in the North Sea, the Minch, and Iceland that fecundity increases with both length and age, with a curvilinear relationship between fecundity and length. Additionally, studies on *Merluccius merluccius* by Kjesbu et al. (1998), Macchi et al. (2005), and El Habouz et al. (2011) demonstrated that batch fecundity positively correlates with both somatic weight and total length, reinforcing the notion that batch fecundity is influenced by these factors.

The observation of a certain increase in the diameter of hydrated oocytes (Table 2) measured monthly throughout the year in the present study is an indicator of fecundity determinability. Murua and Saborido-Rey (2003) also reported that the increase in oocyte diameters during the reproductive period was indicative of fecundity determinability. The frequency distribution of oocytes within the ovaries of multiple spawning fish is crucial for determining whether the species exhibits determinate or indeterminate fecundity (Hunter and Macewicz, 1985; Hunter et al., 1985; Murua and Saborido-Rey, 2003). The presence of a hiatus between vitellogenic oocytes and hydrated oocytes in the oocyte frequency distribution indicates that batch fecundity is determinate (Hunter and Macewicz, 1985; Lowerre-Barbieri et al., 1996; Macchi et al., 2005; Murua and Motos, 2006). The monthly distribution of oocyte diameters observed in the present study (Figure 6) reveals such a hiatus, indicating that the batch fecundity of whiting in the Southern Black Sea is determinate. A Previous report indicate that whiting is iteroparous, group-synchronous, fecundity determinate, and a batch spawner (Murua and Saborido-Rey, 2003). These criteria should be considered when determining the spawning stock biomass of whiting.

The length at first sexual maturity ( $L_{m50}$ ) is another crucial parameter that plays a key role in the sustainability of fish stocks (Tsikliras and Stergiou, 2013). Compared to previous studies reporting the length at first sexual maturity of whiting in the Black Sea, the corresponding values obtained in the present study were found to be lower (Table 3).

Table 3. The values of the length at first sexual maturity ( $L_{m50}$ ) reported in the previous studies conducted in the Black Sea

Researchers	First sexual maturity length ( $L_{m50}$ )	Research area
İşmen (1995)	Female:14.7 cm. Male:12.5 cm	Black Sea
Samsun (2005)	Female:13.8 cm. Male:12.9 cm	Black Sea
Bilgin (2012)	Female:14.6 cm. Male:13.9 cm	Black Sea

The t-test analysis comparing the length at first sexual maturity values obtained in the present study with those from previous studies (İşmen, 1995; Samsun, 2005; Bilgin et al., 2012) revealed a significant difference among females ( $p < 0.05$ ), while no significant difference was found among males ( $p > 0.05$ ). Despite a long-standing ban on bottom trawling in the eastern Black Sea, comparisons of first sexual maturity parameters suggest that there has been no recovery in whiting fish stocks. Previous studies on the length at first sexual maturity (Tsikliras and Stergiou, 2013; Hunter et al., 2015; Lappalainen et al., 2016) indicated that environmental fluctuations (such as temperature and nutrition) and high levels of fishing exploitation could negatively affect these parameters. To conserve stock biomass and ensure the sustainability of fisheries, it is recommended that fish be allowed to spawn at least once during their lifetime (Beverton and Holt, 1957). The fundamental parameter for maintaining a population is the spawning stock. Sustainable fishery management should be established by determining the spawning stocks of the whiting fish population in the Black Sea and considering the criteria determined in this study and other studies.

## Conclusion

It is expected that banning all types of fishing activities on the whiting population during periods of intensive reproduction will contribute to the sustainability of the whiting stocks and fisheries. It is believed that banning all fishing activities during the peak spawning periods of whiting populations would contribute to the sustainability of whiting stocks and fisheries. Therefore, banning all fishing

during at least one of the peak spawning periods (particularly May and August, as they overlap with the spawning periods of other fish) should be considered by fisheries management agencies.

For a more accurate assessment of recruitment, the fecundity of whiting should be estimated based on hydrated oocytes. The biomass of the spawning stocks of the whiting population in the southern Black Sea is currently unknown. Batch fecundity should be taken into account when determining the spawning stock biomass of whiting with multiple spawning and determinate fecundity.

The fisheries management to be implemented on the whiting population should be planned according to the determined spawning stocks. The length values at first sexual maturity obtained in this study showed a decrease compared to the values of previous studies. The presence of individuals with smaller sexual maturity length in the spawning stock may mean a decrease in egg production, decrease in quality and decrease in recruitment. In order to ensure a healthier recruitment, The whiting fishery should be adjusted to a minimum size of 14 cm so that individuals larger than the minimum landing size can remain in the spawning stock.

### **Ethical Statement**

Ethical approval is not required for this study.

### **Conflict of Interest**

The authors declare that there are no conflicts of interest.

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### **Author Contributions**

YC: Conceptualization, Methodology, Formal Analysis, Visualization, Writing—Original Draft Preparation. The author developed the research idea, designed the methodology, and performed the data analysis and initial manuscript writing. CS: Supervision, Investigation, Writing—Review & Editing. The author supervised the research process, contributed to data collection, and revised the manuscript. EO: Data Curation, Validation. The author contributed to organizing and validating the data and preparing materials for the study.

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