



# Variability in total egg production and implications for management of the southern stock of European hake

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## ABSTRACT

Stock recruitment (SR) relationships aim to predict the recruitment based on a given level of reproductive stock. Though the spawning stock biomass (SSB) is commonly used to fit such SR models, there is increasing evidence that the total egg production (TEP) is a more relevant stock reproductive potential index. Indeed, the TEP takes into account both the size-dependent capacity of females to produce eggs and the demographical structure of the spawning stock. This paper presents a method to assess the TEP based on the fecundity and egg quality based on the age as well as spawning fraction for the southern stock of European hake (*Merluccius merluccius*). The fecundity and egg quality increased significantly as the female size increased. The spawning fraction also increased with female size. Larger females concentrated their reproductive period during the first quarter of the year, whereas smaller ones spawned throughout the year. The stock's demographic structure showed a clear increase in the proportion of younger females and a reduction of older ones through the 1982–2007 time-series, which led to a decrease of egg production per unit of female SSB. Our results showed a significant contribution of older individuals to the reproductive output. The relevance of using TEP as an index of stock reproductive potential is discussed.

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## 1. Introduction

The stock recruitment (SR) relationship is a key tool for fisheries managers. These relationships are used to predict the recruitment (i.e., the number of young fish entering the fished population) for given levels of progenitors and to anticipate the response of the stocks to exploitation. The SR models are used to derive reference points for fisheries' stock management (Needle, 2002). Though SR models were originally based on egg production (Ricker, 1954; Beverton and Holt, 1957), estimates of spawning stock biomass (SSB) generated from Virtual Population Analysis (VPA) have been commonly used instead of reproductive potential. This assumes a direct proportionality between egg production and SSB. However, this assumption is increasingly criticised (Marshall et al., 1998, 2003; De Lara et al., 2007). First, for many exploited species, no distinction is made between males and females in the reproductive stock (Marshall et al., 2006). Second, the egg quality and quantity is known to vary according to the female's size (Vallin and Nisling, 2000) and spawning experience (Trippel, 1998). The SSB does not take into account the demographic struc-

ture of the reproductive stock and, consequently, does not reflect the variability in the total egg production (TEP) and recruitment. Marshall et al. (1998) reported that the TEP is an improved index of recruitment potential compared with estimates of spawning biomass.

Southern European Hake (*Merluccius merluccius*) is one of the exploited species for which the combined sex SSB is used to define reference points for management purposes (ICES, 2009). Southern European Hake has been declared to be overexploited and unsustainably harvested (ICES, 2007), and it is currently under a management plan for recovery. The main management tools for complying with the reference points are the limitation of the Total Allowable Catches (TAC) as well as the regulation of the fishing effort (in kilowatt-days) and the characteristics of fishing gears (ICES, 2009). However, these measures do not take into account the relative contribution of the age groups of the reproductive stock to the recruitment (age group 0), although large individuals are likely to be considerably beneficial to the reproductive output (Birkeland and Dayton, 2005). More and more importance is given to the stock reproductive potential, defined by Trippel (1999) as the "annual variation in a stock's ability to produce viable eggs and larvae that may eventually recruit to the adult population or fishery". For many species, recommendations are made to preserve large individuals (Berkeley et al., 2004; LaPlante and Shultz, 2007). An appropriate stock management requires a good understanding of the repro-

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ductive biology to adequately preserve the stock of genitors and to optimise its demographic composition in terms of contribution to egg production (Marshall et al., 2003).

Previous studies conducted on hake reproduction have shown that this species is an asynchronous and indeterminate spawner (Murua and Motos, 2006), but very little work has been done about the influence of female features on the fecundity and egg quality for this species. Indeed, the success of the recruitment depends on the number of eggs released and their capacity to reach viable larval and juvenile stages, as well as the suitability of the environmental conditions (Meiners-Mandujano, 2007; Ottersen et al., 2006). Demographic time-series data including size-age composition, maturation and sex ratio have been available for the southern European hake stock since 1982, but information on fecundity is more limited to recent years. By combining historical and contemporary data, it is possible to develop new indices of reproductive potential, such as female only SSB (FSSB) and TEP. This present study aims to estimate a TEP time-series with respect to female attributes and to investigate its variability with regard to the reproductive stock structure to assess the contribution of each age group to the egg production as well to ascertain the implications of the finding in stock management.

## 2. Materials and methods

### 2.1. Sampling effort, biological sampling and histological classification

This study combined two hake datasets. The first one covered the period from 1982 to 2007 and gathers the biological information required for stock assessment routines (individual length, age, sex and maturity) available from ICES WG (ICES, 2008). Maturity ogives for this period were taken from the analysis carried out on the same dataset by Dominguez-Petit et al. (2008). The second dataset gathers information on individual reproductive biology (microscopic maturity stage, fecundity and egg quality) and was available for the years 1999, 2000, 2003 and 2004, although egg quality information was available only for 2003 and 2004 (see Section 2.2). The methods used to produce the data, and the database it self, is the same as that used in Dominguez-Petit et al. (2010), Korta et al. (2010) and Dominguez-Petit and Saborido-Rey (2010).

Ovary stages were identified from histological sections. Females were identified as immature if their ovaries presented only oocytes in the primary growth stage. Females presenting ovaries with signs of maturity (such as cortical alveoli, vitellogenesis, migratory nucleus, hydrated oocytes or post-ovulatory follicles (POF)) (Saborido-Rey and Junquera, 1998; Dominguez-Petit, 2007) were identified as active mature females. Among the total number of fish sampled (3312) for the reproductive biology database, 2201 individuals were classified as active mature females (309 in 1999, 309 in 2000, 845 in 2003 and 738 in 2004).

The determination of actively spawning females (Brown-Peterson et al., in press) was based either on the dominant presence of hydrated oocytes (spawning is imminent) or the presence of recent POFs (female having spawned within the 24 past hours). Indeed, Picquelle and Stauffer (1985) and Dominguez-Petit (2007) indicated that hydrated females may be vulnerable to the fishing gear and may be oversampled. Therefore, the average incidence of hydrated females and females with a presence of recent POF was likely to be more representative of the spawning component at the sampling time. Following these criteria, 620 females were considered to be actively spawning through the 4 years of study. These individuals, together with the rest of the active mature females, were later used to estimate the daily spawning fraction.

### 2.2. Egg quality

The hydrated oocyte dry mass and diameter were used as indicators of egg quality and, hence, of reproductive success (Kjorsvik et al., 1990; Brooks et al., 1997). A total of 243 females with ovaries in the hydrated stage from 2003 and 2004 were selected. Three sub-samples were extracted from the anterior, middle and posterior thirds of the right lobe of the hydrated ovaries, and about 35 hydrated oocytes from each sub-sample were manually separated. All oocytes were then pooled, divided into two replicates and preserved in labelled Eppendorf tubes with 4% formaldehyde. To remove formalin, the oocytes were rinsed twice in a sieve with distilled water for 2 min (1 min for each dip). The oocytes were collected from the sieve and placed in a watch glass with clean distilled water for at least 20 min before the image analysis to allow rehydration (20 min has been shown to be the time for oocyte diameter stabilisation). The oocyte diameter (expressed in  $\mu\text{m}$ ) and the number of oocytes in each replicate were obtained through image analysis following the same methodology as Dominguez-Petit (2007). Mean dry weights were determined by drying the two replicates for 24 h at 110 °C and then dividing the weight by the number of oocytes per replicate. Although we are aware of previous work (Hislop and Bell, 1987) that this procedure to determine oocyte dry weight and diameter is biased due to losses whilst stored in formaldehyde it was the only option for ovaries preserved at sea and the best available for our application.

### 2.3. Estimation of spawning fraction and fecundity

The spawning stages of the hake ovary were classified histologically as described above and used to estimate the proportion/fraction of spawning females. The spawning fraction (SF), i.e., the proportion of spawning females per day, was estimated following the same methodology as in Korta et al. (2010). The mean and variance of spawning fraction were calculated according to Picquelle and Stauffer (1985). To estimate the spawning fraction, samples from 1999, 2000, 2003 and 2004 were available (Table 1). First, to illustrate the spawning pattern of hake along the year, the mean daily spawning fraction per month for this 4-year period was calculated as in Korta et al. (2010). Then, the mean spawning fraction per length class was estimated per quarter. Females were grouped by 2-cm class intervals (2 cm was chosen to have an interval that is still descriptive of the SF pattern within the length range whilst having enough individuals in each group to estimate a representative SF). The four-quarterly linear regressions of spawning fraction versus length class were used to model the total egg

**Table 1**

European hake number of samples used for the spawning fraction and number of actively spawning females used for the batch fecundity.

Month	Spawning fraction (number of samples)				Batch fecundity (number of females)			
	1999	2000	2003	2004	1999	2000	2003	2004
1		2	2	3		1	5	7
2	3	2	4	4	12	1	13	23
3	1	2	4	4	3	5	16	18
4	2	1	4	3	1	1	5	20
5	3	2	4	6	3	7	19	13
6	2		2	1	4		10	1
7		1	2	1		1	10	4
8	1		2	1	1		1	1
9	1		1	1				2
10	1		2	2			2	1
11	1		1	1			1	
12			3	2			2	
Total	15	10	30	29	24	16	84	90

production. The inverse of SF gives the average batch interval of the mature females.

As the presence of recent POFs in hydrated females indicates that part of the batch had ovulated, only ovaries with hydrated oocytes and without evidence of recent spawning (Murua et al., 2003) were selected for the estimation of batch fecundity (BF) and relative BF (BF<sub>rel</sub>). BF was estimated by the gravimetric hydrated oocyte method following the same methodology as in Korta et al. (2010). The absolute and relative batch fecundity was, thus, estimated in 214 hydrated ovaries (Table 1). BF and SF were estimated by length class and converted to age using ALK, as described above.

#### 2.4. Population parameters

The annual abundances from 1982 to 2007, combined sex maturity ogives and weight at given ages of hake were provided by ICES (2008). The annual mixed spawning stock biomass (SSB) was obtained from the product of the population in numbers, the proportion of mature fish at given age and the mean weight at given ages.

The sex ratios and female maturity ogives were obtained from the 1982–2007 Spanish biological database, including a total of 35,125 records among which 18,695 were females. Due to a low number of observations in 1987 and 1992, the sex ratios from these 2 years were replaced by the mean values of their two adjacent years. The female maturity ogives (updated from Dominguez-Petit et al., 2008) were fitted with annual logistic regression by age. The annual female spawning stock biomass (FSSB) was obtained by combining the stock abundances, sex ratios, female maturity ogives and mean individual weights at given ages for each year in the period of 1982–2007. The pattern of this index was compared to the traditional SSB (ICES, 2008). The effect of the stock structure on the subsequent TEP was investigated by testing the relationship between the TEP produced per unit of female SSB (TEP/FSSB) and the proportion of females of each age within the active mature stock during the period of 1982–2007.

The age-length key (ALK) for females followed a similar pattern as the combined sex ones, but they were less robust due to the lower number of individuals, especially at larger sizes. Therefore, the combined sex ALK was used to estimate the mean length at age as in the ICES Working Group for Southern Stock of Hake (ICES, 2008), i.e., based on an annual ALK, except for the period of 1982–1993, when annual ALK were not available and the mean ALK for the period of 1994–1998 was used instead. For the last decade, the age estimation of European hake has been seriously questioned (Pineiro and Sainza, 2003), and its growth is believed to have been underestimated (de Pontual et al., 2006). However, current southern hake stock assessment still uses the ages from the historical ALK (ICES, 2009), so we had no alternative but to adopt the historical ALK.

#### 2.5. Estimation of total egg production (TEP)

The TEP index was estimated for each age group (a) by, quarter (q) and year (y) based on the assumption that the batch fecundity is proportional to the realised fecundity (Murua et al., 2003):

$$TEP_{a,q,y} = N_{a,y} \times SR_{a,y} \times M_{a,y} \times BF_a \times SF_{a,q} \times Q_a$$

where

$N_{a,y}$  is the number of individuals at a given age from 1982 to 2007 at the beginning of the year, obtained from VPA (ICES, 2008);  $SR_{a,y}$  is the sex ratio at a given age for the period of 1982–2007, estimated as explained in Section 2.4;

$M_{a,y}$  is the expected proportion of mature females at a given age from annual logistic regressions for the period of 1982–2007, estimated as explained in Section 2.4;

$BF_a$  is the pooled mean absolute batch fecundity at a given age obtained from linear model based on observations from the 1999, 2000, 2003 and 2004 combined data, as described in Section 2.3;

$SF_{a,q}$  is the mean spawning fraction by quarter obtained from the four-quarterly linear regressions based on observations from 1999, 2000, 2003 and 2004 combined, as described in Section 2.3; and

$Q_a$  is the mean hydrated oocyte dry mass at a given age obtained from a linear model based on observations from 2003 and 2004 together, as described in Section 2.2.

Thus, the TEP for a given year is expressed as

$$TEP_y = \sum_{q=1}^4 \sum_{a=0}^i TEP_{a,q,y}$$

Note that this TEP index does not represent the real egg production because the oocyte dry mass at a given age as obtained from the linear model is included as a factor in the model.

#### 2.6. Statistical analysis

Prior to the analysis, data were examined for normality as well as heterogeneity and log-transformed when necessary. Generalised Linear Models were used to examine relationships between female attributes (length and age) and reproductive output (egg quality and quantity). The parameters of the linear models fitted for fecundity, oocyte dry mass and spawning fraction versus female length were used to model the TEP. Pearson parametric correlation was used to test the relationships between oocyte diameter and oocyte dry as well as wet mass. All of the analyses were conducted with R® software.

### 3. Results

#### 3.1. Egg quality

The females used to assess egg quality ranged in length from 44 to 76.5 cm. Hydrated oocyte dry mass ranged from 0.0323 to 0.0565 mg and averaged at 0.0463 mg (S.D. = 0.0043). There was a positive and significant relationship between the female length and the oocyte dry mass (Fig. 1a,  $p < 0.01$ ) as well as female age (Fig. 1b,  $p < 0.05$ ), although the female size poorly explained the variability in the hydrated oocytes dry mass ( $r^2 < 0.1$ ), as observed by the large scatter around the middle length range (Fig. 1a). The relationship was still significant but improved only slightly ( $r^2 = 0.23$ ) when the mean dry mass was estimated by a 1-cm size class (Fig. 1c).

Hydrated oocyte diameter ranged from 790 to 1223  $\mu\text{m}$  and averaged at 1056  $\mu\text{m}$  (S.D. = 78). There was a positive and significant relationship between the oocyte diameter and the female length (Fig. 2a,  $p < 0.001$ ) as well as the female age (Fig. 2b,  $p < 0.01$ ). However, as with dry mass the female size poorly explained ( $r^2 < 0.1$ ) the variability in the oocyte diameter (Fig. 2a). The relationship improved notably ( $r^2 = 0.63$ ) when the mean diameter was estimated by size class (Fig. 2c). The diameters of the hydrated oocytes were well correlated with their wet masses ( $r^2 = 0.88$ ), whereas they were poorly correlated ( $r^2 < 0.1$ ) with the dry masses (Fig. 3).

For dry mass there were no significant differences between 2003 and 2004 for any of the relationships analysed (ANCOVA,  $p > 0.1$ ), and no clear seasonal patterns were detected either, i.e., the mean dry mass estimated by quarter remained constant along the year

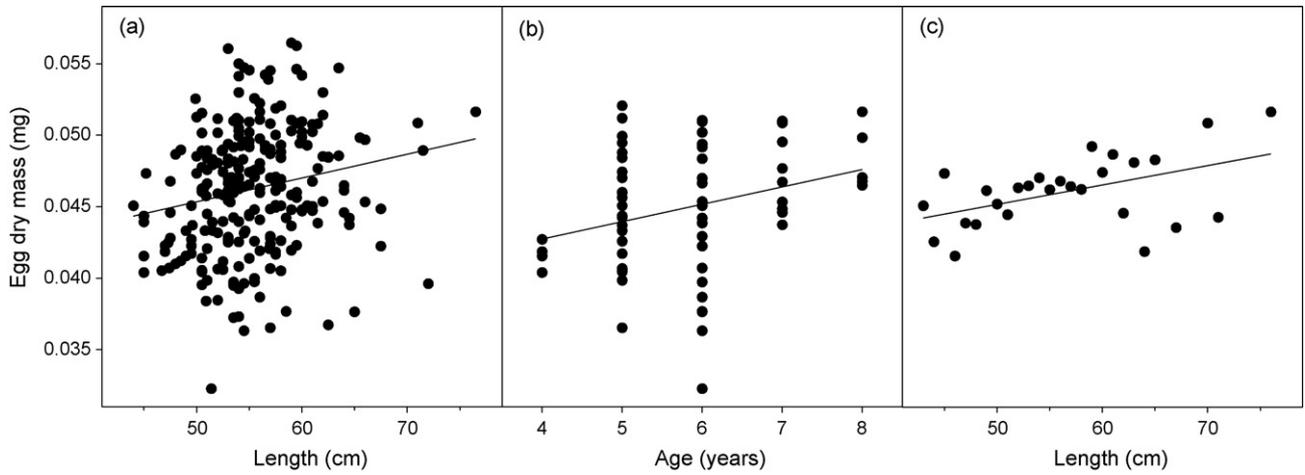


Fig. 1. Hydrated oocyte mean dry mass versus (a) female length, (b) age and (c) female length class (cm) of southern stock of hake for the period 1982–2007.

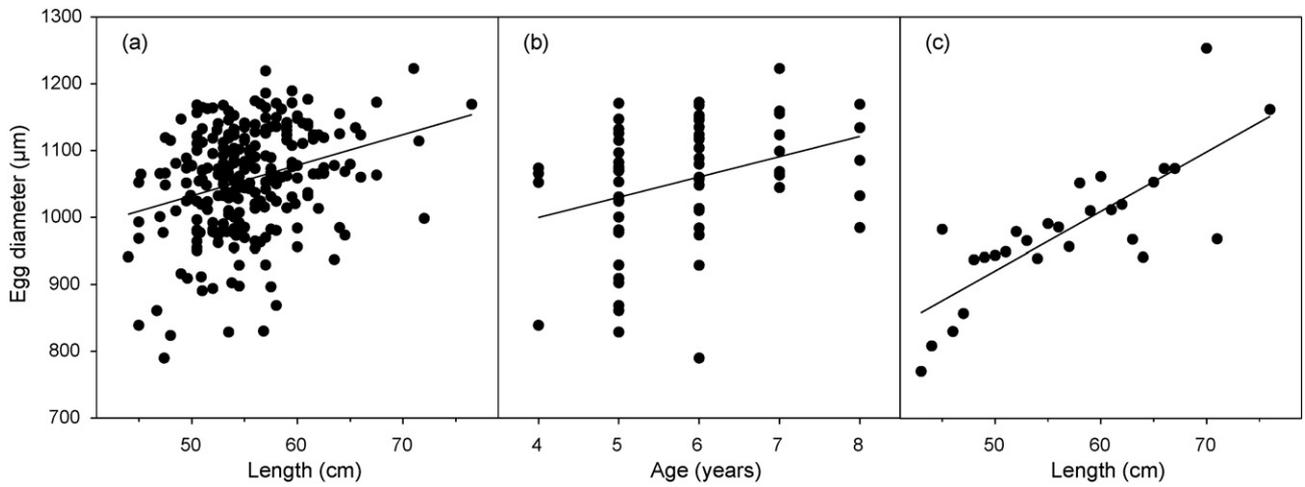


Fig. 2. Hydrated oocyte mean diameter versus (a) female length, (b) age and (c) female length class (cm) of southern stock of hake for the period 1982–2007.

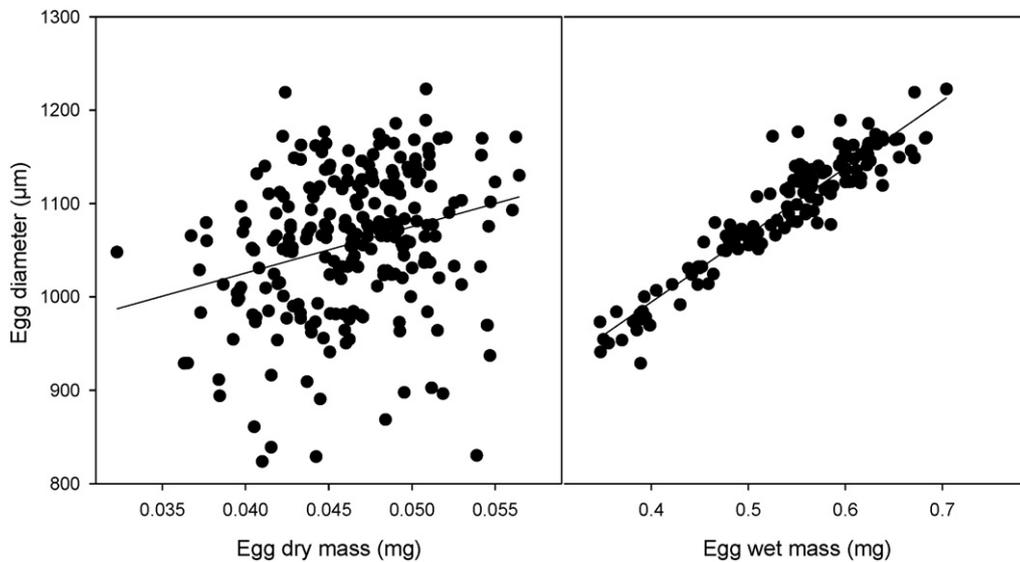


Fig. 3. Relationships between hydrated oocyte dry and wet mass and hydrated oocyte diameter of southern stock of hake.

(ANOVA,  $p > 0.1$ ). However, significant differences were observed in oocyte diameter by length and age between 2003 and 2004 (ANCOVA,  $p < 0.0001$ ). In 2003, there was no seasonal variation in the mean diameter, but in 2004 the mean diameter significantly decreased along the year (ANOVA,  $p < 0.01$ ).

### 3.2. Spawning fraction

The spawning fraction was obtained using 84 samples for 38 months of the study period of 1999–2000 and 2003–2004, although only the last period showed good temporal sample coverage (Table 1). The average monthly variation of the spawning fraction showed a main peak of spawning activity in February and a secondary peak in May–June; in October, a tendency for a third peak was also present, with the lowest spawning activity observed during the third and fourth quarters (Fig. 4). However, clear annual differences existed. Thus, in 2000 the main February peak was attenuated, with the maximum SF recorded in May; in contrast, in 2004 only one peak was detected in winter (December–April), with a larger average SF than in the rest of the years (Fig. 4).

Our results indicate that, on average at the population level, southern European hake spawned once every 4.7 days in February, once every 7.0 days in May and once every 84 days in November, the latter being when the lowest spawning activity was recorded. The spawning fraction computed by 2-cm length class showed a

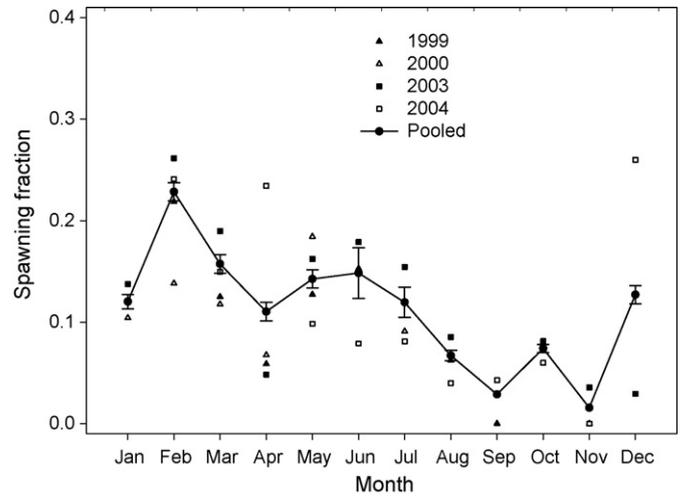


Fig. 4. Average spawning fraction of southern stock of hake by month for each year sampled and pooled for all years. Bars represent the mean variance from the annual SF.

significant increase as the female length increased for the two first quarters ( $p < 0.005$ ,  $r^2 = 0.41$  and  $0.49$  for quarters 1 and 2, respectively), but the relationship was not significant ( $p > 0.5$ ) for the third and fourth quarter (Fig. 5). The ANCOVA analysis indi-

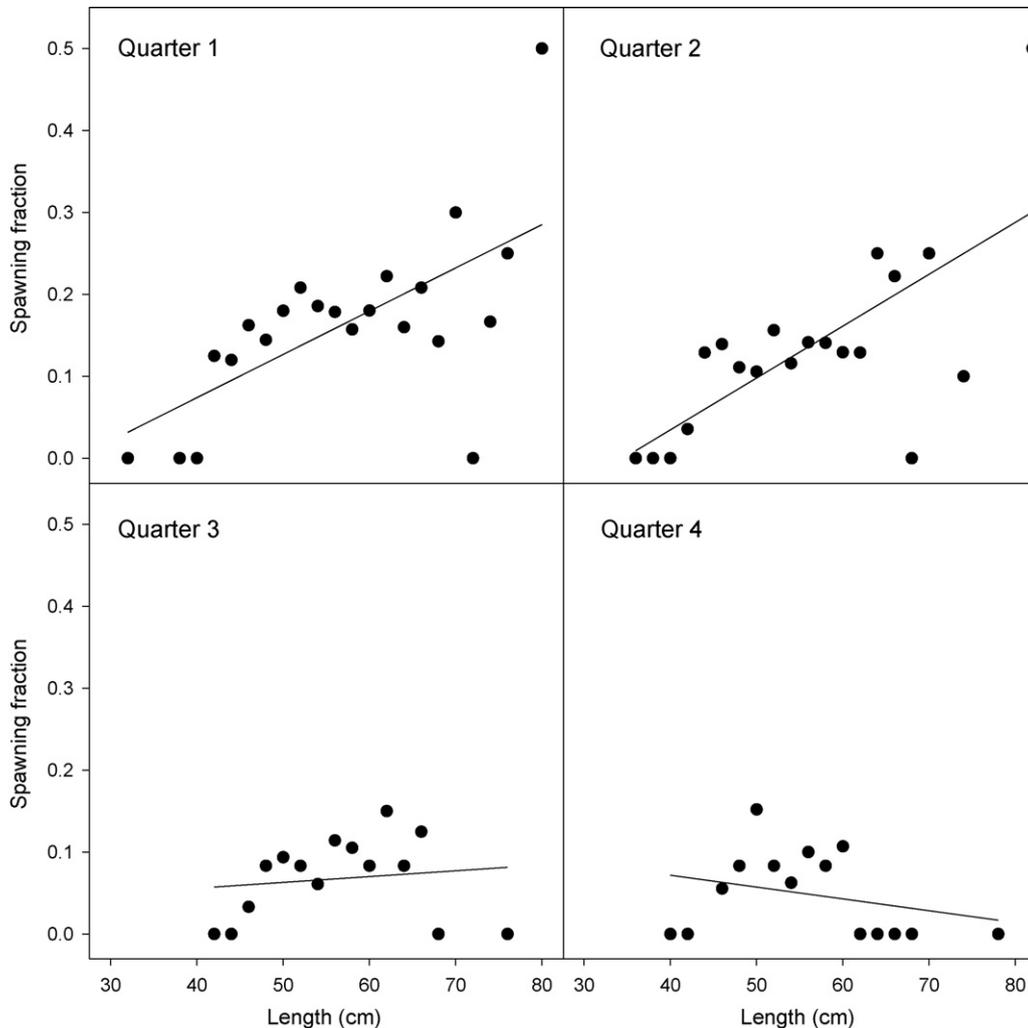


Fig. 5. Mean spawning fraction at length of southern stock of hake estimated by quarter and pooled for all years.

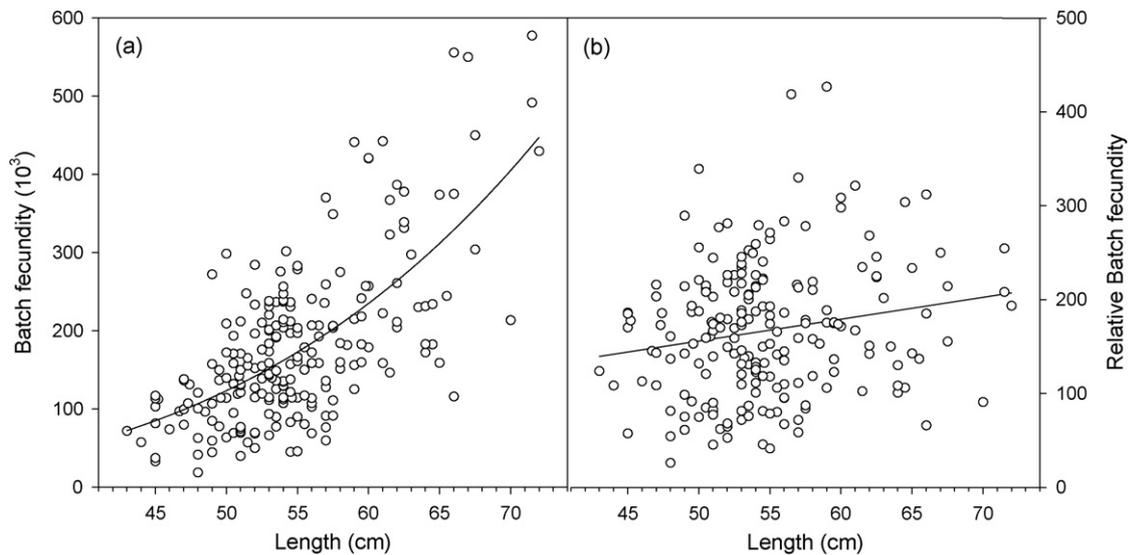


Fig. 6. Absolute (a) and relative (b) batch fecundity versus female length of southern stock of hake.

cated that the regressions of the spawning fraction versus female length from quarters 1 and 2 were not significantly different ( $p=0.060$ ).

### 3.3. Batch fecundity

A total of 214 females were sampled for batch fecundity (BF). The absolute batch fecundity ( $BF_{abs}$ ) ranged from 18,710 to 577,302 oocytes. The relationship between BF and female length was fitted to a power model (Fig. 6a) that showed a significant positive relationship ( $r^2=0.38$ ,  $p<0.001$ ). The power coefficient of the  $BF_{abs}$ -length relationship was 3.618 ( $se=0.318$ ), differing from the expected value of 3 if the fecundity follows a cubic relationship to biomass. The length-weight relationship of the sampled females was defined by  $weight\ (g)=0.026 \times length\ (cm)^{2.650}$ . The power coefficients from these two relationships were significantly different at the 0.05 level ( $2.650 \notin [2.995; 4.241]$ ). This indicates that larger females produce many more eggs that predicted by their biomass (weight).

This was also supported by the fact that the relative batch fecundity showed a significant ( $p=0.01$ ) increase as the female length increased (Fig. 6b). The relative batch fecundity ( $BF_{rel}$ ) ranged from 26 to 426 hydrated oocytes per g of gutted weight female (total length between 43 and 72 cm), with an average of 167 oocytes  $g^{-1}$ .

The analysis of covariance indicated no significant difference between the regression lines of the 4 years of batch fecundity data. However, a seasonal pattern was detected, with a significantly higher batch fecundity during the first quarter (ANCOVA,  $p<0.001$ ) (Fig. 7).

### 3.4. Temporal trend of stock demographic structure

Both SSB and FSSB showed overall decreases since the early 1980s (Fig. 8). Both were highly correlated ( $r^2=0.96$ ) but did not follow the same pattern. Before 1990, SSB was, on average, only 1.5 times larger than FSSB, i.e., mature females contributed proportionally more than males to SSB; after 1990, the contribution of mature females decreased notably, with SSB on average of 2.5 times larger and showing a peak of 3.7 in 1997.

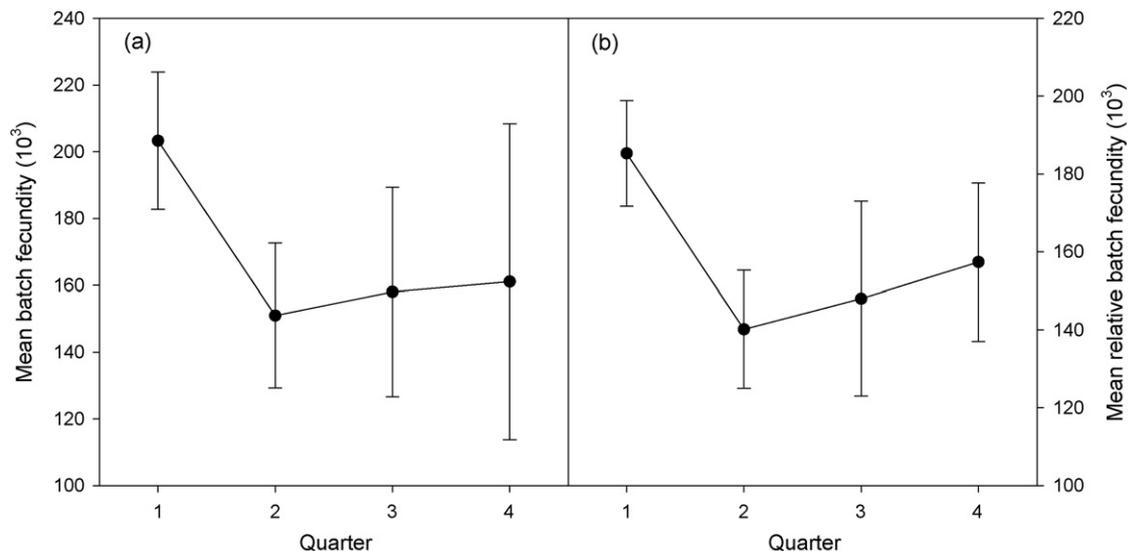
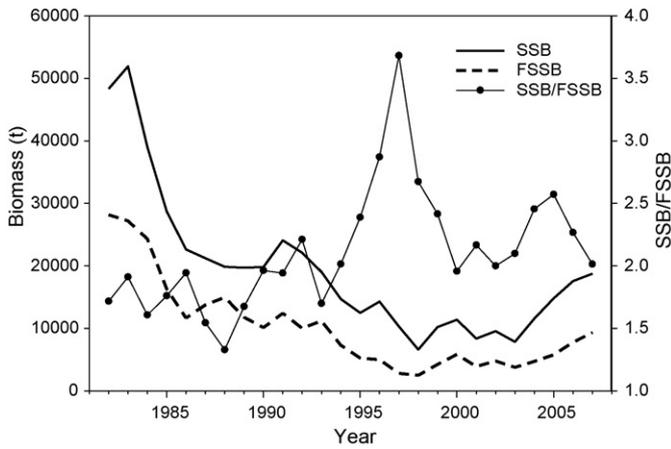


Fig. 7. Mean absolute (a) and relative (b) batch fecundity of southern stock of hake estimated by quarter. Bars indicate 95% confidence intervals.



**Fig. 8.** Temporal trends of SSB, FSSB and the ratio SSB/FSSB of the southern stock of hake for the period 1982–2007.

The age structure of the female population in numbers showed variations of the proportions of age groups through the period of 1982–2007 (Fig. 9). The proportion of mature females at ages 3 and 4 showed an increasing trend (from 0.3% in 1982 to 11.7 and 21.9%, for ages 3 and 4 respectively, in 2007), whereas the proportion of age 5 was around 5% from 1982 to 1984 and increased to an average of 23% during the period of 1985–2007. The proportions of females of age 3–5 increased significantly during the period analysed ( $p < 0.001$ , except for age 5  $p < 0.05$ ). Age 6, 7 and 8+ showed a significant ( $p < 0.001$ ) decreasing trend from 35.9, 32.2 and 26.0%,

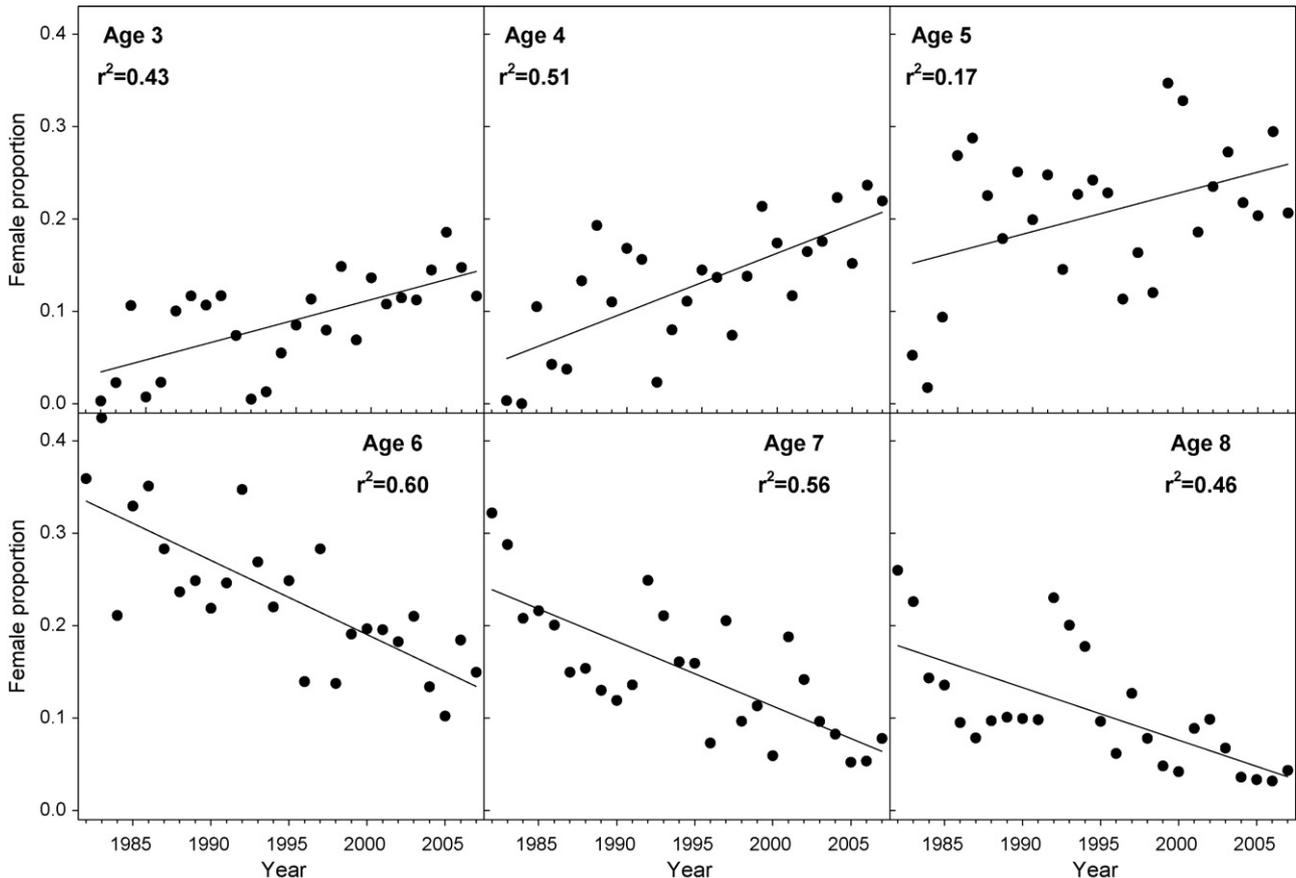
respectively, in 1982 to 14.9, 7.8 and 4.3%, respectively, in 2007 (Fig. 9).

The mean age of the females in the mature stock decreased by 2.5 years from 1982 to 2007 (range of 6.8–4.3), with a minimum value of 3.7 in 1996.

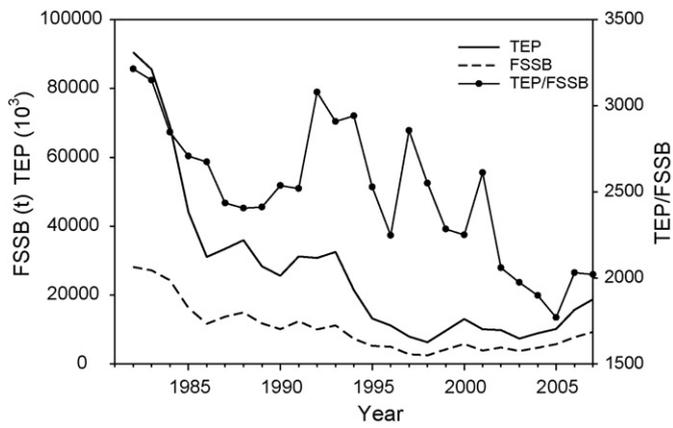
**3.5. Egg production**

The trend of the TEP was highly influenced ( $r^2 = 0.97$ ) by the mature female population size (Fig. 10). To capture the pattern of egg production independently of the stock size along the study period, the ratio of TEP/FSSB was used (Fig. 10). This ratio allowed the focus to be on the effect of the population structure on the TEP. The TEP/FSSB shows an overall and significant ( $r^2 = 0.58$ ,  $p < 0.001$ ) decreasing trend from 1982 to 2007. This ratio was significantly ( $p < 0.001$ ) and positively related with the proportion of ages 6, 7 and 8+ in the female mature stock, whereas it was significantly ( $p < 0.005$ ) and negatively related to the proportion of ages 3 and 4 in the mature stock (Fig. 11).

The TEP was relatively well related to the subsequent recruitment ( $r^2 = 0.41$ ,  $p < 0.001$ ), estimated at age 0 from VPA. However, if excluding the last 2 years (2006 and 2007) when the recruitment estimation by VPA is normally less reliable, the model yielded a better relationship ( $r^2 = 0.65$ ,  $p < 0.001$ ) (Fig. 12). The mean contribution of each age class to the TEP was investigated (Fig. 13). During the period of 1982–1998, the group of 8+ had the highest contribution to the TEP (46.7%), though the proportion in the mature stock was not the highest (26.3% for group 8+ and 29.0% for group 6) (Fig. 13), whereas during the following period of 1999–2007, age 5 had the highest contribution (24.3%) and the contribution of group



**Fig. 9.** Temporal trends of the proportion (in numbers) of each age class (from 3 to 8+) within the stock of mature females for the southern stock of hake.



**Fig. 10.** Temporal trends of FSSB, TEP and the ratio TEP/FSSB of southern stock of hake.

8+ to TEP dropped to 17.8% (Fig. 13). A shift of contribution to the TEP toward younger individuals was observed, i.e. the contribution to TEP of the group 6+ decreased significantly ( $r^2 = 0.70$ ,  $p < 0.001$ ) along the time-series (Fig. 13).

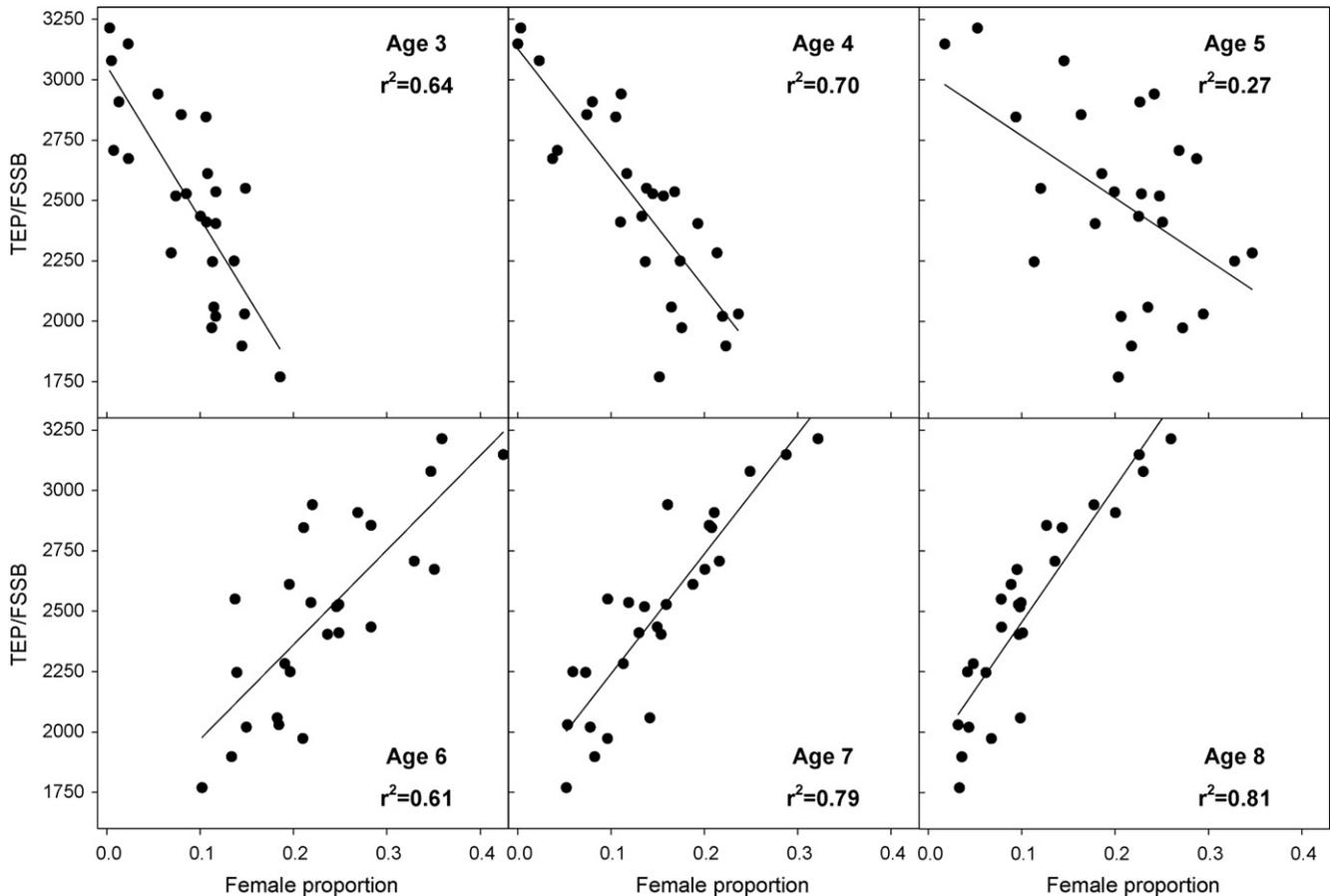
**4. Discussion**

The length range of the females sampled in 2003 and 2004 to analyse the hydrated oocytes' quality (244 individuals from 44 to 76.5 cm) covers 96% of the mature females observed during survey and laboratory random samples for the same years. Because of the unavailability of ovaries' data for all past years, we considered the

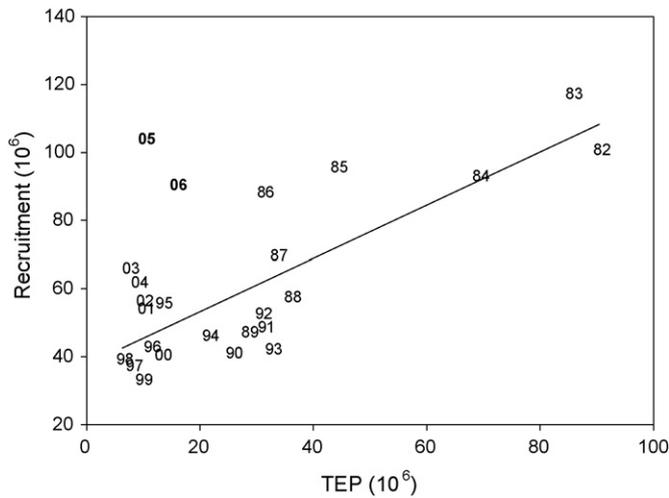
variability in egg quality to be constant and equal to the 2003–2004 average for the whole time-series period. We also acknowledge that the dry weights are only indicative of relative trends because dry weight may be reduced by up to 30% due to leaching during formaldehyde storage (Hislop and Bell, 1987).

The present study indicates that the egg quality, defined here by the hydrated oocyte dry mass and diameter, increases significantly with female size. These results are in accordance with observations made for other teleost species (Vallin and Nissling, 2000; Almatar et al., 2004).

The variability in dry mass is mainly due to the yolk reserve of the oocytes (Kjesbu and Kryvi, 1993). Studies on early life stages showed that the yolk reserves are positively correlated with the survival of the larvae during the first days after hatching and the growth rate (Saborido-Rey et al., 2003; Nissling et al., 1998). It has been found for some gadoid species that larger larvae result from heavier and bigger eggs and have more successful early life history (Trippel, 1998; Marteinsdottir and Steinarsson, 1998; Rideout et al., 2005). We found the oocyte diameter to be strongly correlated to their water content, which will determine the density of the released egg. By consequence, depending on the physical (e.g., temperature) and chemical (e.g., salinity) characteristics of the surrounding water (Sundby, 1991), the egg diameter will contribute to its buoyancy (Vallin and Nissling, 2000). Therefore, the egg diameter will determine its position in the water column, and possible advection depending on the hydrography of the spawning area which is likely to play a major role in the fertilisation success of the eggs as well as the larval survival (Nissling et al., 1994). Indeed, Włodarczyk and Horbowa (1997) found in the Baltic Sea that larger cod eggs were distributed in shallower depths, where the oxygen conditions are better, and small eggs were found in denser water,



**Fig. 11.** Relationships between the proportions of females from age 3 to 8+ within the mature female stock of the southern stock of hake and the ratio TEP/FSSB.



**Fig. 12.** Linear relationship between the TEP and the recruitment of the following year (regression line excludes years 2005 and 2006, in bold).

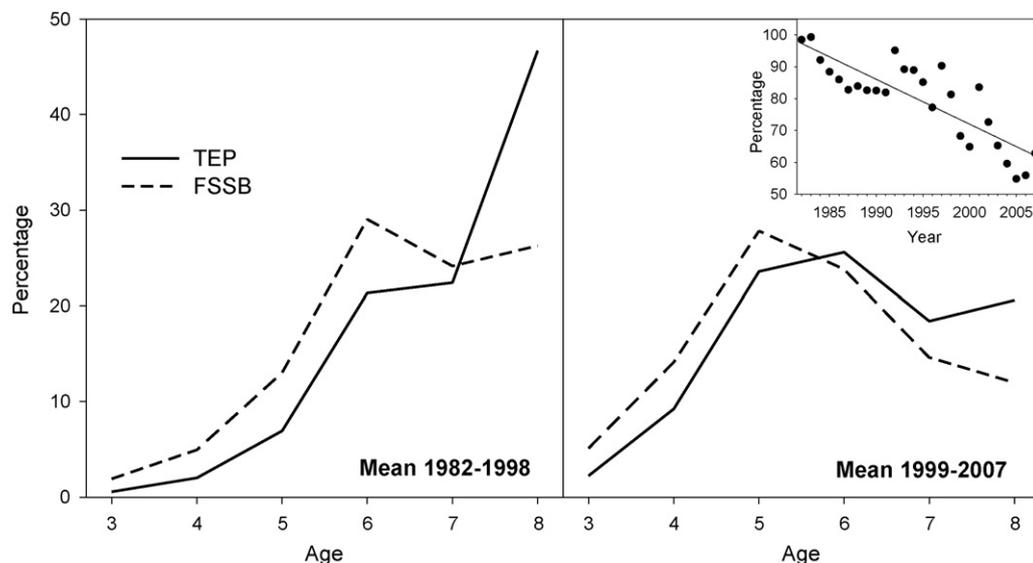
i.e., closer to the bottom. Vallin and Nissling (2000) also suggested that larvae hatching from bigger eggs in shallow depths are likely to find food more easily, as the abundance of plankton preys are more abundant on the surface.

Since one of the objectives of our study was to investigate the effect of maternal attributes on the reproductive output of European hake, we considered hydrated oocytes as the best available proxy of released eggs. However, our dataset is characterised by a non-negligible noise. Several sources of variability can be pointed out:

- (1) All of the fish used in this study were sampled from the wild, and consequently their reproductive history remains unknown. For other species such as cod, it has been demonstrated that second-time spawners produce more and better quality eggs than first-time spawners (Trippel, 1998). This information can only be obtained from individuals kept in captivity, and the data used in this study do not provide this information.

- (2) For the same sampling reason, the data do not provide information on the batch number. However, it has been observed that in gadoid species such as cod, the egg diameter and dry mass decrease as the individual spawning season progresses, i.e., as the batch number increases (Trippel, 1998; Saborido-Rey et al., 2003); nevertheless, this statement is not systematic since Włodarczyk and Horbowa (1997) observed larger eggs at the end of the spawning season. European hake is an indeterminate species, recruiting oocytes all along the spawning period (Murua and Motos, 2006; Dominguez-Petit, 2007), and the determination of batch number, or the moment within the individual spawning period, is still not achievable. This later variable, which is likely to affect egg quality and fecundity, is not taken into account in that analysis.
- (3) Though we considered both the hydrated oocyte dry mass and the diameter to be criteria related to quality, these two were weakly correlated in our study. Other authors found clear, positive correlations between released cod egg diameter and their dry mass (Ouellet et al., 2001) and larval dry mass (Trippel, 1998). However, Kjesbu and Kryvi (1993) showed in cod that yolk and water uptake also occurs during the final maturation stage, i.e., preceding ovulation. In our study, we only know that the hydrated oocytes used are in the final maturation stage (i.e., hyaline; Kjesbu et al., 1996), but we do not know how close they are to the ovulation and, consequently, how close their yolk and water are to their final contents. If, for hake as well, the uptake of yolk and water is significant during the final maturation stage, the moment at which females are sampled is likely to be partly responsible for the variability in egg quality, as well as for the weak correlation between the dry mass and diameter (or water content). Nevertheless, because samples were stored in formaldehyde, dehydration occurred at different degrees of magnitude and oocytes were rehydrated until they reached stabilisation.

Even if it is well accepted that egg quality contributes to the success of the early larval stage (Brooks et al., 1997), the primary condition for egg development is its fertilisation. This present study does not cover this area, but some authors have found that, for cod, the fertilisation and hatching rates in captivity were higher for second- than for first-time spawners, though it



**Fig. 13.** Mean contribution (in percentage) of each age group to the TEP and female SSB for the periods 1982–1998 and 1999–2007. Inset: relative contribution to TEP of females older than 5 years.

is unclear if that is due to egg quality or male performance (Trippel, 1998).

Despite these possible sources of variability that could not be avoided in our study, we found consistent trends indicating that larger females are responsible for significantly better quality eggs. Most of the studies conducted on egg quality converge toward the conclusion that heavier and larger eggs result in larger larvae that have higher survival probabilities due to intrinsic (higher development rate) and extrinsic (hatched in favourable surrounding conditions) characteristics (Marteinsdottir and Begg, 2002, and references therein). Though further investigations are required on the survival likelihood of those oocytes for the European hake specifically, we infer that larger oocytes will result in better quality larvae and may consequently contribute more to the recruitment.

Though southern European hake presents a protracted spawning season at the population level (females in spawning conditions have been found in all months of the study period), the bulk of the reproductive activity occurs during the first and second quarter, with a major peak in February and a secondary one around May. This result is in accordance with observations made for the northern stock of hake in the Bay of Biscay (Lucio et al., 2000).

Having observed quarterly variation in the batch fecundity, the pattern of the spawning fraction with length was investigated at the same temporal resolution. During the first and second quarter larger rather than small females contribute significantly more to the spawning activity. This indicates that from approximately January–June, the larger mature females have a higher probability of being in a spawning condition. This suggests that they spawn a higher number of batches and consequently have either a longer spawning season or a shorter batch interval than smaller females. In the third and fourth quarter, the opposite trend was observed but was not significant. Thus, at the third quarter, the pattern changes to a non-significant relationship between female length and spawning fraction. This relationship becomes negative during the last quarter, with a similar spawning fraction level for small individuals (50–60 cm) as for the three other quarters but a drastic decrease of spawning fraction for large individuals (above 60 cm). At the population level, the spawning season of females ranging in length from 50 to 60 cm is extensively protracted, whereas larger females seem to concentrate their spawning period during the first and second quarter of the year. The smallest individuals (<41 cm) did not apparently participate in spawning but were apparently starting to mature. Since the maturation occurs as the fish grows, it is likely that the small females will commence spawning in the following quarter, by which time they will be larger than 40 cm. One could argue that we should consider females that already have spawned at least once in their life in the regression model since virgin maturing females are equivalent to immature ones. However, since each individual will spawn for the first time at a distinct length, it is impossible to determine a knife-edge length from which the spawning fraction is actually representing the fish in the spawning stage among individuals that potentially can spawn or already have spawned. If we ignore the null spawning fractions at small lengths, we conclude that along the last quarter of the year, the spawning fraction decreases as the female length increases.

Similar conclusions on indeterminate fish species were reached by several authors, agreeing that larger females have higher spawning fraction (Claramunt et al., 2007), especially at the beginning of the reproductive season (Almatar et al., 2004). Macchi et al. (2004) found a higher spawning fraction for large females of *Merluccius hubbsi*. They also found that individual larger females have longer spawning seasons than younger ones. If larger fish of *Merluccius merluccius* start egg production earlier in the season than younger fish, further investigations would be relevant to appropriate management to determine the recruitment success with regards to the spawning date.

European hake recruit oocytes into vitellogenesis all along the spawning season (indeterminate fecundity; Dominguez-Petit, 2007; Murua et al., 2006, and as a consequence the potential fecundity cannot be a relevant proxy of the number of eggs a female releases. Instead, the number of hydrated oocytes to be spawned (batch fecundity) in combination with the spawning fraction is used as an indicator of realised fecundity. The realised fecundity is the result of the number of batches a female produces in a spawning season and the batch fecundity of each batch. The number of batches increases with female size, and batch fecundity may vary among batches for a single female. However, for the individuals used in this study, none of this information was available, and only the batch fecundity can be used to compare the reproductive achievement among individual fish. The range of relative batch fecundity found in the study mentioned above (from 26 to 426 oocytes  $g^{-1}$ , with a mean of 167 oocytes  $g^{-1}$ ) is in accordance with what was found previously (Murua et al., 1996, 165 oocytes  $g^{-1}$  on average). The batch number is known to be a parameter affecting the batch fecundity, which decreases as the batch number increases (Trippel, 1998). The unknown batch number is likely to be a source of variability in the number of oocytes released. However, both the absolute and relative batch fecundity show a similar trend, which indicates that females produce relatively more eggs per batch as they become larger. In other words, a female of 70 cm will produce on average 1.36 more eggs per unit of body weight than a 45-cm female. This is an indication that the biomass of mature females is not directly proportional to the total number of eggs produced, and this is supported by observations made for other stocks (Marshall et al., 2003).

Though SSB and FSSB follow similar overall trends, their annual patterns are different. This is mainly due to the estimation of FSSB from annual maturity ogives of female only and sex ratios. Indeed, it is important to consider the relative proportion of males and females because, at large sizes, females are significantly predominant (Lucio et al., 2000). Consequently, we believe that the use of the stock reproductive potential (SRP) indices is more relevant if they are based on the female stock only, which it is not the case in current stock assessment procedures. The age composition of the mature female stock shows important changes in the proportion of each age group along the time-series. With time, the youngest mature females tend to represent larger proportions rather than the bigger females, which are less and less represented in the spawning stock. The decrease of the mean age in the female mature population is a phenomenon observed in many exploited stocks (Ottersten et al., 2006). Our study suggests that the bigger (or older) females tend to have larger relative contributions to the TEP. A reduction in the mean age of the female reproductive stock is likely to negatively affect the offspring survival success, independently of the stock size. This phenomenon is well reflected by the decrease of the TEP per unit of FSSB. For a given level of SSB but two distinct stock age compositions, the resulting egg productions are likely to be different. This is observable by comparing the years of 1994 and 2006. In 1994, the FSSB was 7289 tonnes with a TEP of  $21.4 \times 10^5$ , whereas in 2006, though the FSSB was higher (7746 tonnes), the TEP was lower ( $15.7 \times 10^5$ ). Though the FSSB is low for these 2 years, 1994 is characterised by a relatively higher proportion of older-age fish compared to 2006, and this is responsible for the difference in TEP. Older fish, such as the ones from group 8+, have a major contribution to the TEP though their proportion in the FSSB is not the highest. Similar conclusions were reached for the Argentine hake *Merluccius hubbsi* (Macchi et al., 2004). In other words, the TEP is not directly proportional to the spawning stock biomass, and oldest individuals play a more influential role in increasing the TEP than younger ones, in terms of both the quantity and the quality of the eggs produced. However, further investigations are required to assess the fertilisation rate of the eggs, which is a crucial step

before the larval stage. Indeed, the mechanisms occurring between hake spawning events and recruitment remain poorly understood. The TEP could explain the subsequent recruitment for the period of 1982–2007 relatively well. However, the recruitment estimates in 2006 and 2007 are above the values expected by the model. This can be due to the uncertainty in estimating the last year's abundances by the VPA.

Therefore, the TEP appears to be an SRP index that is more relevant than any SSB index (female or combined sexes) because it takes into account the demographic structure of the reproductive stock as well as the contribution of each age class to the egg production. Our results support the hypothesis that larger females contribute relatively more to the SRP than smaller ones.

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