Fisheries Research 204 (2018) 147–155

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Is the spawning frequency of Argentine hake, *Merluccius hubbsi*, affected by maternal attributes or physical variables?

Gustavo J. Macchi[a,b,*], Karina Rodrigues[a,b], Ezequiel Leonarduzzi[b], Marina V. Diaz[a,b]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Investigaciones Marinas y Costeras (IIMyC), Rodríguez Peña 4002, B7602GSD Mar del Plata, Buenos Aires, Argentina

[b] Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo Nro. 1, B7602HSA Mar del Plata, Buenos Aires, Argentina

ARTICLE INFO

Handled by George A. Rose

Keywords:
Merluccius hubbsi
Spawning frequency
Female size
Female age
Temperature
Egg production

ABSTRACT

Spawning frequency (SF) is a parameter that represents the number of spawning events per time unit, used to estimate the reproductive potential of many fish populations. We analyzed the relationship between SF and size and age of Argentine hake females from the Patagonian stock. We also studied the influence of female nutritional condition and physical variables on spawning and estimated the monthly egg production in relation to size structure. Samples were collected during the spawning season between 2000 and 2014. This study revealed that the size and age of spawners had a positive effect on the SF of the stock. During the reproductive season this parameter ranged between 13 and 3 days for females within a total length (TL) interval of 35–80 cm. A positive relationship between the spawning activity and the water temperature was also observed, suggesting that this variable could has an influence on the growth and maturation of oocytes. Towards the end of the reproductive period SF was higher than at the beginning of the spawning, but batch fecundity was lower. This could be associated with the poor nutritional condition of females at the end of seasonal spawning. Our results reinforce the hypothesis of a significant maternal effect in Argentine hake, characterized by a higher reproductive output in larger females (> 50 cm TL), which in general is not considered in the traditional stock assessment.

1. Introduction

In iteroparous fish species with indeterminate annual fecundity, the pattern of oocyte development indicates that unyolked oocytes continuously recruit and are spawned throughout the reproductive season (Hunter et al., 1992). Therefore, to estimate the annual fecundity of these species, it is necessary to determine the number of oocytes spawned per batch (batch fecundity) and to estimate the number of spawning events during the reproductive season (Hunter et al., 1985; Murua and Saborido-Rey, 2003; Murua et al., 2003). The number of spawning events depends on the length of the reproductive season and on the time elapsed between subsequent egg batches, or spawning frequency (SF). For most species, spawning seasonal is reported in months using subjective criteria. However, a quantitative method has recently been suggested that could be used to obtain an objective indicator of the spawning period for some small pelagic fish, based on the gonadosomatic index and the proportion of active females (Claramunt et al., 2014). Nevertheless, individual differences in duration of the spawning period may exist, depending on the size and age of the females, as was reported for several species (Fitzhugh et al., 2012).

Spawning frequency represents the number of spawning events per time unit, and may be estimated at the population level from the daily spawning fraction, i.e., the proportion of females spawning per day (Hunter and Goldberg, 1980; Ganias et al., 2014). Daily spawning fraction is usually estimated through the proportion of spawning stages determined from random ovary samples. The most commonly used methodology estimates the proportion of mature females with post-ovulatory follicles (POFs) per sample, which corresponds to a daily spawning cohort (Ganias, 2012). Another criterion also used is the proportion of imminent pre-spawning females, detected by the presence of ovaries with hydrated oocytes or in nuclear migration (DeMartini and Fountain, 1981; Priede and Watson, 1993; Hunter and Macewicz, 2003; Ganias et al., 2014). However, in some species it was shown that these stages are not randomly distributed in the reproductive area, because their incidence depends on the capture hour, which may produce some bias in the estimates (Hunter and Goldberg, 1980; Ganias, 2012). The main requisites to estimating SF are having representative ovary samples from the spawning stock and being able to identify daily spawning classes (or cohorts) from those samples.

Spawning frequency is a parameter also used to estimate spawning

* Corresponding author at: Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo Nro. 1, B7602HSA Mar del Plata, Buenos Aires, Argentina.
E-mail addresses: gmacchi@inidep.edu.ar (G.J. Macchi), krodrigues@inidep.edu.ar (K. Rodrigues), eleonard@inidep.edu.ar (E. Leonarduzzi), mdiaz@inidep.edu.ar (M.V. Diaz).

https://doi.org/10.1016/j.fishres.2018.02.011
Received 25 August 2017; Received in revised form 16 November 2017; Accepted 12 February 2018

biomass by the Daily Egg Production Method (DEPM), which was developed mainly for small pelagic fish (Parker, 1980). This method assumes that the daily spawning fraction remains constant over the sampling interval from which this variable is estimated (Parker, 1985; Picquelle and Stauffer, 1985; Claramunt et al., 2014), and is considered independent of female size structure. Nevertheless, for some species it was reported that SF may be influenced by female length or weight, as in *Thunnus albacares* (Schaefer, 1998) and in small pelagic fishes, *Sardinops sagax* and *Engraulis ringens*, from Chilean waters (Claramunt et al., 2007). Those authors found that the daily spawning fraction increases with the size and weight of females, affecting the proportionality between spawning biomass and egg production.

As previously mentioned, SF depends on the proportion of mature females with evidence of recent or imminent spawning, determined by detection of POFs or oocytes in final maturation, respectively. In several species, it was demonstrated that the duration of ovary maturity stages may be influenced by temperature, because this parameter can accelerate POF degradation (Fitzhugh and Hettler, 1995; Schaefer, 1996; Macchi et al., 2003) or oocyte growth (Bye, 1984, 1990; Stacey, 1984). Therefore, in addition to female size structure, SF may also be affected by annual or spatial variations in water temperature. Moreover, it is also possible that the nutritional condition of females could have an influence on growth or quality of the oocytes, affecting the reproductive potential, as was recently reported for the Northern stock of Argentine hake, *Merluccius hubbsi* (Rodrigues et al., 2018).

Argentine hake inhabits the waters of the Southwest Atlantic Ocean between 22°S and 55°S, at depths ranging from 50 m to 500 m (Cousseau and Perrota, 1998). This is the main fishery resource for the Argentine bottom-trawler fleet, with an approximate total catch of 283,000 tons reported in 2016.[1] The Patagonian stock, distributed from 41°S to 55°S, is the most abundant, accounting for approximately 85% of the fishery (Aubone et al., 2000). Argentine hake is a batch spawning fish with indeterminate annual fecundity. Individuals of the Patagonian stock reproduce during the austral spring and summer, between November and April, with the main peak in January (Macchi et al., 2004; Pájaro et al., 2005). Spawning frequency of this species was estimated as a mean monthly value (Macchi et al., 2004; Rodrigues et al., 2015), but whether there is a relationship between this variable and female size was not evaluated. This is an important topic, taking into account that in a recent review it was reported that in many species the SF and the extension of the reproductive period increased with size and age of the spawner (Fitzhugh et al., 2012). In Argentine hake, analysis of the length-frequency and age-frequency variation during different months of the reproductive season suggested that old females ($>$ 5 years old) have a longer spawning period than younger ones (Macchi et al., 2004; Pájaro et al., 2005). Therefore, it is important to know whether the number of spawning events per year can vary with the size or age composition of Patagonian Argentine hake stock, because it would affect the stock-recruitment relationship estimated during the population assessment.

The main objective of this study was to analyze the relationship between SF and female size and age of Argentine hake from Patagonian waters. The spawning fraction was estimated from ovarian samples that were collected during different months of the spawning season, and this information was correlated to the length, age and nutritional condition of females. Physical data from the reproductive area were incorporated in order to establish possible relationships between sea temperature and hake spawning. In addition, batch fecundity values were estimated during the same sampling period, and the relative egg production analyzed in relation to the size structure of the stock.

[1] Ministerio de Agroindustria. 2016 Desembarques de capturas marítimas totales—por especie y flota. Subsecretaría de Pesca y Acuicultura, Secretaría de Agricultura, Ganadería y Pesca, Ministerio de Agroindustria, Buenos Aires, Argentina. [Available from website, accessed July 2017.]

2. Material and methods

2.1. Data collection and histological processing

Samples of *M. hubbsi* were obtained from bottom trawls during 21 research surveys, which were carried out in the northern Patagonian area between 2000 and 2014 by the National Institute of Fisheries Research and Development (INIDEP). These surveys were performed in the austral summer (December–March), during the spawning season of the Patagonian hake stock, covering the main reproductive area of this population (Fig. 1). The fishing trawls were distributed in transects perpendicularly oriented to the coastline, separated by approximately 20 nmi, and which extended to depths of between 50 m and 120 m. Captures were obtained using a bottom trawl with a mouth width of approximately 20 m, a height of about 4 m, and with a 20 mm mesh at the inner lining of the cod-end. At each sampling station, salinity and temperature data were collected using a Sea-Bird 19 CTD (conductivity–temperature–depth profiler). Data series were filtered and reduced to values of temperature and salinity according to a 1-db interval. Since Argentine hake is a demersal species that aggregates near the sea bottom during the day, when the fish samples were taken, we used only data obtained from this stratum of the water column for oceanographic information.

A total of 17,898 adult females were sampled for histological analysis of the ovaries (Table 1). From each specimen, total length (TL) in centimeters and total weight (TW) in grams were registered, and the ovaries were removed and preserved in 10% neutral-buffered formalin, which were later weighed (GW) to the nearest 0.1 g. Only otoliths from females sampled in January 2001, 2004, 2005 and 2006 (n = 2757) were obtained for age determination, and therefore the relationship between SF and age was estimated only for this month of the spawning season.

Since gutted weight data were not available, total weight without ovaries (TW*) was used to assess female nutritional condition. For the samples collected during each survey, the relative condition factor (Kn) was calculated, expressed as a proportion of the observed TW* to TW* determined by the TW* vs TL relationship estimated from all data collected, which is described by the following equation:

$$TW^* = 0.012 \times TL^{2.84}\ (R^2 = 0.96,\ n = 17898,\ P < 0.01)$$

A portion of the fixed ovaries (about 2.0 g) was removed, dehydrated in ethanol, cleared in xylol and embedded in paraffin. Sections were cut to a thickness of 5-μm and stained with Harris's hematoxylin, followed by eosin counterstain. Histological staging of ovaries was based on the stage of oocyte development and on the occurrence of postovulatory follicles (POF) (Hunter and Goldberg, 1980; Brown-Peterson et al., 2011). Postovulatory follicles were classified as day-0 (elapsed time from spawning $<$ 24 h) and day-1 (elapsed time from spawning $>$ 24 h but $<$ 48 h), according to the description reported by Hunter and Macewicz (1985) for *Engraulis mordax*, and later applied to the Argentine hake (Macchi and Pájaro, 2003; Macchi et al., 2004; Ganias et al., 2014).

2.2. Data analysis and estimation of spawning frequency and fecundity

Previous analysis of seasonal and inter-annual egg production of the Patagonian stock of Argentine hake demonstrated that batch fecundity and SF showed more variation between different months of the reproductive season than between years (Macchi et al., 2004; Pájaro et al., 2005). Therefore, the variation of the proportion of POF (day-0 and day-1) by length class during different months of the spawning season (December–March) was analyzed, pooling all data collected from 2000 to 2014. A generalized linear model (GLM) was used to determine if month, TL, Kn, bottom temperature (BT) or bottom salinity (BS) significantly influenced the probability of spawning, defined as the

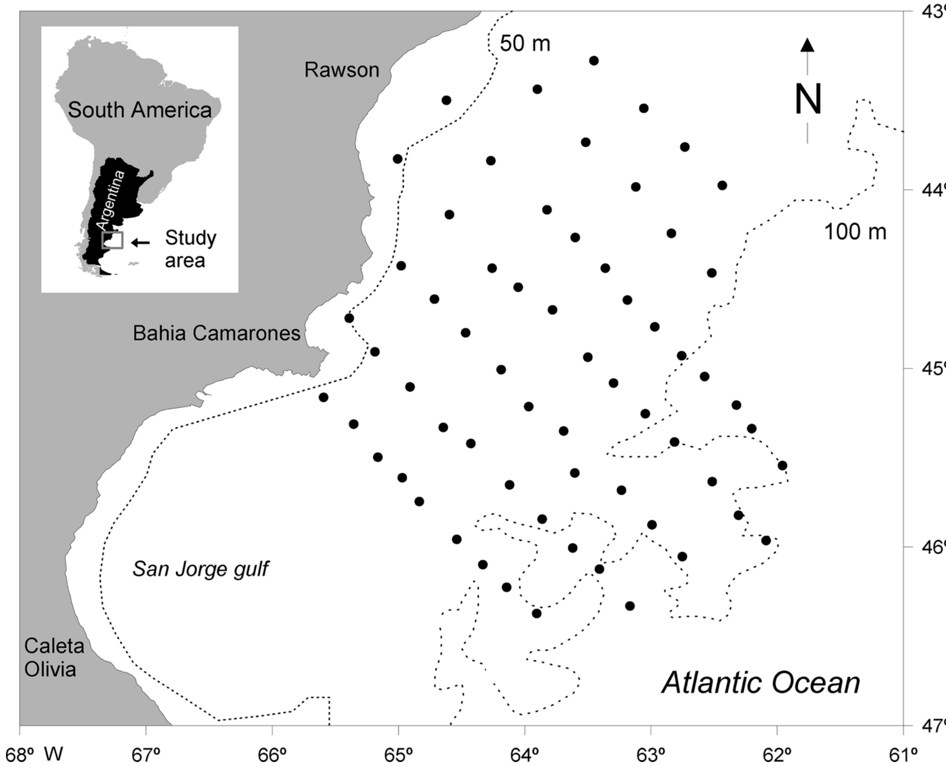

Fig. 1. Sample locations of *Merluccius hubbsi,* collected during the spawning season (December–March) of the Patagonian stock, in the waters of Chubut Province – Argentina, between 2000 and 2014.

probability that a female has day-0 or day-1 POFs. We followed the data exploration protocol proposed by Zuur et al. (2010) to avoid problems related with high correlations between explanatory variables. Therefore, variables with variance inflation factors higher than 3 were not considered in the analysis. A forward stepwise regression procedure was used to determine the set of fixed factors that explained a significant portion of the observed variability. Factors terms were selected for final analysis by the χ^2 test ($p \leq 0.05$). Month was modeled as a categorical variable and TL, Kn, BT and BS were modeled as continuous variables. The model had a logit link function and a quasi-binomial error structure. Pseudo r^2 values were calculated in order to compare the proportion of the deviation accounted for monthly TL, Kn, BT and BS. This pseudo coefficient was estimated as the deviance in the model, containing only the intercept (null deviance) minus the deviance after adding the main factors, divided by the null deviance. In order to show the effect of different variables in the model, the probability of spawning was plotted versus monthly TL, Kn, BT and BS. Finally, a pairwise comparison among the mean values of spawning probability was estimated for different months using a Turkey's test. All statistical analyses were conducted using R software (R Development Core Team, 2010).

Table 1
Number samples of Argentine Hake, *Merluccius hubbsi*, adult and mature females, and number of ovaries collected for fecundity estimations during research surveys carried out in different months of the spawning season of the Patagonian stock of the species between 2000 and 2014.

Year	Month	Adult females	Mature females	Fecundity
2000	December	472	472	58
2001	January	1035	931	102
	February	849	784	85
	March	542	378	16
2002	March	467	277	18
2004	January	851	813	54
	December	826	826	72
2005	January	961	961	90
	February	940	894	79
2006	January	1079	1035	95
	December	1060	1008	65
2007	January	914	914	78
2008	January	966	904	81
	December	708	604	81
2009	January	907	875	91
	March	946	946	70
2010	January	933	933	102
2011	January	640	640	80
2012	January	1144	1144	98
2013	January	870	870	83
2014	January	788	788	77

To estimate the daily spawning fraction (*S*), this study only considered the proportion of females with day-1 POFs (between 24 and 48 h from spawning) within the group of mature females (Table 1), following the criterion of Hunter and Goldberg (1980). Mature ovaries were those with yolked oocytes. According to the terminology suggested by Brown-Peterson et al. (2011), mature females corresponded to spawning-capable individuals (i.e., able to spawn during the current cycle) or to those females in the regressing phase, with evidence of recent spawning (i.e., with POFs or atresia of yolked oocytes). The SF, or the time elapsed between spawning events, was calculated as $1/S$ (Ganias et al., 2014). For example, a short time elapsed between spawning events indicates a high SF. To evaluate the influence of female size on SF, we pooled all data by month and used standard regression analysis to describe the relationship between SF and TL. These relationships were estimated after verify the residuals of the models taking into account the leverage and Cook's distance values, in order to detect the presence of influential data points. Comparison of the models obtained for each month (from December to March) was performed using a Krustal-Wallis Test and Dunn's Multiple Comparison Test. In addition, using regression analysis, we estimated the relationship between SF and age from data collected in January.

Batch fecundity (BF i.e., number of oocytes released per spawning) was also estimated monthly, pooling all data collected between 2000 and 2014. We used the hydrated oocyte method on fixed ovarian samples (Hunter et al., 1985), selecting only ovaries with hydrated oocytes and without any evidence of recent spawning (i.e., no POFs) (Table 1). The methodology used to estimate BF for Argentine hake was described by Macchi et al. (2004): 3 pieces of an ovary, approximately 0.1 g–0.2 g each, were removed from the anterior, middle and posterior parts of one gonad, weighed (± 0.1 mg), and the number of hydrated oocytes counted. Batch fecundity for each female was the product of the mean number of hydrated oocytes per unit weight and the total weight of the ovaries. The relationship between BF and TL for each month was described using standard regression. An analysis of variance using log-transformed data was applied to compare the coefficients of the BF versus TL relationships, estimated for each month of the spawning season. The months sampled were compared in pairs after overlapping the range of lengths of the females.

Monthly fecundity by length class was obtained by multiplying the BF value by the TL class for the number of spawning events by the TL class, estimated for each month of the spawning season.

3. Results

3.1. Seasonal variation in female size composition

The size-frequency distributions of mature female hake, obtained after pooling all data collected during the sampling period, showed monthly variation during the spawning season (Fig. 2). In December and January, mature females of the Patagonian hake stock were mainly dominated by individuals of the intermediate length classes, which ranged between 40 cm and 50 cm in TL (Fig. 2). In February and March, the incidence of mature females that had a TL smaller than 50 cm decreased, but larger individuals maintained the same percentages than during the spawning peak (Fig. 2). These results suggest that adult females with small or intermediate length classes can finish their spawning activity earlier in the season in comparison to larger specimens (> 50 cm TL). However, during March, a group of mature females with a modal size of 40 cm TL was also observed, which may be individuals that had reached their first sexual maturity at the end of the spawning season (Fig. 2).

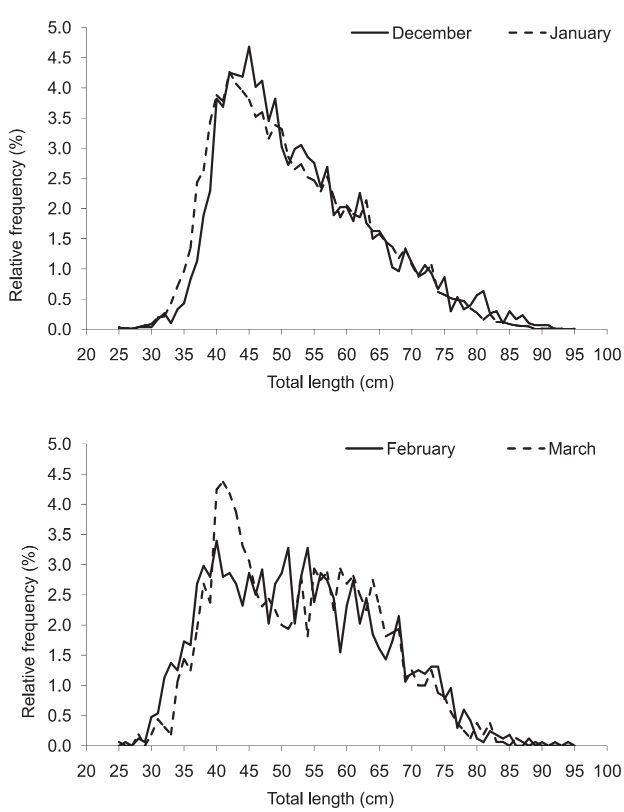

Fig. 2. Length frequency distributions of mature females of *Merluccius hubbsi*, sampled in different months of the spawning season of the Argentine Patagonian stock, between 2000 and 2014.

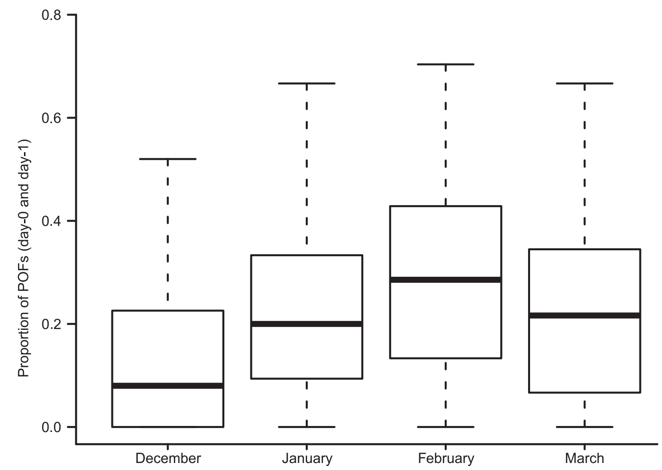

Fig. 3. Boxplot of the proportion of mature females of *Merluccius hubbsi* with post-ovulatory follicles (POFs, day-0 and day-1) estimated for the different months of the spawning season of the Argentine Patagonian stock between 2000 and 2014. The line in the box represents the mean value and the dotted line the standard error.

3.2. Spawning in relation to maternal and physical variables

The monthly variation of spawning activity, estimated from the proportion of day-0 and day-1 POFs, showed highly significant differences ($P < 0.001$) between December and the other months of the spawning season. At the beginning of the reproductive period (December) there was a low proportion of mature females with POFs, compared to hake samples collected later in the spawning season (Fig. 3). The peak of spawning activity was observed in February, with an average of approximately 28% of mature females with POFs.

All explanatory variables analyzed in the GLM were tested and no variance inflation factor values higher than 3 were observed. The stepwise construction of the model on the probability of spawning activity is summarized in Table 2, after using a forward selection of explanatory variables. The model showed that TL, Kn, BT and BS influence the probability of spawning, expressed as the proportion of POFs, during different months of the reproductive season of the Argentine hake from the Patagonian stock. The relationship between these variables was statistically significant, although the amount of variation explained by the model was low, approximately 11% of the variability in the probability of spawning (Table 3). Total length and temperature had a positive effect in the model, showing an increased probability of spawning in larger females and in waters with higher temperatures

Table 2
Deviance analysis of explanatory variables for the binomial step of the GLM applied to spawning activity of Argentine hake, *Merluccius hubbsi*. Factors were added to the model if they were significant according to a χ^2 test ($p < 0.05$). Df: degrees of freedom.

Variable	df	Residual Df	Residual Deviance	Deviance reduction	Cumulative explained deviance (%)	p $\|\chi^2\|$
Null model	NA	712	3239.39			
+Month	3.00	709.00	3070.78	168.61	5.21	< 0.001
+BT	1.00	708.00	2974.96	95.82	8.16	< 0.001
+TL	1.00	707.00	2912.52	62.43	10.09	< 0.001
+BS	1.00	706.00	2887.95	24.57	10.85	0.010
+Kn	1.00	705.00	2871.46	16.50	11.36	0.036

Table 3
Results of the Generalized Linear Model predicting the probability of spawning of the variables month, total length (TL), relative condition factor (Kn), bottom temperature (BT) and bottom salinity (BS) in Argentine Hake, *Merluccius hubbsi*, from the Patagonian stock.

Variable	Coefficients	Standard error	t value	*p*
Intercept	19.295	10.252	1.882	0.06
January	0.617	0.122	5.061	< 0.001
February	0.747	0.164	4.544	< 0.001
March	0.643	0.154	4.165	< 0.001
BT	0.114	0.020	5.651	< 0.001
TL	0.028	0.006	4.287	< 0.001
BS	−0.661	0.311	−2.123	< 0.05
Kn	−1.522	0.729	−2.088	< 0.05

(Fig. 4). In contrast, the effect of Kn on the probability of spawning trended negatively, indicating that females with a higher proportion of POFs were characterized, in general, by a poor condition (Fig. 4). Salinity also showed a significant negative effect in the model (Table 3).

Comparison between different months of the spawning season showed the same previously described pattern, with the lowest values of the proportion of POFs in December, compared with the models estimated for January–March (Fig. 4).

3.3. Spawning frequency in relation to female size and age

The SF in relation to female size was estimated for each month of the spawning season using the proportion of day-1 POFs by length class. In all cases, significant negative relationships were obtained between SF and TL (Fig. 5), which indicates a decrease in the recovery time between egg batches, or a higher SF for larger hake females. The proportion of variability explained by each model ranged between 0.14 and 0.46. Comparing the models estimated for each month of the spawning season, there was a highly significant difference ($P < 0.0001$) between December and the rest of the reproductive period. In December, the time elapsed between spawning events was higher than in January–March, indicating a lower SF at the beginning of reproductive season. We estimated that during December 35 cm TL females may have a spawning event every approximately 13 days, and 80 cm TL females every 7 days (Fig. 5). In January and February, SF for females between 35 cm and 80 cm TL ranged approximately from 7 to 4 days, and in March from 7 to 3 days. Basically, SF increased for larger Argentine hake females with the progression of the spawning season.

Spawning frequency also showed a negative trend with the age of spawners (Fig. 6), indicating a shorter time between spawning events in older females. In January, a SF range of 5–8 days was estimated for females between 13 and 2 years old, respectively.

3.4. Batch fecundity and egg production in relation to female size

Batch fecundity also increased with female size, and fitted to a power relationship for all samples collected in the different months of the spawning period (Fig. 7). Comparison of these models showed highly significant differences ($P < 0.001$) between BF values estimated in March with respect to other months of the reproductive season. In contrast to that observed for spawning frequency, BF in March was

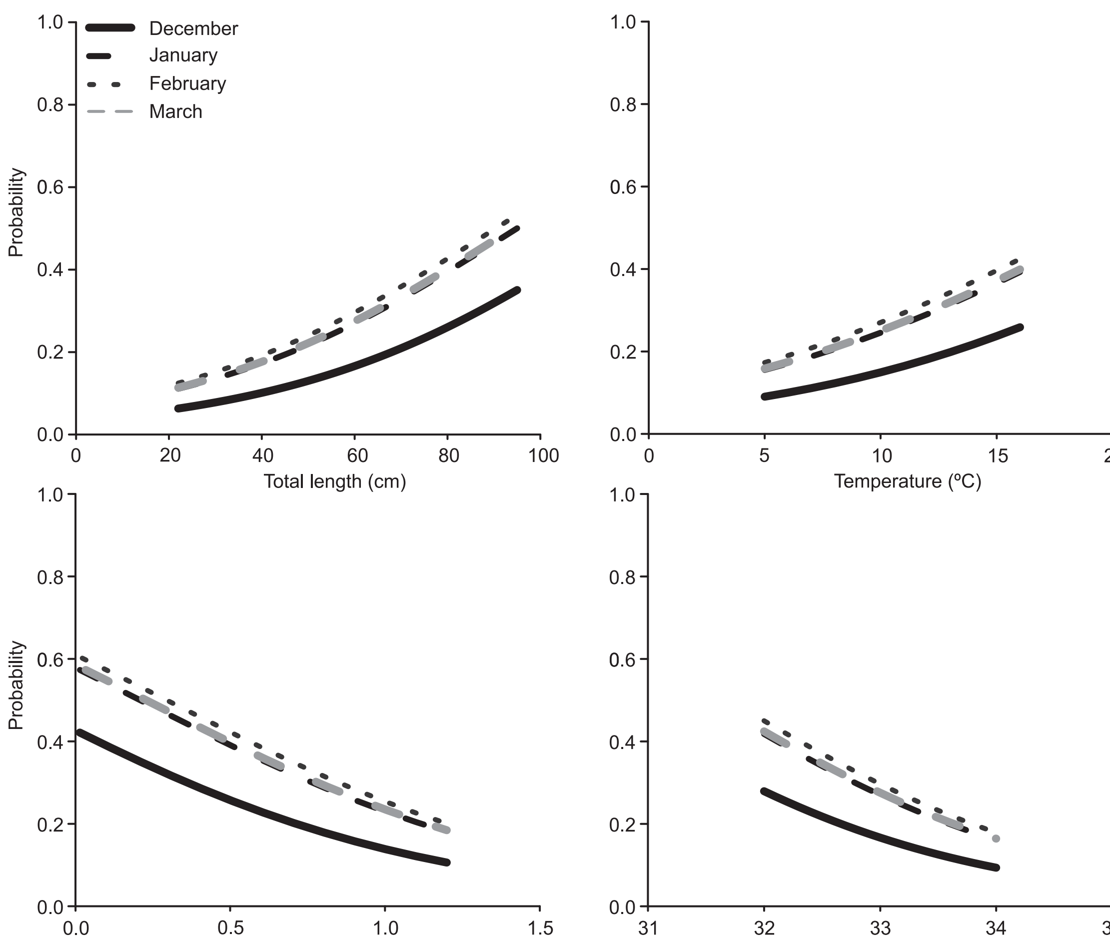

Fig. 4. Probability of spawning in females of *Merluccius hubbsi* from the Argentine Patagonian stock in relation to total length, bottom temperature, relative condition factor (Kn) and bottom salinity, estimated for different months of the spawning season during 2000 and 2014.

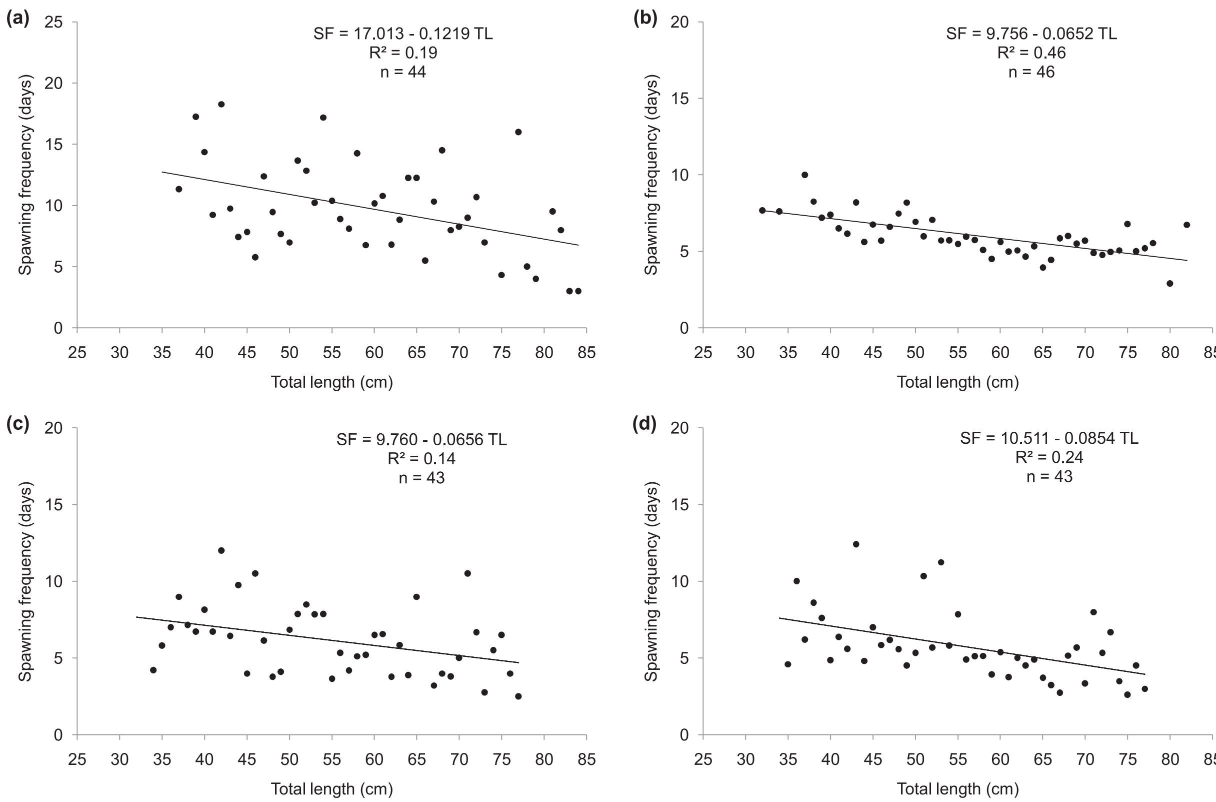

Fig. 5. Spawning frequency (SF) as a function of total length obtained for females of *Merluccius hubbsi* from the Argentine Patagonian stock in different months of the spawning season: a) December, b) January, c) February and d) March, during the period 2000–2014.

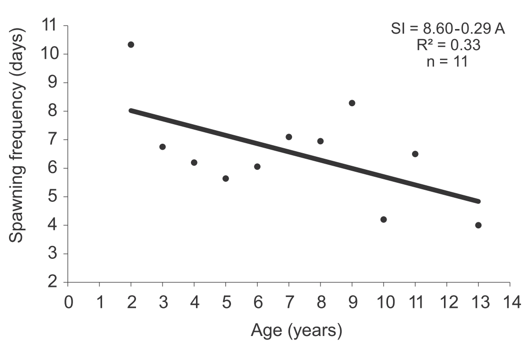

Fig. 6. Spawning frequency (SF) as a function of age obtained for females of *Merluccius hubbsi* from the Argentine Patagonian stock with samples collected in January 2001, 2004, 2005 and 2006.

lower than from December to February, indicating that the number of oocytes produced per batch diminished at the end of the spawning season. The BF ranges estimated by the models were approximately 140,000–1,800,000; 119,000–1,830,000; 95,000–1,735,000 and 100,000–1,180,000 hydrated oocytes in December, January, February and March, respectively, for females between 35 and 80 cm TL. These results indicate that the BF values at the end of the spawning period showed a sharp decrease, mainly in larger females, in contrast to the pattern described for SF. Nevertheless, when both BF and SF variables were combined, the monthly fecundity by size was similar in smaller adult females, but changed for sizes greater than 50 cm TL (Fig. 8). Larger females (> 50 cm TL) produced a low number of oocytes in December compared to January–March, with January and February being the months with the highest values of fecundity.

The relative egg production by length class showed a coincident pattern for December and January, characterized in general by a similar reproductive output for females between 45 cm and 80 cm TL (Fig. 9). However, during these months the size frequencies were dominated by small- and intermediate-length classes between 40 cm and 50 cm TL (Fig. 2). During February–March, relative egg production mainly depended on females larger than 60 cm TL, with a sharp decrease in production by individuals smaller than 50 cm TL.

4. Discussion

The monthly variation of the proportion of POFs during the reproductive season of the Patagonian Argentine hake showed highly significant differences between December and the rest of the analyzed months (January, February and March). Using this variable, i.e., the monthly incidence of spawning females, the highest reproductive activity was recorded during February. However, considering the abundance of mature females in the reproductive area, the spawning peak of Argentine hake in Patagonian waters would be one month earlier, according to Macchi et al. (2004). As the reproductive season progresses, adult females that have already finished spawning move towards deeper waters, producing a decreasing trend in the abundance of Argentine hake in the main spawning area (Macchi et al., 2007). Therefore, if the peak of reproductive activity is defined as the moment of highest egg production, we should not only consider the proportion of spawning females obtained in the sampling, but also the abundance of these specimens in the spawning area. The size distribution of mature females showed that the smallest adult specimens (< 50 cm TL) finish their reproductive activity first, having a shorter spawning season than larger females, as was previously reported for the Patagonian hake stock (Macchi et al., 2004; Pájaro et al., 2005). Larger females stayed longer in the spawning area, as reflected in the size frequency distributions of February and March (Fig. 2). It has been suggested that the longest spawning period could be associated with the income of energy during the reproductive season, evidenced by the feeding activity of the larger hake females at the spawning peak of the Patagonian stock (Macchi et al., 2013).

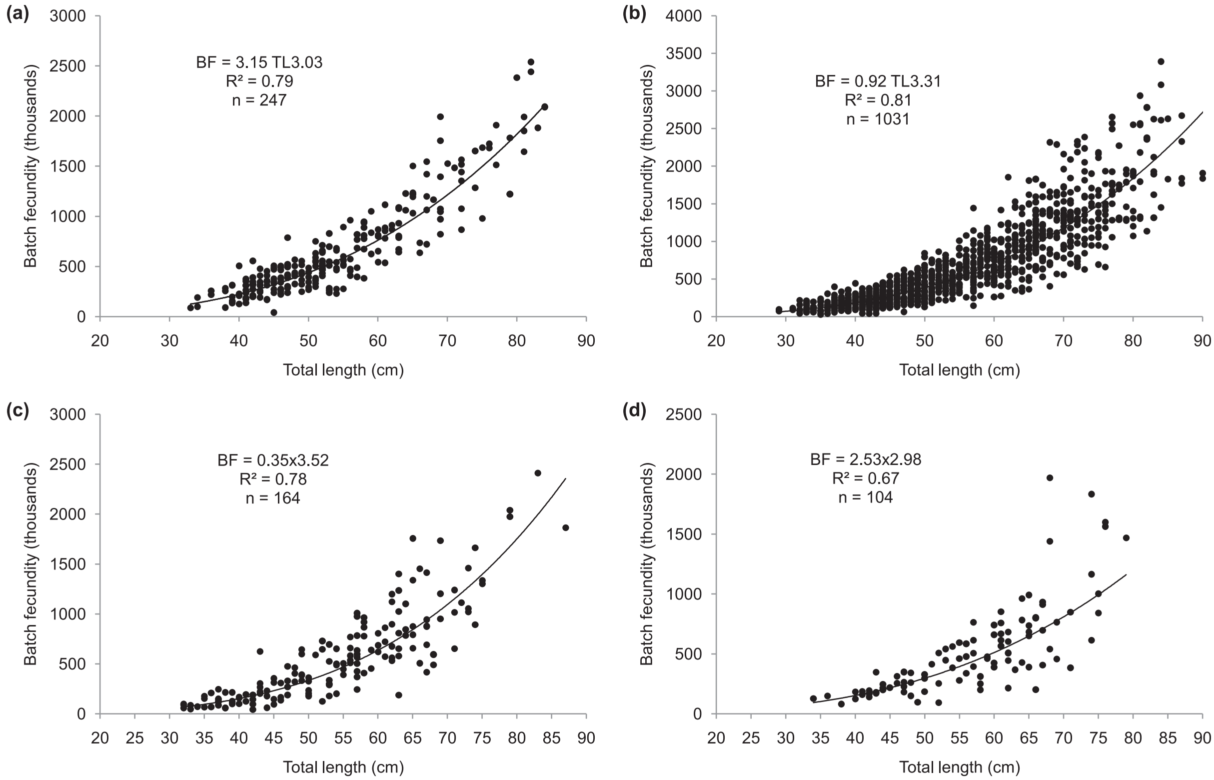

Fig. 7. Batch fecundity (BF) as a function of total length obtained for females of *Merluccius hubbsi* from the Argentine Patagonian stock in different months of the spawning season: a) December, b) January, c) February and d) March, during the period 2000–2014.

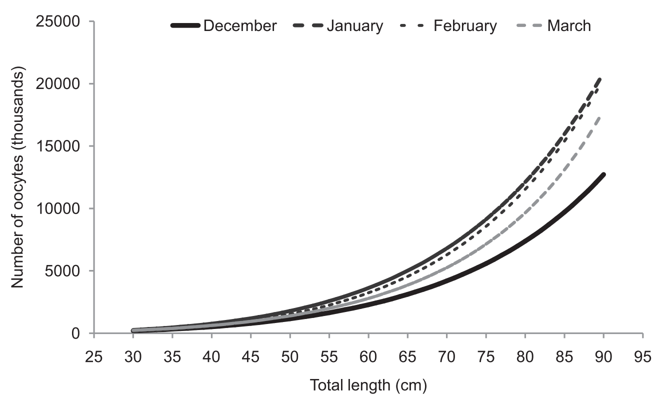

Fig. 8. Monthly fecundity as a function of total length obtained for females of *Merluccius hubbsi* from the Argentine Patagonian stock in different months of the spawning season during the period 2000–2014.

The GLM analysis showed a positive relationship between the spawning activity, represented by the proportion of females with POFs, and TL. This result suggests that SF may be influenced by female size. The regression analysis between both variables supported this hypothesis, showing significant relationships between SF and TL for all sampled months during the spawning season. The SF of Argentine hake from the Patagonian stock ranged between 13 and 3 days for females within a length interval of 35 cm–80 cm TL. The highest SF values (spawning every 3–7 days) for this size range were obtained in March, and the lowest values (spawning every 7–13 days) were from December. The SF also showed a negative trend with the age of spawners, suggesting higher reproductive frequencies in older females. In other species it has been reported that SF depends on the size or weight of the spawners, in particular in pelagic species with indeterminate annual fecundity (Schaefer, 1998; Claramunt et al., 2007). These results are highly relevant because there exists a common

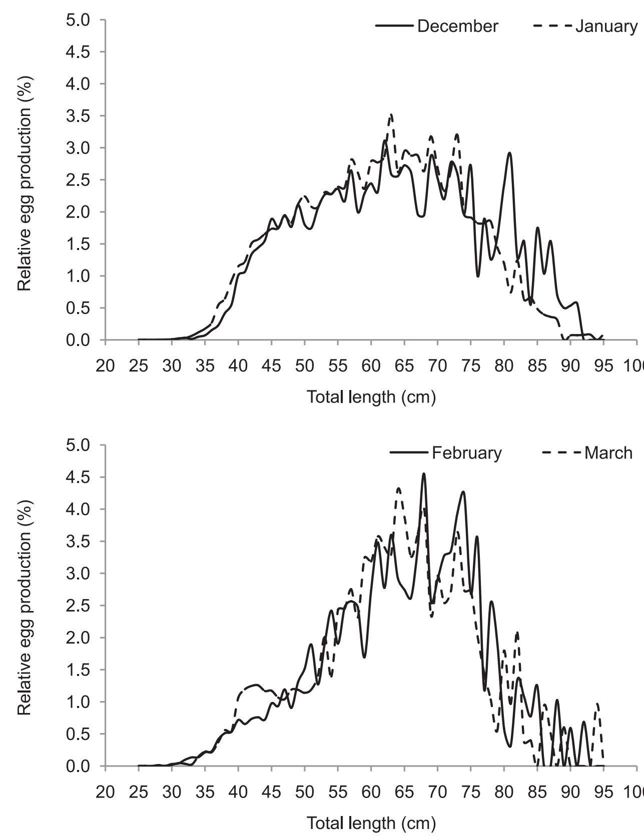

Fig. 9. Relative egg production by total length class estimated for females of *Merluccius hubbsi* from the Argentine Patagonian stock in different months of the spawning season during the period 2000–2014.

assumption that the SF of a stock is constant during the entire reproductive season, and in general the relationship between the number of spawning events and the size of the spawners is not analyzed (Fitzhugh et al., 2012). The incorrect assumption about the annual egg production of a fish stock could lead to substantial bias in the estimation of their reproductive potential and consequently would affect their assessment and management.

The increase of SF with the size and age of the spawners observed in the Patagonian hake, combined with the longer reproductive period reported for larger females (Macchi et al., 2004), corroborates the BOFFFF (Big, Old, Fat, Fecund Female Fish) hypothesis (Berkeley et al., 2004) suggested by Macchi et al. (2017) for this stock. Therefore, egg production by biomass unit of the Argentine hake in Patagonian waters would depend on the size-age structure of the stock, affecting the assumption of proportionality between spawning biomass and recruitment. The analysis of monthly relative egg production, obtained from the combination of BF and SF, showed that at the end of the reproductive season of the Argentine hake, most of the eggs are produced by females larger than 60 cm TL. However, considering that abundance of this stock in the spawning area decreases during February and March (Macchi et al., 2004), absolute egg production at the end of the spawning season should be much lower than from December to January.

Batch fecundity and SF showed opposite patterns after analysis of the monthly variation of these variables with female size. The relationships between BF and TL that were estimated for December–February were similar, but in March the fecundity values were lower. In contrast, SF showed higher values in March, at the end of the reproductive season, while December was the month with lower SF. It is possible that the opposite trends of these variables observed during the reproductive season may be due to the effect of different factors. For example, the GLM showed a positive relationship between spawning activity and water temperature, which increases at the end of the reproductive season. These results suggest a positive influence of temperature on the growth and maturation of oocytes, which could reduce the elapsed time between egg batches or increases the proportion of spawning females, affecting SF in Argentine hake, occurring mainly in March. Supporting this hypothesis, several studies indicate a positive relationship between temperature and oocyte maturation (Bye, 1984, 1990), as well as with the degradation time of the FPOs (Fitzhugh and Hettler, 1995; Macchi et al., 2003). In *Engraulis ringens* from northern Chile, a negative trend was observed between SF and the temperature; but in this case, the authors mentioned a possible negative influence of El Niño in the region during the period analyzed (Claramunt et al., 2007). However, the relation between SF and temperature observed in Argentine hake and in other species is a topic that deserve further research in the future, in order to know which are the physiological mechanisms affecting oocyte maturation. We also observed a negative trend between spawning and bottom salinity, but it is possible that this relationship may be caused by the location of the spawning aggregations, mainly sited in the inshore area, where salinity values in general are lower (Romero et al., 2006).

In contrast to SF, BF values estimated for the Patagonian Argentine hake at the end of the spawning season (March) were lower than in the period December–February, as was observed previously for this stock (Macchi et al., 2004) and also for other species (Dickerson et al., 1992; Macchi et al., 2002). In this case, it is probable that the number of oocytes per batch could be affected by another variable; in fact, the GLM analysis demonstrated a negative trend between the spawning and the nutritional condition of females, with lower Kn values at the end of the reproductive season. In March, the northern Patagonian area is dominated by Argentine hake females in post-spawning stage, which were characterized by a nutritionally deficient condition after finishing the seasonal spawning (Leonarduzzi et al., 2012). The poor condition observed in females of this species in March could affect mainly BF and oocyte quality, as was recently reported for the Northern stock of Argentine hake, after analysis of the proximate composition of different tissues of mature specimens (Rodrigues et al., 2018).

In conclusion, towards the end of the reproductive period of the Patagonian Argentine hake, the proportion of spawning females is higher, which positively affects spawning frequency, but batch fecundity and oocyte quality are lower. Therefore, to establish the real effect of the nutritional and physiological condition of the spawning females on the number and quality of their eggs, it is necessary to assess survival and condition of early developmental stages of larvae collected during the reproductive season.

This study revealed that the size and age of spawners had a positive effect on the SF of the stock. The results herein obtained are of great relevance for the assessment of a fishing stock, since an incorrect assumption of constant spawning frequency, independent of the age-size of the population, would lead to an overestimation of the biological reference points used for the assessment model, increasing the uncertainty of the stock-recruitment relationships.

Funding

This work was supported by the INIDEP, Consejo Nacional de Investigaciones Científicas y Técnicas [CONICET; grant number: PIP 112 201201 00047] and Fondo para la investigación Científica y Tecnológica [FONCyT; grant number: PICT-2013-1484].

Acknowledgments

We wish to thank M. Estrada and H. Brachetta for their work in preparation of the histological sections of the Argentine hake specimens and the technical staff of the Hake Assessment Group of the INIDEP for age determination of the specimens. We wish to express our gratitude for the corrections made by reviewers that greatly improved the manuscript. This article is referenced by INIDEP as contribution N° 2104.

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