The use of Hepatic and Somatic Indices and Histological Information to Characterize the Reproductive Dynamics of Atlantic Sardine Sardina pilchardus from the Portuguese Coast

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The use of hepatic and somatic indices and histological information to characterize the reproductive dynamics of Atlantic sardine *Sardina pilchardus* from the Portuguese coast

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**Abstract**

Samples of Atlantic sardine *Sardina pilchardus* (also known as European pilchard) were collected bimonthly from 2004 to 2008 off the central west coast of Portugal to describe the reproductive activity of this indeterminate batch-spawning species; compare the seasonality of somatic growth, condition, and feeding; and evaluate differences between sexes. Monthly assessments of individual biological information for both males and females were complemented by histological analysis of ovaries during 1 year and liver tissues (both sexes) at different times of the reproductive cycle. The temporal patterns of the gonadosomatic index and various histological indices (most advanced oocyte stage, atresia incidence and prevalence, and spawning activity) indicated that Atlantic sardine were reproductively active mainly from October to March and that residual activity occurred in the remaining months. For both sexes, condition indices (hepatosomatic index, relative weight, and amount of fat stored) increased mainly during spring, reached a maximum at the end of summer just before the subsequent spawning season began, and then decreased during autumn and winter, declining to minimum levels at the beginning of spring coincident with a significant reduction in reproductive activity. Somatic growth took place mainly during spring and early summer for both sexes. The observed seasonal patterns in these biological properties suggest a seasonal transition from a period in which energy resources are allocated to reproduction (autumn and winter) to a period in which resources are allocated to growth and fat deposition (spring and summer). The only exception was the distinct hepatosomatic index pattern and the histological differences in hepatocytes between males and females during the spawning season, which may be related to the dual function of the liver in females (lipid metabolism and yolk precursor synthesis).

Fish reproductive investment is the result of essential life history trade-offs in resource allocation (Stearns 1992). Energy that is surplus to the essential standard metabolic requirements (i.e., maintenance, locomotion, predation avoidance, and feeding activity) is allocated to somatic growth, energy storage, or reproduction after the fish reaches sexual maturation. The priority with which this surplus energy is allocated to each of the above biological functions differs among fish species (Calow 1985). In the case of North Sea plaice *Pleuronectes platessa*, Rijnsdorp (1990) proposed a hypothetical mechanism, postulating that energy in excess is first prioritized into the building of reserves, then into reproduction, and finally into somatic growth if surplus exceeds a certain threshold.
The interaction between reproduction and growth is one of the most important trade-offs in fish (Stearns 1992) because most reproductive traits (especially female fecundity) and growth are a function of body size (Wootten 1998). Moreover, animals that have indeterminate growth (e.g., fish) must consider the survival costs and the available energy for reproduction and must make an allocation decision between current and future reproduction as an adaptation to the fluctuating environmental conditions. For instance, an increase in growth during the current spawning period would be associated with a decrease in offspring production during that period, but the resulting larger size of the fish could confer a benefit of increased fecundity in the subsequent reproductive season (Heino and Kaitala 1999; Tsikiras et al. 2007).

The trade-off pattern between growth and reproduction might differ depending on the reproductive strategy of a fish species. For instance, most clupeiform fishes have indeterminate fecundity (the Atlantic herring *Clupea harengus* is an exception) and are characterized by a small size, high growth rates, a relatively short life span, and late maturity occurring at a large size relative to adult size such that energy is first allocated to growth and then to reproduction. On the contrary, in many gadiform fishes, fecundity is determinate (the European hake *Merluccius merluccius* is an exception) and these fish are characterized by a large size, low growth rates, a relatively long life span, and early maturation at a small size; thus, reproduction starts well before the growth rate declines (Rochet 2000; Murua and Saborido-Rey 2003).

The Atlantic sardine *Sardina pilchardus* (also known as the European pilchard) is a small, pelagic clupeid that is distributed in the northeast Atlantic from the North Sea to Senegal and along the Azores archipelago of Portugal (Parrish et al. 1989). Similar to most other clupeid fishes, it is a fast-growing species with a relatively short life span (Sinovic 1986; Alemany and Álvarez 1993; Voulgaridou and Stergiou 2003; Silva et al. 2008) and early maturation (up to the second year of life; Silva et al. 2006). These characteristics, in addition to important interannual fluctuations in population biomass and recruitment, make Atlantic sardine stocks especially difficult to manage (Cole and McGlade 1998; Schwartzlose et al. 1999; Borges et al. 2003; Carrera and Porteiro 2003; Silva et al. 2009). The Atlantic sardine is a batch spawner with indeterminate fecundity, releasing consecutive batches of pelagic eggs during a protracted spawning season (Muzzinic 1954; Abad and Giráldez 1993; Amenzouzi et al. 2006; Garias et al. 2007b; Stratoudakis et al. 2007). Atlantic sardine are known to feed year-round (intensity peaks in winter–spring; Garrido et al. 2008a) but grow and accumulate fat mainly in spring and summer (Bandarra et al. 1997; Silva et al. 2008).

The Atlantic sardine has historically been a very important commercial fish resource in Iberian waters and is targeted mainly by the purse-seine fishery (Mendes and Borges 2006). This species currently represents around 40% of the fish landed along the Portuguese coast (DGPA 2008). Since the early 20th century, the Atlantic sardine has been the subject of many studies focusing on its general biology (Ramalho 1927; Pinto and Barraca 1958), reproduction (Pinto and Andreu 1957; Dias et al. 1973; Pérez and Figueiredo 1992; Zwolinski et al. 2001; Garias et al. 2007a), condition and fat content (Figueiredo and Santos 1988; Bandarra et al. 1997; Garrido et al. 2008b), feeding behavior (Garrido et al. 2007, 2008a), and growth (Silva et al. 2008). However, there has not yet been a comprehensive study to assess the interactions between reproductive activity and somatic growth, changes in condition, and feeding during an annual cycle for Atlantic sardine off the Portuguese coast.

Because the Atlantic sardine is an indeterminate spawner, the number of eggs produced annually is not fixed at the beginning of the spawning season and instead is the result of continuous recruitment of new batches of unyolked oocytes in the ovary; these oocytes accumulate yolk, mature, and are spawned repeatedly during the reproductively active period (Murua and Saborido-Rey 2003). Production of this quantity of eggs during such an extended period requires a considerable amount of energy resources that can be obtained (1) from energy reserves accumulated prior to spawning, (2) directly from food input during the spawning season, or (3) from both sources (Hunter and Leong 1981). Establishing the functional relationships governing the seasonal allocation of resources between somatic and gonadal growth in Atlantic sardine could help to increase our understanding of and our ability to predict how population reproductive productivity responds to changes in environmental conditions. In particular, the role of the liver in terms of energy storage has seldom been investigated in a high-fat fish species with indeterminate fecundity, and the dynamics of the liver during an annual cycle have not previously been examined for Atlantic sardine in this geographical area.

The objective of the present work was to use several biological indices and histological information from market samples collected in the purse-seine fishery to (1) describe the reproductive activity of Atlantic sardine off the central portion of Portugal’s western coast; (2) compare the seasonality of the reproductive activity in relation to the temporal dynamics of somatic growth, changes in condition, and feeding activity; and (3) assess differences between males and females in the development of the gonads and other organs. The results are then discussed in terms of the possible functional relationships between these different organs in relation to the physiological transfer of energy resources and their allocation to reproduction, growth, and condition.

**METHODS**

**Fish sampling and laboratory protocols.**—Atlantic sardine samples were collected twice per month from purse-seine landings off the central west coast of Portugal (Peniche Harbor) from January 2004 to December 2008 (Table 1). In total, 116 samples of 37–167 individuals were obtained, following a length-stratified design with 10 fish per 0.5-cm total length class. All
Table 1. Summary of the number of purse-seine fishery samples collected and the total number of Atlantic sardine sampled per year along the west coast of Portugal during 2004–2008. For each sex (F = females; M = males), the number of fish sampled per year and the annual mean values estimated for all macroscopic biological indices (with minimum and maximum values in parentheses are presented) GSI = gonadosomatic index; HSI = hepatosomatic index; Wr = relative weight; FI = fat index; StF = stomach fullness index; StC = stomach color index). See methods for definition of indices.

| Year | Samples | Fish n | Sex | Fish n per sex |
|------|---------|--------|-----|----------------|
| 2004 | 26      | 1,946  | F   | 1,034          |
|      |         |        | M   | 912            |
| 2005 | 24      | 2,117  | F   | 1,183          |
|      |         |        | M   | 934            |
| 2006 | 21      | 1,635  | F   | 865            |
|      |         |        | M   | 770            |
| 2007 | 23      | 1,529  | F   | 822            |
|      |         |        | M   | 707            |
| 2008 | 23      | 1,578  | F   | 848            |
|      |         |        | M   | 730            |

 Samples were used to obtain macroscopic indices of reproductive activity, condition, and growth (described below); in addition, ovaries from a subset of samples collected during an annual cycle (from July 2005 to July 2006) were analyzed histologically to complement the description of female spawning dynamics. A portion of liver tissue of mature and immature individuals was also stored for histological analyses (described later) to obtain a broad description of liver cytological changes in relation to reproductive activity. Apart from the description of growth, only mature individuals were used in the analyses; fish were classified as mature if they were at least 16 cm total length (the average length at 75% maturity reported for Atlantic sardine off the Portuguese west coast in the last two decades was around 15 cm; Silva et al. 2006). Total length thus ranged from 16.0 to 24.0 cm (mode = 19.5–20.0 cm) for the 8,805 individuals (4,752 females and 4,053 males) investigated.

Total length (nearest 0.1 cm), total and gutted weights (nearest 0.1 g), sex, macroscopic maturity phase, gonad and liver weights (nearest 0.01 g), visceral fat stage, stomach fullness and color, and age were recorded for all fish. Macroscopic maturity phase was determined using the five-phase macroscopic key of Afonso-Dias et al. (2007; where 1 = immature/regenerating; 2 = developing; 3 = spawning capable; 4 = spawning; 5 = regressing). The amount of mesenteric fat was assessed macroscopically according to the four-stage classification key developed by Furnestin (1943; where 1 = no fat visible; 2 = thin thread of fat surrounding part of the gut; 3 = thick layer of fat partially surrounding the gut; 4 = thick layer of fat completely surrounding the gut). Stomach fullness, used as an index of feeding intensity, was evaluated according to the four-stage key developed and calibrated by Cunha et al. (2005; where 1 = almost empty; 2 = half-full; 3 = full; 4 = bursting). Stomach color, reflecting the relative proportion of phytoplankton and zooplankton in the stomach contents, was evaluated according to the three-stage scale presented by Cunha et al. (2005; where 1 = beige, indicating an empty stomach; 2 = orange/brown, indicating a predominance of zooplankton; 3 = green, indicating a predominance of phytoplankton). For age determination, otoliths were extracted from the fish, cleaned, mounted with Entellan in black plastic plates, and observed under a dissecting microscope; age (years) corresponded to the number of complete translucent rings formed during the slow growth period of the species (Soares et al. 2007).

During the 2005–2006 annual cycle, ovaries were collected from females obtained from one of the monthly samples (n = 416), and liver samples from a subsample of these females
were also collected in August and December 2005 and March and April 2006. These samples were complemented by liver tissue of mature and immature males and females collected off the northern portion of Portugal’s west coast (Matosinhos Harbor) in July, August, and September 2009, resulting in a total of 31 liver samples from females and 12 liver samples from males. Ovaries and liver tissues were preserved in a 4% solution of buffered formaldehyde and were stored in a 70% solution of ethanol for histological analysis. The samples were subsequently dehydrated, cleared with xylol, and embedded in paraffin. Histological sections of 3–5 μm were cut and stained with hematoxylin and eosin yellow (Kiernan 1999).

Liver sections were analyzed microscopically, mainly focusing on the histomorphological characteristics of the hepatocytes. Ovary sections were investigated and scored for the following histological parameters: oocyte stage of the most advanced batch, presence and age of postovulatory follicles (POFs), and atresia prevalence and incidence. The classification of oocyte stages was based on that developed by Ganias et al. (2004) for Mediterranean Atlantic sardine and is composed of eight stages (where 1 = primary growth oocyte; 2 = cortical alveolar oocyte; 3–5 = yolked oocyte; 6–7 = oocyte maturation with germinal vesicle migration; 8 = oocyte maturation with oil droplets, yolk coalescence, and hydration). This oocyte stage scoring system was used to confirm the macroscopic maturity phase of the ovary. The attribution of age to POFs (daily age-classes from day 0 to day 3+) followed the methodology developed by Ganias et al. (2007a) and employed both histomorphological criteria (overall aspect of the POFs and degree of degeneration of the granulosa cells) and metric criteria (cross-sectional area of POFs). The different stages of atresia (alpha-, beta-, gamma- and delta-atretic follicles) were identified according to the descriptions by Hunter and Macewicz (1985) for the northern anchovy Engraulis mordax and by Pérez and Figueiredo (1992) for Atlantic sardine from the Iberian coast.

**Macroscopic indices.**—To evaluate the ovarian, hepatic, and somatic condition of the fish, the following indices were calculated: the gonadosomatic index (GSI), hepatosomatic index (HSI), and relative weight (W_r), respectively. The GSI and HSI were obtained as the ratio of gonad weight (W_gonad) or liver weight (W_liver) to gutted weight (W_gutted):

\[ \text{GSI} = \frac{W_{\text{gonad}}}{W_{\text{gutted}}} \times 100 \]

and

\[ \text{HSI} = \frac{W_{\text{liver}}}{W_{\text{gutted}}} \times 100. \]

To validly use the GSI with the present data, it was necessary to examine the independence of the GSI and fish somatic weight by testing for the isometry of ovarian development (Somarakis et al. 2004). The ovaries collected from July 2005 to July 2006 and analyzed histologically were used. Ovaries were subdivided into two groups depending on the most advanced oocyte stage: (1) those with unyolked oocyte stages (stages 1 and 2) and (2) those with yolked oocyte stages (stages 3–6). The relationship between ovary weight and W_gutted was modeled for these individuals by using a linear model with the form:

\[ \log_e(W_{\text{gonad}}) \sim \log_e(W_{\text{gutted}}) \times \text{Ooc}, \]

where Ooc is the most advanced group of oocytes considered. The slopes obtained from the regressions (for each of the two ovary groups) were then compared with 1.0 (for isometry) by using a Student’s t-test (Zar 1999). No ovaries with oocyte stages 7–8 were present in the samples used. Nevertheless, Somarakis et al. (2004) showed that the relationship between Atlantic sardine ovary weight and body weight is not isometric at the hydrated oocyte stage (stage 8); consequently, females with hydrated oocytes (macroscopic maturity phase 4) were excluded from the calculation of GSI in this study. Although a similar effect has not been studied for males, a disproportionate increase in W_gonad from macroscopic maturity phases 3–4 was observed in preliminary data analysis. Therefore, macroscopic maturity phase 4 males were also excluded from the calculation of GSI.

The W_r was calculated as follows (Cone 1989):

\[ W_r = \frac{W_{\text{gutted}}}{W_t} \times 100, \]

where W_gutted is the fish gutted weight and W_r is the corresponding predicted weight for a fish with the same length as obtained from a linear regression of \( \log_e(W_{\text{gutted}}) \) as a function of \( \log_e(\text{total length}) \) estimated from all fish sampled during 2004–2008.

Monthly estimates of the above biological indices were calculated as arithmetic means of individual fish values. For mesenteric fat and stomach fullness, two categories were created; stages 1 and 2 (low mesenteric fat and low stomach fullness) of these indices were assigned a value of 0, and stages 3 and 4 (high mesenteric fat and high stomach fullness) were assigned a value of 1. The proportion of fish with a value of 1 (i.e., fish with high mesenteric fat or high stomach fullness) was used to estimate the fat index (FI) or stomach fullness index (SFI) in each month, whereas the proportion of fish with green stomachs (stomach color index) was obtained monthly from the stomach color data. To describe seasonal growth, mean length at age (for age in months) was calculated separately for males and females from individual fish data for the 2003–2005 cohorts. Because Atlantic sardine growth is limited after the third year (Silva et al. 2008), only the first 3 years of life were considered for each cohort to avoid the confounding effects of sampling error in the seasonal growth pattern.

Since sampling was length stratified, unbiased means should be obtained by raising length-class means to monthly length frequency distributions of the catch (Morgan and Hoening 1997).
However, a preliminary analysis of a subset of the samples showed negligible differences between monthly means obtained by each method. It was subsequently decided to use the monthly mean index values obtained directly from individual data, allowing for a more straightforward analysis of comparisons and correlations (see below).

To compare biological indices between the two sexes, each index was modeled against month, sex, and the sex × month interaction by using linear models (LMs) or generalized LMs (Crawley 2007). Quasitil and residual plots suggested that a generalized LM with a gamma error distribution and an identity link was the most appropriate for the analysis of GSI and HSI. The \( W_r \) was better fitted with an LM (with a normal error distribution). For the FI and StF, the proportion of fish with a value of 1 (see above) was modeled against the same covariates by using a generalized LM with a binomial error distribution and a logit link function. An analysis of deviance of each fitted model was then performed to test which model terms (notably sex) were significant. To compare mean values of the biological indices between sexes in a particular month or between successive months for the same sex, the Wilcoxon–Mann–Whitney two-sample test (hereafter, Wilcoxon–Mann–Whitney test) was applied between pairs of data from the same month to the length-stratified samples (Zar 1999). Moreover, to confirm that sex-specific differences in GSI were not due to differences in \( W_{\text{gutted}} \) between sexes, the relationships between \( W_{\text{gutted}} \) and \( W_{\text{gutted}} \) by sex and month were compared by using analysis of covariance (Crawley 2007).

Cross-correlation function (CCF) analyses were undertaken to describe seasonal relationships among reproductive activity, condition, and somatic growth. The CCF coefficients were calculated for each pair of indices (sexes separated) at time lags from 0 to 9 months. The 95% tolerance limits (corresponding to ±2 SEs of the CCF coefficients) were used to assess significant departure from the null hypothesis of zero cross-correlation (Diggle 1990).

All calculations and statistical analyses were carried out in R software version 2.8.1 (R Development Core Team 2008).

**Histological indices.**—For each monthly sample, two ratios were calculated with the intent of “quantifying” the cytological changes in the ovary over time and at the population level and subsequently comparing the latter with other macroscopic indices. The first ratio (FA2) was the proportion of females with ovaries containing cortical alveolar oocytes or more advanced oocytes (oocyte stage ≥ 2). The second ratio (FA3) was the proportion of females with ovaries containing vitellogenic oocytes (oocyte stage ≥ 3). The incidence of spawning was obtained as the proportion of females presenting signs of recent spawning. Spawning fraction \( S \) (i.e., the fraction of females spawning per day) was estimated with the following formula (ICES 2008):

\[
S = \frac{n\text{POF}_1 + n\text{POF}_2}{2 \times ([n\text{POF}_1 + n\text{POF}_2/2] + n\text{POF}_3 + n\text{noPOF})},
\]

where \( n\text{POF}_i \) is the number of females in the sample with POFs of age \( i \) (day 0 to day 3+) and \( n\text{noPOF} \) is the number of females without POFs.

The prevalence of alpha atresia in vitellogenic oocytes \( (\text{Pr}_\alpha v) \) was calculated as the proportion of females presenting this resorption stage among the total number of females containing vitellogenic oocytes in each sample. The prevalence of atresia in nonvitellogenic oocytes \( (\text{Pr}_\alpha n) \) was determined by simple profile counting as the percentage of alpha-atretic vitellogenic oocytes among the total number of vitellogenic oocytes (healthy and atretic). Simple profile counting is usually not the most accurate method to estimate atresia intensities from histological sections, as the tools provided by modern stereology are more appropriate (Andersen 2003). However, in the present study, the objective was not to obtain precise estimates of atresia intensity but rather to evaluate the seasonal trend for this variable; therefore, the simple profile method was considered to be adequate within this context.

**RESULTS**

The LM showed that the ovary weight and \( W_{\text{gutted}} \) exhibited a significant linear relationship \( (R^2 = 0.81, F = 568.3, P < 0.01; \text{Figure 1A}) \), and the slopes for the two ovary groups did not differ significantly from 1.0 (Student's \( t \)-test, ovaries with unyolked oocyte stages: \( t = 1.3474, n = 204, P = 0.16 \); ovaries with yolked oocyte stages: \( t = -1.0119, n = 209, P = 0.24 \)), indicating that GSI is independent of fish somatic weight and that ovarian development is isometric for the oocyte stages considered. Additionally, macroscopic and histological data collected during the 2005–2006 annual cycle showed that the GSI increased throughout oocyte development (Figure 1B); thus, GSI could be validly used in this study as an indicator of female reproductive state. Monthly variation in the GSI during 2004–2008 showed that Atlantic sardine were reproductively active mainly in autumn and winter (Figure 2A). The maximum GSI values (9.6–12.3%)—assumed here to represent peak spawning—were reached in December–February depending on the year, while the minimum values (0.3–0.9%) were usually observed in July–August. Spawning activity was synchronous between sexes. However, males showed a significantly higher mean GSI than females during the main spawning season, particularly from November to January, whereas during the resting period (July and August) the opposite was observed (Figure 2A; Table 2). Sex-specific differences in GSI were due to higher or lower \( W_{\text{gutted}} \) in males than in females with the same \( W_{\text{gutted}} \) rather than to differences in \( W_{\text{gutted}} \) between sexes, as indicated by analysis of covariance (results not shown). The main spawning season remained fairly constant, ranging from 5 to 7 months in duration across the study period.
The GSI values at peak spawning ranged from a minimum in 2006 (females [mean ± SD]: 6.24 ± 2.51%; males: 8.17 ± 3.48%) to a maximum in 2008 (females: 8.57 ± 3.19%; males: 12.29 ± 3.48%) and demonstrated no clear temporal pattern. Histological data obtained from July 2005 to July 2006 supported the seasonal reproductive pattern described by the GSI for females. Cortical alveolar oocytes appeared in the ovaries in September, and concurrently all indices of atresia dropped nearly to zero. Vitellogenesis started in October, coinciding with a sharp rise in GSI, and lasted mainly until March, when GSI dropped to a low level (variations in FA2 and FA3 are shown in Figure 3A; see also Figure 4A, 4B). The decline in FA2 and FA3 from March onwards indicated that both cortical alveolar and vitellogenic oocytes started to experience atretic degeneration (Figure 4C). This was confirmed by a significant increase in both Prαv and Intαv and by a significant increase in Prαnv (Figure 3C). Cortical alveolar and vitellogenic oocytes were almost completely eliminated from the ovaries by June. During summer, when the GSI values were at their minimum, the Prαnv remained high and the ovaries were mainly composed of primary growth oocytes and atretic follicles at different stages of resorption (Figure 4D). The value of S closely followed the pattern described for the GSI: S increased in October, peaked in December (14%), and then decreased sharply from February onwards (Figure 3B). The incidence of spawning (presence of POFs) showed an identical pattern, particularly in December, when S was at a maximum as approximately 80% of the females were actively spawning at this time. Overall, the pattern observed for FA3 and S indicated that from October to February, most females were able to accumulate yolk in the oocytes, mature, and spawn.

Small increases in the GSI were often observed outside the main spawning season (between April and June) in both sexes. Although these increases were not statistically significant (Wilcoxon–Mann–Whitney test: P < 0.01), they may reflect weak pulses of reproductive activity, as supported by the histological observations carried out during the annual cycle of 2005–2006. In this period, a fraction of the females continued to produce vitellogenic oocytes in April–May, as indicated by the stable FA3 (Figure 3A), followed by a small increase in the GSI in June. Moreover, a significant increase in both Prαv and Intαv was again observed in May.
FIGURE 2. Monthly patterns in various indices for Atlantic sardine during 2004–2008: (A) gonadosomatic index (GSI), (B) hepatosomatic index (HSI), (C) relative weight (Wr), (D) proportion of fish with a high fat content (mesenteric fat stages 3 and 4; i.e., fat index), and (E) proportion of fish with high stomach fullness (stomach fullness stages 3 and 4; i.e., StF index). Data are presented as means (±SD) for females (red) and males (blue); months are numbered sequentially from 1 to 60 (1 = January 2004; 60 = December 2008).
The HSI was similar in males and females outside the reproductive season, but within the spawning period a distinct pattern was observed and values were consistently higher in females (e.g., in January–February, HSI ≈ 1.1–1.5%; see also Table 2). Figure 5 further illustrates the difference in liver dynamics between the two sexes. Among inactive individuals (macroscopic maturity phase 1), HSI values presented a similar distribution for both sexes, whereas in individuals with developing, active, or regressing gonads (macroscopic maturity phases 2–5), the HSI was significantly lower for males than for females (Wilcoxon–Mann–Whitney test: P < 0.01). In males, the HSI was at a minimum level in January–February (HSI ≈ 0.5–0.8%) soon after the peak reproductive period, increased to a maximum in May–July (HSI ≈ 1.6–2.5%), and then decreased during most of the summer and autumn (Figure 2B). Not surprisingly, the HSI showed a significant inverse correlation with the GSI (Figures 2A, 2B, 6A). In females, a seasonal pattern in HSI was not obvious; the HSI increased from March to June, then decreased during summer, but increased again in September–October and then (depending on the year) stayed more or less stable or decreased slightly during the spawning season (Figure 2B). Thus, despite an inverse relationship between the HSI and GSI outside the spawning season, both indices increased during the first half of the spawning season for females, resulting in a relatively weak CCF coefficient between GSI and HSI (Figure 6A). The HSI values at peak spawning varied between years and demonstrated no clear trend across the study period; the lowest HSIs at peak spawning were observed in 2008 (males [mean ± SD]: 1.59 ± 0.39%; females: 1.52 ± 0.94%), and the highest values were observed in 2005 (males: 2.78 ± 1.39%; females: 2.41 ± 1.62%).

The histological analysis of the liver tissue samples showed some differences in the histomorphological characteristics of hepatocytes between sexes and according to the reproductive activity. During the reproductively active period, the cytoplasm...
of hepatocytes in the livers of females was deeply stained with hematoxylin (Figure 7A), whereas towards the end of the spawning season the cytoplasm became less stained and some “empty” vacuoles were present, suggesting changes in the cellular activity. In contrast, for reproductively active males, the cytoplasm of the hepatocytes appeared lighter or slightly eosinophilic such that the nucleus was more conspicuous (Figure 7B). During the recovering or resting period, hepatocytes in the livers of males and females were characterized by a less stained cytoplasm with a granular aspect, and females sometimes presented empty vacuoles (Figure 7C). In immature individuals of both sexes, the hepatocytes were histomorphologically similar to those in reproductively recovering or resting fish (Figure 7D).

The two condition indices \((W_r\) and \(FI\)) showed a similar seasonal pattern, and they were highly synchronized and strongly correlated (CCF coefficient at a lag of 0 months = 0.87–0.89; Figure 6C). Both variables decreased from October to March, when minimum values were observed \((W_r \approx 0.9; FI \approx 0.0)\), and increased from April to September, when they attained their maximum annual values \((W_r \approx 1.1; FI \approx 1.0; Figure 2C, D)\). Males and females showed identical seasonal variations in \(W_r\) and \(FI\) (Figure 2C, D; Table 2). Overall, these results suggest that body reserves are accumulated concomitantly in muscles and around viscera and that males and females build reserves in a similar way. The somatic indices were significantly negatively correlated with the GSI for both sexes, and their maximum values lagged in time by approximately 4–5 months; thus, the maximum condition occurred in August–September, whereas the peak spawning period was generally observed in December–February (Figures 2A, 2C, 2D, 6A). The HSI was significantly and positively correlated with both \(W_r\) and \(FI\), although the seasonal patterns in HSI and somatic indices were not synchronized (Figure 6C). The HSI usually reached maximum values in spring, 2–3 months before the occurrence of maximum condition values in August–September (Figures 2B, 2C, 2D, 6C). The range of values observed for \(W_r\) and \(FI\) varied interannually and displayed no temporal pattern; similar to the HSI, the lowest values of these variables during the study period were observed in 2008.

Data describing feeding intensity, expressed as the amount of food contained in Atlantic sardine stomachs (i.e., StF), were not available for the whole period investigated, but StF appeared to be higher in 2006 and maximum values occurred in winter (December, January, or February) and to a lesser extent in spring (March and May; Figure 2E). Generally, males and females presented very similar trends in StF, indicating that the two sexes had similar seasonal feeding behavior (Table 2). In relation to the previous macroscopic indices, the StF seemed to weakly
correlate with the reproductive activity (GSI) and the hepatic and somatic condition indices (Figure 6B, D). The correlation between \( W_s \) and StF was significant with a time lag of approximately 5 months because feeding intensity seemed to be more important in winter–spring and \( W_s \) peaked at the end of summer (Figures 2C, 2E, 6D).

Regarding Atlantic sardine growth, mean monthly fish length indicated that in the first year after recruitment, growth took place mainly during spring months between March–April and exhibiting a possible extension from September to June. The GSI values were usually low for both sexes; \( W_{gonad} \) represented a maximum of 5% of the body weight in three-fourths of the individuals. In teleost fishes, the size of the testes may relate to the mode of fertilization, whereas ovarian size partly reflects differences in the temporal pattern of egg development, fecundity strategy, and spawning dynamics. The GSI in asynchronous, indeterminate batch spawners is usually lower than that in synchronous, determinate, total-spawning species (Tyler and Sumpter 1996; Wootton 1998; Rinchard and Kestemont 2003). During the spawning period, both Sp and spawning incidence closely followed the pattern in GSI. Although these indices are to be considered with some caution (as they were obtained from a single monthly sample), they suggest that from October to December, either (1) the fraction of active females was higher in the population or (2) females were spawning more frequently. Spawning fraction was also reported to vary seasonally in other sardine species (Brazilian sardine *Sardinella brasiliensis*:

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**DISCUSSION**

The seasonal reproductive pattern of Atlantic sardine off the central west coast of Portugal was described by monthly variations in GSI over a 5-year period from 2004 to 2008. The GSI was shown to be independent of fish somatic weight (prior to hydration) and to reflect the ovarian oocyte composition; it could thus be used as an indicator of female reproductive state. The significant increase in GSI corresponded primarily with the beginning of vitellogenesis in October, as vitellogenesis and hydration in pelagic eggs are the mechanisms responsible for massive growth of oocytes in teleosts (Tyler and Sumpter 1996). From October to February, all females analyzed had healthy vitellogenic oocytes; however, the GSI continued to increase during this period, probably due to a reduction in female mean body weight (related to a decrease in \( W_s \)) or to an increase in batch fecundity, as was previously demonstrated for Atlantic sardine off the coast of Portugal (Zwolinski et al. 2001). The sharp decrease in the GSI during March corresponded to an important decrease in the FA3 and increases in Pr\( \alpha \) and Int\( \alpha \). High proportions of alpha atresia are considered to forecast the cessation of reproduction near the end of the spawning season in indeterminate batch spawners (Hunter and Macewicz 1985) and therefore corroborate data indicating that in 2005–2006, Atlantic sardine off the Portuguese west coast reproduced mainly from October to March. This is in accordance with the findings of Stratoudakis et al. (2007), who defined the Atlantic sardine spawning season along the Portuguese coast as occurring mainly from November to April and exhibiting a possible extension from September to June.

The GSI values were usually low for both sexes; \( W_{gonad} \) represented a maximum of 5% of the body weight in three-fourths of the individuals. In teleost fishes, the size of the testes may relate to the mode of fertilization, whereas ovarian size partly reflects differences in the temporal pattern of egg development, fecundity strategy, and spawning dynamics. The GSI in asynchronous, indeterminate batch spawners is usually lower than that in synchronous, determinate, total-spawning species (Tyler and Sumpter 1996; Wootton 1998; Rinchard and Kestemont 2003). During the spawning period, both Sp and spawning incidence closely followed the pattern in GSI. Although these indices are to be considered with some caution (as they were obtained from a single monthly sample), they suggest that from October to December, either (1) the fraction of active females was higher in the population or (2) females were spawning more frequently. Spawning fraction was also reported to vary seasonally in other sardine species (Brazilian sardine *Sardinella brasiliensis*:

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![Figure 5](https://bioone.org/journals/Marine-and-Coastal-Fisheries:-Dynamics,-Management,-and-Ecosystem-Science)
FIGURE 6. Cross-correlation function (CCF) analysis between monthly average index values for each sex of Atlantic sardine (red = females; blue = males): (A) gonadosomatic index (GSI) versus the hepatosomatic index (HSI) and relative weight (Wr), (B) GSI versus the stomach fullness index (Stom. Cont.), (C) Wr versus the HSI and fat index (Fat), (D) stomach fullness index versus the HSI and Wr, and (E) mean fish total length versus the GSI and Wr for the 2005 cohort only (results for the 2003 and 2004 cohorts not shown). Dashed horizontal lines represent a CCF coefficient (corr. coeff.) equal to 0 and the upper and lower 95% tolerance limits under the assumption that the CCF coefficient is 0 at all lag times.
Isaac-Nahum et al. 1988; Chilean sardine *Sardinops sagax sagax*: Claramunt et al. 2007) and in other small clupeids (Parrish et al. 1986).

During the period investigated, Atlantic sardine showed a very stable seasonal reproductive pattern, although depending on the year the peak of activity varied from December to February and the duration of the spawning season fluctuated between 5 and 7 months. According to Coombs et al. (2006) and Stratoudakis et al. (2007), seawater temperature is the main factor determining Atlantic sardine spawning seasonality in northeast Atlantic waters, presumably through modulation of the physiological processes and endocrine regulation (determining temperature preferences for the species) or as a reproductive strategy that has evolved in response to temperature, upwelling events, primary productivity, or a combination of these factors (Roy et al. 1989; Basilone et al. 2006). However, other physical factors (temperature and upwelling events) and biotic factors (food availability, food quality, and intraspecific competition for food acquisition) might also regulate reproductive activity through their effects on fish body condition (which varied interannually), as has been reported for other small clupeids (Roy et al. 1989; Tsuruta and Hirose 1989; Abad and Giráldez 1993; Parrish and Mallicoate 1995; Millán 1999; Kreiner et al. 2001; Takasuka et al. 2005; Amenzouzi et al. 2006; Basilone et al. 2006; Gáñias et al. 2007b; Gáñias 2009). Therefore, if temperature has a major role in determining the spawning season duration, the end of the main reproductive activity in March could also have resulted from a synergistic effect of this physical factor and the exhaustion of stored energy.

The patterns of several indices (variations in the GSI, FA3, spawning incidence, and atresia; Figures 2A, 3) suggested that in some years (e.g., 2006), some residual reproductive activity took place after March. In sardines and in other clupeids, there are often two “peaks” in spawning activity, of which one is usually much more pronounced (Alheit 1989); two peaks occur because two different portions of the population are spawning at different times or because an improvement in feeding conditions allows for additional reproductive activity. In Iberian waters, smaller Atlantic sardine are reported to have shorter spawning seasons but their maximum spawning activity is synchronized with that of the larger fish (Silva et al. 2006; Stratoudakis et al. 2007). In the present case, it is thus reasonable to consider that some extra energy resources obtained directly from food input may have provided the “fuel” necessary for fish to produce additional batches of eggs (Hunter and Leong 1981; Gáñias 2009). Garrido et al. (2008b) also observed a similar small increase in GSI during April–May in Atlantic sardine from the same area, and this increase was correlated with an increase in feeding intensity. In the present study, higher feeding intensities (i.e., StF), which were often linked to a higher fraction of phytoplankton
ingested (stomach color index; results not shown), occurred mainly in winter and spring. Winter and spring are not usually considered to be seasons of high productivity in Iberian waters; however, along the west coast of Portugal, nutrient inputs caused by increasing river run-off in conjunction with some thermohaline stratification can promote significant plankton productivity in winter and spring during certain years (Moita 2001; Santos et al. 2007). It could therefore be suggested from these findings that an increase in primary productivity and food input during late winter and spring of certain years could potentially provide dietary energy and nutrients (including some essential fatty acids that are produced by phytoplankton and that are important for reproduction; Sorbera et al. 2001; Tocher 2003; Garrido et al. 2008b) at a time when Atlantic sardine are at their minimum physical condition (and most likely when reserves are depleted), thus sustaining the continuation of vitellogenesis and production of gametes. This implies that Atlantic sardine would be able to rely on both stored energy (as occurs in capital breeders) and direct food input (as occurs in income breeders) for the production of gametes, as was previously suggested by
Hunter and Leong (1981) for northern anchovy in California and by Ganias (2009) for Atlantic sardine from the Mediterranean Sea.

During the period investigated, the GSI and the somatic condition factors \( W_r \) and FI showed inverse seasonal trends for both sexes. This observed seasonal transition, in which resource allocation shifts from reproduction to fat deposition and condition improvement (i.e., a “storage strategy”), is a very recurrent characteristic in other Atlantic sardine populations (Hickling 1945; Tomasic et al. 1989; Abad and Giráldez 1993; Voulgaridou and Stergiou 2003; Amenzoui et al. 2006; Ganias et al. 2007b; Sinovcic et al. 2008; Mustac and Sinovic 2009), other sardine species (Roy et al. 1989; van der Lingen and Hutchings 2004; Cubillos and Claramunt 2009), and other small, pelagic clupeids (Cubillos et al. 2001). Indeed, clupeid fish do not always spawn during periods of major productivity or high food abundance (e.g., linked to upwelling events). However, during these periods, they accumulate energy that is not used immediately but is stored as fat (sometimes for several months) for later reproductive activity (Roy et al. 1989; Cubillos et al. 2001). This strategy has the benefit of guaranteeing the production of eggs under unpredictable conditions (changeable environment) or limited food conditions (oligotrophic areas) and simultaneously favoring the survival of eggs and larvae (e.g., by limiting the offshore transport of these early life stages in certain upwelling zones; Roy et al. 1989; Lluch-Belda et al. 1991; Ganias et al. 2007b).

The present results also showed that \( W_r \) and FI were very strongly related. Correspondingly, Mustac and Sinovic (2009) reported that in Atlantic sardine from the Adriatic Sea, the lipid content of mesenteric fat and of the body tissues (mainly muscle) had identical temporal trends. In Iberian Atlantic sardine, Bandarra et al. (1997) reported that the total amount of lipids greatly varied seasonally, following the same trend observed for FI in this study. Overall, these results suggest that although the FI scale is only semiquantitative, it seems to reflect quite accurately the condition of individual Atlantic sardine. These findings also indicate that in Atlantic sardine, the accumulation of fat around the gut (mesenteric fat) and the improvement in condition (by building up muscle mass and accumulating fat within the muscle and between skin and muscle) take place in parallel. In clupeids and scombrids, muscle and viscera are in fact the main lipid storage compartments, whereas in gadoids the lipids are predominantly stored in the liver. For instance, in Atlantic cod Gadus morhua, the HSI is a very good indicator of total energy reserves and fish condition (Lambert and Dutil 1997).

In contrast, the present results showed a relatively weak correlation between the HSI and the condition indices \( W_r \) and FI. However, these three characteristics had similar interannual variability, suggesting that although the liver is not the main energy storage compartment in Atlantic sardine, it presumably has some relation to fish condition and feeding activity, playing a role in nutrient transfer between the different organs (in particular, lipids of dietary origin that are not used immediately could be transferred from the liver to the muscle and viscera and may later be used in reproduction). The decrease in HSI observed during summer in both males and females, in contrast to the increasing trends for \( W_r \) and FI, could be attributed to the important role of the liver in nutrient metabolism, which is linked to higher food abundance (Santos et al. 2007) and higher metabolic rates (due to higher temperatures) in summer (Wootton 1998).

The present results agreed with those of a previous study of Atlantic sardine from the Iberian coast (Silva et al. 2008) in that somatic growth primarily took place once the main spawning season was concluded. The significant positive relationship between growth and each of the condition indices \( W_r \) and FI also suggested that Atlantic sardine grow and improve condition at approximately the same time (spring and summer), although growth seemed to slow down in summer (growth is known to be stimulated by higher temperatures, but more energy may have been diverted to condition improvement during these months). Cubillos et al. (2001) suggested that the seasonal growth pattern observed for the Peruvian anchoveta Engraulis ringens and Araucanian herring Strangomera benticki off the Chilean coast was the result of a long-term reproductive adaptation to the seasonal upwelling conditions. Here, it is hypothesized that the greater investment by Atlantic sardine in somatic growth and accumulation of energy reserves during spring and summer occurs not because more food is usually available or ingested during these months (Santos et al. 2007; Garrido et al. 2008a; present study: negative correlation between \( W_r \) and StF) but because during this reproductive resting period, individuals do not mobilize most of their energy into the production of gametes and energy thus becomes available for the other biological functions.

During this study, some differences between males and females during an annual cycle were recognized. Although spawning activity was synchronous between the sexes, the GSI was systematically higher for males than for females during the reproductively active period. This difference between sexes has also been reported for Atlantic sardine along the Moroccan coast (Amenzoui et al. 2006); it does not, however, conform to the commonly accepted view that testes in teleost fish frequently represent a lower proportion of the body weight than the ovaries (Wootton 1998). The \( W_r \) and FI each showed almost identical trends for males and females, suggesting that the way in which energy was stored was the same for both sexes of Atlantic sardine along the west coast of Portugal during the period considered. Similarly, Caponio et al. (2004) found no significant difference in lipid content between male and female Atlantic sardine in the Ionian Sea, whereas Mustac and Sinovic (2009) showed that female Atlantic sardine in the Adriatic Sea often had more fat than males. The present results also indicate that males and females have similar seasonal feeding behavior, corroborating the findings of Garrido et al. (2008a) that feeding intensity and diet composition were similar between male and female Atlantic sardine from Portuguese waters.
The most notable distinction between males and females in this study appeared in the seasonal dynamics and histomorphology of the liver during the spawning period. For both sexes, the liver plays a major role in digestion, nutrient metabolism, storage, and detoxification processes. However, in females, the liver has a dual function during the reproductive season as it is also responsible for the synthesis of vitellogenin (protein; the plasma precursor of yolk), which is then taken up by oocytes during vitellogenesis (Lubzens et al. 2010). This role of the liver in females would thus explain why in October, when vitellogenesis started, the HSI increased again for females and remained relatively stable during the main spawning season, whereas for males the $W_{\text{liver}}$ decreased during most autumn and winter months (probably related to the loss of body condition). The enormous cellular activity of protein synthesis during reproduction could also explain the histomorphological modifications observed in hepatocytes during this study, which were also reported by Rinchard and Kestemont (2003) for three other fish species. Moreover, the period of intense hepatic activity seems to depend on the fish fecundity strategy, thus corroborating the role of the liver in vitellogenesis. For indeterminate batch spawners (like Atlantic sardine), oocytes are continuously recruited during the spawning season and the hepatocyte activity remains intense throughout this period, whereas for determinate batch spawners (like Atlantic cod) the vitellogenic activity of the liver decreases during the reproductive season due to the completion of vitellogenesis prior to spawning (Dahle et al. 2003; Rinchard and Kestemont 2003).

The present work confirmed that the spawning season of Atlantic sardine caught along the west coast of Portugal extends throughout the autumn and winter months. Somatic growth, condition improvement, and fat accumulation take place primarily during the reproductive recovery period in spring and summer, in a process characterized by alternating periods of resource allocation to reproduction and resource allocation to fat deposition, growth, and condition improvement. It could thus be hypothesized that a physiological shift in allocation from reproduction to growth and condition would occur every year at a given time in Atlantic sardine and that this shift would be regulated by endogenous rhythms, external physical and biotic factors, or some combination thereof. Atlantic sardine seem to feed mainly in winter and spring, although the most productive plankton season in this area is reported to occur frequently corresponding to spring and summer months. Therefore, the hypothesis proposed is that the occurrence of maximum growth and condition in spring and summer is not attributable to the maximized resources in adult prey fields but rather to the fact that no resources are being diverted to reproduction, thus illustrating the significance of resource allocation trade-offs. Additionally, these findings illustrate the importance of also analyzing other body organs (liver, muscle, and gut) while studying the sexual cycle of a species in order to understand how the fish can presumably adapt its reproductive strategy in response to varying environmental conditions. Male and female Atlantic sardine showed very similar temporal trends for all macroscopic indices except the HSI, thus indicating that both sexes have equivalent seasonal reproductive patterns, growth periodicities, feeding behavior, fat accumulation, and condition improvement strategies.

The distinct seasonal dynamics and histomorphology observed for the liver are most likely related to the dual function of this organ in females during the spawning season (i.e., the synthesis of yolk precursors). On the other hand, the role of the liver in the overall condition of Atlantic sardine and in the transfer of nutrients and energy between gut, gonads, and muscle could not be clearly determined based on these results. Although the GSI was always higher for males than for females during the spawning season, the results did not clarify whether males and females invest the same amount of energy into the production of gametes. However, as allocation to reproduction can be the main factor limiting adult growth (Kozlowski et al. 2004), we could hypothesize that if male and female Atlantic sardine have different energy demands and costs in reproduction and distinct strategies for making trade-offs between reproduction and growth, such differences could have a long-term impact on growth dynamics between males and females.

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