ISSN: 0001-5113
AADRAY

ACTA ADRIAT.,
62 (2): 183 - 198, 2021

ORIGINAL SCIENTIFIC PAPER

Reproductive traits of the European hake, *Merluccius merluccius* (L. 1758), in the Adriatic Sea

Barbara ZORICA*, Igor ISAJLOVIĆ, Nedo VRGOČ, Vanja ČIKEŠ KEČ, Damir MEDVEŠEK, Vedran VULETIN, Ivana RADONIĆ, Ratko CVITANIĆ, Ivana LEPEN PLEIĆ and Marija ŠESTANOVIĆ

Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovića 63, 21000 Split, Croatia

***Corresponding author, e-mail:** *zorica@izor.hr*

Within this paper, reproduction traits of the Adriatic population European hake, Merluccius merluccius *(Linnaeus, 1758), were investigated. Specimens of the target species were monthly collected (N=1173) during 2019 along the eastern Adriatic Sea with commercial bottom trawlers. Overall, European hake total body length varied between 15.5 and 49.5 cm (mean±SD=27.5±5.0 cm) and domination of females was noted in larger length classes (TL>31.0 cm). The sexual ratio of all specimens was m/f=0.64; male prevalence was established over the year, except during the spawning peaks (December, June) when the sex ratio was in favour of the females. According to European hake's maturity stages, gonad weights, gonadosomatic indexes and histological analysis of the gonad tissues, this species in Adriatic spawn twice in one year; the spawning period was from December to February, while second smaller signs of spawning were observed at the beginning of the summer, in June. 50% of the European hake male and female specimens sexually matured at 20.7 cm and 22.4 cm of total length, respectively.*

Key words: Sex ratio; Maturation; Spawning; Early life stages; Hake, Mediterranean

INTRODUCTION

Nowadays, according to the last and available stock assessments for the Mediterranean and the Black Sea the majority of exploited fish stocks are currently fished at biologically unsustainable levels (FAO, 2018). Those stock assessments, as well as the status of their stocks, were defined due to the values of the fishing mortality and the size of the spawning stocks (SSB-spawning stock biomass) over the decades. One of the overexploited stocks that inhabit Adriatic Sea (Geographical sub-area (GSA): 17 and 18) is the stock of European hake, *Merluccius merluccius* (Linnaeus, 1758). This fish is considered as one of the major demersal commercial species, which official annual average catches from 2008 to 2018 in the Mediterranean as well as in the Adriatic were 18779.4±2583.4 t and 856.6±135.2 t (FAO, 2019), respectively.

Generally, European hake is a benthopelagic species, widely distributed in the north-eastern Atlantic (from Norway in the north to the Guinea Gulf in the south), Mediterranean as well as the Black Sea (CASEY and PERIERO, 1995; COHEN *et al*., 1990; FISCHER *et al*., 1987; MURUA, 2010). As a demersal fish species, it occurs mostly between the depths of 70 m and 370 m, although it can be found in both shallower (30 m) and deeper (1000 m) waters (KORTA *et al*., 2015). In the Adriatic Sea, it is largely distributed along the whole area, except in the area north of the mouth of the Po river (FRATTINI and CASALI, 1998; JUKIĆ and ARNERI, 1984; PICCINETTI *et al*., 2012), while its bathymetric distribution goes from several meters in coastal areas to 800 m in the South Adriatic Pit (JUKIĆ-PELADIĆ *et al*., 1999; ŽUPANOVIĆ and JARDAS, 1987). Over the year, European hake individuals migrate within the Adriatic Sea either for food pursuit or for spawning. Namely, European hake juveniles migrations are driven by the search for food, hence during the spring they move towards the shallow areas, while during the winter months they are found in the area of Pomo/Jabuka Pit (PICCINETTI *et al*., 2012; VRGOČ *et al*., 2004) as this area in that period is known as an upwelling or nutrient rich area (ZAVATARELLI *et al*., 1998). European hake adults in the winter period migrate towards the area of Pomo/Jabuka Pit for spawning and it's first spawning peak appears (ŽUPANOVIĆ and JARDAS, 1987). Afterwards, in early spring, those specimens turn to shallower coastal areas where spawning invents continue but with lower intensity (UNGARO *et al*., 1993). The second peak of spawning occurs during the summer months and it also happens in the area of Pomo/Jabuka Pit (JUKIĆ and PICCINETTI, 1981; UNGARO *et al*., 1993; ŽUPANOVIĆ and JARDAS, 1987). This species is a multiple batch spawner, with length at first maturity oscillating within the total body length range of 20-33 cm and 21.5-43.0 cm in Adriatic (VRGOČ *et al*., 2004) and Mediterranean (CARBONARA *et al*., 2019), respectively. In the Adriatic Sea, European hakes are mainly caught with bottom trawlers and beam trawlers. Over the last two decades, European hake catches (landing and discard) and the values of assessed females spawning stock biomass in the whole Adriatic Sea (GSA 17 and GSA 18) fluctuated almost in the same manner; a decreasing trend within the mentioned period have been observed (STECF, 2019; 2020). Due to adverse European hake stock status, as well as status of Norway Lobster, the General Fisheries Commission for the Mediterranean (GFCM) establish fisheries restricted areas (FRA) in 2017 (FAO, 2017) that refers to Pomo/Jabuka Pit, an area known as the nursery and spawning area of mentioned species.

European hake reproductive parameters like sex ratio, size at sexual maturity, spawning period and fecundity were scarce and/or partly studied in the past (mostly in the second half of the last century - JUKIĆ and PICCINETTI 1981; ŽUPANOVIĆ and JARDAS 1986; UNGARO *et al*., 1993). Bearing in mind the present status of this stock as well as the fact that obvious climate-regiment shift did happened in Adriatic by the end of the last century (GRBEC *et al*., 2014) the main aim of this study was set. Namely, due to previous studies (DOMÍNGUEZ-PETIT *et al*., 2008; DOMÍNGUEZ-PETIT and SABORIDO-REY, 2010; MURUA, 2010) changes in reproduction traits might be expected due to observed changes in population biomass levels and environmental conditions in the study area. Hence, within this study, essential reproductive parameters of European hake are going to be improved and updated in order to improve existing management measures in the Adriatic Sea and in general.

MATERIALS AND METHODS

European hake specimens were monthly collected from commercial bottom trawlers, operating within the Croatian fishing ground (eastern Adriatic Sea, Fig. 1) from January to December 2019. Each month samples of investigated species were sampled either onboard (N=9) of commercial bottom trawl or on its landing place (N=17), put on ice and transported to the laboratory for the analysis.

A total of 1 179 European hakes were analysed in the laboratory upon arrival. Total length (TL, cm) and weight (W, g) of each specimen were measured with the precision of ± 0.1 cm and ±0.01 g, respectively. Length frequency distribution of the collected European hakes was compared to the one obtained throughout scientific survey MEDITS (International bottom trawl survey in the Mediterranean) which was done in the same year to check for possible differences. The length-weight relationship was calculated separately for each sex by applying the exponential regression $W = a\mathrm{TL}^b$, (W = total fish weight in g; TL = total length in cm; a = proportionality constant; and b = allometric growth parameter). The hypotheses of isometrical growth, and abbreviation between male and female allometric growth parameter, were tested using a Student's t-test ($P < 0.05$).

Sex and gonad maturity stages were assessed macroscopically according to MEDITS protocol. Used maturity scale has 8 maturity stages and its description is taken from MEDITS Handbook (AAVV, 2017) and given in Table 1. The overall sex ratio (males: females), sex ratio by size intervals (1.0 cm) and sex ratio by month were obtained by dividing the number of males by the total number of the individuals.

Over the study period, each month, gonads of 10 adult female individuals were taken for histological analysis. Histology was carried out not only to confirm macroscopically determined stages of gonads but also to endorse European hake as indetermined batch spawner. Gonads were fixed in 4% formaldehyde solution. Afterwards, the gonads were dehydrated in alcohol and embedded in paraffin wax. Longitudinal or cross sections were made with a microtome. The section thickness was 10 µm. Sections were stained with haematoxylin and eosin. Obtained histological slides of gonads were examined under a stereomicroscope (Zeiss Discovery. V12; magnification 150x). Oocytes were classified according to (BROWN-PETERSON *et al*., 2011)

Each histological slide of female gonad tissue was photographed by a camera coupled to Zeiss binocular microscope and a PC. By image analysis and processing that was carried out using Zeisse-software the maximum and minimum diameters of oocytes in different development stages were measured and then were averaged to obtain the mean size of each oocyte. Microscopic maturity staging of ovaries was done according to CARBONARA *et al.* (2019) who provided European hakes oocites development for each macroscopical maturity stage in the MEDITS scale (Table 1).

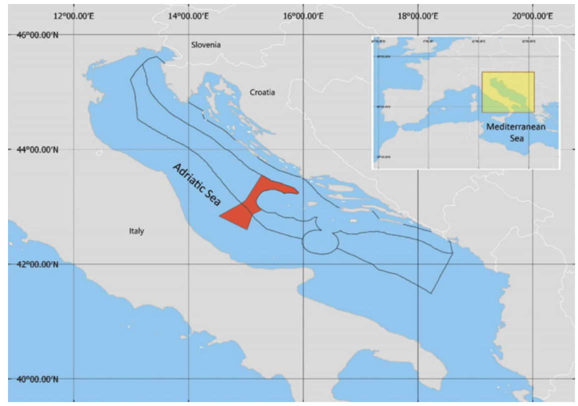

Fig. 1. Map of the Adriatic Sea showing the study area – lines are surrounding Croatian fishing ground while red area refers to FRA Jabuka/Pomo pit

The spawning seasonality of the European hake was described based on the data of the monthly changes in gonadosomatic index (GSI: the proportion of monthly gonad weight (Wg, g) in the total body weight (W, g)) and the fluctuations of maturity stages. GSI values by each maturity stage were compared and statistically analysed.

For the determination of the lengths at maturity (L_{50}, the length at which 50% of the individuals in population are mature) only specimens exclusively collected during spawning were used. Length at maturity was determined by proportion of spawning fish (gonads staged as 2b, 2c, 3, 4a and 4b) in each 1 cm size class, fit into a logistic model:

$$P(x) = \frac{a}{1 + e^{b+cx}}$$

where P is the percentage of mature fish at length x and a, b and c are constants.

All statistical analyses were performed with SPSS 5.5 software package and a level of significance of α=0.05 was accepted.

RESULTS

Among all European hakes, collected along the eastern side of the Adriatic Sea from January till December 2019, 749 were males, 424 were females and for 6 individuals' sex was not determined due to the poor condition of their

Table 1. Description of macroscopic maturity scale provided by MEDITS protocol (AAVV, 2017) along with the female microscopic appearance provided for each MEDITS maturity stage given by CARBONARA et al. (2019)

Stage	**Maturation state**	**Macroscopic characteristics**	**Microscopic characteristics**
0	UNDETERMINED	Sex not distinguished by naked eye. Gonads very small and translucid, almost transparent. Sex undetermined.	
1	IMMATURE-VIRGIN	Small pinkish and translucent ovary shorter than 1/3 of the body cavity; eggs not visible by naked eye. Thin and whitish testis shorter than 1/3 of the body cavity.	Only OO and PG through the perinuclear stage are present. The ovarian wall is thin and little space between oocytes are observed.
2a	VIRGIN-DEVELOPING	Small pinkish/reddish ovary shorter than ½ of e body cavity; eggs not visible by naked eye. Thin whitish testis shorter than 1/2 of the body cavity.	
2b	RECOVERING	Pinkish-reddish/ reddish-orange and translucent ovary long about ½ of the body cavity. Blood vessels visible; eggs not visible by naked eye. Whitish/pinkish testis, more or less symmetrical, long about ½ of the body cavity.	Only PG and CA oocytes are visible.
2c	MATURING	Ovary pinkish-yellow in colour with granular appearance, long about 2/3 of the body cavity. Eggs are visible by naked eye trough the ovary tunica, which is not yet translucent. Under light pressure eggs are not expelled. Whitish to creamy testis long about 2/3 of the body cavity. Under light pressure sperm is not expelled.	PG, CA, Vtg1 and Vtg2 oocytes are observed. No evidence of POFs. Some atresia can be present.
3	MATURE/SPAWNER	Ovary orange-pink in colour, with conspicuous superficial blood vessels, long from 2/3 to full length of the body cavity. Large transparent, ripe eggs are clear visible and could be expelled under light pressure. In more advanced conditions, eggs escape freely. Whitish-creamy soft testis long from 2/3 to full length of the body cavity. Under light pressure, sperm could be expelled. In more advanced conditions, sperm escapes freely.	All stages present: PG, CA, Vtg1, Vtg2, Vtg3 oocytes and/or oocytes undergoing hydration (GVM, GVBD, HO). If spawning is already started, POFs are observed. Atresia (AO) of vitellogenic and/ or hydrated oocytes may be present.
4a	SPENT	Reddish ovary shrunken to about 1/2 length of the body cavity. Flaccid ovary walls; ovary may contain remnants of disintegrating opaque and/ or translucent eggs. Bloodshot and flabby testis shrunken to about ½ length of the body.	Atresia of vitellogenic oocytes and POFs can be visible. PG and CA present.
4b	RESTING	Pinkish and translucent ovary long about 1/3 of the body cavity. Eggs not visible by naked eye. Whitish/pinkish testis, more or less symmetrical, long about 1/3 of the body cavity.	Only OO and PG oocytes (sometimes with circumnuclear oil droplets) are observed. The ovarian wall is thicker and there is more space, interstitial tissue and capillaries around PG oocytes than those observed at the immature stage.

gonads, hence, they were excluded from further analysis. Minimum and maximum values of male total body length were 15.5 cm and 42.0 cm, respectively (Fig. 2; Table 2). Mean monthly total body lengths of European hake males varied over the investigated period and the highest values were recorded during the summer months (June – September, Table 2), while on the yearly basis the mean total body length of all male specimens was 26.4 ± 4.1 cm. In general, European hake females were larger than males showing total body length range and mean yearly value of 16.5 – 49.5 cm (Fig. 2; Table 2) and 29.3 ± 6.8 cm, respectively. As well as in males, somewhat larger females were also caught during the warmer part of the year (April – September). European hake male and female length frequencies within the whole investigated period were significantly different (Kolmogorov-Smirnov Test, P<0.001); all specimens with TL > 42.0 cm were exclusively female. Furthermore, the length distribution of fish species collected within this study (15.5 cm < TL < 49.5 cm; January – December, 2019) was significantly different (Kolmogorov-Smirnov Test, P<0.001) from European hake length distribution obtained throughout MEDITS survey that happened in the same year and area (10.0 cm < TL < 78 cm; Croatian fishing ground, June – July 2019; (STECF, 2020)). As far as total body weight, in both sexes, it followed the observed length trends. The total body weight range for male and female European hakes fit within the values of 23.18 – 507.20 g and 27.00 – 753.98 g, respectively (Table 2). Overall, the mean total body weight for male was 130.76 ± 62.20 g, while for females it was slightly higher with the value of 187.16 ± 108.06 g.

The growth of the European hake males ($W=0.0049TL^{3.0892}$; $r^2=0.958$; Fig. 3A), as well as females ($W=0.0061TL^{3.0275}$; $r^2=0.961$; Fig. 3B), proved to be isometrical as the values of parameter b did not statistically differ from 3 (Student's t-test (P<0.05)). Therefore, the hypothesis of isometric growth for this species was accepted for both sexes, despite that monthly mean values of allometrical coefficients varied within the investigated period for both sexes. Namely, the allometrical coefficient of males ranged from 2.729 (September) to 3.114 (December), while for females its range was slightly wider and goes from 2.612 (April) to 3.460 (December).

During the investigated period European hake sex ratio was statistically significant in favour of males (m/f=0.64; χ^2=56.54, d.f=1, P<0.05). The sex ratio of studied species varied with size since males were represented at smaller length sizes, while females were dominant at larger length classes (Fig. 4). Namely, in the length classes below 31.0 cm, the domination of males was obvious with its mean values of m/f = 0.73. A greater proportion of females was observed in higher length classes, precisely from total body length class of 31.0 cm onwards. Monthly variations of sex ratio, which are shown in Fig. 5, revealed the prevalence of females only in two months (June and December) of the year. Afterwards, the sex ratio was dominated by the males.

Histology of the collected ovaries (N=87) reveals the differences between macro- and microscopic staging. Maturity stages 2a and 4a were macroscopically correctly identified (Table 3). Macroscopic maturity stage 2b, if it was incorrectly determined, was usually replaced by the maturity stage 4b. Maturity stage 2c and 3 were mostly replaced by maturity stage 2b and 4a, respectively. In the ovaries of the specimens that were prepared to spawn or just started to spawn histological analysis reveals the presence of almost all oocyte stages (PG, CA, Vtg1,

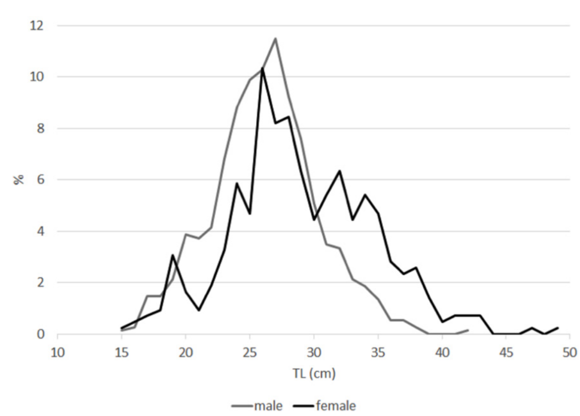

Fig. 2. Total length (TL, cm) frequency distribution of European hake male and female specimens collected with commercial bottom trawler along the eastern Adriatic Sea, January – December 2019

Table 2. Overview of the monthly collected male and female European hake specimens along the eastern side of the Adriatic Sea from January to December,2019; regarding its number (N), total body lengths (TL, cm), total body weights (W, g), gonad weights (Wg, g) as well as its monthly mean values with their standard deviations (SD) values

Month	N	Range TL (cm)	Mean TL(cm)±SD	Range W(g)	Mean W(g)±SD	Range Wg (g)	Mean Wg(g)±SD
Males							
January	109	15.5 – 38.5	23.8 ± 5.2	23.18 – 343.80	98.63 ± 70.17	0.02 – 4.87	0.59 ± 0.75
February	69	17.0 – 36.5	25.5 ± 4.4	28.66 – 367.50	114.27 ± 68.86	0.04 – 4.56	1.12 ± 1.11
March	110	17.0 – 35.5	25.6 ± 3.9	28.67 – 301.90	116.67 ± 53.90	0.05 – 3.59	0.79 ± 0.64
April	38	23.0 – 35.0	28.3 ± 2.9	70.81 – 260.08	148.45 ± 44.53	0.02 – 1.42	0.67 ± 0.38
May	70	21.0 – 36.0	26.5 ± 2.9	63.90 – 313.50	126.80 ± 45.67	0.12 – 1.97	0.60 ± 0.43
June	19	26.0 – 37.0	30.8 ± 2.9	118.70 – 340.72	199.24 ± 59.50	0.11 – 1.77	0.68 ± 0.50
July	59	21.5 – 33.5	27.7 ± 3.2	72.81 – 278.95	151.12 ± 51.32	0.05 – 1.74	0.49 ± 0.44
August	43	25.0 – 38.0	28.5 ± 3.2	108.72 – 387.70	163.85 ± 66.50	0.17 – 1.83	0.82 ± 0.49
September	39	26.5 – 42.0	29.5 ± 2.8	117.73 – 507.20	175.80 ± 62.39	0.28 – 2.12	0.79 ±0.46
October	88	20.0 – 37.5	26.9 ± 3.2	49.56 – 377.21	140.84 ± 54.77	0.07 – 2.70	0.55 ± 0.39
November	75	17.0 – 35.0	26.1 ± 3.9	29.44 – 278.62	123.79 ± 54.87	0.03 – 2.92	0.55 ± 0.56
December	30	23.0 – 29.0	25.7 ± 1.6	87.15 – 181.99	122.58 ± 24.75	0.11 – 3.65	1.10 ±0.79
Females							
January	39	16.5 – 42.0	24.4 ± 6.9	27.00 – 473.15	120.50 ± 109.28	0.04 – 16.28	1.61 ± 3.46
February	13	19.0 – 37.0	28.1 ± 5.9	35.11 – 329.49	156.08 ± 90.57	0.06 – 10.23	2.97 ± 2.93
March	35	19.5 – 39.0	27.7 ± 4.5	39.26 – 373.03	154.26 ± 70.96	0.04 – 14.11	2.08 ± 3.53
April	38	25.5 – 49.5	31.3 ± 5.5	106.61 – 753.98	215.08 ± 122.95	0.31 – 6.24	1.08 ± 1.01
May	50	21.0 – 43.5	31.1 ± 5.5	56.80 – 635.27	220.01 ± 114.53	0.14 – 2.84	0.86 ± 0.64
June	21	26.5 – 39.0	32.7 ± 4.1	135.68 – 391.70	243.25 ± 82.22	0.21 – 1.56	0.78 ± 0.33
July	48	24.0 – 41.5	30.8 ± 4.1	88.30 – 458.18	212.35 ± 85.77	0.14 – 9.67	1.40 ± 2.14
August	21	25.0 – 37.5	32.4 ± 3.8	111.66 – 377.30	239.25 ± 75.66	0.13 – 1.66	0.65 ± 0.39
September	31	26.5 – 43.5	33.1 ± 5.4	126.55 – 489.10	252.25 ± 108.92	0.25 – 6.17	1.37 ± 1.07
October	54	22.0 – 47.0	28.5 ± 4.7	57.06 – 643.12	168.44 ± 96.99	0.18 – 11.46	1.72 ± 2.50
November	34	18.0 – 35.0	26.2 ± 3.9	35.57 – 251.95	121.20 ± 52.05	0.04 – 2.57	0.58 ± 0.53
December	40	23.0 – 35.0	27.7 ±3.3	87.15 – 344.60	167.39 ± 75.22	0.13 – 27.44	6.08 ± 7.85

Vtg2, Vtg3, AO; Fig. 6). Throughout histological analysis, diameter of 11 311 oocytes (N=60) were measured with the lowest and highest values obtained at 12.69 µm (PG) and 596.0 µm (AO), respectively. Diameter ranges of all observed and measured oocyte stages are shown in Fig. 7.

According to obtained values of the monthly percentage composition of gonad maturity stages and the mean monthly gonadosomatic indices, a trend in spawning seasonality was noted (Table 2, Fig. 8). The gonads in maturity stages defined as maturing (2c), mature (3) and spent (4a) were present almost during the whole year, their higher percentages were noted from December to March. The slight increase of spent specimens was also noted in summer month, precisely in August. The most abundant maturity stage over the year was 2b – gonads in the stage of recovering. Regarding the values of gonadosomatic index it was noted that female had much higher values of this parameter than males. The highest values of gonadosomatic index were reached during the winter months (December – February), particularly in December (male: GSI=0.915; Wg=1.10 g; female: GSI=2.779; Wg=6.08 g). Whereupon, values of the gonadosomatic index with slight oscillation decreased till the end of the summer (Fig. 8). Slightly increase of GSI values were observed in June for males and July for females. The lowest values of gonadosomatic index were reached in

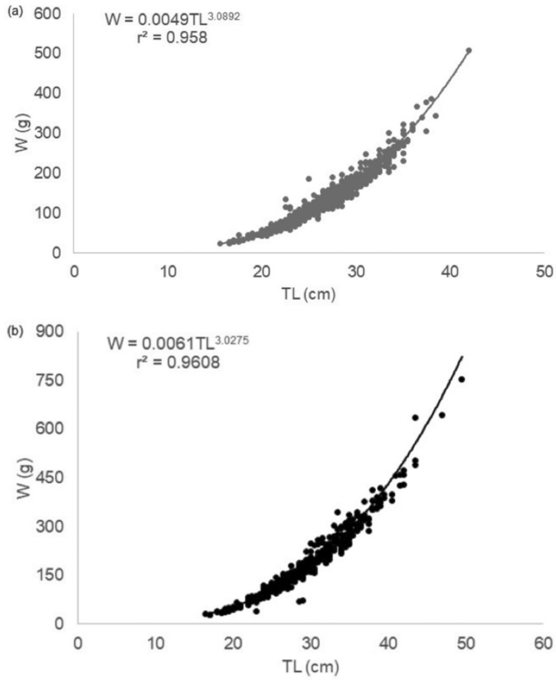

Fig. 3. The length (TL) - weight (W) relationship of male (a) and female (b) European hake specimens caught with commercial bottom trawlers along the eastern Adriatic Sea, January - December 2019

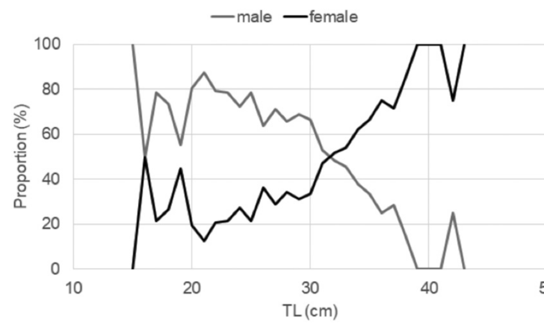

Fig. 4. Proportion of the sex ratio by length size classes of European hake specimens caught with commercial bottom trawlers along the eastern side of Adriatic Sea, January – December 2019.

July and August for male and female specimens, respectively. By the beginning of the autumn, the values of the two reproductive parameters started to increase. Calculation of the GSI values for each maturity stages pointed out that the highest and the lowest values were obtained for the gonads which were determined as spawning or just spawn and gonads in the resting/recovering stage (Fig. 9), respectively. This was also confirmed by the statistically analysis (Krusak-Wallis ANOVA, H=453.038, d.f.=6; $P<0.05$), while Post-hoc test indicated that only GSI values of the resting and recovering stages were not statistically significant difference (Fisher LSD Post-hoc test; $P>0.05$), while among the other stages difference in their GSI values were pronounced.

During the main spawning season (December – February, Fig. 8) the smallest mature European hake male had a total body length of 17.5 cm, while the smallest mature females showed highest total body length of 22.5 cm. According to the proportion of matured specimens within the spawning season estimated total body length at which 50% of males and females were matured was 20.74 cm (a=1.01; b=13.32; c=-0.64; r^2=0.966) and 22.38 cm (a=1.01; b=26.70; c=-1.19; r^2=0.992) (Fig. 10), respectively.

DISCUSSION

In this study length frequency distribution of both sexes was unimodal with the peaks reached at the length classes of 24.0 cm for males and 26.0 cm for females (Fig. 2). Length range of

Table 3. Comparison of the maturity stages determined macroscopically and microscopically for the same gonad

		Macroscopic identification (%)					
Microscopic identification	N	2a	2b	2c	3	4a	4b
2a	1	**100.0**					
2b	70		**74.3**	2.9		1.4	21.4
2c	9		22.2	**55.6**	11.1	11.1	
3	6				**66.7**	33.3	
4a	1					**100.0**	

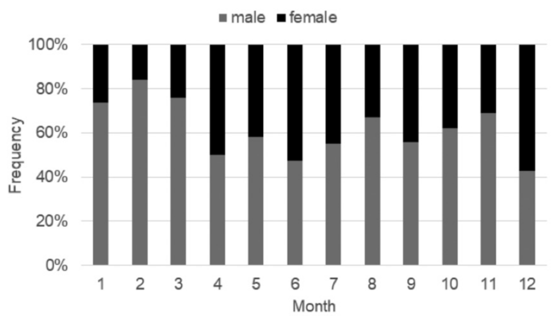

Fig. 5. Monthly sex ratio fluctuations of European hake specimens caught with commercial bottom trawlers along the eastern side of Adriatic Sea, January – December 2019.

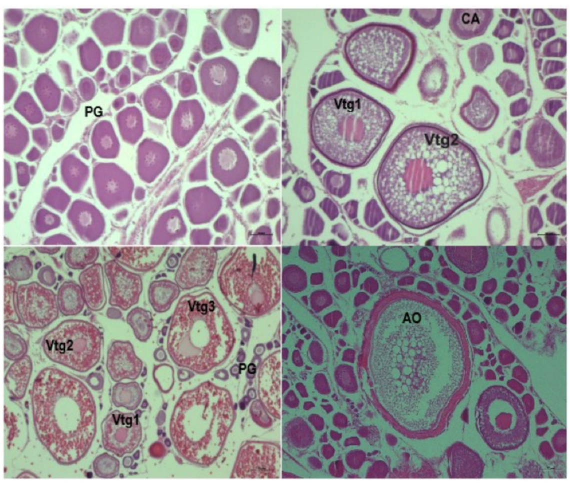

Fig. 6. Images of European hake ovary tissue and oocytes that were observed within it: PG – primary growth oocyte, CA – cortical alveolar, Vtg1 – primary vitellogenic oocyte, Vtg2 – Secondary vitellogenic oocyte, Vtg3 – Teritary vitellogenic oocyte, AO – Atretic oocyte

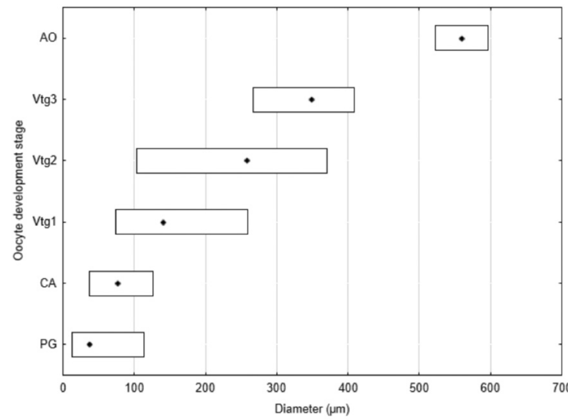

Fig. 7. Measured oocytes diameter ranges for each oocyte development stages observed in European hake ovary histological slides, January – December 2019

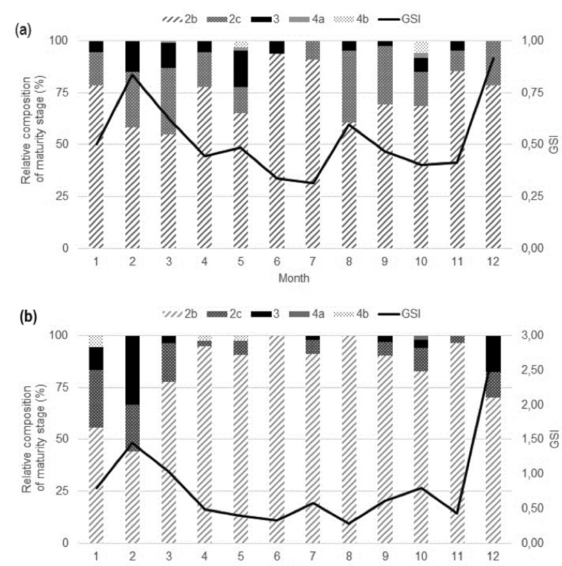

Fig. 8. Monthly oscillation of male (a) and female (b) gonad macroscopically determined maturity stages and mean monthly gonadosomatic index (GSI) of European hake specimens collected on the eastern side of Adriatic Sea from January to December 2019

the collected European hake specimens (15.0 cm < TL < 49.0 cm) via commercial bottom trawler was in accordance with the earlier findings obtained through the project "DemMon" (VRGOČ *et al.*, 2004), which also analysed the catches of commercial bottom trawlers in Croatian fishing ground from 2002 – 2003. Furthermore, the length distribution of studied fish species was significantly different from its length distribution obtained throughout MEDITS survey (June – July 2019). Within this scientific survey somewhat higher amount of smaller as well as the larger specimens were caught. The reasons for this lie in the fact that according to Croatian law, whole commercial bottom trawling is under spatial-temporal legislation and area of Pomo/Jabuka Pit known as nursery/spawning area of this species is established as Fisheries Restricted Area with a non-take zone (NN, 2016; 2019). Hence, slight differences observed were expected since scientific survey, with smaller mesh size net, try to cover the whole fishing area and areas where commercial vessels are not allowed.

Analysis of the length-weight relationship

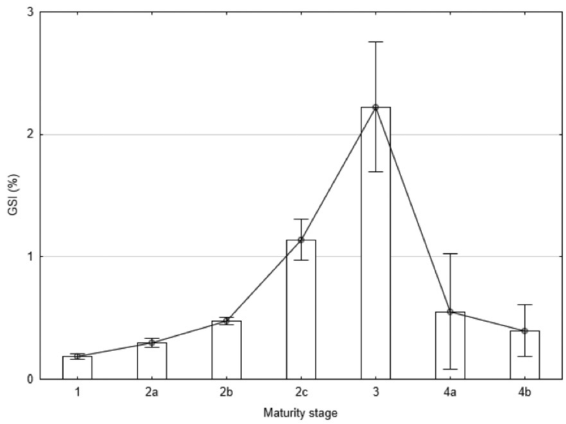

Fig. 9. Oscillation of gonadosomatic index (GSI) regarding the macroscopic maturity stages of European hake specimens collected on the eastern side of Adriatic Sea from January to December 2019

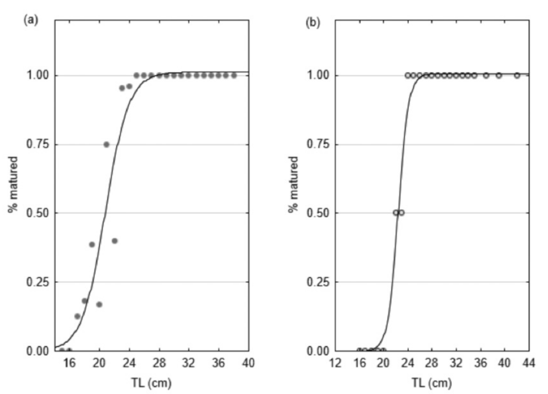

Fig. 10. Maturity ogives of European hake male (a) and female (b) specimens caught within it spawning season (December – February, 2019) with bottom trawlers along the eastern side of Adriatic Sea

of male and female European hake individuals proved to be isometrical (Fig. 3), although according to present data it was obvious that female gained in weight more than males over their life span. Namely, the average body weight of females was higher than males from the same length classes (25.0 cm < TL < 35.0 cm; W♂=155.28±44.55g; W♀=182.42±57.05g). Overviewing the literature and data regarding the length-weight parameters of European hake inhabiting (VRGOČ *et al*., 2004; SOYKAN *et al*., 2015; UZER *et al*., 2019), minor oscillations between all reported values of allometrical coefficient (*b*) were noted, but in general results of this study corresponds with the ones previously reported in a review of SARTOR *et al.* (2017). Those slight allometrical coefficient deviations were expected since length – weight relationship as well as the derived parameters are closely related to geographical areas, sampling strategy, degree of stomach fullness, gonad maturity, sex, size range, health and general fish condition and preservation techniques (FROESE, 2006; JU *et al*., 2016; TESCH, 1971).

Throughout this study, statistically significant prevalence of male European hake was observed (Figs. 4; 5). Domination of female individuals was pronounced in larger length classes (TL>31.0 cm) and only during June and December. The greater abundance of males at smaller total body length is well known for this species. Namely, studies of PIÑEIRO and SAÍNZA (2003), HABOUZ *et al*. (2011) and MELLON-DUVAL *et al*. (2017) proved that European hake males grow slower than females, which resulted in a greater proportion of females in larger length classes.

Histological observation of ovary tissues confirmed European hake as an indetermined batch spawner with asynchronous oocyte development since all oocytes development stages (Fig. 6) were present at the same time during the season of highest spawning activity (MURUA *et al*., 2003). These findings were in consistency with previous ones given for the Mediterranean (CARBONARA *et al*., 2019; RECASENS *et al*., 2008) and Atlantic (MURUA and MOTOS, 2006) European hake population. The values of the oocytes diameter for different development stages obtained throughout this study (Fig. 7) fitted within their ranges already published by RECASENS *et al.* (2008) and CARBONARA *et al.* (2019), while PHILIPS and RAGHEB (2013) reported slightly higher values of ova diameter (280 – 840 µm) for European hakes inhabiting Egyptian Mediterranean waters; most probably due to the fact that they did not measured ova diameter on histological slides of gonad tissues. Microscopical staging due to presence of postovulatory follicles (POF) and atresia (Fig. 6) allowed easier segregation of ovaries that were spent from those that were in the recovery phase. Macroscopic and microscopic scaling of maturity stages show discrepancy that has been noted as well as in the other studies dealing with the same species (CARBON-

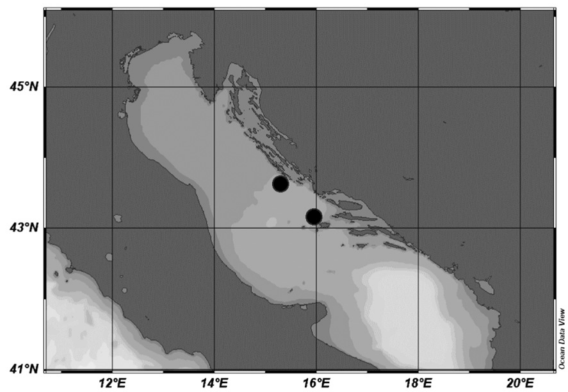

Fig. 11. Sampling stations where European hake eggs were found during the additional ichthyoplankton sampling done along the eastern Adriatic (Croatia) during the MEDITS surveys in July 2019 and July 2020

ARA *et al.*, 2019). Generally, it seems that macroscopic disjunction of spent and recovering maturity stage for European hake is a still difficult task that can be resolved by the implementation of histology as it can detect the presence of POF and atretic oocytes characteristic for spent maturity stage. Misleading results of macroscopic gonad staging might result in incorrect values of length at first maturity, however this is not the case in this study, since in estimation of mentioned length values all stages, beside immature, were included in percent calculation of matured fish. Analysis of GSI values by each maturity stage pointed out that weight of gonads, which was significantly different between almost all observed maturity stages, except between resting and recovery stages, in this study (Fig. 9) and others (CARBONARA *et al.*, 2019; RECASENS *et al.*, 2008), might be one of the parameters that can facilitate macroscopic determination.

According to the values of GSI and proportion of maturity stages over the year in this study (Fig. 8), it was obvious that Adriatic population of European hake mostly spawn during the colder part of the year (December – February) and reached their peak of spawning in December. Furthermore, a slight increase of gonad weights and GSI values along with the appearance of matured stages (2c, 3) in wormer part of the year (May- August) suggested that this species tend to actively spawn once again within the same year. This summer spawning activity is also sustained by the fact that during the last two MEDITS surveys (July 2019, July 2020) European hake early life stages, precisely it's eggs, were collected through an additional ichthyoplankton sampling. Namely, on 07th July 2019 (sampling station: 43° 37.5'N, 15° 18'E at 18:20 hours) and on 19th July 2020 (sampling station: 43°9.9'N, 15°56.8'E at 18:20 hours) two eggs of European hake, genetically confirmed (GenBank accession numbers: MW980055, MW980057), were collected in the open sea area of the middle eastern Adriatic including the area of Jabuka/Pomo pit (Fig. 11). Observed prolonged spawning period, as well as the fact that European hake most probably spawn twice in a year, coincided with previous findings obtained for the same species in the Adriatic (ŽUPANOVIĆ and JARDAS 1989; UNGARO *et al.*, 1993; VRGOČ *et al.*, 2004; CARBONARA *et al.*, 2019).

Furthermore, given that the mature/spent stages were observed throughout the year, although in lower percentages, we can assume that this species spawns throughout the whole year. This is in line with the findings of European hakes inhabiting Mediterranean. Namely, according to review provided by SARTOR *et al.* (2017; and references within it) Mediterranean population of European hake spawns throughout the whole year or they have one or more peaks of spawning in a year. Obviously, this species has the ability to change its spawning strategy in order to accommodate environmental conditions (like prey availability, changes in sea temperature or salinity etc.) of the geographical area (FERRER-MAZA *et al.*, 2014; CARBONARA *et al.*, 2019). Furthermore, by the extension of spawning season and the fact that this species is a batch spawner, somehow ensured itself with a higher probability to find suitable conditions for larval survival, thereby a higher level of recruitment success as previously reported (JAMES and PITCHFORD, 2003).

In this study, European hake lengths at first maturity for both sexes were rather low (male: L_{50}=20.74 cm; female: L_{50}=22.38 cm; Fig. 10) but still within the values reported so far for the Adriatic Sea (VRGOČ *et al.*, 2004). Overviewing the literature, the range of the reported lengths at first maturity for this investigated species was quite wide going from 21.5 cm (Cantral Aegean Sea – (SOYKAN *et al*., 2015)) to 43.0 cm (Gulf of Lion – ALDEBERT and CARRIES (1989)). Bearing in mind, that majority of the European hakes in the eastern Adriatic Sea are distributed and caught on the continental shelf and/or near its edge as well as the fact that the dominant length classes of those specimens goes from 20.0 cm to 30.0 cm obtained vales were expected. Obtained values of length at first maturity in this paper match the best with the values reported by PHILIPS and RAGHEB (2013) and SOYKAN *et al*. (2015). In both mentioned studies, length-frequency distribution of analysed European hakes was quite similar (13.0 cm < TL < 53.0 cm – (PHILIPS and RAGHEB, 2013); 9.0 cm < TL < 45.5 cm – (SOYKAN *et al*., 2015)) to one obtained within this study (15.0 cm < TL < 49.0 cm). Additionally, obtained lower values of length at first maturity fits with the findings of DRAZEN and HAEDRICH (2012) who reported that the mentioned value in demersal fish species increase with depth. Namely, European hakes caught below 200 m, as in this study, where feeding intensity according to VELASCO and OLASO (1998) is higher has to reach its first maturity earlier and at smaller length size than the hakes caught in deeper areas of Mediterranean or Atlantic. Obviously, due to a wide length at first maturity range reported for this species within the literature, European hake tends to change its reproduction pattern in the different geographical region. Besides, the observed divergence between the length at first maturity values is often associated with species level of exploitation (LAPPALAINEN *et al*., 2016) or climate changes. From this study, it was noted that male European hakes reached its maturity at slightly lower length values than its females, but this pattern is general for most teleosts (HELFMAN *et al*., 1997).

Results of the present study indicated that the Adriatic population of European hake has similar reproductive pattern as their populations distributed along the whole Mediterranean. Observed discrepancies pointed out that European hake spawning phenology is most probably driven by their environmental conditions. Here observed outcomes will, for sure, improve stock assessments and consequently the management of this highly important demersal fish species. Nevertheless, according to TSIKLIRAS and STERGIOU (2014) fish reproductive parameters and its changes in future should be monitor through longer time period in order to understand and elucidate the reasons (phenotypic variability, sampling bias) for their variations that at the end effect assessments of fish species biomass levels and its management.

ACKNOWLEDGEMENTS

This study was supported throughout the scientific project "Exploration of ecologically sensitive areas with special emphasis on growth, development and protection of commercially important maritime organisms (ESAmar)" founded by Croatian Science Foundation and research project "*Data Collection Framework (DCF)*" founded by the Ministry of Agriculture of the Republic of Croatia. Thanks to the PhD Ezgeta-Balić for her help with picture preparation. Finally, many thanks to anonymous reviewers for their useful comments on the manuscript.

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Received: 9 June 2021
Accepted: 8 September 2021

Reproduktivna biologija oslića, *Merluccius merluccius* (L. 1758), u Jadranskom moru

Barbara ZORICA*, Igor ISAJLOVIĆ, Nedo VRGOČ, Vanja ČIKEŠ KEČ, Damir MEDVEŠEK, Vedran VULETIN, Ivana RADONIĆ, Ratko CVITANIĆ, Ivana LEPEN PLEIĆ i Marija ŠESTANOVIĆ

Kontakt e-pošta: zorica@izor.hr

SAŽETAK

U okviru ovog rada je istražena reproduktivna biologija jadranske populacije oslića, *Merluccius merluccius* (Linnaeus, 1758). Uzorci istraživane vrste su prikupljeni iz uzoraka lovina ostvarenih komercijalnom pridnenom mrežom koćom s područja istočnog Jadrana (Hrvatsko ribolovno more) jednom mjesečno (N = 1173) tijekom 2019. godine.

Raspon totalnih dužina tijela oslića se kretao između u rasponu od 15,5 cm do 49,5 cm (srednja vrijednost ± SD = 27,5 ± 5,0 cm) te je pri većim dužinskim razredima zabilježena dominacija ženki (TL> 31,0 cm).

Omjer spolova (m/ž) je iznosio 0,64; prevalencija mužjaka je utvrđena tijekom čitave godine izuzev razdoblja mrijesta (prosinac, lipanj) kada je omjer spolova bio u korist ženki. Analizom stadija zrelosti, mase gonada, gonadosomatskog indeksa te histoloških preparata tkiva spolnih žlijezda utvrđeno je da se ova vrsta u Jadranu mrijesti dva puta u jednoj godini; utvrđeno je razdoblje mrijesta od prosinca do veljače, dok je drugo razdoblje mrijesta nešto slabijeg intenziteta utvrđeno početkom ljeta, točnije u lipnju.

Prva spolna zrelost svih analiziranih jedinki oslića je nastupila pri totalnoj dužini tijela od 20,7 cm za mužjake odnosno 22,4 cm za ženke.

Ključne riječi: omjer spolova; sazrijevanje; mriještenje; rani razvojni stadiji; oslić; Sredozemlje