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Original Article

Fecundity regulation in horse mackerel

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Egg production methods have been used successfully in the provision of advice for fisheries management. These methods need accurate and unbiased estimates of fecundity. We explore the reproductive strategy of horse mackerel and estimation of fecundity. Fecundity and fecundity regulation in relation to condition was investigated over a number of years. Fulton's *K*, lipid content, and hepatosomatic index increased after the start of spawning, though decreased again at the end of spawning. The increase in the gonadosomatic index, fecundity, and body condition after the onset of spawning suggests that horse mackerel utilizes food resources during the spawning season and might be an income breeder. However, the decline in *K* and lipid before the spawning season suggests that the first batch of oocytes is developed on stored energy. Fecundity varied between years and within a spawning season. Over latitude, variations in fecundity were small. *K* and lipid content are not reliable indices as proxy for fecundity. Batch fecundity appears to be heterogeneous across the spawning season but homogeneous across latitude. The homogeneity of batch fecundity over latitude could indicate that the daily egg production method is an appropriate approach for estimating the abundance of a wide ranging species, as horse mackerel.

Keywords: batch fecundity, body condition, egg production method, indeterminate, lipid content, *Trachurus trachurus*.

Introduction

Egg production methods (EPMs) are an important tool to estimate fish biomass in many fish stocks (Armstrong and Witthames, 2012; Bernal *et al.*, 2012; Kraus *et al.*, 2012). Despite its large cost, EPMs provide fisheries-independent estimates of the spawning-stock biomass (SSB) of the targeted commercial species as well as information on the spawning biology and habitat of non-commercial species spawning (Fives *et al.*, 2001; Ibaibarriaga *et al.*, 2007; Valavanis, 2008).

Different EPMs have been developed to cope with the range of reproductive strategies in fish. The annual EPM (AEPM) requires reliable estimates of the total egg production over the whole spawning season and the total fecundity (the total number of vitellogenic oocytes in the ovary over the whole spawning season; e.g. Lockwood *et al.*, 1981; Armstrong *et al.*, 2001; Damme *et al.*, 2009). In capital breeders, potential fecundity (standing stock of vitellogenic oocytes before spawning) is a good estimate of total fecundity. The daily EPM (DEPM) has been used to estimate the SSB of small pelagic fish (e.g. Lasker, 1985; Macewicz and Hunter, 1993; Stratoudakis *et al.*, 2006; Ward *et al.*, 2011). The DEPM requires a reliable estimate of daily egg production and batch fecundity (the number of oocytes spawned in a single batch). Batch fecundity can be estimated in both capital and income breeders. For EPM to be used as a reliable tool in fisheries management, it is necessary to have a good understanding of the reproductive strategy of the targeted species.

Reproduction is a core process in life and it requires a high investment of energy (Rijnsdorp, 1990; Smith *et al.*, 1990). Fish need to balance their surplus energy (energy available above the maintenance level) between somatic growth and reproduction (Rijnsdorp,

1990). Income breeders use their current uptake of energy for their current reproductive investment (Stephens *et al.*, 2009). But insects and marine zooplankton have been shown to use stored energy to produce a first batch of eggs and are able to utilize the current food supplies for the production of subsequent batches to increase their reproductive output (Varpe *et al.*, 2009; Wessels *et al.*, 2010). It has been suggested that large organisms can cope with large energy storage, while small organisms cannot store and carry large energy reserves and are thus income breeders (Klaassen, 2002).

Horse mackerel, *Trachurus trachurus*, is a medium-sized pelagic fish, which matures around 20 cm at between 2 and 4 years of age (Abaunza *et al.*, 2003; ICES, 2010). In the northeastern Atlantic (Figure 1), the horse mackerel population has an 8-month long spawning season (Abaunza *et al.*, 2003; Dransfeld *et al.*, 2005), although the duration of an invidual's spawning period is unknown. Spawning in the population starts in late winter along the Portuguese coast and moves progressively northwards until it finishes in the summer west of Scotland (ICES, 2011). It is considered to be an income breeder and to have an indeterminate fecundity type (Karlou-Riga and Economidis, 1997; Gordo *et al.*, 2008; Ndjaula *et al.*, 2009). Fish adopting such a spawning strategy augment their fecundity (the standing stock of vitellogenic oocytes in the ovary) from the previtellogenic oocyte population (*de novo* vitellogenesis) during the spawning season (Greer Walker *et al.*, 1994; Murua and Saborido-Rey, 2003). In this situation, potential fecundity is an underestimate of total fecundity.

Due to uncertainties in the assessment, the management of horse mackerel is difficult (ICES, 2010). Every 3 years, an ICES coordinated survey is conducted that covers the whole horse mackerel spawning area and spawning season (Figure 1; ICES, 2011). To

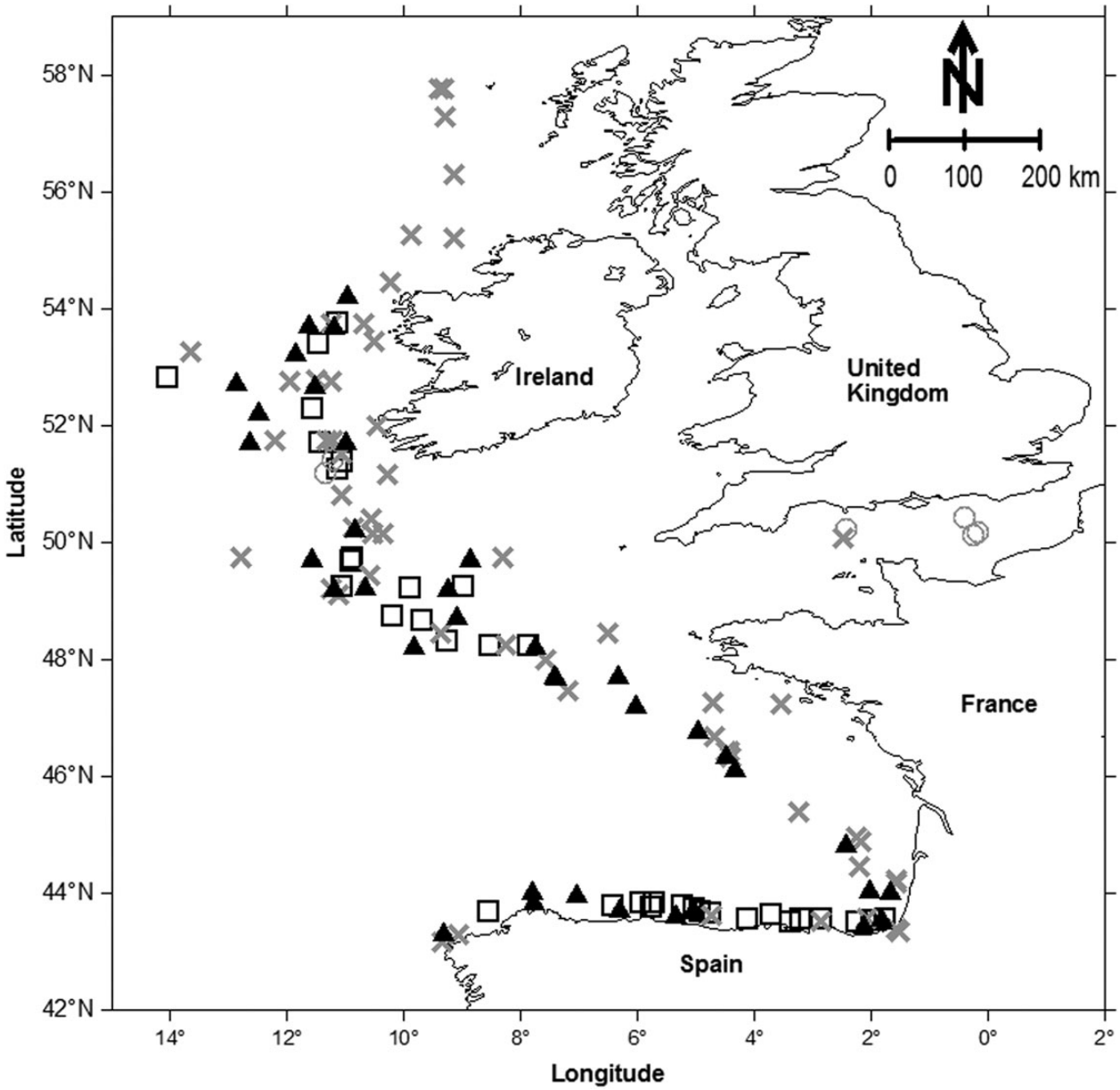

Figure 1. Sampling stations of horse mackerel in the western area in 2004 (open square), before the spawning season 2007 (open circle), 2007(multiplication symbol), and 2010 (filled triangle).

cover the entire spawning area and season, the survey is carried out by 9 or 10 European institutes and fecundity samples are analysed by five different institutes (ICES, 2011). A survey involving many participating institutes in collecting and analysing samples needs a good inter-calibration between the institutes. Before the surveys and analyses, international workshops are organized for the inter-calibration (ICES, 2006, 2009). The ICES coordinated survey is directed at an AEPM for mackerel *Scomber scombrus* (Lockwood *et al.*, 1981; ICES, 2011). Since the potential fecundity of the indeterminate-spawning horse mackerel is an underestimate of the total fecundity, the AEPM cannot be used to reliably assess horse mackerel SSB. The triennial ICES survey does not target the sampling of spawning adult fish, thus the traditional DEPM assessment, which estimates batch fecundity and spawning females from hyaline oocytes and post-ovulatory follicles (POF's) cannot be carried out (Lasker, 1985).

The current stock assessment method uses only the total annual egg production as a relative and imprecise index of SSB, because of the absence of reliable fecundity estimates (ICES, 2010). This approach implies that fecundity is constant over time. This assumption of unvarying fecundity is likely to be incorrect, especially for income breeding species such as horse mackerel (ICES, 2002). So to improve the information used to provide fisheries advice, greater understanding of the reproductive strategy and the variability in fecundity through space and time, at various resolutions, is required.

Body condition and surplus energy content are important factors that regulate fecundity and it is likely that jointly they determine the total annual fecundity of a female (Rijnsdorp, 1990; Damme *et al.*, 2009a, b). It has been suggested that these factors may be used as an index for interannual variation in horse mackerel fecundity (ICES, 2003). The relationship between the proxies and fecundity needs to be strong, well described, and based on a time-series before the use of the proxies will improve the management advice (De Oliveira *et al.*, 2006).

Body condition of horse mackerel in the Bay of Biscay varies over time but does not appear to change during the spawning season (Lucio and Martin, 1989). This contrasts with other pelagic fish (e.g. herring *Clupea harengus* and *Sardinella*, ter Hofstede *et al.*, 2007; Davidson and Marshall, 2010). This is possibly due to the replacement of fat by water, whereby the total weight of the fish will not change. The total amount of lipid content might, therefore, give a better indication of fecundity. It has been shown in horse mackerel in captivity that lipid content increases with oocyte development from the cortical alveoli to the migratory nucleus stage (Ndjaula *et al.*, 2009).

This study investigates inter-calibration of fecundity estimation among the different institutes. It then considers the oocyte and fecundity development, in relation to several metrics associated with body condition, before and during the spawning season in wild northeastern Atlantic horse mackerel. Variation in fecundity and body condition in space and time over the spawning area is studied. Finally, observations on total body mass, lipid content, and feeding intensity were assessed in relation to the spawning season to assess the value of these factors as indices of total individual egg production.

Methods

Sample collection

During the 2004, 2007, and 2010, international triennial mackerel and horse mackerel egg survey samples were collected from freshly caught horse mackerel in pelagic trawls (Damme *et al.*, 2005; ICES, 2007, 2009). For each female horse mackerel sampled, total fish length (L_T, 0.1 cm), total body weight (W_T, 1 g), gutted weight (W_G, 1 g), liver weight (W_L, 0.1 g), and ovary weight (W_O, 0.1 g) were measured. Ovaries were extracted and samples of 26 μg were taken with a solid displacement pipette (Drummond Scientific Company Wiretrol II) inserted into the ovary, through a cut in the tunica wall (Hunter *et al.*, 1989; Damme *et al.*, 2005; Witthames *et al.*, 2009). Duplicate pipette samples were taken from each fish and preserved separately in 2 ml of 3.6% buffered formaldehyde. Stomachs were removed from the fish and the stomach fullness was estimated. Fullness was divided into four categories: empty (i), partially full (ii), full (iii), and stuffed (iv). After this procedure, the whole fish, including intestines and the remains of the ovaries, were frozen separately for lipid content analysis in the laboratory.

From October 2006 until February 2007, horse mackerel females were collected from pelagic freezer trawlers. All fish were frozen; hence, no fecundity samples could be collected but body condition measurements were carried out.

Body condition measurements

Body condition was calculated as Fulton's condition factor K (Heincke, 1908):

$$K = \frac{W_T}{L_T^3} \quad (1)$$

where W_T is the total weight and L_T the total length. The lipid content analysis was carried out during the 2004 and 2007 surveys. In the laboratory, the whole fish (the carcass including head, skin, bones, intestines, and remains of the ovaries) was shredded. The remains were homogenized and duplicate samples were analysed for lipid and water content according to Bligh and Dyer (1959) or its adaptation by Smedes (1999). The gonadosomatic index (GSI, %) was calculated as:

$$\mathrm{GSI} = 100 \times W_O \times (W_T - W_O)^{-1} \quad (2)$$

The hepatosomatic index (HSI, %) was calculated as:

$$\mathrm{HSI} = 100 \times W_L \times W_T^{-1} \quad (3)$$

Fecundity analysis

The duplicate pipette samples were randomly distributed and analysed by different institutes participating in the ICES triennial survey, AZTI (Spain), IEO (Spain), IMARES (the Netherlands), IMR (Norway), and MI (Ireland). One of the duplicate samples was sent to one institute and the second was send to another institute, e.g. sample 1A was analysed by institute 1, 1B was analysed by institute 2, 2A was analyses by institute 3, 2B was analysed by institute 4, etc. In addition, some images of oocytes and samples were sent around for direct comparison of oocyte diameter measurements and fecundity estimates.

Fecundity is determined by counting the number of vitellogenic oocytes in the ovary sample. Distinguishing between previtellogenic and vitellogenic oocytes is possible by measuring the oocyte diameter. For this study, the oocyte diameter threshold for vitellogenic oocytes was set at 185 μm (Witthames and Greenwood, 2002;

ICES, 2005; Ndjaula *et al.*, 2009). In the pipette samples, all vitellogenic oocytes were measured and counted in whole mount analysis using ImageJ (Rasband, 1997–2008). The larger share of the oocytes was detected by automatic particle analysis, whereas the remaining oocytes were counted manually. Oocytes in early vitellogenesis are transparent and could be difficult to analyse by automatic particle analysis. To enhance the contrast, oocytes in the 2004 survey samples were therefore coloured using PAS staining (Periodic acid followed by Schiff's reagent) or toluidine blue. The PAS staining is more intense when the oocytes are more advanced in maturation, whereas in toluidine blue, all oocyte stages are coloured equally. The oocyte counts and measurements of the stained oocytes were compared with the results of unstained oocytes. The results were comparable and it was decided not to stain the 2007 and 2010 oocyte samples (ICES, 2006).

Data from the whole mount analysis were used to estimate leading cohort (LC), defined as the diameter of the 10% biggest oocytes and fecundity (the total number of vitellogenic oocytes) was calculated using the formula:

$$F = \frac{N}{s} \times W_O \tag{4}$$

where F is the fecundity, N the total number of vitellogenic oocytes in the pipette subsample, and s the subsample weight (26 μg).

Relative fecundity F_R was calculated as:

$$F_R = \frac{F}{W_T - W_O} \tag{5}$$

Only samples which showed no sign of spawning were used for the analysis. Macroscopically, it can be difficult to assign the maturation stage. The whole mount pipette samples were checked under the microscope for spawning markers, hyaline oocytes, or POF's. Whenever spawning markers were encountered, the samples were rejected for fecundity estimation.

Usually, batch fecundity is estimated from counts of hyaline oocytes. However, in our material very few samples contained hyaline oocytes, and batch fecundity was estimated from the number of oocytes with diameters >450 μm, which were clearly separated from the main vitellogenic oocyte stock (Figure 2). Batch fecundity F_B was calculated as:

$$F_B = \frac{N_B}{s} \times W_O \tag{6}$$

where N_B is the number of vitellogenic oocytes >450 μm in a batch.

Statistical analysis

The comparison of body measurements between years and months as well as the comparison of oocyte measurements and fecundity estimations between the institutes was done by a multiple factor ANOVA. Adjusted p-values were estimated with the Tukey *post hoc* test (Tukey HSD in R) and shown in the results section.

Effects of biotic and abiotic parameters on fecundity were tested with GLM. Since lipid content was only measured in 2004 and 2007, models with and without lipid content were tested. Length was collinear with weight and fecundity showed collinearity with the GSI, both weight and the GSI were not included in the GLM analyses. The same starting models were used for fecundity and batch

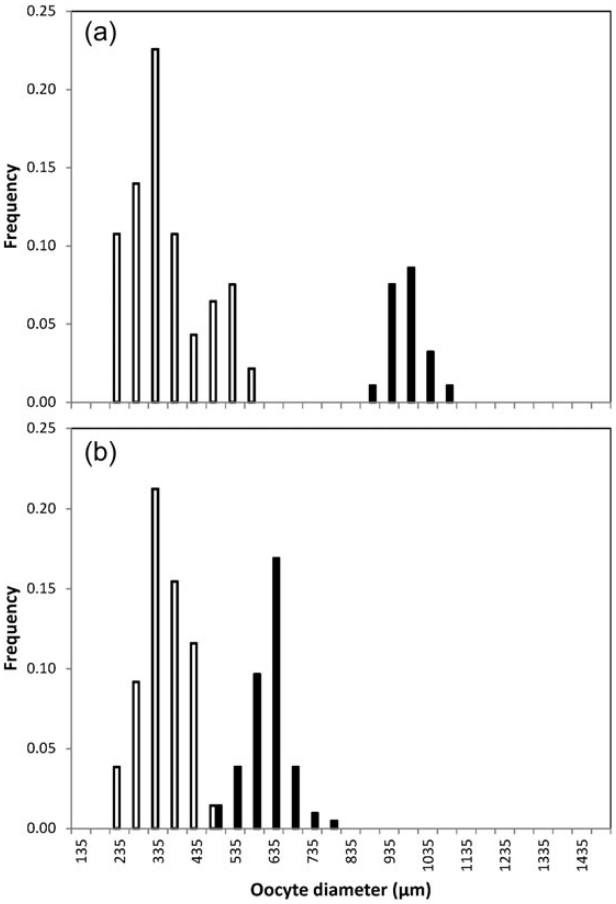

Figure 2. Examples of batch fecundity estimation based on vitellogenic oocytes in horse mackerel in (a) a female with a clearly separated batch and (b) a female where the batch is visible but not clearly separated from the newly developed vitellogenic oocytes. White bars are the stock of newly developed vitellogenic oocytes, and black bars are the maturing vitellogenic oocytes which will be spawned in one batch.

fecundity. The model without lipid content was

$$\begin{aligned}\ln F_R = {} & \beta_0 + \beta_1 \ln L_T + Y + M + \beta_2 K + \beta_3 K \times Y \\ & + \beta_4 K \times Y \times M + \beta_5 K \times M + \beta_6 \mathrm{HSI} + \beta_7 \mathrm{HSI} \times Y \\ & + \beta_8 \mathrm{HSI} \times Y \times M + \beta_9 \mathrm{HSI} \times M + \varepsilon,\end{aligned} \tag{7}$$

where Y is the year and M the month. The model with lipid was

$$\begin{aligned}\ln F_R = {} & \beta_0 + \beta_1 \ln L_T + Y + M + \beta_2 K + \beta_3 K \times Y + \beta_4 K \times Y \times M \\ & + \beta_5 K \times M + \beta_6 \mathrm{HSI} + \beta_7 \mathrm{HSI} \times Y + \beta_8 \mathrm{HSI} \times Y \times M \\ & + \beta_9 \mathrm{HSI} \times M + \beta_{10} L + \beta_{11} L \times Y + \beta_{12} L \times Y \times M \\ & + \beta_{13} L \times M + \varepsilon,\end{aligned} \tag{8}$$

where L is the lipid content. LC was added in Equation (7) to test the effect of this on fecundity, and lastly, latitude was added to both Equations (7) and (8) including the interactions with K, GSI, and lipid content. Model selection was based on the AIC information

criterion using the stepwise backward selection approach. Statistical analyses were performed in *R* (Team, 2011).

Results

Body condition

In total, 1252 females were sampled before (250 females) and during (1002 females) the spawning season in the different survey years (Table 1) covering the whole spawning area (Figure 1). The mean total fish length differed between the years ($p < 0.001$), fish caught in 2010 were larger compared with 2004 and 2007. *K* differed significantly between the years (*K*: all years $p < 0.002$), *K* differed significantly in almost all months ($p < 0.001$) except January and May–June, March and April–July, April and July, May and June, and June and July (Figure 3a). *K* varied between 0.65 and 1.12 and was high from October till January but was lowest in February just before the spawning season (Figure 3a). During the spawning season, *K* increased till June but dropped again at the end on the spawning season in July (Figure 3a). Lipid content was significantly lower in 2004 ($p < 0.001$), and in the months February–June, lipid content was significantly lower compared with the other months ($p < 0.05$). Lipid content was high from October till December but decreased in January and February with the lowest lipid content just before the spawning season (Figure 3b). At the end of the spawning season, in June and July, lipid content increased again (Figure 3b). It was not possible to collect the livers from the frozen females that were collected outside the spawning season. The HSI was significantly higher in 2010 compared with 2004 and 2007 ($p < 0.001$). During the spawning season, the HSI increased (Figure 3c) and the HSI was significantly lower in March and April compared with the other months ($p < 0.008$). The GSI was significantly different between the years ($p < 0.001$). The GSI was low and showed only a slight increase before the start of the spawning season from October till February and the difference is not significant (Figure 3d). During the spawning season, the GSI showed a big increase but dropped again at the end of the spawning season (Figure 3d); the GSI was the same in March, April, and July, but in May and June, GSI was significantly higher compared with the other months ($p < 0.001$).

Body condition was also variable over the latitudinal range of the spawning area (Figure 4). *K* over latitude and year showed that 2004 was significantly different from 2007 and 2010 ($p < 0.03$). *K* was significantly different over the latitudinal range especially the low latitudes compared with the middle latitudes (43–44°N compared with 48–53°N, $p < 0.05$), and the middle latitudes were different from the high latitudes (50–51°N compared with 57°N, $p < 0.05$). *K* decreased from south to north, though the most northern transect has the highest but also the most variable *K* values (Figure 4a). Lipid content increased from south to north (Figure 4b) and was significantly different over the lower transects (43–49°N) compared with the higher latitudes (50–57°N; $p < 0.04$). The HSI was highly variable and seemed to be highest in the middle of the

Table 1. Numbers of horse mackerel females and average total fish lengths (s.d. in parenthesis) sampled during the surveys.

	Numbers			Fish length (cm)		
Month	**2004**	**2007**	**2010**	**2004**	**2007**	**2010**
October	–	50	–	–	26.9 (2.4)	–
November	–	50	–	–	25.4 (2.2)	–
December	–	50	–	–	27.8 (3.1)	–
January	–	50	–	–	27.1 (1.4)	–
February	–	50	–	–	29.1 (1.3)	–
March	122	45	5	28.5 (3.1)	27.2 (3.3)	30.9 (1.8)
April	163	182	55	28.0 (2.6)	28.3 (4.2)	30.8 (3.7)
May	30	139	80	28.3 (2.0)	28.9 (3.6)	31.4 (2.8)
June	60	60	8	30.5 (3.1)	27.5 (2.9)	28.6 (2.3)
July	–	39	16	–	27.7 (1.8)	33.5 (2.1)

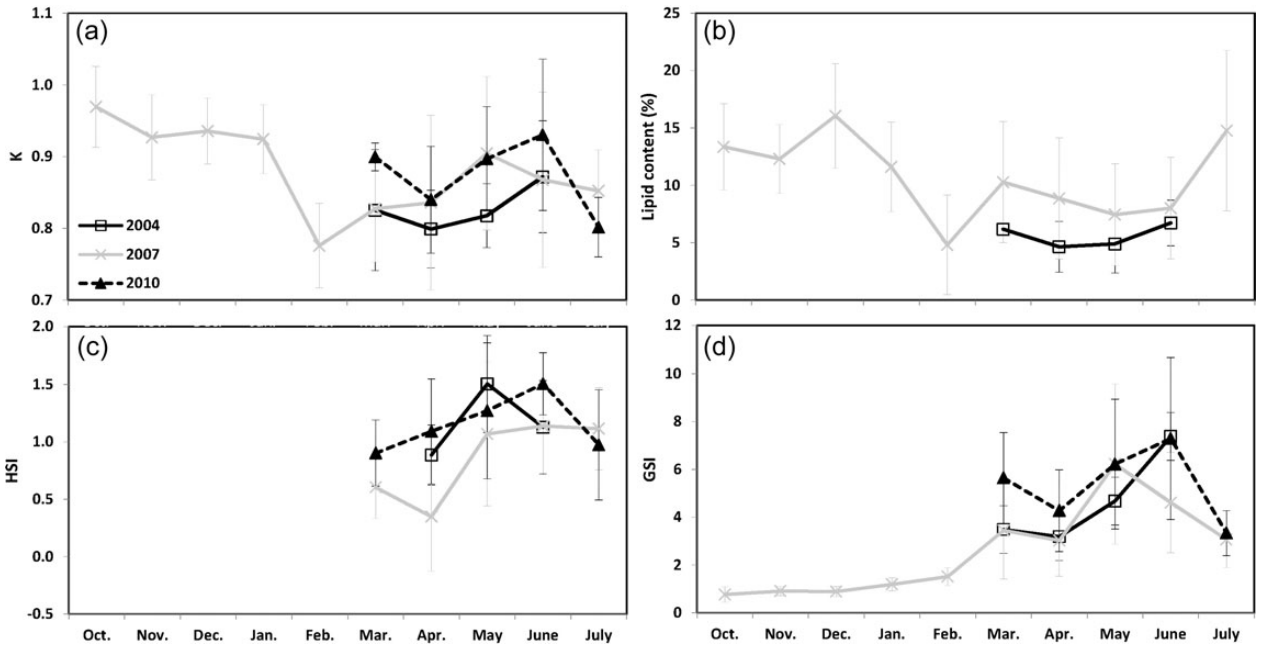

Figure 3. Body condition of horse mackerel; (a) Fulton's condition factor *K*, (b) lipid content, (c) HSI, and (d) GSI in 2004 (open square), 2007 (multiplication symbol), and 2010 (filled triangle).

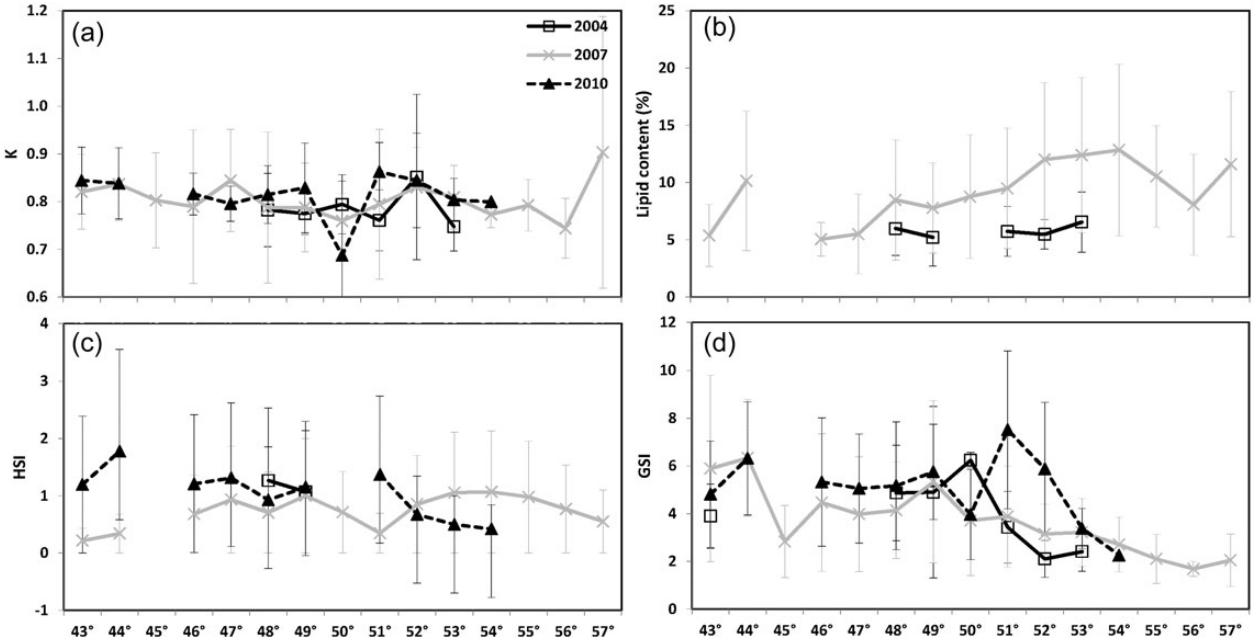

Figure 4. Body condition of horse mackerel over the latitudinal range; (a) Fulton's condition factor *K*, (b) lipid content, (c) HSI, and (d) GSI in 2004 (open square), 2007 (multiplication symbol), and 2010 (filled triangle).

Table 2. Stomach fullness of spawning horse mackerel.

Year	Month	Empty	Partially full	Full	Stuffed
2004	March	0.95	0.05	–	–
	April	0.97	–	0.03	–
	May	0.48	0.33	0.15	0.04
	June	0.82	0.15	0.03	–
2007	October	0.22	0.68	0.10	–
	November	–	0.38	0.62	–
	December	–	0.14	0.86	–
	January	–	0.06	0.94	–
	February	–	0.26	0.74	–
	March	0.81	0.19	–	–
	April	0.28	0.67	0.04	–
	May	0.20	0.47	0.29	0.04
	June	0.51	0.44	0.05	–
	July	0.92	0.08	–	–
2010	May	0.43	0.36	0.14	0.07
	June	0.25	0.25	0.50	–

spawning latitudinal range (Figure 4c) but only in 2010 was the HSI significantly higher in March and April compared with the other months ($p < 0.001$). The GSI over the latitudinal range was significantly different in 2010 from the other years ($p < 0.001$), and the GSI at 43°N was significantly different from the northern latitudes, >50°N ($p < 0.05$), and GSI at 46–49°N was significantly higher compared with the northern latitudes, >50°N ($p < 0.05$; Figure 4d).

Before the spawning season, most stomachs were partially full or full, while empty stomachs were only found in October (Table 2). During the spawning season, stomachs were either empty or partially full (Table 2). Empty stomachs were found in all months but highest numbers were found at the beginning (March) and the end (July) of the spawning season (Table 2). Full stomachs were rare during the spawning season. Fully stuffed stomachs were rare both before and during the spawning season (Table 2). During sampling, no signs of regurgitation were found. 75% of females with spawning markers, POF's or hyaline oocytes, had an empty, while 22% had a partially full stomach.

Fecundity

In 2004, images of ovary samples of 8 females and ovary subsamples of 29 females were sent round the analysing institutes for comparison of the oocyte image analysis method (Table 3). Based on the images, no difference was found in oocyte counts between the institutes (ANOVA, $p = 0.780$). No significant difference was found in the estimation of fecundity of the 29 females between the institutes ($p = 0.051$), but there was, however, a significant difference in the oocyte diameter measurements ($p = 0.001$). All other duplicate ovary subsamples were analysed randomly by the different institutes. Hence, every institute analysed samples from the whole spawning season and spawning area; thus, a comparison of the means of oocyte diameter, LC, and fecundity was expected to show similar results between the analysing institutes. However, oocyte diameter measurements and fecundity estimates differed between the analysing institutes (Table 4). The mean oocyte diameter, LC, and F_R differed significantly between institute 2 and 5 and the other institutes (mean diameter: $p < 0.004$; LC: $p < 0.004$; F_R: $p < 0.001$). In 2007, the fecundity estimates of institute 2 were much higher compared with the other institutes, whereas in 2010, the fecundity estimates of institute 5 were much lower compared with the other institutes (Table 4). Since these differed considerably, it was decided not to use these estimates in the fecundity, oocyte diameter, and LC analysis.

Of the 1002 females sampled during the spawning season, 33 contained spawning markers and were not used for the fecundity analysis. All females which had spawning markers were caught during the night.

F and F_R were significantly higher in 2010 ($p < 0.001$; Figure 5 and Table 5). F and F_R increased significantly from the start (March and April) of the spawning season, but decreased again

towards the end of spawning, and in May and June, F and F_R were significantly higher compared with March, April, and July ($p < 0.001$). F_R was significantly different at transects 44 and 53°N compared with the other transects ($p < 0.04$; Figure 6). F_B was not significantly different between the years (Figure 5 and Table 5). However, relative F_B was significantly higher in 2007 ($p < 0.001$; Figure 5d). Similarly, like F, F_B increased significantly from the start of the spawning season (March and April) and decreased again to the end of the spawning, and in March, April, and July, F_B was significantly lower compared with May and June ($p < 0.001$; Figure 5c and d). On transects 43–44°N and 51–54–55°N, F_B differed significantly from each other ($p < 0.05$).

The backward selection of Equation (7) for F_R and relative F_B showed that all parameters and interactions were significant and the resulting model with the lowest AIC was Equation (7) (Table 6). However, when the model including lipid content [Equation (8)] was tested for both F_R and relative F_B lipid content and the interactions were dropped from the model and the resulting model with the lowest AIC was again Equation (7). Testing Equation (7) with LC showed that LC did not have a significant effect on fecundity. Latitude has a significant effect on F_R, but when the model with latitude was tested for relative F_B latitude was dropped from the resulting model. The resulting model with the lowest AIC for F_R was

$$\ln F_R = \beta_0 + \beta_1 \ln L_T + Y + M + \mathrm{Lat} + \beta_2 K + \beta_3 K \times Y + \beta_4 K \times \mathrm{Lat} + \beta_5 \mathrm{HSI} + \beta_6 \mathrm{HSI} \times M, \quad (9)$$

where Lat is the latitude (Table 7).

Table 3. Comparison between institutes of horse mackerel oocyte diameter measurements and fecundity estimates (s.d. in parenthesis) based on ovary subsamples of 29 females.

Institute	Mean oocyte diameter (μm)	F_R (oocytes per g)
3	364.7 (53.1)	648.0 (226.3)
4	439.4 (62.9)	779.8 (399.7)
5	393.4 (55.9)	568.6 (225.0)

Both F and F_B increased with the size of the females as was shown by the positive effect of T_L in the models (Figure 7). Relative F and F_B seem to decrease with increasing oocyte diameter size (Figure 8), though there is no significant effect of LC found on fecundity. Fulton's K has a significant positive effect on fecundity (Tables 6 and 7 and Figure 9). Lipid content has no significant effect on fecundity (Figure 9).

Discussion

Our study has shown that horse mackerel exhibits variable reproductive characteristics and that body condition changes over space and time. Body condition and lipid content decreased just before the spawning season but both increase again after the start of spawning, though body condition decreases again at the end of spawning. The HSI increased during spawning but dropped at the end of the spawning season. The GSI was very low before the onset of spawning but increased during the spawning season and decreased at the end of spawning. F and F_B increased after the onset of spawning and decreased at the end of the spawning season. Relative F and F_B seem to decrease with increasing oocyte diameter size in 2007 suggesting down-regulation (a reduction in the numbers of vitellogenic oocytes; Óskarsson *et al.*, 2002; Kurita *et al.*, 2003) of fecundity. F and F_B increased with in increasing K, but no relationship was found with lipid content. Bigger females had a more vitellogenic oocytes and produced bigger oocyte batches.

Although the trials with exchanged images and subsamples showed no significant difference in oocyte counting between the analysing institutes, significant differences in oocytes diameter measurement and fecundity estimates were found between the analysing institutes when comparing the final analysis. Part of this can be explained by methodological approaches. The final fecundity analysis was carried out on different subsamples of the fish. Pipette sampling is carried out on board the survey vessels in varying weather conditions and by different people. Pipette sampling needs to be done carefully with no fluid or air trapped in the pipette. Small differences in the subsamples taken from one fish can lead to big differences when the subsample estimate is raised to the total ovary size to estimate the total number of vitellogenic oocytes. It is also important that the analyses of fecundity samples are correctly inter-calibrated between the analysing institutes.

Table 4. Comparison between institutes of horse mackerel oocyte diameter measurements and fecundity estimates based on all analysed ovary subsample analyses.

Year	Institute	Number of subsamples analysed	Mean oocyte diameter (μm)	LC (μm)	F_R (oocytes per g)
2004	1	–	–	–	–
	2	83	307.0 (45.4)	464.7 (125.6)	607.2 (305.0)
	3	158	361.5 (68.2)	564.8 (94.9)	685.3 (358.2)
	4	48	430.8 (61.7)	—	666.1 (420.3)
	5	191	391.4 (71.1)	594.9 (98.5)	648.5 (376.0)
2007	1	–	–	–	–
	2	121	331.1 (69.2)	525.4 (137.3)	1 131.0 (402.7)
	3	236	387.5 (75.1)	387.5 (75.1)	676.6 (354.5)
	4	137	370.3 (50.9)	573.1 (72.6)	635.0 (290.9)
	5	223	449.1 (90.3)	650.5 (149.1)	537.6 (273.5)
2010	1	32	335.1 (48.4)	599.1 (36.6)	1 036.6 (462.3)
	2	39	270.0 (47.5)	561.1 (35.2)	1 080.0 (467.2)
	3	68	316.4 (41.6)	592.3 (31.9)	1 112.1 (505.2)
	4	55	341.1 (44.5)	619.8 (33.5)	1 106.4 (450.0)
	5	105	373.9 (45.0)	599.6 (30.4)	743.2 (375.6)

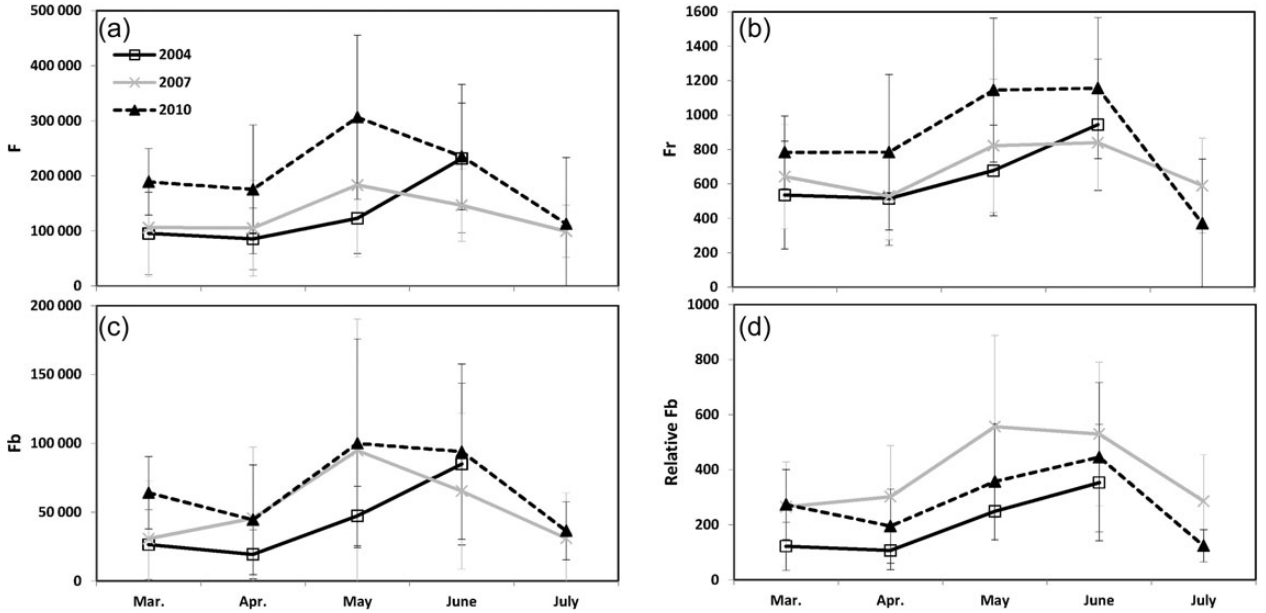

Figure 5. Fecundity development over the spawning season (a) total number of vitellogenic oocytes, (b) relative fecundity, (c) batch fecundity, and (d) relative batch fecundity in 2004 (open square), 2007 (multiplication symbol), and 2010 (filled triangle).

Table 5. Horse mackerel fecundity and oocyte diameters in the different years (s.d. in parenthesis).

Year	Mean oocyte diameter (μm)	LC	Total fecundity (number of oocytes)	Relative fecundity (number of oocytes per g)	Relative batch fecundity (number of oocytes per g)
2004	352.1 (69.4)	543.3 (115.3)	11.5×10^4 (9.6×10^4)	603.5 (342.1)	193.4 (172.7)
2007	385.9 (90.6)	509.7 (159.0)	13.3×10^4 (10.4×10^4)	671.1 (337.3)	406.2 (275.7)
2010	316.9 (54.2)	590.2 (37.4)	23.8×10^4 (14.4×10^4)	956.8 (457.4)	284.6 (199.3)

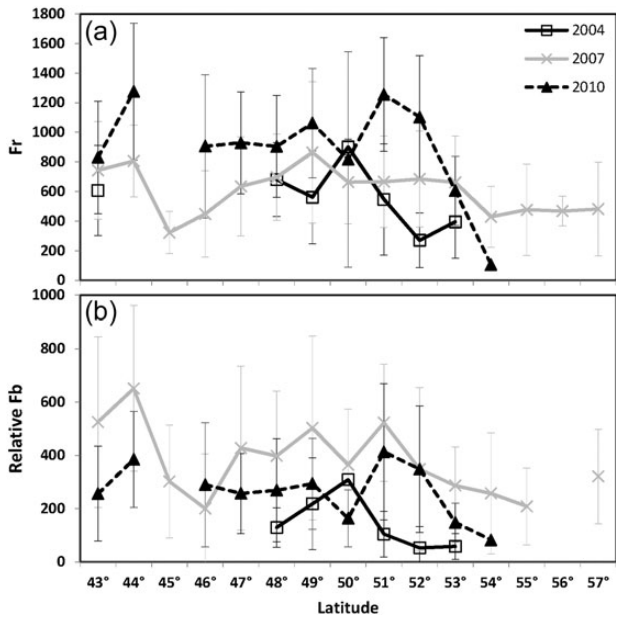

Figure 6. Fecundity development over the latitudinal range (a) relative fecundity and (b) relative batch fecundity in 2004 (open square), 2007 (multiplication symbol), and 2010 (filled triangle).

Although inter-calibration workshops were held before each survey apparently two institutes found different results for fecundity compared with the other institutes. For such a survey covering a large area and period and carried out by many institutes, inter-calibration should be carried out and the scientist that carry out the sampling and analysis should participate themselves in the calibration workshops.

All females with spawning markers were caught during the night, suggesting that horse mackerel spawn at night-time. Other studies of horse mackerel do not report spawning time of horse mackerel, but jack mackerel, *Trachurus symmetricus*, are also night-time spawners (Macewicz and Hunter, 1993).

Before the spawning season, most females showed signs of feeding; only few empty stomachs were found. At the start and the end of the spawning season, large numbers of empty stomachs were found. Oocyte final maturation, spawning, and mating require high energy and oxygen demands restricting other activities (Rijnsdorp and Ibelings, 1989; Kjesbu *et al.*, 1998). Hence, fish cease feeding during actual spawning. The stomachs containing food items during the spawning season suggest that in between the spawning of oocyte batches horse mackerel does feed. Earlier studies on horse mackerel diet in the Atlantic, North Sea, and Adriatic show that many stomachs are empty, especially during the spawning season (Sahrhage, 1970; Dahl and Kirkegaard, 1986, 1987; Cabral and Murta, 2002; Jardas *et al.*, 2004). These studies also show a clear diurnal feeding pattern. No such pattern was found in this study, probably as most sampling of females occurred during the daytime.

Condition factor *K* and lipid content dropped significantly just before the onset of the spawning season. During the spawning season *K*, lipid content and the HSI increased. This is in contrast to Lucio and Martin (1989), who did not find a change in body

Table 6. Parameter estimates and the *p*-value of the selected models for F_R and relative F_B [Equation (7)].

	Estimate	SE	Significance[a]
F_R			
Intercept	2.181	1.604	.
$\ln L_T$	0.418	0.228	**
Y (2007)	1.898	0.670	*
Y (2010)	1.781	0.877	
M (April)	−2.102	1.293	
M (May)	−0.111	1.225	
M (June)	1.245	1.242	
M (July)	−0.867	1.558	
K	4.861	1.802	**
HIS	−1.141	0.801	
K × *Y* (2007)	−4.665	1.327	***
K × *Y* (2010)	−2.668	1.047	*
K × *M* (April)	0.776	1.860	
K × *M* (May)	−1.161	1.912	
K × *M* (June)	−2.463	1.686	
K × *M* (July)	−3.111	2.239	
HSI × *Y* (2007)	2.163	0.884	*
HSI × *Y* (2010)	0.450	0.457	
HSI × *M* (April)	1.365	0.853	
HSI × *M* (May)	1.194	0.858	
HSI × *M* (June)	1.104	0.788	
HSI × *M* (July)	2.719	1.046	**
K × *Y* (2007) × *M* (April)	2.414	1.234	.
K × *Y* (2007) × *M* (May)	1.321	1.358	
K × *Y* (2007) × *M* (June)	1.737	1.138	
K × *Y* (2007) × *M* (July)	5.101	1.436	***
K × *Y* (2010) × *M* (April)	0.574	0.942	
K × *Y* (2010) × *M* (May)	0.597	1.050	
HSI × *Y* (2007) × *M* (April)	−2.394	0.955	*
HSI × *Y* (2007) × *M* (May)	−1.475	0.946	
HSI × *Y* (2007) × *M* (June)	−1.782	0.878	*
HSI × *Y* (2007) × *M* (July)	−3.809	1.131	***
HSI × *Y* (2010) × *M* (April)	−0.379	0.585	
HSI × *Y* (2010) × *M* (May)	−0.271	0.555	
Relative F_B			
Intercept	4.246	3.734	
$\ln L_T$	0.688	0.477	
Y (2007)	1.506	1.608	
Y (2010)	0.473	1.869	
M (April)	−6.173	3.216	.
M (May)	−4.757	3.010	
M (June)	−2.034	3.040	
M (July)	−4.352	4.003	
K	1.899	4.061	
HSI	−2.675	1.374	
K × *Y* (2007)	−5.380	2.676	*
K × *Y* (2010)	−2.500	2.152	
K × *M* (April)	1.239	4.204	
K × *M* (May)	3.924	3.582	
K × *M* (June)	−0.322	3.727	
K × *M* (July)	6.457	5.175	
HSI × *Y* (2007)	2.482	1.769	
HSI × *Y* (2010)	1.465	0.831	.
HSI × *M* (April)	4.443	1.556	**
HSI × *M* (May)	1.616	1.115	
HSI × *M* (June)	2.374	1.318	.
HSI × *M* (July)	−0.389	1.263	
K × *Y* (2007) × *M* (April)	6.637	2.384	**
K × *Y* (2007) × *M* (May)	0.872	1.656	
K × *Y* (2007) × *M* (June)	4.060	2.056	*
K × *Y* (2007) × *M* (July)	−0.256	1.832	
K × *Y* (2010) × *M* (April)	3.951	1.785	*
HSI × *Y* (2007) × *M* (April)	−4.871	1.975	*
HSI × *Y* (2007) × *M* (May)	−0.386	1.594	
HSI × *Y* (2007) × *M* (June)	−2.355	1.737	
HSI × *Y* (2010) × *M* (April)	−2.877	1.166	*

[a]Significance levels: ., $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

condition during the spawning season of horse mackerel in the Bay of Biscay. Horse mackerel in captivity did show an increase in *K*, lipid content, and HSI during oocyte development (Ndjaula *et al.*, 2009). The GSI only showed a slight increase before the start of the spawning season and showed a big increase at the start of spawning. The study of captive horse mackerel also showed an increase in the GSI with growth of the oocytes (Ndjaula *et al.*, 2009). This seems to suggest that oocyte development and growth before the spawning season is minimal and major oocyte development occurs during the spawning season. Horse mackerel are also able to increase their body condition during the spawning season, suggesting that they utilize the food resources during the spawning season directly for oocyte development. Major oocyte growth and utilization of food during the spawning season is not clear evidence but does support the idea of horse mackerel having an income breeding strategy and indeterminate fecundity. Other field studies in the Bay of Biscay (Gordo *et al.*, 2008) and the Mediterranean (KarlouRiga and Economidis, 1996) and a study of captive western horse mackerel (Ndjaula *et al.*, 2009) indicated an indeterminate fecundity. The drop in *K* and lipid just before the start of spawning could indicate that the first batch of oocytes is developed from stored energy. This has not been shown before in fish, although it has been observed in insects and marine zooplankton (Varpe *et al.*, 2009; Wessels *et al.*, 2010).

Relative F_B varied from 193 oocytes per g female in 2004 to 406 oocytes per g female in 2007. Batch fecundity found in the southern horse mackerel off Portugal was on average 200 oocytes per g female (Abaunza *et al.*, 2008; Goncalves *et al.*, 2009) and 205 oocytes per g female in the Mediterranean (Karlou-Riga and Economidis, 1997). These studies used the histological method to identify hydrated oocytes and POF's for estimating batch fecundity. The average batch fecundity in 2004 was comparable with the batch fecundities found in the Portuguese and Mediterranean studies, but in 2007 and 2010, the mean batch fecundity was much higher. F_B of the 33 females that contained POF's or hydrated oocytes did not differ from the F_B of the females without spawning markers. However, POF's and hyaline oocytes are difficult to identify using the image analysis method and can only be reliably identified using histology (P. Goncalves, pers. comm.). Although batch fecundity estimates in this study are higher, the (in this study not significant) decrease in batch fecundity with increasing latitude is also found by Abaunza *et al.* (2008).

Bigger females have a higher batch fecundity. This increase with increasing size has been shown in other studies (Karlou-Riga and Economidis, 1997; Abaunza *et al.*, 2008).

Lipid content peaked in December before spawning. Lucio and Martin (1989) showed a sharp increase in lipid content from October to November, with the peak occurring in November, for horse mackerel in the Bay of Biscay. Lipid content decreased before the onset of spawning and increased during the spawning

Table 7. Parameter estimates and the *p*-value of the selected models for F_R [Equation (9)].

F_R	Estimate	SE	Significance[a]
Intercept	−1.934	1.641	
$\ln L_T$	0.774	0.226	***
Y (2007)	4.450	0.924	***
Y (2010)	4.725	1.100	***
M (April)	−0.020	0.283	
M (May)	0.537	0.266	*
M (June)	0.952	0.300	**
M (July)	0.814	0.358	*
Lat (44°)	0.105	1.325	
Lat (46°)	0.952	1.783	
Lat (47°)	0.740	1.476	
Lat (48°)	1.942	1.267	
Lat (49°)	0.061	1.240	
Lat (50°)	−0.370	1.638	
Lat (51°)	−1.849	1.461	
Lat (52°)	−2.889	1.299	*
Lat (53°)	−4.137	1.524	**
Lat (54°)	2.113	3.980	
Lat (55°)	−2.950	1.770	.
Lat (56°)	−1.956	5.222	
Lat (57°)	−0.569	1.312	
K	6.091	1.775	***
HSI	0.633	0.320	*
K × *Y* (2007)	−5.217	1.160	***
K × *Y* (2010)	−5.464	1.362	***
K × Lat (44°)	0.096	1.582	
K × Lat (46°)	−0.907	2.185	
K × Lat (47°)	−1.151	1.746	
K × Lat (48°)	−2.336	1.521	
K × Lat (49°)	0.134	1.474	
K × Lat (50°)	0.743	2.046	
K × Lat (51°)	2.374	1.720	
K × Lat (52°)	3.524	1.540	*
K × Lat (53°)	4.827	1.840	**
K × Lat (54°)	−3.548	5.091	
K × Lat (55°)	3.228	2.174	
K × Lat (56°)	1.925	6.956	
K × Lat (57°)	−0.557	1.548	
HSI × *M* (April)	−0.234	0.345	
HSI × *M* (May)	−0.380	0.324	
HSI × *M* (June)	−0.628	0.345	
HSI × *M* (July)	−0.731	0.382	

[a]Significance levels: ., $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

period. No significant relation is found between lipid content and fecundity. Lipid content will be difficult to use and should not be considered as a reliable proxy for fecundity in a stock assessment. Fecundity seems to increase with increasing *K* but *K* is not highly significant in the fecundity models. *K* is probably not a reliable proxy to use for fecundity either. Thus, it is unlikely that either lipid content or Fulton's *K* can act as a proxy for fecundity, especially when considering the assertion of De Oliveira *et al.* (2006) that the correlations must be strong. Recent studies have shown that Fulton's *K* is not a good proxy for muscle fat (Davidson and Marshall, 2010) or mesenteric fat content (McPherson *et al.*, 2011). There is also no standard unit for Fulton's *K* and thus as a metric is it not transferable between fish (C. Minto, GMIT, Ireland, pers. Com.). Body condition should be validated against a direct biochemical measurement before they can be used as a proxy (Davidson and Marshall, 2010; McPherson *et al.*, 2011).

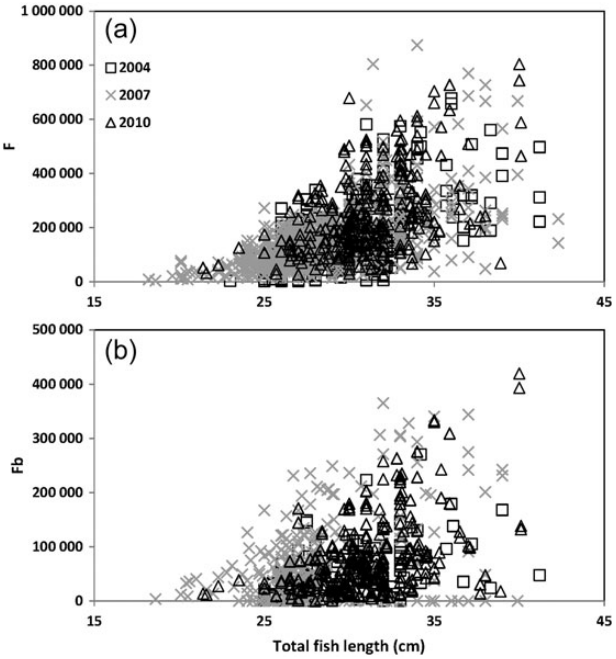

Figure 7. Horse mackerel total number of vitellogenic oocytes and batch fecundity in different length classes during the spawning season in 2004 (open square), 2007 (multiplication symbol), and 2010 (filled triangle).

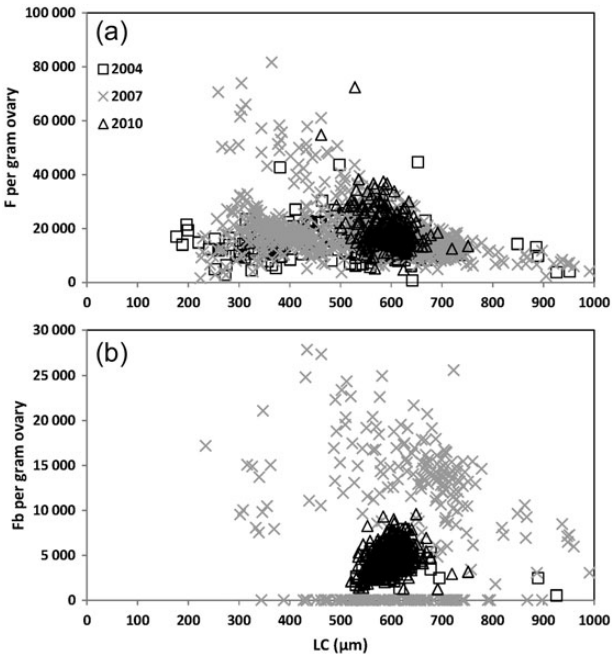

Figure 8. Fecundity down-regulation in horse mackerel in (a) relative fecundity and (b) relative batch fecundity in 2004 (open square), 2007 (multiplication symbol), and 2010 (filled triangle).

The DEPM has been used to estimate the SSB of small pelagic income breeders (Lasker, 1985; Somarakis *et al.*, 2004; Ward *et al.*, 2009). However, it has been shown that the DEPM is vulnerable to changes in batch fecundity (Somarakis *et al.*, 2004; Stratoudakis

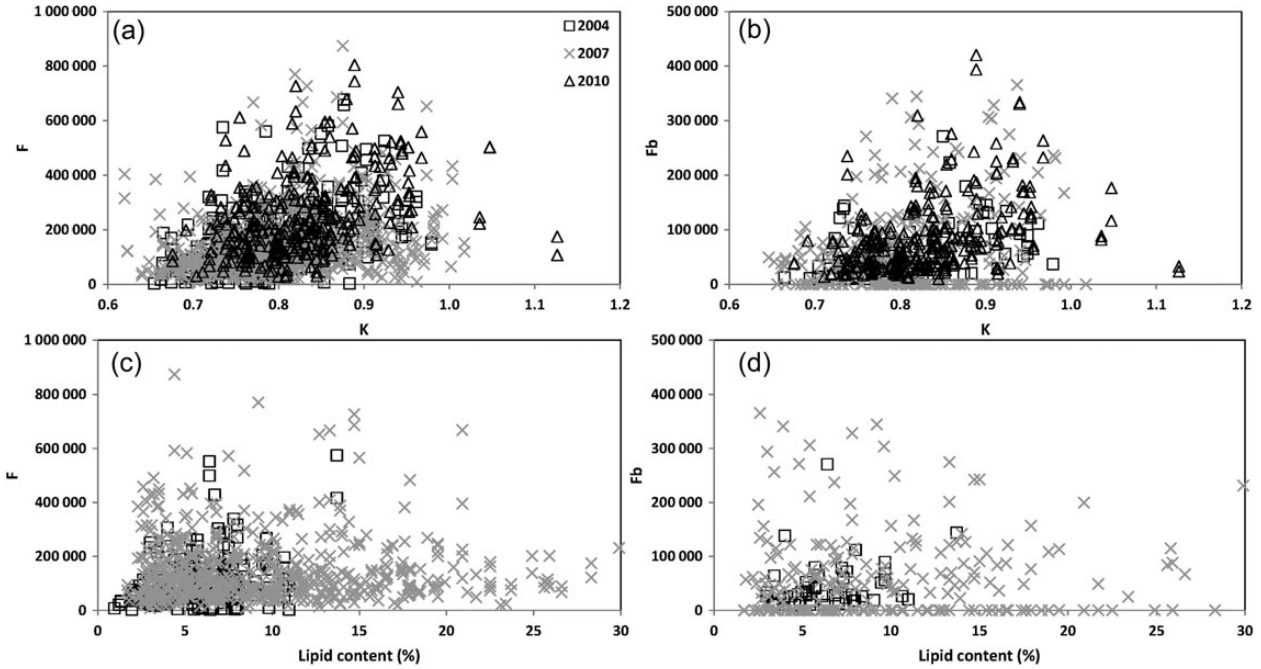

Figure 9. Body condition, (a and b) *K* and (c and d) lipid content, regulating the total number of vitellogenic oocytes and batch fecundity during the spawning season in 2004 (open square), 2007 (multiplication symbol), and 2010 (filled triangle).

et al., 2006). Peak spawning in horse mackerel occurred in June in all years (ICES, 2012). F_B of horse mackerel varies within a spawning season and on the southern and northern transects was F_B significantly different. Relative F_B was significantly different in 2007 compared with the other years. The pelagic species for which the DEPM has been used to estimate SSB all have a considerable smaller spawning area, especially when compared with western horse mackerel. But batch fecundity has been found to also vary over time and between stocks (Somarakis *et al.*, 2002; Ganias *et al.*, 2004; Stratoudakis *et al.*, 2006). This suggests that an *ad hoc* approach to sampling spawning adults is not appropriate. Under the current sampling regime, the DEPM could be a reliable method for estimating SSB of stocks, such as horse mackerel. However, because of the variation in time and space, we advise that before using this method in management strategies, an extensive analysis of an SSB time-series should be carried out to assess the reliability of the DEPM for Atlantic horse mackerel.

Horse mackerel spawning occurs at night. The first batch of oocytes probably develops using stored energy as suggested by the sharp decline in *K* and lipid content before the spawning season. During the spawning season, GSI, *K*, and lipid content increase and drop again at the end of spawning. *K* and lipid content are not reliable indices to use as a proxy for fecundity in assessments. The homogeneous distribution of batch fecundity over space and time suggests that the DEPM is probably an appropriate approach for estimating the abundance of a wide ranging species, such as horse mackerel. However, to apply the DEPM, much greater and more carefully targeted sampling of spawning adults would be required as well as an extensive analysis of SSB estimations based on the available survey data.

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