



PROOF

Spawning Frequency of *Trachurus mediterraneus* (Carangidae) in The Sea of Marmara

Nazlı Demirel^{1,*}, Ahsen Yüksek¹

¹ Istanbul University, Institute of Marine Science and Management, 34116, Vefa, Istanbul, Turkey.

* Corresponding Author: Tel.: +90.212 4400000; Fax: +90.212 5268433;
E-mail: ndemirel@istanbul.edu.tr

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Abstract

In this study the spawning frequency of *Trachurus mediterraneus* was estimated for the first time in the Mediterranean basin. The presence of the late-migratory nucleus stage oocytes and post ovulatory follicles (POFs) in the same ovaries were observed continuously during the sampling period. The daily percentage of spawning females with ovaries containing: late migratory nucleus (MN) stage, POFs (present up to 12-24h from the spawning event) was calculated as 11.7% and 18.6% respectively. The average percentage of females presenting one of the two different states was 15.3% which indicated that Mediterranean horse mackerel had high spawning rates in the Sea of Marmara. Our results indicated that *T. mediterraneus* is a multiple spawner and females spawn approximately every 6.6 days, therefore 20 times in the spawning period May-August 2009.

Keywords: Spawning frequency, post ovulatory follicles, Mediterranean horse mackerel

Marmara Denizi'nde Bulunan *Trachurus mediterraneus* (Carangidae) Türünün Üreme Sıklığı

Özet

Bu çalışmayla Akdeniz havzasında ilk defa *T. mediterraneus* türünün üreme sıklığına ilişkin değerlendirme ortaya konmuştur. Örnekleme dönemi boyunca aynı ovaryumda ileri çekirdek göçü evresindeki (ileri ÇG) oositlerle birlikte yumurtlama sonrası foliküllerin (YSF) bir arada bulunduğu gözlenmiştir. İleri çekirdek göçü evresi, yeni YSF (12 saate kadar) ve eski YSF (36 saate kadar) içeren ovaryumlara sahip yumurtlayan dişilerin yüzdesi sırasıyla %11,7, %20,9 ve %16,4 olarak hesaplanmıştır. Üç farklı oosit yapısına sahip ovaryumlu dişilerin ortalama yüzdesi Marmara Denizi'nde sarıkuyruk istavrit balığının %16,3 ile yüksek yumurtlama sıklığına sahip olduğunu göstermektedir. Çalışmamızın sonuçları, *T. mediterraneus* türünün çoklu yumurtlayıcı olduğunu ve Mayıs-Ağustos 2009 üreme döneminde dişilerin ortalama her 6 günde bir olmak üzere yaklaşık 20 defa yumurtladığını göstermektedir.

Anahtar Kelimeler: Üreme sıklığı, yumurtlama sonrası folikülleri, sarıkuyruk istavrit balığı

Introduction

Knowledge on reproductive strategy of fish species provides important information for estimation of spawning stock biomass (SSB). Estimated SSB can be a critical component of an effective fisheries management allowing prediction of stock biomass for the following years. The term reproductive strategy includes several reproductive characteristics such as, ovarian organization, fecundity type and spawning pattern of females, according to development and recruitment of oocytes (Murua and Saborido-Rey, 2003). Many commercial marine fishes are multiple

spawners, which mean that the females spawn only a portion of the oocytes per batch (Murua and Saborido-Rey, 2003; Murua *et al.*, 2003). Multiplying the number of oocytes per batch with the number of batches in a spawning season gives the reproductive potential of single female (Murua *et al.*, 2003; Hunter and Macewicz, 1985).

Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner, 1868), Atlantic horse mackerel *Trachurus trachurus* and blue jack mackerel *Trachurus picturatus* are three species of the genus *Trachurus* in the Mediterranean Sea. Mediterranean horse mackerel is a schooling species, widely

distributed in Turkish waters. It is one of the most important fishery stocks, together with horse mackerel and small pelagics such as anchovy, sprat and sardine in Turkey. Despite the fact that Black Sea represents the most significant part in Turkish fishery, the main fishing area of this species is the Sea of Marmara. Although its importance in fisheries and the decreasing trends of its stocks, observed in the last 10 years (TÜİK, 2012), very limited information on its reproductive biology (Demirel and Yüksek, 2013) is available in Turkish waters. Even more, very limited researches were performed on reproductive strategy of this species (Cautis, 1979; Yankova *et al.*, 2010; Yankova, 2011 for the Black Sea; El-Gharabawy and Abdel-Aziz, 1988 for the East Mediterranean Sea; Viette *et al.*, 1997 for the West Mediterranean Sea) in the entire Mediterranean basin. According to previous

studies this species was defined as a batch spawner with asynchronous ovarian organization and indeterminate fecundity (Demirel and Yüksek, 2013) in the Sea of Marmara.

The aim of this study is to estimate the number of batches of Mediterranean horse mackerel during the spawning season. We histologically examined female gonads and identified the stages of oocytes in the ovaries. Our study presents, an estimation of spawning frequency of Mediterranean horse mackerel.

Materials and Methods

Samplings were performed by commercial fishing boats in the north-eastern part of the Sea of Marmara (Figure 1) biweekly in May-August 2009

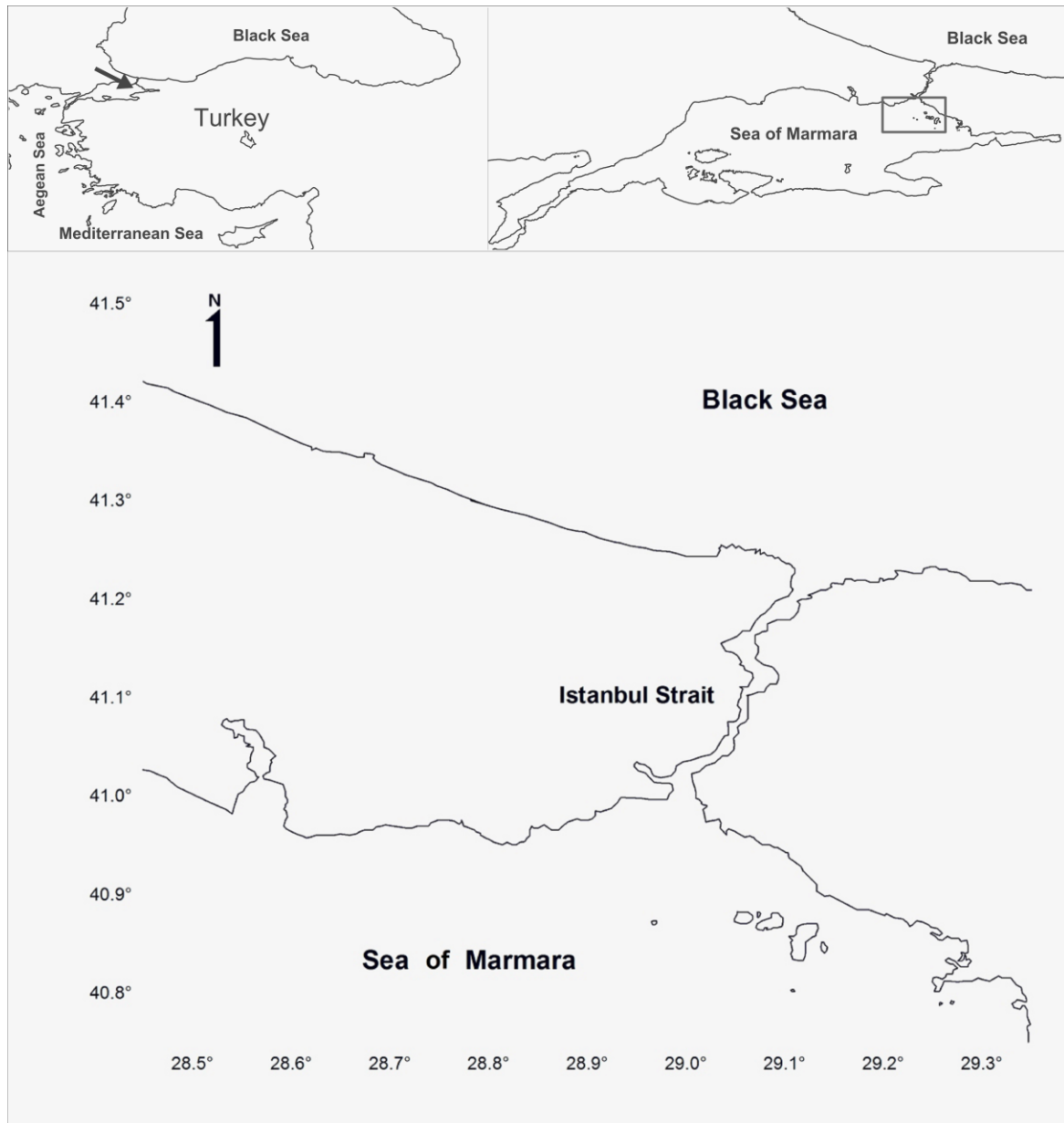


Figure 1. Sampling region.

which was considered as a peak spawning period of *T. mediterraneus* in the Sea of Marmara (Demirel and Yüksek, 2013). Mediterranean horse mackerel samples were randomly chosen as 50-75 individuals in each sampling (Table 1). In the sampling region fishing is generally performed via purse seines between 25-65 m depths and samples were taken in the early morning around 06.00 am. Sea surface temperatures were recorded and ranged between 19.8 °C and 22.9 °C during the sampling period.

Spawning frequency is defined as the ratio of mature females which spawns per day to all female. Thus, we applied post-ovulatory follicle (POF) method for the estimation of spawning frequency (Hunter and Macewicz, 1985). The method is based on recording the proportions of daily spawning classes considering the female ovaries with late migratory nucleus stage (late MN), hydrated oocytes (HO) and POFs.

Developmental stage of female gonads was evaluated with naked eye based on the general macroscopic criteria (Murua and Saborido-Rey, 2003). Mature ovaries were removed and preserved in 10% buffered formalin immediately after capture for further analyses. Histological analyses were performed on mature female ovaries and only ovaries in advanced developmental stages were considered to estimate spawning frequency. Tissues were removed from each ovary, dehydrated and cleared in xylene before embedding in paraffin. Each tissue was sectioned in 5 µm thick slices and stained with Mayer's hematoxylin and eosin. Each section was examined under light microscope between 10x-40x magnification by the Leica image analysis software.

Histological characteristics and developmental stages of the oocytes and age of POFs were defined from the slides of ovarian tissues. POFs in the ovary were defined as the degeneration levels of follicles. The estimated classification according to the criteria (Hunter and Macewicz, 1985) that was modified for *T. trachurus* (Karlou-Riga and Economidis, 1997) were indicated three states: (i) Spawning will start in 24 h; presence of late MN or HO, (ii) Spawning occurred within 12-24 hours; presence of new post ovulatory follicles (n-POFs) in the ovary, (iii) Spawning occurred 36 hours before; presence of the old post ovulatory follicles (o-POFs) in the ovary. On the contrary there is no information on duration of

MN, HO and POFs for Mediterranean horse mackerel. However we have some information for late MN and HO that last 12-24 hours in other species of *Trachurus* genus *T. trachurus* (NE Atlantic by Eltink, 1991; Gonçalves *et al.*, 2009; Aegean Sea by Karlou-Riga and Economidis, 1997) and Pacific jack mackerel *T. symmetricus* (NW Pacific by Macewicz and Hunter, 1993). Therefore we assumed that Mediterranean horse mackerel shows similar patterns for oocyte development and POFs ageing. Thus, we followed the illustrative instructions recorded for anchovy (Hunter and Macewicz, 1985), Pacific jack mackerel (Macewicz and Hunter 1993), Atlantic horse mackerel (Karlou-Riga and Economidis, 1997) and sardine (Ganias *et al.*, 2003).

The significance among each ovarian state which used to estimate spawning frequency was tested by one-way ANOVA. Statistical analyses were performed using STATISTICA® (v. 8.0).

Results

Within this study, a total of 437 specimens were collected between May and August 2009 from which 177 females were examined. Specimens ranged between 13.5 and 19.5 cm in total length, 18 and 64 g in weight.

Ovaries with different spawning states were considered for the estimation of spawning frequency (Table 2). Average fraction of Mediterranean horse mackerel female with less than 36 h POFs were calculated 0.15 (S.D. = 0.05). The daily percentage of spawning females with ovaries containing (Figure 2): late migratory nucleus (MN) stage, POFs (present up to 12-24h from the spawning event) was calculated as 11.7% and 18.6% respectively. The average percentage of females presenting one of the two different states was 15.3%. According to our results, we evaluate that females spawn every 6.6 days and approximately 20 times in the spawning period May-August 2009 in the Sea of Marmara.

We found significant differences among the percentages of females with different ovarian states (ANOVA; $F = 8.05$, $d.f. = 2$, $P < 0.001$) but no difference was detected among months (ANOVA; $F = 0.82$, $d.f. = 3$, $P = 0.584$).

Table 1. General information of the sampling dates, coordinates of the sampling area and the sample sizes

Dates	Coordinates	N	Female	Male
04.05.2009		50	14	32
22.05.2009		54	27	27
05.06.2009		55	25	30
18.06.2009	40° 51' 54" N 29° 03' 45" E & 40° 50' 30" N 29° 03' 52" E	66	29	37
01.07.2009		50	21	29
20.07.2009		55	14	29
03.08.2009		51	25	27
17.08.2009		47	22	24

Table 2. Proportion of females with different ovarian states of (Mn, spawning occurs in 24 h.; n-POFs, new post ovulatory follicles, spawning occurred within 12-24 h.; o-POFs, old post ovulatory follicles, spawning occurred 36 h. before). SD: standard deviation

Months	Stage of oocytes and POFs						Mature females N
	Mn		n-POFs		o-POFs		
	n	%	n	%	n	%	
May	5	12.2	6	14.6	5	12.2	41
June	6	11.1	10	18.5	8	14.8	54
July	6	17.1	11	31.4	9	25.7	35
August	3	6.4	9	19.1	6	12.8	47
Total	20		36		28		177
Mean %		11.7		20.9		16.4	
S.D.		2.2		3.6		3.1	

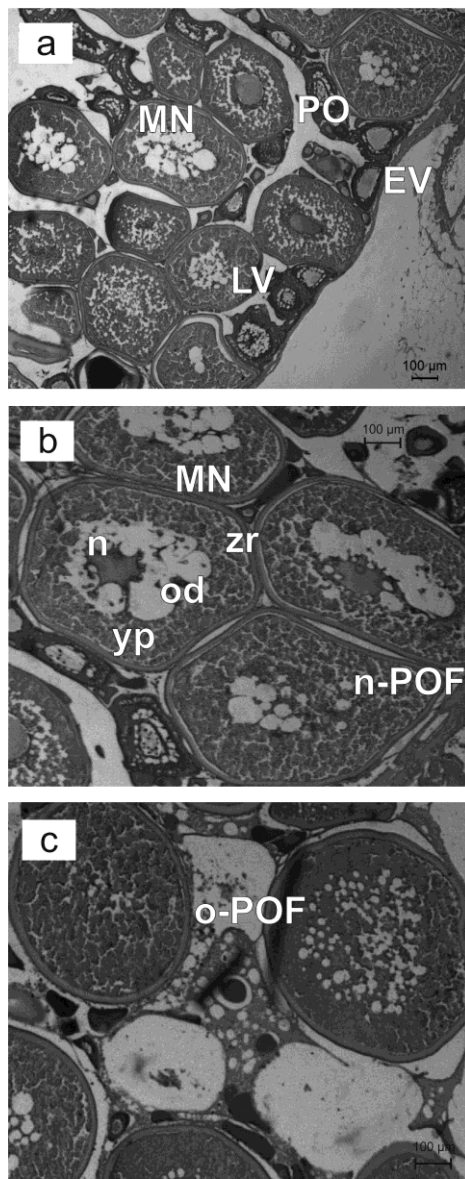


Figure 2. Different stages of oocytes and POFs in mature females ovaries. a) General view of oocytes with 4 developmental stages. b) Oocytes with late migratory nucleus stages and new POFs. c) Old-POF, mean age: 36h. EV: Early vitellogenesis, LV: Late vitellogenesis, MN: Migratory nucleus, PO: Primary oocytes, POF: post ovulatory follicle, n: nucleus, od: oil droplet, yp: yolk plate, zr: zona radiate

Discussion

Reproductive strategy of marine fishes differs among species. The patterns of reproduction provide information about life cycle of given species and better management of its exploitation. Although reproductive rate in bony fishes were emphasized with no relation with fecundity (Froese and Luna, 2004), estimation of spawning stock biomass for a multiple spawner still implies the estimation of spawning frequency and batch fecundity. In light of these issues, we obtained, for the first time, an estimation of spawning frequency of *T. mediterraneus*. The presences of the late-migratory nucleus stage oocytes and POFs in the same ovaries were observed continuously during the sampling period, confirming the species as multiple spawner (Hunter and Macewicz, 1985).

Although no information was available on the spawning frequency for *T. mediterraneus* in the entire Mediterranean basin, previous researchers assumed that *Trachurus* genus is generally characterized by high spawning frequency (Macewicz and Hunter, 1993). According to the results of previous studies, spawning frequency of other species of *Trachurus* genus, *T. trachurus*, was estimated as 8.3% for the eastern Atlantic (Eltink, 1991), 17.1% and 20.9% for Saronikos Gulf and Aegean Sea (Karlou-Riga and Economidis, 1997) and 10-30% for the Portuguese coasts (Gonçalves *et al.*, 2009). Our results for *T. mediterraneus* indicated similarities to the spawning frequencies of *T. trachurus*.

The number of Mediterranean horse mackerel females with different ovarian states showed significant differences in the sampling period. Females whose ovaries contain late MN oocytes were represented with lower number in the samples. The short duration of migratory nucleus stage (Murua *et al.*, 2003) may be the reason of the lower percentage of this stage compared to the others, indicating the probability of the biased estimation and the necessity of a higher sample numbers. Moreover, Hunter and Goldberg (1980) indicated that spawning frequency estimation is based on the assumption that the spawning population is stable in the area during the spawning season. Additionally some researchers pointed out that a biased sampling may be the results of the fish behavior (Gonçalves *et al.*, 2009). As an example of *T. trachurus*, females immediately disperse after spawning and because of that sampling effort were considered to represent a good number of females with each ovarian state (Gonçalves *et al.*, 2009). We detected differences between the proportion of females with new-POFs that indicate spawning occurred in 12 h and females with old-POFs that indicate spawning occurred in 36 h. It was reported that the duration of POFs affected by water temperature (Hunter and Macewicz, 1985). In our study, sea surface temperatures were ranged between 19.8 °C and 22.9 °C in the sampling period. However,

due to the lack of knowledge on Mediterranean horse mackerel oocyte development, POFs duration and atresia formation in female ovaries, we considered water temperature values as descriptive features of sampling area for further studies.

In this study we defined spawning frequency of this species by ovaries with three different states of the Mediterranean horse mackerel. Differences among methods on estimation of spawning frequency demonstrated the need for more sampling as well as laboratory experiments to improve knowledge on oocyte duration and POFs ageing in future studies.

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