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Research Article

Modeling the trajectories of growth and reproduction in European Hake (*Merluccius merluccius*, L. 1758) from the Sea of Marmara, Turkey

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Abstract

The study evaluated to model the growth and reproduction aspects of the hake, *Merluccius merluccius*, in the Sea of Marmara. On-board sampling was conducted on the commercial beam trawl fishery from February 2016 to January 2017. For the growth trajectories, using monthly length frequency data set, different methods were employed to improve parameter optimization. The variation of reproductive intensity was modelled using Generalized Additive Models (GAMs). The growth was fast and parameterized as $L\infty$=65.9 cm, K=0.16 year^{-1} according to the Electronic Length Frequency Analysis (ELEFAN) with genetic algorithm optimization approach. Seasonal variation in growth, fitted a seasonally oscillating VBGF (soVBGF), indicated intense seasonality. The onset of the positive phase of the growth oscillation was observed around October proofed by a high fish condition that the growth starts accelerate after the summer period. A high gonadosomatic index (GSI) was evident over most of the year except between May and July, captured also the time component in the GAM modeling. The total length and fish condition have the highest impact on the GSI among the modeling parameters evaluated while temperature has low partial effect. The length at the first sexual maturity for females and males was calculated at 26.5 and 22.0 cm, respectively, lower than that of the Atlantic and Mediterranean waters. It is believed that this study allows deeper and new conclusions for the management of hake stock in the Sea of Marmara and Mediterranean Basin.

Keywords: *Merluccius merluccius*, Age, Growth, Spawning, Sea of Marmara

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Introduction

The European hake (*Merluccius merluccius*, Linnaeus, 1758) is widely distributed from the Northeast Atlantic shelf to the Mediterranean Sea (Casey and Pereiro, 1995). It is a benthopelagic species, range extends from 30 to 1000 m (Froese and Pauly, 2019). It is generally found in muddy bottom habitat in continental shelf basin. This fish is mostly piscivorous, i.e. feeds mostly on fish, and also eats crustaceans, molluscs etc. (Domínguez-Petit, 2007). It carries out daily vertical feeding migrations, staying close to the bottom in the daytime and rising off the bottom to adopt a midwater habit at night (García-Rodríguez and Esteban, 1995).

The hake represents a basic component of the demersal communities and its high market value ranks it among the most commercially important species in the Mediterranean Sea (Ligas *et al*., 2011). Due to its socio-economic importance and significant source of protein, various aspects of its bio-ecology (Alegria Hernandez *et al*., 1982), distribution patterns (Orsi-Relini *et al*., 2002; Maravelias *et al*., 2007), growth (e.g. Piñeiro and Sainza, 2003; De Pontual *et al*., 2006), genetics (Roldán *et al*., 1998), fisheries and exploitation (Martin *et al*., 2002) have been studied. However, studies from the Sea of Marmara (from now on simply as SEM) have contributed little to Mediterranean hake research as a basin scale. The first historical information was generated by Beverton and Holt (1959) on the growth parameters of hake in the SEM. Asa basic scale, its growth (Kahraman *et al*., 2017a; Gül *et al*., 2019a,b) and reproduction biology (Kahraman *et al*., 2017b) in the SEM has just been a hot spot for the researchers in the recent years.

The European hake is commercially important species for all Turkish waters. Due to this economic importance, the European hake is caught by different fisheries techniques such as beam trawl, nets, and longlines. According to official Turkish Fisheries Statistics, there has been a clear decrease in landings from the SEM in the last 15 years. Unfortunately, the unit stocks of hake in the SEM and Aegean Sea have not been settled yet. Moreover, no stock assessments or quotas have been assigned to hake in Turkish fisheries. The minimum landing size is the only output control objected to hake in the current Turkish legislation. However, data of the reproductive and growth biology of the hake in the SEM is scarce and has, to a wide extent, not been modelled. Advanced biological knowledge is the main tool for sustainable management of the European hake fishery in the SEM. An overview of existing literature on hake also will be helpful when comparing the novel findings of this study and also remark the points where more research is practical in the SEM and adjacent seas.

Materials and methods

Study area

The Sea of Marmara is a small basin with a surface area of ~11,500 km^2 and a maximum depth of 1390 m. This basin is a unique intercontinental sea located where the continents of Europe and Asia meet. The hydrography of this small basin is chiefly generated by the Mediterranean and Black Seas (Beşiktepe *et al*., 1994) creating a unique two-layer marine ecosystem (Sorokin, 1983). The surface layer of the Sea of Marmara above 25 m comprises Black Sea waters of relatively low salinity (‰18), while high-salinity (‰38.5) Mediterranean waters form the deep layer below 25 m (Beşiktepe *et al*., 1994). For this reason, the benthic assemblages differ between the shallow and deep waters in the Sea of Marmara (Yildiz, 2020).

Fishing by shrimp beam trawling in the SEM started from 1969 (Zengin and Akyol, 2009), but two years later, the bottom trawl fishery was banned in the entire SEM in 1971. After those years, beam trawls have become the principle gears to catch economic demersal species such as shrimps and European hake (Deniz *et al*., 2020). In addition, European hake stocks have been caught by gillnets once in a year between March and the end of May in depths of 50-70 m (Göktürk *et al*., 2016). However, in the SEM the highest hake abundances are located at approximately 90 m (Yildiz, 2020). Beam trawlers primarily target the *Parapenaeus longirostris*, which is the most abundant in benthic fauna of the Sea of Marmara, but European hake is mostly captured as a main by-catch species (Yazici, 2004; Zengin *et al*., 2004; Öztürk, 2009). The main beam trawling fishing area in the SEM is located around Kapıdağ Peninsula in the western part of the SEM (Öztürk, 2009).

The length overall and horse power (HP) of 147 beam trawl vessels engaged in the study period ranged between 7 and 24 m (12 m±3 SD) and 70 and 448 HP (178 m±92 SD), respectively.

Sampling

Commercial beam trawlers were used, whose length was 2.5 m and cod end was 44 mm due to the reasons listed above and the limitations of other fishing gears (fishing depth and duration of season). Beam trawl hauls were conducted at a depth of 50-150 m, at a speed of 2.0-2.5 knots. In the closed season (15 April-31 August and 1-31 January), a special fishing permission was approved from the ministry. Monthly biological data were collected from February 2016 to January 2017 in the Şarköy and Hoşköy districts in Tekirdağ locality on the Northwest part of the SEM (coordinates in decimal degrees (DD), 40.6850, 27.2739; 40.5035, 27.2709; 40.5086, 27.7128).

Length-weight relationship (LWR)

Total length (TL) and total weight (TW) of 698 specimens were recorded to the nearest 0.1 mm and 0.1 g, respectively. Difference in mean length and weight between sexes was tested with Wilcoxon-test. The Chi-square test was used to compare sex ratios from the expected 1:1 (♂/♀) ratio. The sexes determined by macroscopic examination of the gonads. The LWR was calculated by Le Cren’s (1951) equation. Analysis of covariance was performed to test the differences in the LWRs between sexes (Zar, 1999). A test of whether the fish in a population exhibit isometric growth or not can be calculated by noting that *b* is the estimated slope from fitting the transformed length-weight model (Ogle, 2019). Hypothesis tests regarding model parameters can be estimated with a t-test using the below formula:

$t = (\hat{\beta} - \beta_0)/Se_\beta$

Where $\hat{\beta}$, $SE_{\hat{\beta}}$ and the df are from the linear regression results and β_0 is the specified value in the H_0: Isometric growth.

Growth functions

In most cases, growth characteristics are assessed by using otolith reading to estimate the age. In fact, bias in age estimation can be caused by accuracy and/or precision issues (Campana, 2001; De Pontual *et al*., 2006). Hake otoliths are difficult to interpret due to the complexity of the macrostructure and growth variability that has been related to the long spawning season (Vitale *et al*., 2019) and the age estimation method has not been validated (Piñeiro and Sainza, 2003; De Pontual *et al*., 2006). For above-mentioned reasons, we used the length frequency analysis (LFA) to obtain the growth parameters. For this analysis, Paulys' most popular technique of ELEFAN I was implemented by using of TropFishR package (Mildenberger *et al*., 2017) through R software program (R Development Core Team, 2018). With this package one can do some arrangement for choosing an appropriate bin width to maximise the Rn score (Taylor and Mildenberger, 2017). The TropFishR package also provides two novel optimization routines for the fitting of VBGF or soVBGF parameters: (i) the 'ELEFAN_SA' function uses the Simulated Annealing package 'GenSA' and (ii) the 'ELEFAN_GA' function uses the Genetic Algorithm package 'GA' (Scrucca, 2013):

$$Lt = L_{inf} (1 - \exp(-(L_t\, L_{inf}\, K(t - t_0) + S(t) - S(t_0)))),$$

Where $S(t)=(CK/2\pi) \sin 2\pi(t - ts)$ C is a constant indicating the amplitude of the oscillation, typically ranging from 0 to 1, and ts is time of Summer point (ts) on the growth sine curve. Indicates a better growth situation at this point compared to the winter point (Taylor and Mildenberger, 2017), L_t is the fish length (cm) at time t (years), L_{inf} is the asymptotic length (cm), k is the growth coefficient ($years^{-1}$), and to (years) is the hypothetical time at which the length is equal to zero. Munro's growth performance index ($\varphi'=\log(k)+2\log(L\infty)$) (Pauly and Munro, 1984) were used to test the accuracy of the growth parameters. All individuals were grouped into size class intervals of 1 cm as to obtain monthly length frequency distributions (LFDs).

Spawning period and length at first maturity

The spawning period of hake was determined with monthly variations of the gonadosomatic index (GSI), [Gonad weight (GW)/ (TW–GW)]×100 (Gibson and Ezzi, 1980) and the condition factor, $K=[(TW–GW)/ (TL)^3]\times100$ (Htun-Han, 1978), were calculated by sex. The size of first maturity (L_{50}) was estimated for both sexes by a logistic function that was fitted to the proportion of sexually mature individuals by each size classes during peak spawning season (between December-February) using a nonlinear regression following formula:

$P=1/\{1+\exp[-r(L-L_m)]\}$

Where P is the proportion mature in each size class, r (-b slope) is a parameter controlling the slope of the curvature and L_m is the length at 50% maturity. L_{50} was estimated considering individuals in stages higher than stage II (Fig. 1). For this analysis, sizeMat package (Josymar, 2019) was used by R programming. Markov Chain Monte Carlo for logistic regression (Bayes GLM), using a random walk Metropolis algorithm, was applied for calculation method.

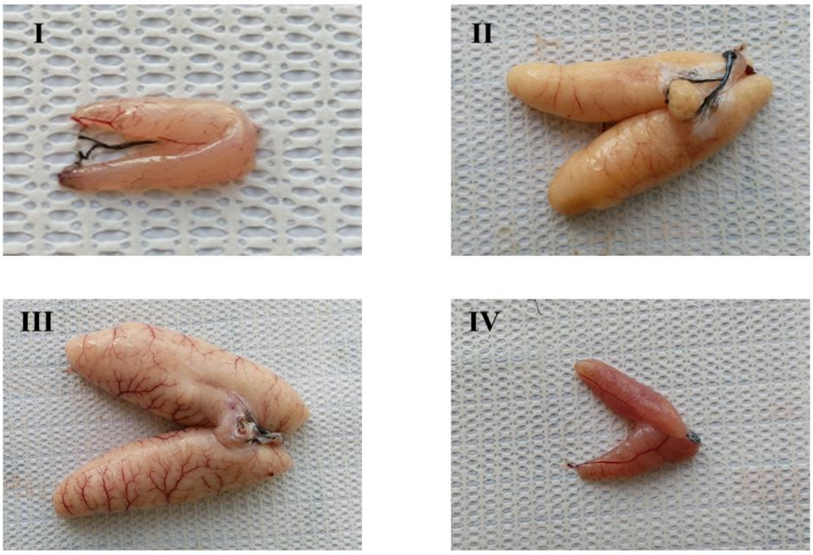

Figure 1: Macroscopic gonadal scale of female hakes at different maturity stages.

Generalized Additive Modeling (GAM) for spawning activity

GAMs were applied to examine the relationship between the hake GSI values and some other factors. GAM generalizes multivariate linear regression by relaxing the assumptions of linearity and normality, replacing regression lines with smooths (Ayón *et al*., 2011). The "mgcv" package in R program was used. Deviance explained, adjusted R^2, Akaike information criterion (AIC), and GCV (Generalised Cross Validation) were calculated as a measurement of the goodness-of-fit. Models with the lowest AIC and GCV scores were selected (Wood, 2006). A correlation matrix was deployed to determine the collinearity between variables and which combinations of predictors could be incorporated as independent variables. Because several variables were highly correlated (>0.3 correlation coefficients), only some sub-models with 3 or 4 variables were assessed. Mean sea water temperate values in monthly basis was taken from Meteorological Service of Turkish State (https://mgm.gov.tr/deniz/deniz-suyu-sicakligi.aspx). These are the systems

(81 Marine Automatic Meteorology Observation Stations (D-OMGI), 13 of which are sea buoys) used to obtain meteorological information in the Turkish seas in order to support maritime activities and to prepare meteorological products and services for the maritime sector.

Results

Sex ratio and length frequency

Six hundred and ninety eight specimens were collected from February 2016 to January 2017 in the Sea of Marmara. The length of the European hake ranged from 8.1 to 44.9 cm with the mean of 21.9±2.47 cm. Length and weight distributions showed significantly differences regarding by sexes. The male: female ratio of the population of the European Hake was 1.00:1.43 which is significantly different from the expected ratio of 1:1 (p<0.05). Male individuals were predominant in small size groups (Fig. 2). While the M:F ratio of the 24 cm length group and smaller individuals is 1: 1.09, this ratio in the 25 cm length group and larger individuals was found to be 1.00: 0.53.

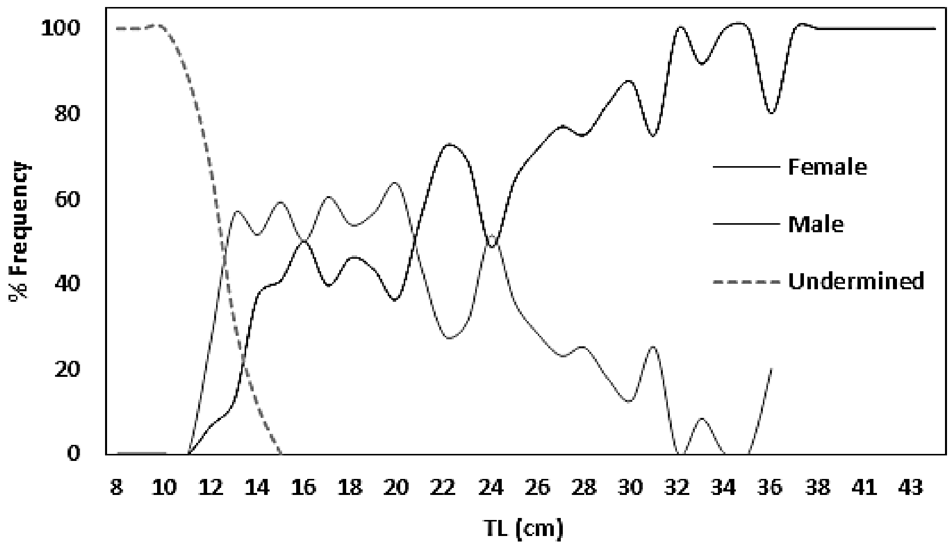

Figure 2: The total length distribution by sexes.

Length-weight relationship

The LWR relationships was observed to be highly significant (P<0.001). The ANCOVA test indicated that there was significant difference between females and males (p<0.05) and R^2 values ranged between 0.94 and 0.99 (Table 1). The total LWR indicates an allometric growth. Positive allometric growth was calculated for females and juveniles whereas males showed isometric growth.

Table 1: The length-weight relationship parameters by sexes.

Sample	N	a	b	95 % Cl b	R^2	Growth model	p
Total	698	0.0049	3.14	3.12-3.16	0.99	+Allometric	<0.05
Female	381	0.0058	3.09	3.06-3.13	0.99	+Allometric	<0.05
Male	266	0.007	3.01	2.96-3.06	0.98	Isometric	>0.05
Unidentified	51	0.0026	3.38	3.14-3.63	0.94	+Allometric	<0.05

Growth parameters

The frequency distributions of monthly length and the growth curves, fitted by ELEFAN, were presented in Figure 3. The growth parameters generated from different methods are given in Table 2. The maximum Rn score was obtained from Genetic Algorithm (ELEFAN_GA). The intensity of the seasonality from two algorithms were very close to each other and assumed a quite remarkable seasonality of the growth rate (C= 0.682 and 0.744).

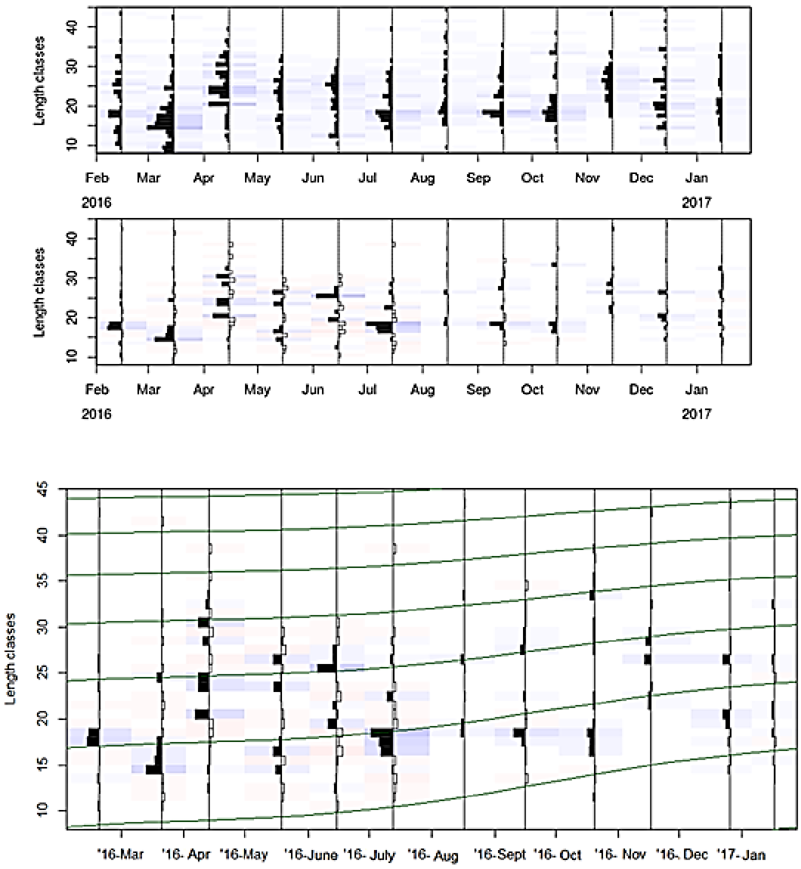

Figure 3: Length frequency data visualized in terms of catches (A) and restructured data (B) with a moving average setting of MA=11 and Graphical fit of estimated growth curves produced through optimizations from ELEFAN_GA plotted through the restructured length-frequency data.

Table 2: von Bertalanffy growth parameters obtained by different methods.

	Powell-Wetherall	K_Scan	RSA	ELEFAN_SA	ELEFAN_GA
L_{inf}	59	58.6	57	60.9	65.9
K	-	0.1	0.1	0.21	0.16
$t_{\_anchor}$	-	0.85	0.70	0.30	0.45
C	-	-	-	0.744	0.682
Ts	-	-	-	0.121	0.759
Ø	-	2.54	2.51	2.90	2.84
Rn Score	-	0.212	0.217	0.223	0.236

Length at first maturity

The L_{50} of European hake was determined 26.5 and 22.0 cm for females and males, respectively. The range of L_{50} was calculated between 26.1-27.0 cm for females and 21.3-22.6 (Confidence intervals at (95%)) for males according to the Bayesian regression analysis (Fig. 4).

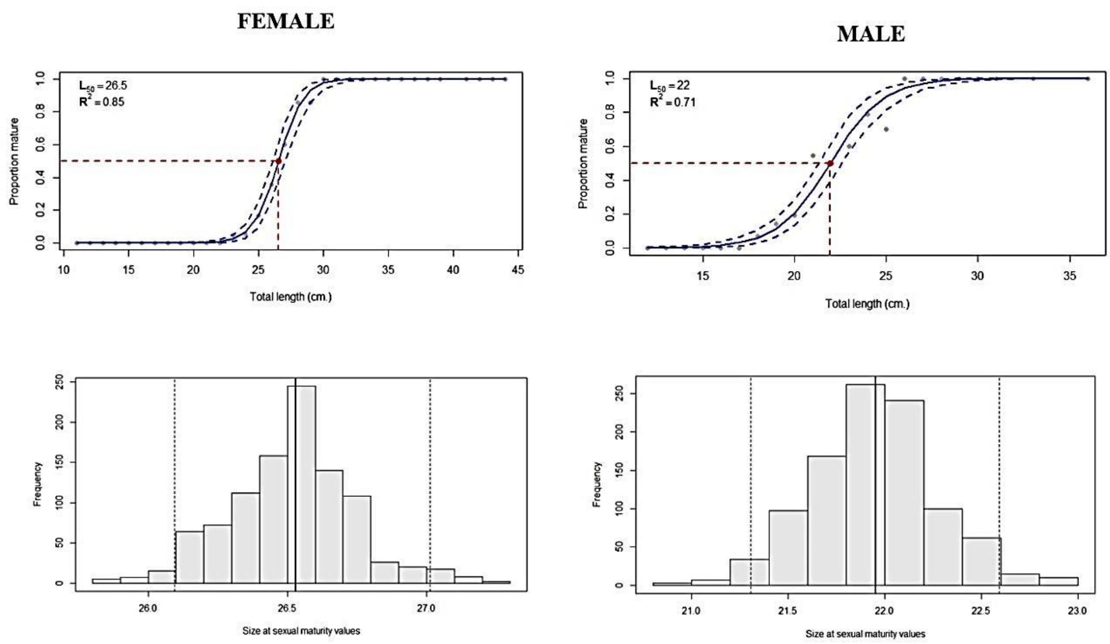

Figure 4: The length at first maturity and ranges for females and males.

Timing of reproduction

Boxplot of GSI by maturity stage (Fig. 5) revealed a gradual increase from the immature stage (0.73±0.03) to the developing (4.06±0.29) and spawning stages (7.16±0.54). Later, the GSI decreased in the post-spawning stage (1.36±0.20). The GSI shows a clear seasonal trend. From Figure 6, it can be perceived that spawning occurs throughout the year except May, June, and July. Mature individuals were observed throughout the year with the highest occurrence between December and February. The lowest conditions were observed in May and June.

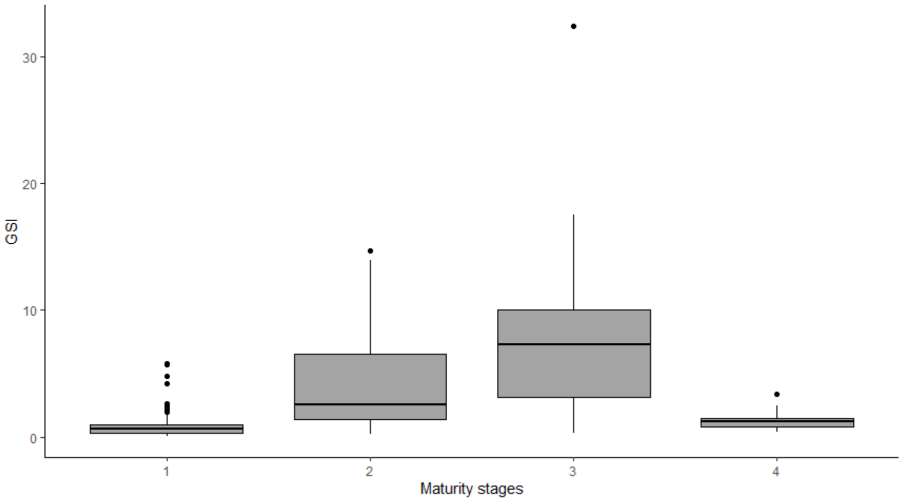

Figure 5: The boxplot of mean gonadosomatic index (GSI) by maturity stage.

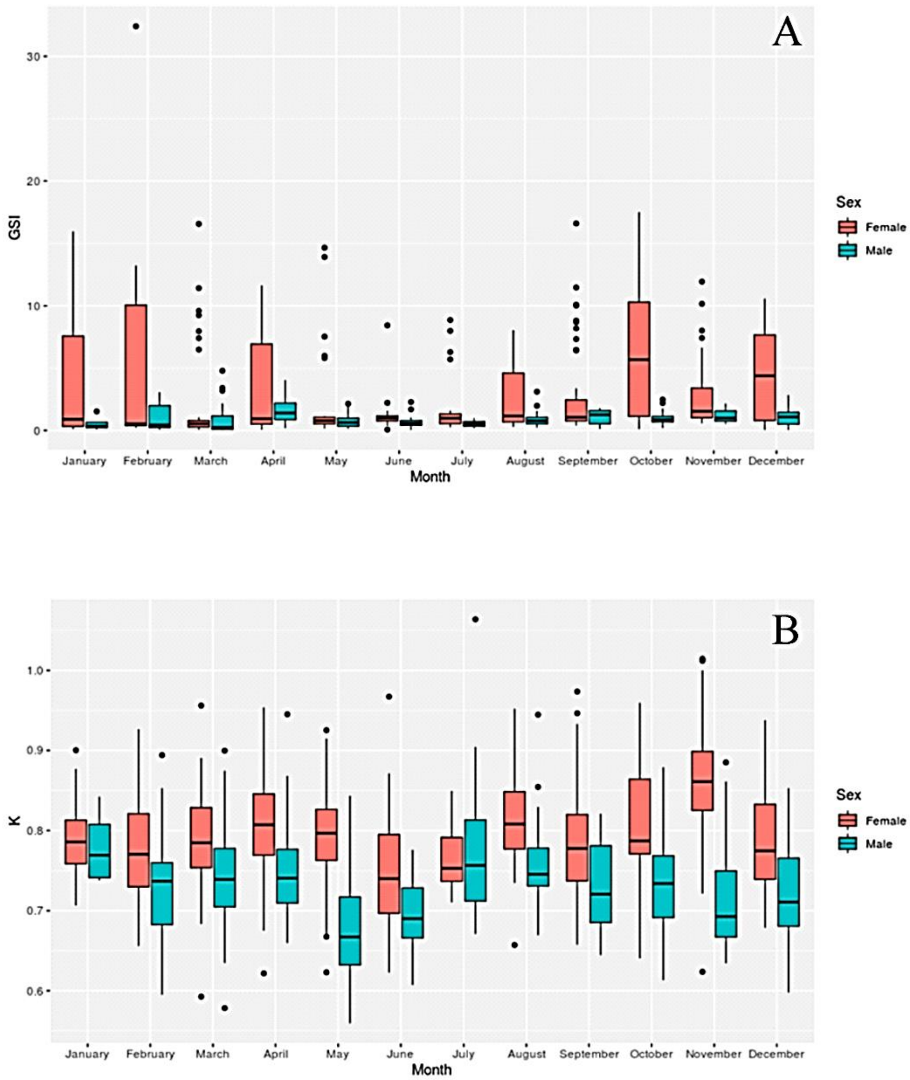

Figure 6: Monthly changes of gonadosomatic index (A) and condition factor (B).

The total length effect was the most important predictor explaining the GSI of the hake, with 53.9 of the deviance explained (Model 01, Table 3). The other single predictors, condition factor (Model 04), day of the year (Model 02), and water temperature (Model 03) explained 7.85, 4.7, and 1.3 of the deviance, respectively. The spline plot of GSI showing the effect of total length indicated that individuals having the highest GSI values around 30-35 cm in length. Then, it decreased sharply with increasing lengths. The GAM results suggest that the highest GSI values associated with the water temperature lower than 10°C (Fig. 7).

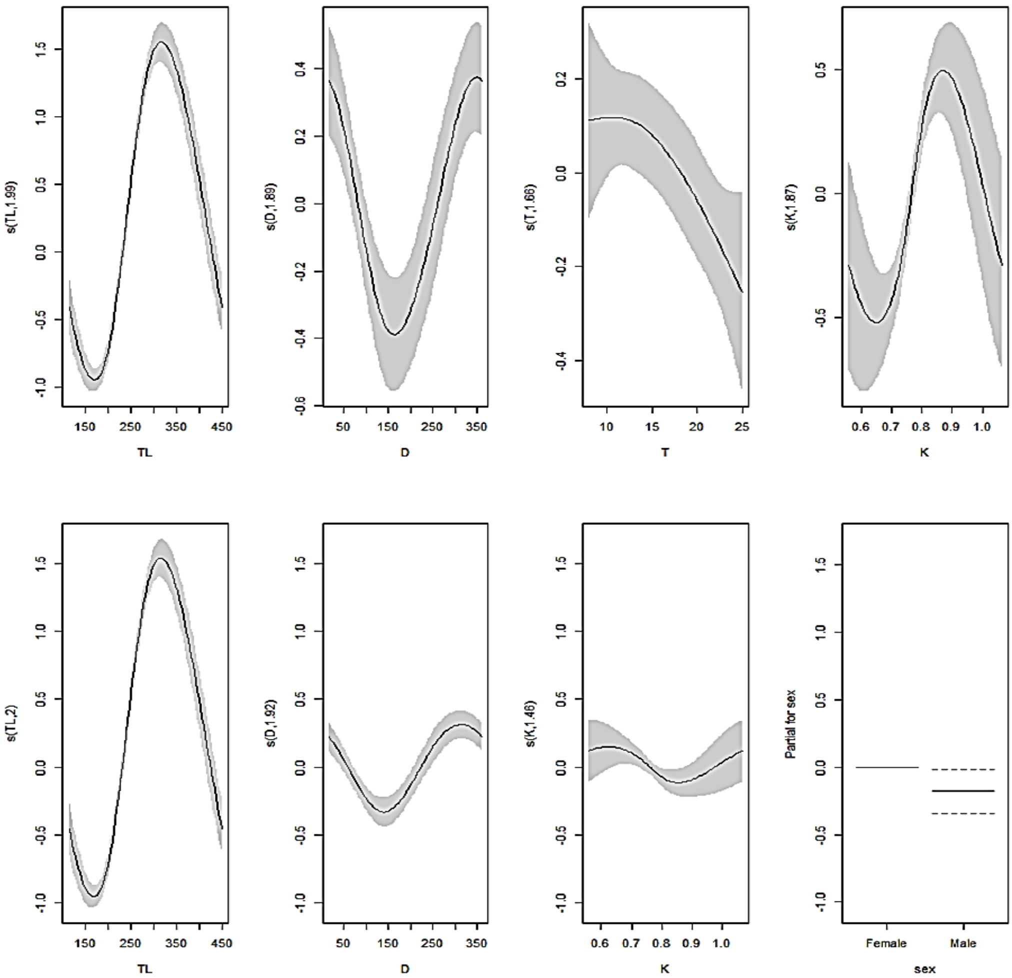

Figure 7: Coefficients of the GAM for European hake against additive terms of (TL, mm) total length, (D) day of the year, T (Average sea water temperature), and K (condition factor) used as single predictor variables (upper board) and additive terms of (TL, mm) total length, (D) day of the year, K (condition factor), and sex used in Model 10. Black lines indicate the GAM coefficient values, and the shadowed regions indicate the 95% confidence interval at *p*<0.05 (lower board).

The correlations among predictor variables revealed high positive correlations (>0.3 correlation coefficients). Hence, the models with more than one variable were evaluated by avoiding the correlation between predictors. Model 10 best explained the GSI, including the effect of total length, day of the year, K and sex (Table 3). The overall performance of the model was adequate, with a R^2 =0.40 and the resulting model explained a 57.1% of the total variance. The effect of all factors was highly significant (p<0.01). According to Model 10, GSI exhibited the maximum effect, with one peaks at approximately 30 cm in TL and end of the year (Fig. 7). Females had higher GSI values than the males.

Table 3: Analysis of deviance explained (DE), GCV, AIC, and R^2 for GAM covariates and their interactions for the best GAM fitted by adding the covariates stepwise. p<0.001: ***

Model	Models	DE (%)	GCV	AIC	R^2
M01	s(TL)***	53.9	0.748	1710.74	0.390
M02	s(Days)***	4.7	1.546	2255.03	0.024
M03	s(Temperature)*	1.3	1.598	2281.16	0.006
M04	s(K)***	7.85	1.493	2227.73	0.043
M05	s(TL)***+ s(Temperature) ·	53.3	0.760	1721.86	0.382
M06	s(TL)***+ s(Days)*** ·	56.4	0.711	1673.72	0.396
M07	s(T)*+ s(K)*** ·	9.24	1.477	2218.85	0.045
M08	s(D)***+ s(K)*** ·	11.3	1.446	2202.02	0.060
M09	s(TL)***+ s(Days)***+ s(K)*	56.7	0.708	1670.76	0.384
M10	s(TL)***+ s(Days)***+ s(K)*+sex*	57.1	0.705	1666.73	0.403

Discussion

In this study, consisting of five main subjects, the length distribution, length-weight relationship, growth, spawning period, and length at first maturity of European hake have been addressed in the SEM. The European hake spawning period was predicted by GAM modeling with incorporated to some biotic and abiotic factors. In addition, length at first maturity was modeled Bayesian approach.

The length distribution of the catch in the SEM shows a large proportion of juvenile individuals in the frequency composition. 49.4% of the total individuals were under the minimum landing size limit (20 cm). According to Demirel *et al*. (2017), the largest individual reported to date in the SEM was 75 cm at the beginning of 1990 (Jica, 1993). For period between 1991 and 1994, the average length of hake was reported 20-24 cm in the SEM (Gözenç *et al*., 1997). From the recent available literature, Kahraman *et al.* (2017a) recorded a length range 10.4-55.3 cm (25.9±0.21 cm) for 2014-2015 period, Gül *et al*. (2019a) reported a length range 8.0-65.0 cm for 2009-2011 period, Gül *et al*. (2019b) also reported 16.2-46.0 cm (26.11±0.39 cm) for 2016, and Yildiz (2020) recorded a length range 10.0-58.8 cm for 2014-2017 period. For remembering, the sampling procedures of these studies are different that some are the fishery dependent while the others are independent studies. As indicted by Gül *et al*. (2019a), the median value of the total lengths of the hake obtained in the 90s was greater than the values obtained at the end of the 2000s. However, the decline in mean length seems not big enough to believe a shift towards to small length classes.

Unfortunately, there is no available literature in the SEM before the 90s.

The common finding of the available literature in consistency with the present study is that sex ratio is skewed towards females in the largest length classes. The sex ratio observed in this study was close to 1 : 1 up to 25 cm. From 25.0 cm TL onwards, the relative percentages of females gradually increased to reach 100% in fish larger than 40.0 cm. A similar trend has been reported from the other parts of the Mediterranean Sea (Uçkun *et al*., 2000; Colloca *et al*., 2003; Ligas *et al*., 2011). Early maturation of males than females at first maturity stage could be the main factor of male dominancy until a certain lengths. Indeed, different explanations have been claimed by some hake researchers: (i) differences in the growth rates of two sexes (Recasens *et al*., 1998; Lucio *et al*., 2000; Ligas *et al*., 2011), (ii) higher natural mortality rate of older males than that of females (Piñeiro, 2011), and (iii) different behavior and consequently different accessibility to the fishing gear (Piñeiro and Sainza, 2003).

In general, the parameters of length–weight relationships (LWRs) formulated in this study are close to those from the other studies. Apparently, hake has a high degree of positive allometry when juvenile sizes then its LWR shift to around isometry. Hence, it can be said that hake grows faster in weight than in length in juvenile stage. The LWR parameters are quite important when one estimate the stock biomass from length data. Consequently, it is needed to justify the sex-specific parameters of LWR into the stock assessment studies. But it should also not be forgotten taht, different numbers of studied samples at length distribution margins, especially in larger sizes, can explain the small differences with values reported in the other studies (Piñeiro and Sainza, 2003).

Although many studies have been published on the age and growth, there is still no consensus by the hake researchers. Hake otoliths have been used to determine the age and growth for a long time period starting from 1933 (Hickling, 1933). However, age estimation of hake still presented problems for older ages (>5), which was a limiting factor for assessments (Vitale *et al*., 2019). The other debate about whether hake is a fast (Bagenal, 1954) or a slow-growing (Hickling, 1933; Meriel-Busy, 1996; Guichet *et al*., 1973) species has been going on since the 1930s (De Pontual *et al*., 2006) and growth rate of the hake is still uncertain (Ligas *et al*., 2011; Vitale *et al*., 2019). In addition to otolith studies, LFA method has been applied successfully on hake (Bagenal, 1954; Aldebert and Morales-Nin, 1992; Gücü and Bingel, 2011). Therefore, this study is the first attempt to analyze the hake growth in the SEM with LFA method. Inherently, our growth parameters (Linf and K) are in consistency with other fast-growth from Mediterranean and SEM research. The K value (0.16) proofed to the fast growth assumption was suggested by the von Bertalanffy growth function fitted to data. This fast growth function clearly shows seasonality a high oscillating pattern (C=0.682). The onset of the positive phase of the growth oscillations was estimated as 0.75 (around early October) supported by a high female condition indicates that the growth starts accelerate after the warmer period (when mean temperature=23.4°C). In summer, hydrographical conditions become unfavorable due to the summer warming, hake are forced to abandon the feeding grounds and, therefore, growth rate slows (Gücü and Bingel, 2011). Concurrently, in the SEM the lowest condition factor was calculated in May, June, and July.

According to the extensive available information, hake is a multiple batch spawner with indeterminate fecundity (Perez and Pereiro, 1985; Murua *et al*., 1998) with a year-round spawning activity characterized by multiple peaks (Recasens *et al*., 1998; Carbonara *et al*., 2020). In their study, Kahraman *et al*. (2017b) has indicated that European hake are featured by asynchronous oocyte development in the SEM similar to other stocks (Murua *et al*., 1998), supporting a protracted spawning period. In this study, we found condition, total length, sea water temperature, and season effect the period and the length of the spawning period of hake. Just recently, Carbonara *et al*. (2020) found that the energy invested in reproduction is derived from energy that is stored during the summer and autumn. Similar to this finding but with one difference, we found that hake in the SEM stores energy during autumn. Although hake has mature or maturing gonads throughout the year, has highest GSI around 10°C of the mean sea water temperature during winter season, sea water temperature has an effect as a single predictor. Similarly, Murua *et al*. (1998) and Alheit and Pincher (1995) found that hake breeds when water temperature reaches 10°C or 12°C.

Values of length at first maturity for females and males are comparable with previous study performed in the SEM by Kahraman *et al.* (2017b) where empirical formulas used for the assessment. As a general manner, length at first maturity for females and males seems lower than those of the SEM. For the management issue, the current minimum landing size for the hake is implemented as 20 cm (TL) in Turkish territorials waters. However, between 2012 and 2016 it was applied as 25 cm in Turkish waters (Bsgm, 2016). For the sustainable hake fishery management, it should be at least 28 cm as it was in previous management period. The findings of these papers have contributed significantly to a better understanding of the growth and reproductive biology of the hake in the SEM. For further studies, it is obviously needed that determination of spawning areas, stock reproduction potential on the basis of spawning stock biomass, and recruitment success.

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