ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 69 (3), 2017: 385-392

Sexual Maturity and Reproductive Patterns of European Hake *Merluccius merluccius* (Linnaeus, 1758) (Actinopterygii: Merlucciidae) from the Sea of Marmara, Turkey

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Abstract: The study deals with the sexual maturity and reproductive patterns of European hake *Merluccius merluccius* (L.) from the Sea of Marmara, Turkey. Totally, 777 specimens were sampled monthly between October 2014 and September 2015. Total lengths of all sampled individuals ranged from 10.4 cm to 55.3 cm. The sex ratio (♂/♀) was 0.56. A total of 341 ovaries were obtained and histologically examined to determine the reproductive traits and developmental stages of oocytes. The gonadosomatic index values for females revealed two main (November and December) and one minor (June) spawning period. By applying an empirical expression and using the maximum lengths in the sample, the length at the onset of sexual maturity for females and males was estimated at 29.9 cm and 22.5 cm, respectively.

Key words: European hake, sexual maturity, reproduction, Sea of Marmara, Mediterranean Sea

Introduction

From the point of view of the fishing activities, European hake *Merluccius merluccius* (Linnaeus, 1758) is a significant demersal species having a broad distribution across the eastern Atlantic Ocean and the Mediterranean (Froese & Pauly 2016). This species is widely caught in European demersal waters (Casey & Pereiro 1995). It is an essential deep shelf predator in the Mediterranean (Carpentieri et al. 2005).

Merluccius merluccius is generally considered as a batch spawner with asynchronous oocyte development that releases yolk oocytes in several batches over a protracted period during each spawning season (Murua & Sabarido–Rey 2003). Spawning occurs throughout the year, with distinct peaks in the Mediterranean Sea (Recasens et al. 1998) and in the Atlantic Ocean (Murua 2010). Comprehensive data on the sexual maturity and reproductive traits of *M. merluccius* are essential for ensuring appropriate harvest of the stock at least within the classic paradigm of protecting recruits and juveniles (the so called "let's them spawn once" approach). In the last two decades, several authors have published reports on reproduction biology of *M. merluccius* for both Atlantic (Murua et al. 1998, Pineiro & Sainza 2003, Murua & Motos 2006, Murua et al. 2006, Domínguez –Petit et al. 2008a, 2008b, El Habouz et al. 2011, Costa 2013) and Mediterranean (Recasens et al. 2008, Philips & Ragheb 2013, Soykan et al. 2015) stocks.

In European Union, the minimum conservation reference size (MCRS) for *M. merluccius* is 20 cm in total length (EU 2011). It is the same in Turkish waters (BSGM 2016). There is however some evidence that 20 cm is still lower than the usually estimated

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size at sexual maturity. Consequently, it would be interesting to investigate this parameter looking also at the sub-regional differences.

The fisheries management approaches for European hake are lacking adequate details regarding the maturation of this species, particularly from the potential spawning grounds of the Sea of Marmara. In this area, the hake fishery is performed mostly by commercial fishermen using beam trawls (with mesh size of 32 mm) and set gillnets (with mesh size of 52 mm); the trawl fishery is completely forbidden. Hence, the present study aims to determine the length at onset of sexual maturity (L_m), spawning peaks, and developmental stages of oocytes together with the seasonal cycle of sexual maturity based on the gonadosomatic index values. This is the first study that estimates the length at the onset of maturity for European hake from the Sea of Marmara.

Materials and Methods

Totally, 777 specimens were collected from the Sea of Marmara mostly by commercial fishermen using beam trawls and set gillnets between October 2014 and September 2015. Total wet weight (TW, g) and total length (TL, cm) were measured for each specimen. The sexes determined by macroscopic examination of the gonads were recorded as male or female.

The gonadal tissue was extracted and weighed (GW, g). All the females were used for histological analysis; in particular, a subsample of about 1.0 cm width section from the central part of the ovary was preserved in 10% buffered formalin. The tissue sections were washed in a buffer solution, dehydrated in ethanol and *n*-butanol series, and embedded in paraffin, and then 5 µm sections were cut with a microtome and mounted on slides. The sections were stained with haematoxylin-eosin and examined on a light microscope.

The sexual maturity was classified according to ICES (2007) and Costa (2013). Despite the lack of uniform terminology, the following scale has been adopted for the oocytes; stage *a*: immature or virgin, stage *b*: developing, stage *c*: spawning, stage *d*: post spawning. Thereafter, stage *c* and *d* were considered as "sexually mature". In addition, we measured the diameters of the oocytes.

In order to corroborate the identification of the spawning period, the gonadosomatic index, a percentage of the gonad weight in relation to the total weight of the fish, $GSI = [GW / TW] * 100$ (Barber & Blake 2006) and the condition factor, $CF = [TW - GW / TL^3] * 100$ (Htun–Han 1978) were calculated. The empirical relationships for estimating mean length at maturity (L_m) from maximum size (L_{max}) for ray-finned fish were employed. In this study, L_m was estimated for both sexes according to the equation, $\log L_m = -0.1189 + 0.9157 * \log (L_{max})$, formulated by Binohlan & Froese (2009). The overall sex ratio (♂/♀) different from the expected 1:1 ratio was evaluated using the Chi-square test (Sümbüloğlu & Sümbüloğlu 2005). In addition, SPSS 22.0 was used for all statistical analyses.

Results

European hakes (N=777) were collected during the study period. The mean length of all individuals was 25.9 ± 0.21 cm, ranging from 10.4 to 55.3 cm. It was determined that 341 specimens (64%) were females with 27.8 ± 0.33 cm, ranging from 13.4 to 55.3 cm, and 192 specimens (36%) were males with 26.5 ± 0.32 cm, ranging from 15.6 to 40.5 cm. The sex ratio (M/F) was calculated as 0.56; therefore, statisti-

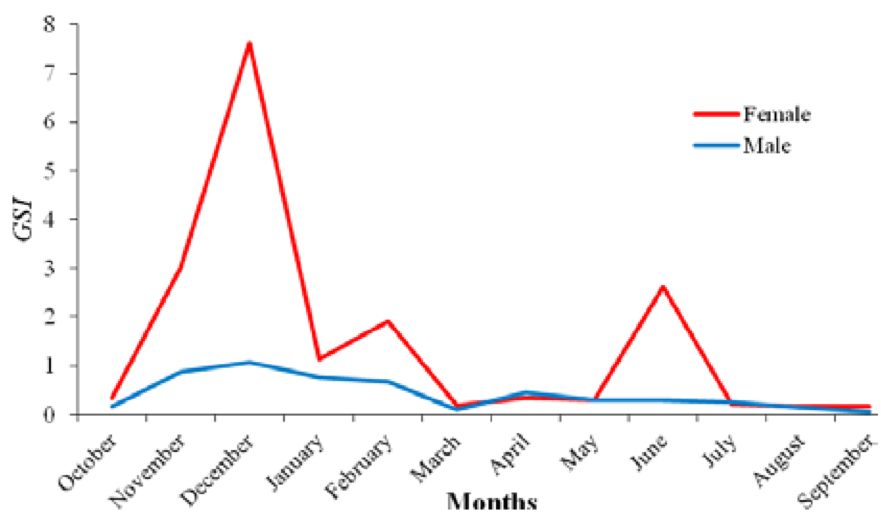

Fig. 1. Monthly changes in the mean gonadosomatic index (*GSI*) by sex of *Merluccius merluccius* from the Sea of Marmara

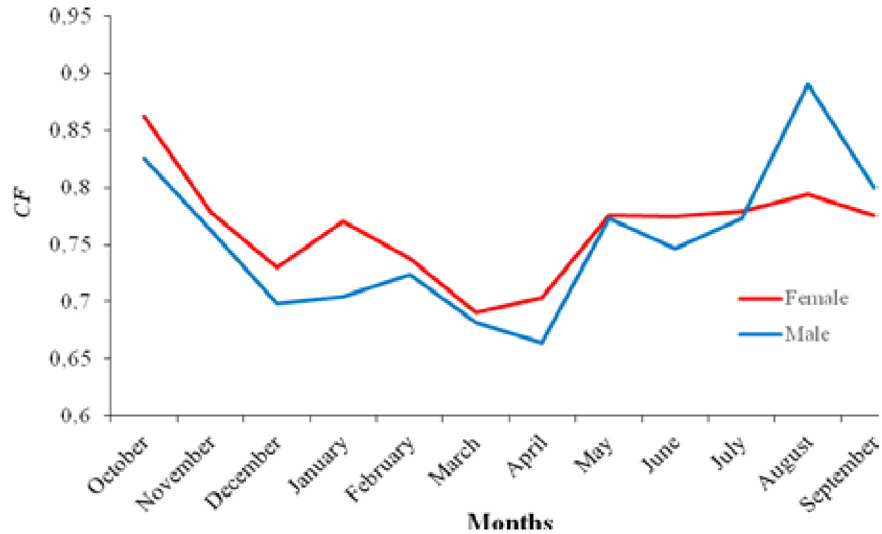

Fig. 2. Monthly changes in the mean condition factor (*CF*) by sex of *Merluccius merluccius* from the Sea of Marmara

cally significant difference (P<0.05) was noticed in general, because females were more represented in all samples. The gonadosomatic index (*GSI*) values calculated monthly for both sexes are presented in Fig. 1. In general, the *GSI* values increased remarkably in the late autumn as well as in the summer, when the reproduction activities were intense. It was observed that these values peaked in November, December, and June; and reached to maximum levels in December. On the other hand, the condition factor (*CF*) values calculated monthly for both sexes are given in Fig. 2, showing the degree of well-being of the fish in their habitat. Analyzing jointly both figures, we found that the *GSI* values increased, while the *CF* values showed a tendency to decrease notably in November, December, March, and April. Thus, it is clear that there was an inverse correlation between *CF* values and ovarian development, and *CF* values also increased in January and August, when the reproduction activities ended.

We determined that there were four different developmental stages of oocytes (Fig. 3). Out of the 341 females, 271 specimens were in stage *a*, 15 specimens in stage *b*, 29 specimens in stage *c*, and 26 specimens in stage *d*. As shown in Table 1, we found that a total of 55 females mostly collected in December were sexually mature (stage *c* and *d*) and all were well over 24 cm. On the other hand, immature or virgin oocytes (stage *a*) were most common in October, January, February, July, and August. Developing and spawning oocytes (stages *b* and *c*) were most prevalent in December and June, suggesting that peak spawning activity of *M. merluccius* occurred during the late autumn and summer. Post-spawning (stage *d*) specimens with opaque and hyaline oocytes absent or residual were first noted in November and December. Accordingly, we concluded that sexual maturity in *M. merluccius* occurs in late autumn. Furthermore, it was determined that the length at onset of sexual maturity (L_m) values was 29.9 cm for females and 22.5 cm for males.

The micrographs of the gonadal cross-sections (Fig. 3) reveal that the ovary of spawning females contained oocytes in all developmental stages. Therefore, it is clear that the oocyte development type is asynchronous also in this stock. In addition, the diameters of oocytes from the immature or virgin (stage *a*) to spawning (stage *c*) were measured at 44.8 µm and 541.3 µm, respectively.

Discussion

We present the first attempt at a histological characterization of oocyte developmental stages and other reproductive patterns of the European hake from the Sea of Marmara. As Costa (2013) indicated, the annual evolution of the maturity stages in this species is a reliable indicator for the spawning periods, and the monthly evolution of gonad weights is another indicator. Actually, we have considered that the two indicators play a major role in determining the season of spawning. In the Bay of Biscay, the main spawning season has a defined spawning peak between January and April (Lucio et al. 2000, Alvarez et al. 2004, Murua et al. 2006). At the Galician Shelf, the spawning period occurs in January and March (Domínguez–Petit 2007). In the northern Tyrrhenian Sea, there were considerable differences in the spawning peaks: the reproductive activity was concentrated between January and May, peak-

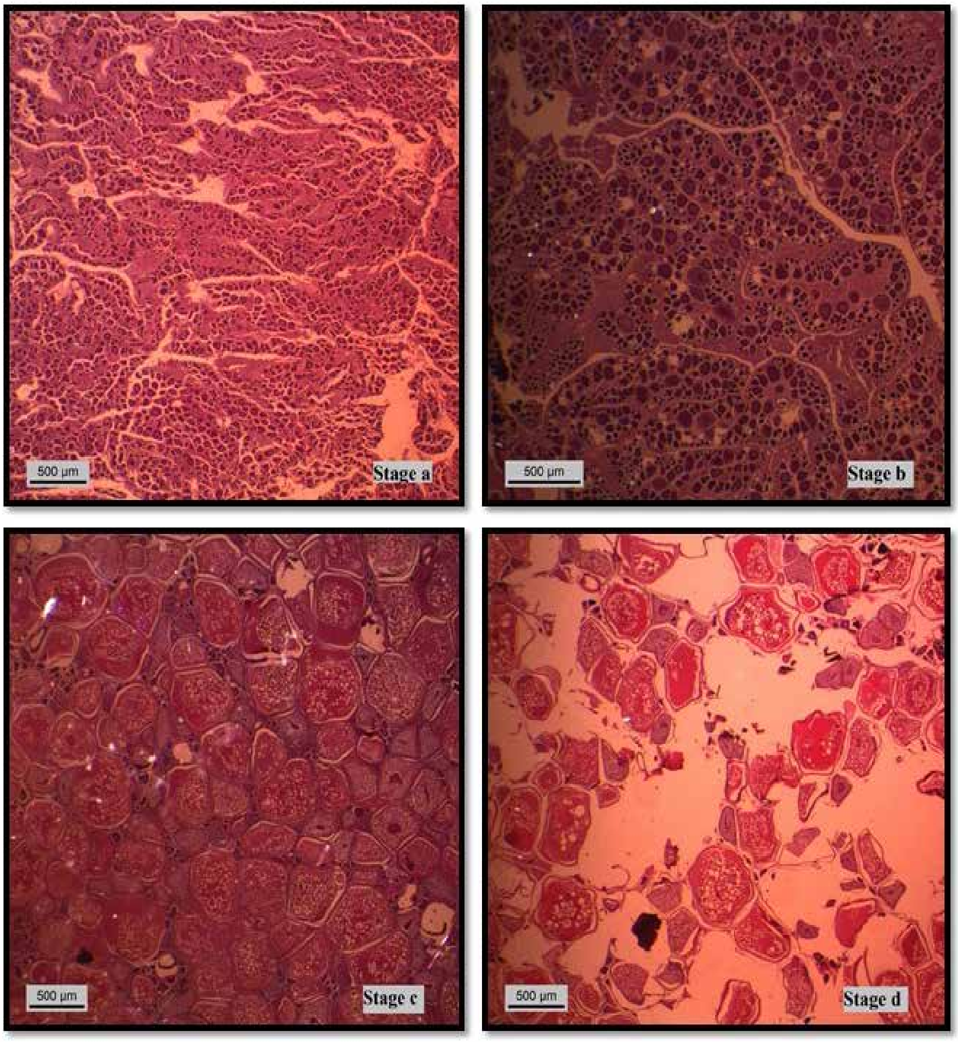

Fig. 3. Micrographs from gonad cross-sections of immature or virgin stage (***a***): Ovary from an immature fish showing only perinucleolar stage oocytes, oocyte diameter: 44.8–61.3 μm, developing stage (***b***): ovary from an active non-spawning specimen with vitellogenic oocytes with yolk granules, oocyte diameter: 74.9–118.6 μm, spawning stage (***c***): ovary from spawning individual with vitellogenic oocytes with enlarged yolk granules, oocyte diameter: 359.0–541.3 μm, post-spawning stage (***d***): ovary from a spawning individual with post-ovulatory follicles.

ing in February and May, while in the Catalan Sea the main reproductive season occurred from August to December, with spawning peaks in September and in December (Recasens et al. 2008). For southern European hake, Mehault et al. (2010) reported that the majority of reproductive activities occurs in February and May. In the eastern central Atlantic, El Habouz et al. (2011) indicated that both females and males were engaged in reproductive activity year-round, with a main spawning peak in winter (January–February) and a secondary concentration in summer (July–August). Along the Portuguese coast, Costa (2013) reported that the highest proportion of mature fish was caught between December and May, and the spawning period occurs three times per year (January–March, May–June and August). Along the Tunisian coast, the number of actively spawning fish peaks in January, April and August (Khoufi et al. 2014). In this study, the spawning periods of *M. merluccius* occurred in November, December and June, when the developing and spawning oocytes (stages *b* and *c*) were predominant. Furthermore, we observed that the *GSI* values increased steadily during these months, peaking in December and June. Accordingly, the spawning seasons suggested in this study are consistent with the reports of previous studies.

In the present study, the photomicrographs of ovarian histology have demonstrated that *M. merluccius* is an indeterminate and asynchronous spawning species (Fig. 3), and this is in accordance with the observations reported by Murua et al. (1998), Murua & Saborido–Rey (2003), Murua & Motos (2006), Recasens et al. (2008) and Domínguez–Petit et al. (2008a). Moreover, we found that the oocyte diame-

Table 1. Oocyte developmental stages related to total length (TL, cm) in immature and maturing female *Merluccius merluccius* as identified by histological sections

			Oocyte developmental stages and the number of fish exhibiting these stages			
Months	**N**	**TL (cm)**	**Stage (a)**	**Stage (b)**	**Stage (c)**	**Stage (d)**
October	35	19.2 – 36.9	33	2		
November	25	17.5 – 43.1	16	2	1	6
December	35	24.2 – 38.8	2	5	21	7
January	38	22.3 – 31.2	34		2	2
February	35	13.4 – 55.3	28		4	3
March	21	18.0 – 21.8	21			
April	16	29.8 – 46.2	15	1		
May	25	29.2 – 41.5	25			
June	39	29.2 – 48.3	25	5	1	8
July	26	23.4 – 35.5	26			
August	28	20.5 – 29.0	28			
September	18	21.6 – 26.3	18			

Table 2. Length at the onset of sexual maturity (cm) for *Merluccius merluccius* from different studies

Authors	**L_m (♀)**	**L_m (♂)**	**Study area**
Pineiro & Sainza, 2003	45.0	32.8	Iberian Atlantic waters
Lahrizi, 1996	41.1	37.8	northern Moroccan Atlantic
El Habouz, 1995	46.5	35	central Moroccan Atlantic
El Habouz, 2011	33.8	28.6	eastern central Moroccan Atlantic
Bouaziz et al., 1998	35.1	21.5	Region of Bou-Ismail (Alger)
Recasens et al., 1998	38.0	28.8	Mediterranean Sea Gulf of Lions
Recasens et al., 2008	35.8	-	Catalan Sea
Recasens et al., 2008	35.1	-	northern Tyrrhenian Sea
Khoufi et al., 2014	29.0	-	Tunisian coast
Soykan et al., 2015	21.4	25.6	central Aegean Sea
This study	29.9	22.5	Sea of Marmara

ters varied between 44.8 µm and 541.33 µm. Murua et al. (1998) and Murua & Motos (2006) reported that the oocyte diameters were between 150 µm and 1,150 µm. Recasens et al. (2008) also indicated that the oocyte diameters for all stages ranged from 20 µm to 1,150 µm. In addition, El Habouz et al. (2011) stated that the diameters of small and hydrated oocytes were 150 µm and over 750 µm, respectively. Accordingly, the diameters found in our study were relatively lower than those of other studies.

In our samples, length at onset of sexual maturity (L_m) was estimated at 22.5 cm for males and 29.9 cm for females. As shown in Table 2, the L_m size for females was relatively close to the findings of other studies carried out along the Tunisian coast: L_m = 29.0 cm (Khoufi et al. 2014), in the eastern central Moroccan Atlantic: L_m = 33.8 cm (El Habouz et al. 2011), in the Region of Bou–Ismail (Alger): L_m = 35.1 cm (Bouaziz et al. 1998), and in the northern Tyrrhenian Sea and in the Catalan Sea: L_m = 35.1 cm and 35.8 cm, respectively (Recasens et al. 2008). Similarly, our L_m value estimated for males was in line with the findings of other studies by El Habouz et al. (2011) and Soykan et al. (2015). However, the L_m sizes for both sexes were far lower than those of other studies (El Habouz 1995, Lahrizi 1996, Pineiro & Sainza 2003).

As has been widely reported, the causes of observed differences in the sex ratio, spawning period, oocyte diameters, *GSI*, *CF*, and L_m values may be attributable to local factors such as temperature, salinity, habitat variation, food availability, maturity stage, fishing season, high fishing mortality, and genetic variation (Ricker 1969, Baganel & Tesch 1978, Recasens et al. 1998, Basilone et al. 2006, Froese 2006, Domínguez–Petit et al. 2010).

Based on the oocyte stages and measurements, sexually mature females were all in excess of 24 cm in length. Moreover, the lengths at onset of sexual maturity (L_m) estimated for females and males were

29.9 cm and 22.5 cm, respectively. In this case, we have concluded that our L_m values for this species are consistent with the findings reported in previous studies. However, the minimum conservation reference size (MCRS) for the European hake is 20.0 cm (TL) in Turkish waters (BSGM 2016), and we have considered that there is no scientific basis for this regulation. Besides, Philips (2014) has suggested that the MCRS for European hake should be 25.0 cm in total length to ensure that fish have the opportunity to spawn. Similarly, we have proposed that the MCRS value for *M. merluccius* should be 25.0 cm as applied in Turkish waters by 2016 (BSGM, 2012).

According to the General Fisheries Commission for the Mediterranean (GFCM), the stocks of *M. merluccius* are overexploited in the Mediterranean Sea (GFCM 2016). Besides, this species has been considered as the Least Concern category in IUCN Red List of Threatened Species, and due to overfishing, its abundance in the Mediterranean has declined remarkably (Fernandes et al. 2016). Similarly, in Turkish waters, particularly in the Sea of Marmara, the European hake populations have been assessed as overexploited, and the excessive fishing effort or intensity on this species is likely to reduce the spawning stock to unsustainable levels. Therefore, it is recommended that an effective recovery plan should be implemented immediately in order to conserve the stocks of European hake in the Sea of Marmara.

Acknowledgements: We are grateful to the artisanal fishermen of the region for their help in collecting the fish samples. This work was supported by Scientific Research Projects Unit of Istanbul University (Project No: 43425).

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Received: 05.12.2016
Accepted: 26.04.2017