

Article

Somatic Condition and Reproductive Potential as a Tandem in European Sardine: An Analysis with an Environmental Perspective in the Northern Adriatic (Gulf of Trieste)

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Abstract: The European sardine's condition is reflected in its reproductive potential, and therefore, in its status as a fishery resource. These values depend on the stock's distribution and resource availability, which is highly determined by environmental characteristics. Sardines from the productive Gulf of Trieste (in the North Adriatic), located in the northernmost section of the most septentrional Mediterranean sub-basin in which sardine exploitation has traditionally been intensive, were analysed. The reproductive cycle and gonadosomatic index (GSI) were studied. Tissue and mesenteric fat values, as well as vacuity (% V), relative condition (Kn), and hepatosomatic (HSI) indices were evaluated due to their potential relationships with reproductive performance. The results suggested opposite patterns between fat reserves and GSI, while Kn showed a relationship neither with GSI, nor with reproductive stage, which led us to conclude that it is more advisable to apply direct lipid indices to project their contribution to reproductive potential. Moreover, the females' condition was generally better than that of the males, added to an advanced gonadal development during spring and summer, albeit males and females reached the spawning season together. Moreover, females' GSIs were significantly higher during active spawning. Furthermore, correlation analyses showed that SST was related with the parameters evaluated, as well as the available portion of productivity for the fish (OPFish), which may explain the sardines' better condition and GSIs than their chlorophyll concentration.

Keywords: fat content; Mediterranean; *Sardina pilchardus*; reproduction; OPFish; pelagic



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1. Introduction

The reproductive potential of fish is defined as the capacity of a fish stock to produce gametes and viable offspring [1,2]. Thus, it is a main aspect in fish conservation, especially when the stock is within the sustainable limits of fish exploitation, or when they are overexploited. Small and medium sized pelagic fish are characterised by short life cycles and small body size, and so early maturation, many eggs per body mass, and batch spawning are common strategies to compensate for their short lifetime fecundity [2]. There is a wide range of evaluations of the reproductive potential in fish, with both qualitative and quantitative approaches that allow us to infer their fecundity and laying season. However, especially in the above-mentioned species, the evolution of their condition and lipid reserves must be taken into account to make an exhaustive analysis of their reproductive potential. In fact, reproductive timing, potential and batch fecundity, and egg quality are related to females' size and condition in capital breeders [3], and so both general condition and energy storage have important implications for recruitment and pelagic ecosystem structures [4–6]. Thus, condition should be carefully studied to be included in assessment

models [7] or models that infer reproductive potential [1] to an effective management of the fishing resource.

The reproductive potential of the small pelagic European sardine (*Sardina pilchardus* (Walbaum, 1792)) depends on its spawning frequency and batch fecundity [8], as it is a multiple spawner species with an indeterminate annual fecundity that serially releases batches of pelagic eggs at intervals (i.e., batch spawner) within the spawning season [9,10]. Its spawning season occurs during the winter [2,11] along its distributional range, although monthly differences exist associated with sardine stock genetic features and environmental characteristics of the area [12]. Production of such a great number of eggs during 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 [13] so that the population's reproductive potential can be influenced by the condition of the fish, which has a direct impact on their recruitment strength [8]. In fact, during the reproductive period, the relative lipid content destined to gonad growth and development may gradually become even more important than growth in length [14]. The European sardine presents a cold-temperate water affinity, and it is distributed throughout the northeast of the Atlantic Ocean, and from the North Sea to Senegal, the Sea of Marmara, the Black Sea, and the Mediterranean Sea [15]. It could be considered one of the most important species within the Eastern Central Atlantic and the Mediterranean Sea not only because of its crucial intermediate trophic level for the ecosystem as a filter feeder [16,17], but also as a human font of essential lipids rich in long-chain polyunsaturated fatty acids (PUFA) and its high-value, easy digestible proteins which contain all essential amino acids necessary for healthy human diets, along with minerals and vitamins [18]. Thus, it represents a great number of catches, being the main contributor to total landings for the whole Mediterranean Sea, together with the anchovy [19]. Therefore, when added to other environmental pressures, a great fishing pressure has caused the biomass of many stocks to be below biologically sustainable levels [20], with a compromised health status. In this sea, its importance could be highlighted, especially in the Adriatic, where sardines represent 41% of total marine catches [21,22]. Sardine fishing covers a great part of the Adriatic basin, but it is mostly concentrated in the northern and central parts in which Italy's main catches come from (between Trieste and Vieste) [21,23]. In this area, a decline in sardine body condition has been observed over the last two decades [24], and reports have documented the sardine's poor state over a large period of time [25].

Sardines primarily feed on small species of zooplankton (copepods, decapods, cirripedes, fish eggs, and cladocerans) and phytoplankton (diatoms and dinoflagellates) whose contribution to individuals' diets varies depending on fish length, season, and region considered [26]. In general terms, lipids are the preferred source of metabolic energy for growth, movement, and reproduction in pelagic fish, and are the first macro-molecules to be catabolised, and so the measurement of lipid content has been preferably used in the study of small pelagic fish conditions, including sardines [5]. Sardine lipid content varies widely with season, which has a direct effect on food availability, water temperature, and, ultimately, the sexual state of the animal [27]. An energy 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 individual reaches sexual maturation, and that is why it can be stated that fish reproductive investment is the result of essential life history trade-offs in resource allocation [13].

Therefore, any factor influencing the energy transfer to gonadal development may have an effect on the reproduction of the individual. Both ovarian maturation and fish fecundity are linked to energy reserves, and hence food supply, since energy availability can cause variations in egg production, with likely impacts on ovarian allometry (i.e., the extent of gonadal growth and development) [28]. Moreover, the fish endocrine system is modulated by external and internal (size and/or age, storage levels, i.e., levels of sugars, amino acids, and lipids) conditions, and thus it can either complete reproductive development and spawning under an optimal environment or delay/abort reproduction under

non-optimal circumstances [29]. Therefore, there exists high variability in terms of fish recruitment, biomass, and distribution, which are mostly dependent on environmental and climatic conditions [12]. In fact, it has been reported that higher catches and larger larval growth rates are found in areas with high chlorophyll concentrations [30], with water stratification and currents among the most important factors that modulate plankton productivity and availability and, therewith, better condition of the planktivorous fish [24]. Further, it should be highlighted that among the variables that could have an effect on this process, water temperatures of $<18\text{ }^{\circ}\text{C}$ have been documented to allow sardines to reach maximum spawning activity [31].

Under the current changing environment, knowledge of the energetic and reproductive physiology of marine resources has gained even more importance, since both can be compromised by the additional environmental pressures linked to this phenomenon, hindering their management inevitably. In a climate change scenario, marine heatwaves have been longer and more frequent during the last century [32], and discharge rates from the relationships between evapotranspiration, precipitation, and river flows during extreme wet and dry years are turning out to be much wider [33], causing changes in productivity due to the alteration of the distribution and availability of nutrients [34,35]. Moreover, climate change may also drive prevailing oceanographic conditions in the spawning habitat of some pelagic fishes out of their optimal environmental window [35]. Therefore, it can be projected that the magnitude of the impact of these factors is diverse and depends on the region and the locality of the pelagic species, its biology, and its ecology. In particular, the incidence of global change may be more pronounced in pelagic individuals inhabiting semi-enclosed seas such as the Mediterranean and their sub-basins, which also are characterized by a high pressure of anthropogenic stressors [36,37]. This could be due to the fact that the Mediterranean is an oligotrophic sea, only accounting for 1% of global primary productivity [38], and so any factor that reduces or hinders access to these resources could pose hazards for pelagic fish. Likewise, pelagic species that are not largely tolerant to increases in temperature, such as sardines, find difficult to migrate northwards or to other areas with lower temperatures in a basin with a limited dispersal potential due to a degree of enclosure of over 99% [39–41].

After considering the above, it is understood that the confluence of different factors, among which environmental variables are decisive, ends up determining the organism's response in terms of somatic condition, energy storage and health status, and, ultimately, the translation of these factors into reproductive dynamics and potential. Besides, it is important to analyse the state of health of the European sardine in highly exploited areas, as well as areas in which the incidence of environmental change may be pronounced, and to monitor these stocks over time. In these terms, the main objectives of this work have been: (1) to analyse the role of different lipid reserves in the capital breeder European sardine in the northernmost section of the most septentrional, semi-enclosed basin of the Mediterranean Sea (the North Adriatic Sea, specifically, in the Gulf of Trieste, relevant in the Italian capture of sardines), and (2) to study the effect of some environmental variables on the physiological and reproductive status of this fish stock.

2. Materials and Methods

2.1. Sampling Collection

Specimens of the European sardine, *Sardina pilchardus*, ($N = 704$) were collected seasonally from 2019 to 2021 along the Gulf of Trieste (coordinates: $45^{\circ}40' \text{ N } 13^{\circ}35' \text{ E}$; surface area: 550 km^2 ; average depth: 18.7 m ; water volume: 9500 km^3) in the North Adriatic coast (Mediterranean Sea, GFCM—GSA 17) by commercial fisheries (Figure 1). Immediately after the purchase, samples were frozen at $-20\text{ }^{\circ}\text{C}$, which has been demonstrated to have no effect on the explained variables [42]. All specimens in this study were the minimum landing size for a sardine (total length (L_T) of $\geq 11 \text{ cm}$) in the Mediterranean Sea, including the Adriatic [25,43].

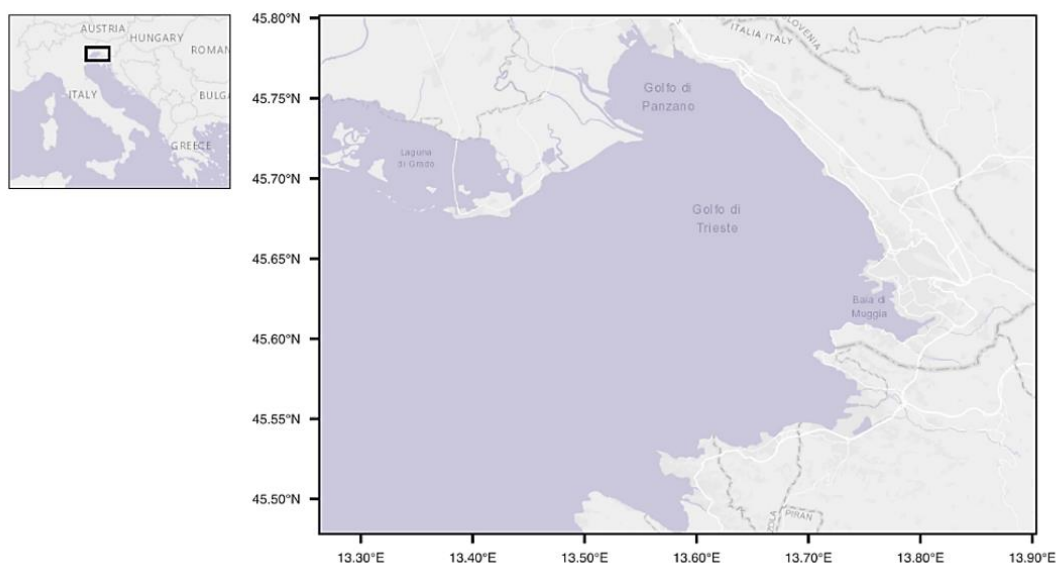


Figure 1. Map of the European sardine sampling area (Gulf of Trieste) and its situation in the Mediterranean Coast.

2.2. Somatic Condition Evaluation

Each sardine individual was measured (total length, L_T , ± 0.1 cm) and weighed (total body weight, W_T , ± 0.01 g; eviscerated body weight, W_E , ± 0.01 g). Gonads (W_G) (± 0.0001 g) and livers (W_L) (± 0.0001 g) were also weighed. A proxy for general somatic condition [5] was obtained by the calculation of the relative condition index (Kn) [44], interpreted as a higher-than-average physical condition for an individual when Kn exceeds 1, and lower condition when it does not reach this figure, as follows:

$$Kn = \frac{W_E}{W_r} = \frac{W_E}{\alpha L_T^\beta}$$

where W_E is the eviscerated body weight of an individual, W_r corresponds to the predicted eviscerated weight of an individual of a given total length, L_T is the total length, and α and β are coefficients obtained by the regression line of the logarithms of length and mass ($\alpha = 0.056101092$, $\beta = 2.1984$). The gonadosomatic index ($GSI = 100 \cdot \frac{W_G}{W_E}$) was calculated as an indirect method to estimate the energy destined to reproduction or reproductive effort [28,45]. The hepatosomatic index ($HSI = 100 \cdot \frac{W_L}{W_E}$) was also obtained.

Regarding the lipidic body condition, tissue fat content (i.e., muscle total lipids) was estimated by the average of both sides along the lateral line of each individual using a fish fat meter (Distell Model FM 992) [46] calibrated for the European sardine. Furthermore, a visual scale for fat mesenteric reserves [47] was applied. The vacuity index ($\% V = 100 \cdot \frac{E}{N}$) was calculated as the number of empty stomachs (E) divided by the total number of stomachs analysed (N).

2.3. Reproduction Analysis

The sex of each specimen was visually and macroscopically determined, and gonads were classified according to the criteria of Brown-Peterson et al. [48] into the following categories: immature (they have not reached sexual maturity); developing (gonads increasing in size with gametes that are beginning to develop); spawning-capable (ready for reproduction, but has not begun to spawn); actively spawning (expelling gametes); regressing (gonads almost empty of gametes); and regenerating (mature but reproductively inactive).

2.4. Statistical Analysis

Analyses were carried out making use of R software version 3.5.1. [49]. Differences among categories were considered as statistically significant if $p < 0.05$. Significance values

were indicated as follows in the Results section: $p < 0.05$ *; $p < 0.001$ **; $p < 0.0001$ ***. When continuous dependent variables were involved, the Shapiro–Wilk test was applied to test the assumption of normality and Levene’s test was executed to prove the homogeneity of variances [50] in all parameters. If both assumptions were met, an independent two-sample t -test, a one-way analysis of variance (ANOVA), or Multi-factor Analysis of Variance tests, when corresponded, were performed. Conversely, if both assumptions of normality and equality of variances were not met, the data were transformed to normality. When only a homoscedasticity assumption was violated, data were analysed with Welch’s t -test. For those parameters in which normal distribution was lacking but homoscedasticity was present, the Kruskal–Wallis analysis of variance was applied. When required, multiple comparison or post hoc tests (Tukey’s range test, Dunn’s method with Bonferroni adjustment, or Games–Howell test, when corresponded) were applied to the identified different categories. When ordinal dependent variables were involved, the Wilcoxon rank sum test with continuity correction was used. For qualitative dependent variables, Pearson’s chi-squared test was performed.

The Spearman’s rank non-parametric correlation test between pairs of variables was used to explore the relationships between Kn, GSI, HSI, and tissue and mesenteric fat content, along with environmental variables such as sea surface temperatures (SST; °C) (NOAA High Resolution SST data [51]), chlorophyll-a concentrations (Chl; $\text{mg}\cdot\text{m}^{-3}$) (NASA combined-satellite [52]), and ocean productivity available to fish (OPFish; %) values, which is an index that characterizes 10–20% of the global phytoplankton production that effectively fuels higher trophic levels [53] (Environmental Marine Information System [54]).

3. Results

3.1. Somatic and Reproductive Condition Analyses. Correlation with Environmental Parameters

The total body length (L_T) of the sardine specimens varied from 10.70 to 16.00 cm (mean \pm SD: 13.37 ± 0.87 cm), while total body weight (W_T) ranged from 11.40 to 35.60 g (19.18 ± 3.76 g), with larger and heavier female sardines compared to males (p *** for both length and weight) (Table 1).

Table 1. Summary of the variables/indices comparing males and females of the European sardine (*S. pilchardus*) in the Gulf of Trieste. L_T : total length (cm); W_T : total weight (g); W_E : eviscerated weight (g); Kn: relative condition index; GSI: gonadosomatic index (%); HSI: hepatosomatic index (%); % V: stomach vacuity index (%). Significance values: $p < 0.05$ *; $p < 0.0001$ ***; NS (not statistically significant).

Variable/Index	Mean \pm SD		Outcome	N	Test	Statistic	p
	Males	Females					
L_T (cm)	13.17 ± 0.80	13.50 ± 0.90	Males < Females	704	One-way analysis of means (not equal variances)	$F = 27.04$	***
W_T (g)	18.07 ± 3.40	19.98 ± 3.80	Males < Females	704	Welch two sample t -test	$t = 7.00$	***
W_E (g)	16.01 ± 2.84	17.64 ± 3.23	Males < Females	704	Welch two sample t -test	$t = 7.11$	***
Kn	0.982 ± 0.113	1.023 ± 0.112	Males < Females	704	Welch two sample t -test	$t = 4.78$	***
GSI	1.906 ± 2.013	2.547 ± 2.268	Males < Females	704	One-way analysis of means (not equal variances)	$F = 15.54$	***
HSI	0.690 ± 0.447	0.785 ± 0.491	Males < Females	704	Welch two sample t -test	$t = 2.62$	*
Tissue fat content	9.468 ± 4.333	10.353 ± 4.469	Males < Females	704	Welch two sample t -test	$t = 2.63$	*
Mesenteric fat	-	-	Males = Females	704	Wilcoxon rank sum test with continuity correction	$W = 60366$	NS
% V	-	-	Males = Females	704	Pearson’s chi-squared test	$\chi^2 = 1.31$	NS

Further significant general differences between the sexes were obtained for tissue fat content (p *), Kn (p ***), GSI (p ***), and HSI (p *) (Table 1), with higher values for females in the parameters mentioned (Figure 2B,D–F). No general differences were recorded for

mesenteric fat content between sexes ($p = 0.891$, NS) (Figure 2C). When reproductive developmental stage was included, significant differences in tissue fat content between the sexes only were reported for the spawning-capable stage (p^*), which were higher in females. A significant interaction has been observed in HSI combining developmental stage and sex (p^*) (Figure 3B). For GSI, significant differences have been seen among developmental stages for this index (p^{***}), with sex differences only at the actively spawning stage (p^{***}) (Figure 3A). Kn did not significantly vary with developmental stage, but it did with month (p^{***}), and had the lowest values registered from December 2019 until May 2020 in both sexes, following an increasing trend towards 2021.

Furthermore, Kn and GSI were not significantly related, even though Kn was positively correlated with both tissue and mesenteric fat content and was more accused with the former in both sexes (Figure 4). Kn was also positively linked to chlorophyll-a in both sexes and was more pronounced in the case of males ($\rho = 0.46^{***}$), while it was lightly related with SST. No significant relationship was observed among Kn and OPFish.

Seasonal trends can be inferred from data, with opposite patterns between GSI and tissue and mesenteric fat content for both sexes (Figure 2), as was confirmed by the Spearman's correlation test ($\rho = -0.5$ for males and -0.56 for females, for tissue fat content; and $\rho = -0.5$ and -0.65 , respectively, for mesenteric fat) (Figure 4). While GSI decreased after winter in both 2020 and 2021, lipid content values started to increase. Tissue fat content values increased in a progressive way from winter, while a steeper slope linked to a later accumulation of mesenteric fat was observed in the late spring of 2020. Moreover, correlations indicated that mesenteric and tissue fat content were strongly related for both sexes ($\rho = 0.830^{***}$ for males and $\rho = 0.813^{***}$ for females). Chlorophyll-a was positively correlated with tissue fat content and more than with mesenteric fat in both sexes. However, a correlation close to -0.2 was seen between chlorophyll-a and GSI. Further, SST was strongly and positively related to tissue and mesenteric fat in a similar way in females and males, and a strong negative correlation when related to GSI ($\rho = -0.72^{***}$ for males, $\rho = -0.68^{***}$ for females) was observed. Values of productivity available to fish (OPFish) were over $\rho = 0.58$ for both tissue and mesenteric fat in both sexes, although higher in the case of females, especially regarding mesenteric fat ($\rho = 0.69^{***}$). At the same time, OPFish was highly negatively correlated to GSI ($\rho = -0.76^{***}$) for both sexes. HSI figures were disparate, although the lowest values of the cycle were detected in the winter months for both sexes, and they were positively related with chlorophyll-a and only slightly with SST in the case of males and with OPFish, showing a correlation over $\rho = 0.21$.

Vacuity index values (% V) by season were 5.56% in Autumn 2019, 46.08% in Winter 2020, 47% in Spring 2020, 11.46% in Summer 2020, 9% in Autumn 2020, 0% in Winter 2021, and 29% in Spring 2021.

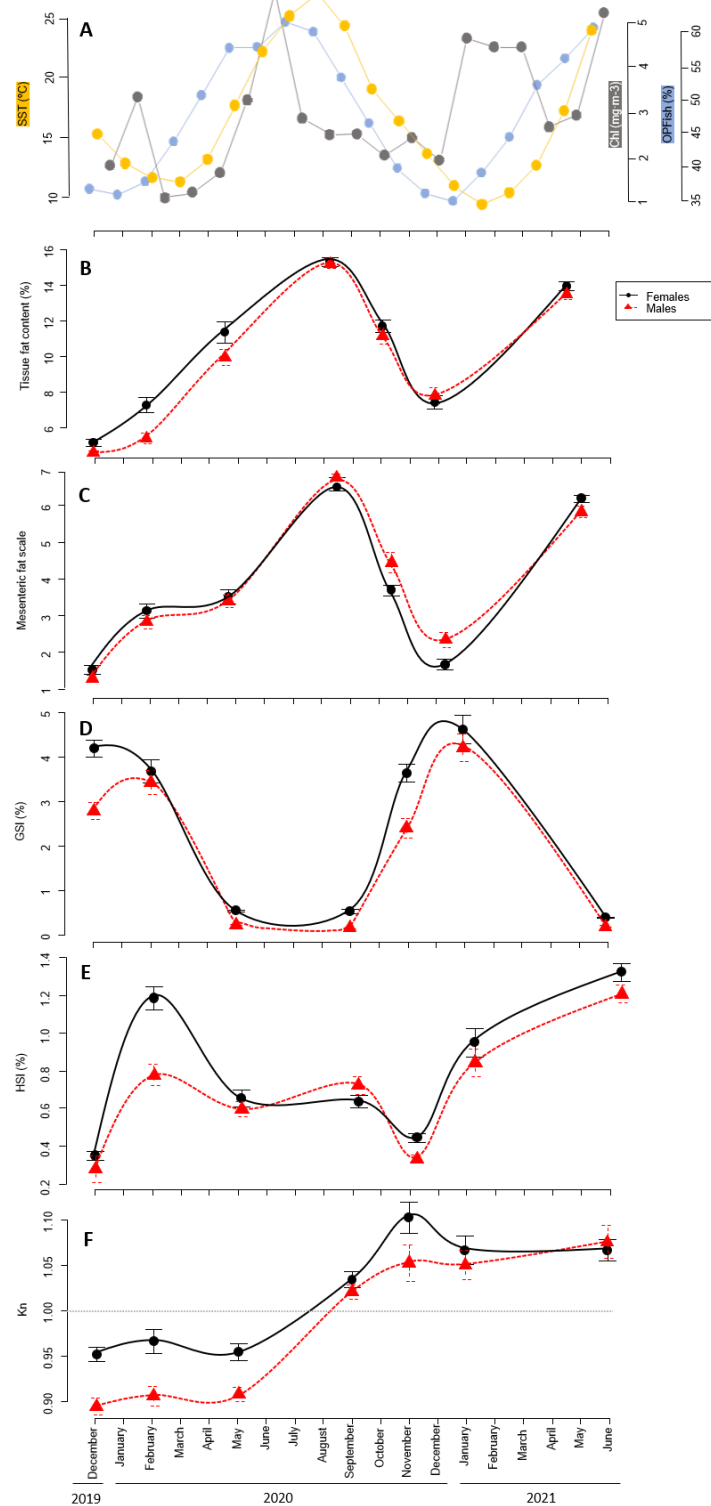


Figure 2. Condition analysis parameters in the European sardine (*S. pilchardus*) and the averages of environmental variables along the Gulf of Trieste (North Adriatic, Mediterranean Sea). (A) Monthly mean sea surface temperature (SST (°C); yellow line), chlorophyll-a concentration (Chl ($\text{mg}\cdot\text{m}^{-3}$); grey line), and available portion of productivity for the fish (OPFish (%); blue line) in the study area over the sampling time (2019–2021). (B) Tissue fat content (%), (C) Mesenteric fat scale, (D) Gonadosomatic index (GSI; %), (E) Hepatosomatic index (HSI; %), and (F) Relative condition factor (Kn) were estimated by sex (females, black line and dots; males, red line and triangles) as averages of the individual measurements.

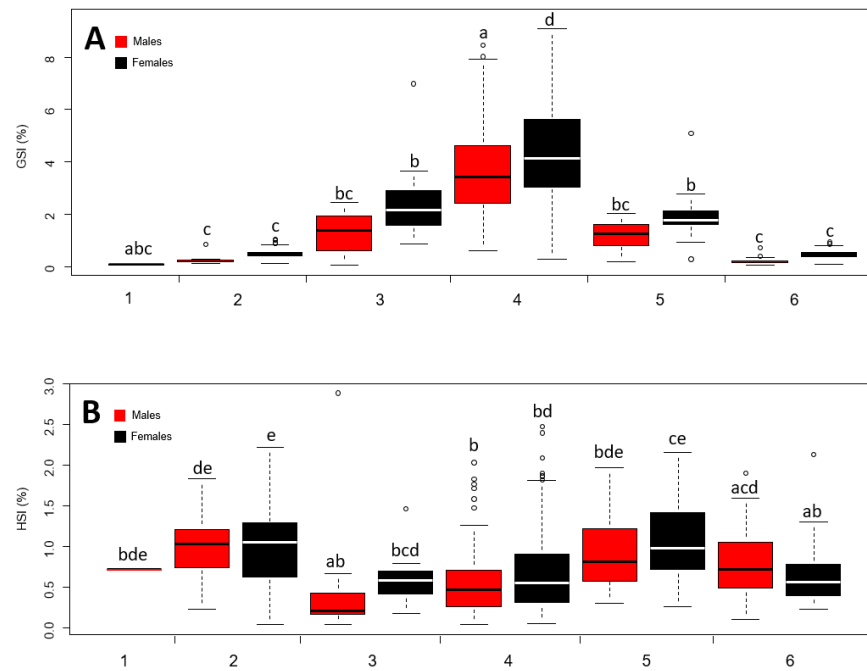


Figure 3. Gonadosomatic and hepatosomatic indices by sex and reproductive developmental stage in the European sardine (*S. pilchardus*). (A) Gonadosomatic index (GSI) by sex and reproductive developmental stage. (B) Hepatosomatic index (HSI) by sex and reproductive developmental stage according to the classification of Brown-Peterson et al. [48]. Different letters on the graph indicate significant differences among stages and/or between sexes. Outliers are marked with a circle (°).

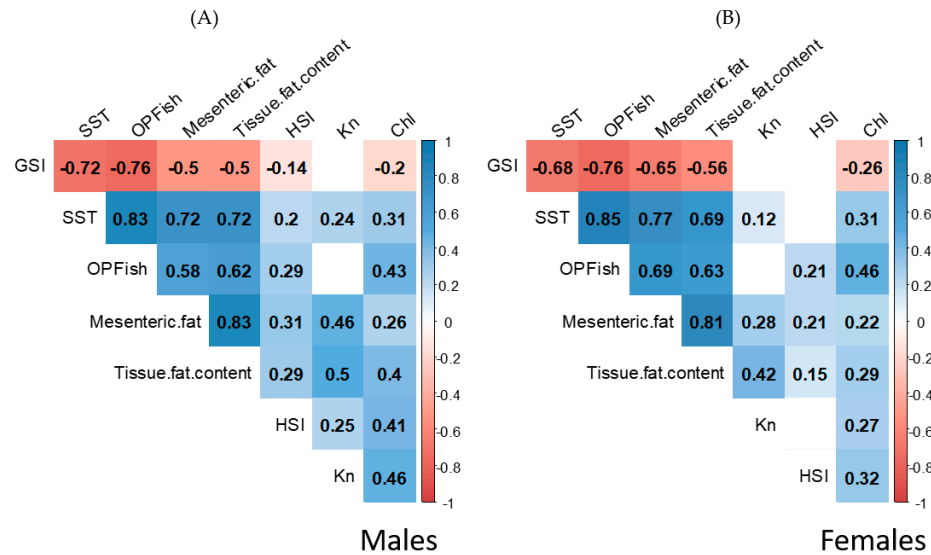


Figure 4. Spearman correlation matrix among the European sardine's (*S. pilchardus*) condition parameters and the environmental variables considered in males (A) and females (B). Relative condition index (Kn), tissue fat content (%), mesenteric fat scale, gonadosomatic index (GSI; %), and hepatosomatic index (HSI; %) were correlated with each other and with the following environmental variables: sea surface temperature (SST; °C), chlorophyll-a concentration (Chl; $\text{mg}\cdot\text{m}^{-3}$), and available portion of productivity for the fish (OPFish; %). The colour gradient from maroon to navy blue corresponds to the correlation with strength, from negative to positive, respectively. The empty squares represent a non-significant correlation according to a p value of <0.05 *.

3.2. Sex Ratio and Reproductive Cycle

The overall sex ratio (m/f = 0.710) deviated significantly from the hypothetical distribution of 1:1 ($\chi^2 = 20.144$, $df = 1$, p^{***}), as more females were observed than males. However, significant differences among sex proportions were not seen monthly.

The early beginning of the reproductive season was recorded in Summer (September 2020) for only one specimen, a female individual. However, the greater percentage of active spawners were observed in the autumn and winter months for both sexes during the different sampled years. Thus, the spawning period in the Gulf of Trieste lasted at least from September–October to March.

During the sampled months of the spring and summer, 2020, and the spring of 2021, a higher percentage of developing individuals corresponded to females, a sign of their more advanced gonad maturation than males over the years, in which a large proportion of them were at the regenerating phase. However, the values during the reproductive seasons were again similar in both sexes (Figure 5).

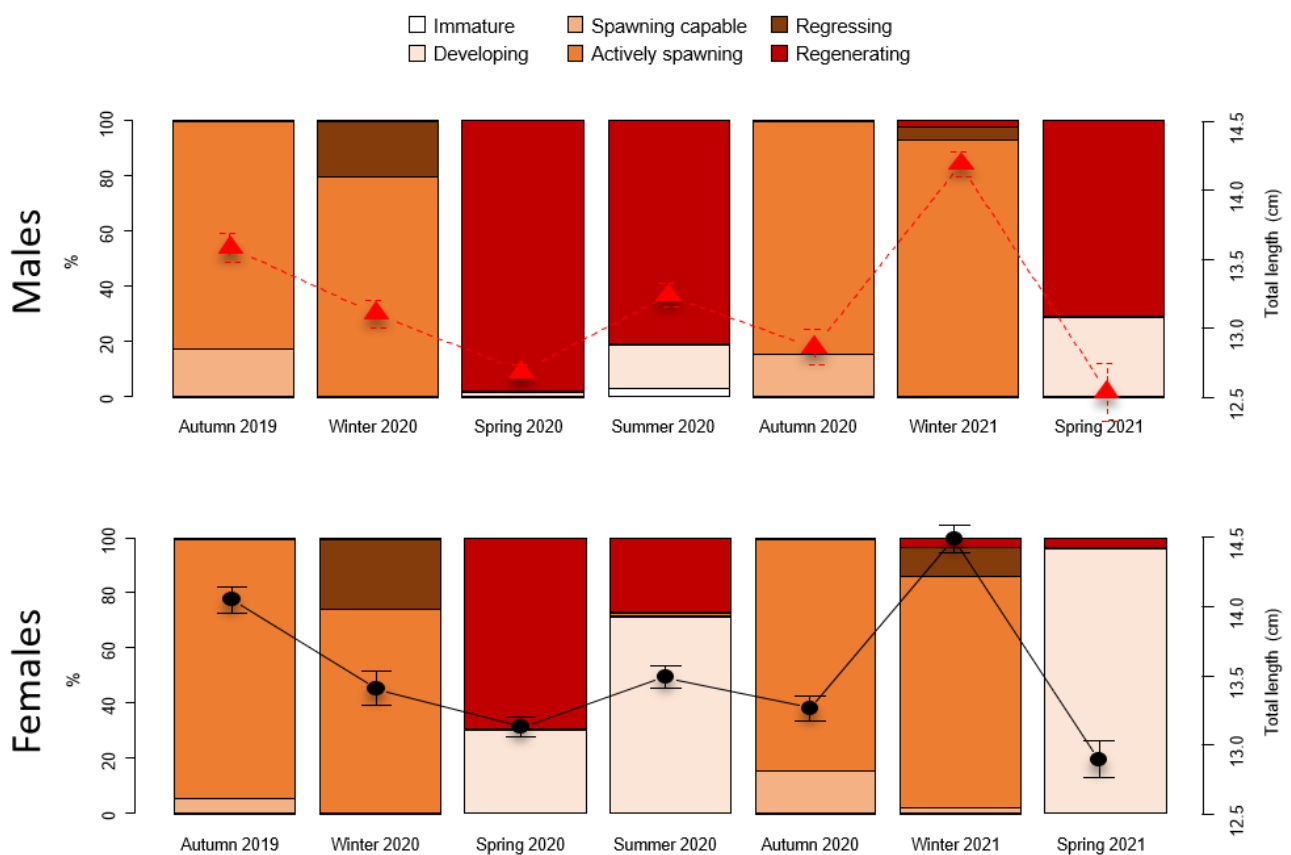


Figure 5. Seasonal analysis of the European sardine's (*S. pilchardus*) reproductive developmental stages in the Gulf of Trieste (North Adriatic). Graphs are presented by sex and reproductive developmental stage according to the classification of Brown-Peterson et al. [47]. The total length (cm) is also reflected for both sexes.

4. Discussion

Several studies suggest the significant effect of spawners' condition on reproductive potential (e.g., [1,3,55]), especially when it comes to the small pelagic species among which the sardine is found [4–6,56]. Therefore, it was considered important not only to estimate the reproductive period seasonality and/or the investment in reproduction (i.e., GSI), but also to assess the lipid storage, somatic condition, and health status of the fishing resource, the European sardine, in the study area.

The Northern Adriatic Sea system is one of the major chlorophyll hot spots in the Mediterranean Sea, and it has been recognized to depend on the water and nutrient discharge from the Po River and a dozen small rivers that flow into the Adriatic Sea north of the Po River delta and in the Gulf of Trieste (i.e., the Isonzo River [57]) [33], with around 40% of the chlorophyll production of the whole Adriatic [58]. These fresh water sources have a major impact on phytoplankton biomass due to the nutrients loads and to local upwelling events and eddies that contribute to spread the discharges offshore and enhance primary production [24]. In fact, it has been reported that the Northern and Central Adriatic are dominated by the pelagic compartment, with especial reference to plankton and small pelagic fish, among which the anchovy and sardine stand out [21]. Evidence of this influence of riverine inputs on the productivity of small pelagic fish has been provided in several studies [30,59,60], reinforced by the fact that sardine catches from inshore waters are generally in better condition than those from offshore waters [61]. However, there is variability linked to seasonality, as zooplankton abundance and feeding opportunities differ due to changes throughout the year. For example, in spring, the optimum feeding condition is inshore, while at the end of summer, the offshore offers larger amounts of zooplankton, which contributes to triggering migratory behaviour in sardines [21].

Direct energy flow from planktonic filter-feeding goes to gonadal development and egg production, implying that in addition to capitalized energy, sardines also use current income for supporting reproduction [62]. In fact, before the spawning period, storage lipids, as well as other nutritional compounds (such as proteins, vitamins, and minerals) in muscles, the liver, and visceral organs, are mobilized to the gonads to ensure maturation [22]. This coincides with the opposite patterns observed in our data between tissue fat and/or mesenteric fat content and GSI curve, which was also confirmed by correlation analyses. These opposite trends among fat reserves and gonad growth have been previously documented in various studies [5,63,64]. Nevertheless, the relative condition results obtained by Kn did not show an apparent relationship with GSI, contradicting our projections, as well as our previous observations [63]. However, they were in line with Campanini et al. [65], which suggests that Kn cannot be considered a good proxy for the energy density of sardines, while fat meter analysis appears to be a suitable method to evaluate the energy content of this species. Further, other authors have suggested that Kn data should be carefully analysed because it is population-/stock-specific [66]. In addition, we can comment that Kn varied from the reproductive season of 2019–2020 to 2020–2021, and we observed Kn average values under 1 in the former and over this figure in the latter winter period, with an increasing trend from the beginning of the sampling. These differences did not seem to be reflected in terms of GSI from one cycle to the other, although in this case, they coincided with more favourable conditions (i.e., a higher average concentration of chlorophyll-a in the area and lower average SST) in winter 2021 than that recorded in winter 2020.

Moreover, according to Ganas et al. [63], the seasonality of spawning did not match the variations of HSI during the sampling period, and no relationship was proven between HSI and GSI, as was also reflected by Somarakis et al. [28]. GSI values in our study were similar to those reported by Mustač and Sinovčić [61] in the Adriatic, since they were the highest when the lowest annual values of sea temperature were recorded. However, despite being correlated, GSI and chlorophyll-a showed a slight negative ρ correlation coefficient, while the proportion of productivity available to fish (OPFish) showed a greater correlation with GSI and with tissue and mesenteric fat content. This suggests that chlorophyll-a and fish were not directly related, but a higher chlorophyll-a concentration might be an indicator of favourable conditions for sardines [67,68]. This could be occurring because, as we should not forget, OPFish values are related to the concentration of chlorophyll-a since they are data derived from chlorophyll-a horizontal gradients [53], taking into account that which would be usable for a species such as the sardine.

In the present study, Figure 2A shows that at the times when amounts of chlorophyll-a are not very large, the proportion of available resources is quite high, occurring in the

months immediately prior to sardine spawning, and decisive for performance during the reproductive time. Moreover, it maintained a more stable and cyclical trend than general chlorophyll-a values throughout the sampling years. Thus, the amount of environmental chlorophyll-a seems to be reflected in K_n , although it does not necessarily translate into the GSI. Thus, the direct available resources (OPFish) (see Supplementary Materials Figure S1 as an example in the study area) seem to better illustrate the direct implication that resources have in reproductive terms, as well as SST, in line with Druon et al. [53], confirming that in order to project sardine production, the impact of abiotic factors (i.e., temperature) on reproduction should be taken into account, as they seasonally affect their distribution. The prevalence of oocyte atresia together with spawning incidence seem to be positively affected by water temperature, whilst the index of zooplankton production significantly correlates with relative fecundity [62]. In addition to the fact that temperature and food availability can shape the intensity of reproduction and quality of the eggs, thereby affecting reproductive potential, they could also condition the survival of the larvae [69]. The amount of yolk in an egg affects the time that larvae can survive without food, and so the effect of temperature on absorption rate should also be considered when relating larval survival to egg quality [70]. Therefore, special attention should be paid to the Gulf of Trieste, where sea surface temperature has increased $0.5\text{ }^\circ\text{C}$ over a period of almost four decades [57], and $0.36\% \text{ year}^{-1}$ in the Northern Adriatic [71].

Garrido et al. [72] commented that the muscle tissue of male and female sardines engaged in spawning showed no significant differences in total fatty acids concentration in sardines from Portugal (Atlantic Ocean). In this regard, our analysis showed that in general, females contained a higher muscle fat content, which was especially reflected during the spawning-capable phase, and during the actively spawning season, these differences were not significant. In this way, significant linear relations have been found between fatty acid concentrations in female sardine muscle and oocytes [73]. Moreover, significant differences between the sexes in GSI were identified only for active spawners (Figure 3A), with larger values observed in females. Our results are similar to those of Basilone et al. [12] for a study in the Central Mediterranean, although they differed from other studies in which males were identified as the individuals with the highest values of GSI [13]. According to Ganas et al. [2], isometric ovarian growth has been shown for all the developmental stages in sardines except for hydration, and so the relative weight of the ovaries (i.e., the gonadosomatic index, GSI) remains stable with body size, except in this phase (i.e., corresponding to hydration and ovulation in females [48]). A similar effect has not been previously analysed for gonad developmental phases in males [13].

Mesenteric lipid content and the total fat content showed similar trends and large positive correlations ($\rho = 0.81\text{--}0.83$), as supported by Mustač and Sinovčić [64] in the eastern Middle Adriatic, which could suggest that the accumulation of fat around viscera, within the muscle, and between skin and muscle takes place in parallel [13]. However, although no significant differences were seen between the sexes, we observed a slightly stronger negative correlation between mesenteric fat content and GSI in females than in males, and higher than the relationship among tissue fat content and GSI. While in males, ρ was equal to -0.5 for both variables, in females we detected a ρ of -0.56 for tissue fat content and a ρ of -0.65 for mesenteric fat. This result is expected because, as we have observed, the investment of females in the reproductive season in gonad growth is greater than that of males (Figures 2D and 3A), being more pronounced in the fall of lipids in these months (Figure 2B,C), and especially regarding mesenteric fat. Most of the energy destined to reproduction is accumulated in the viscera or mesentery [74]. While muscle is a more stable fat store, mesenteric fat is much more labile and likely to be the first fat store to become depleted during gonad maturation, as well as the first fat store to respond to increased food intake [75]. In fact, Krzeptowski [76] suggest that fat in viscera could reflect a much more intensive turnover of body- and energy-producing matter in females during spawning and after it, at the time of recovery of spent gonads. Therefore, the relationship between GSI and fat in females may be higher and even more pronounced when it comes to the

parameter of mesenteric fat. Given these results, our analysis contradicts the results of Somarakis et al. [28], who reported that fat storage stage does not substantially affect GSI. Although chlorophyll-a concentrations in the environment are more strongly related with tissue fat content than with mesenteric, available resources are similarly correlated with both types of fat reserves for both sexes. Observing Figure 2B,C, there was a monthly progressive increase after the reproductive period in tissue fat content, while mesenteric reserves increased rapidly in a short period of time, coinciding with the moment in which the vacuity index dropped from the spring to the beginning of summer to be mobilized rapidly in the development of the gonads. An interesting aspect to highlight is the different vacuity index found in the winter of 2021 (0%) with respect to that registered in the winter of 2020 (46.08%), although high inter-annual variability in feeding intensity has been previously recorded [77]. In fact, the recorded differences may be related to the fishing time, as it has been documented that during summer, *S. pilchardus* feed continually during daytime with a peak at around sunset, while during winter, high feeding rates occurred only in the early night [78], and so it is likely that the catch occurred right after the time of the food intake.

Moreover, sex-biased sex ratios towards females could be highlighted in our results, which were also observed by Zorica et al. [79] in the Eastern Adriatic Sea. The authors documented sardine spawning activity from the beginning of October until the end of April, with a main peak between November and February, over recent decades in the Adriatic Sea [80]. However, spawning sardine stock in the Northern Adriatic has been established by Nejedli et al. [81] from August–September to May. According to our data, in the Gulf of Trieste, we started to identify the bulk of active spawners before November, accompanied by a significant increase in GSI values which continued to grow at least until February. No active spawners were identified in subsequent sampled months. In May, only individuals in a state of regeneration and early development could be observed. In this way, we can deduce that our results do not differ in a global way from those in the Adriatic proposed by Zorica et al. [80]. Differences by sex in the percentage of developing gonads were observed in our study during spring (Figure 5), with more developing females compared to male individuals over the pre-spawning seasons. In spring in the North Atlantic Moroccan area, a higher percentage of females in a more advanced gonadal stage was also reported, even though the percentages of the different stages were equal in the reproductive season (autumn–winter) [82], as shown by our data. It is probably related to size, as Ganas et al. [63] reported that a smaller size may contribute to delayed gonadal maturation, coinciding with the significantly lower sizes for males in our study. Further, the faster recorded recovery of females compared to that of males regarding energetic indices, even after a previous greater investment, could be translated into a higher capacity of the gonadal development and oocyte maturation process.

5. Conclusions

In this study, we intended to shed light on the sardine's somatic condition due to its essential role in reproductive potential and performance within an environmental framework in a highly productive Mediterranean area, which was especially vulnerable to impacts (i.e., fishing pressure and high degree of enclosure, among other factors). Direct lipid measurement indices (tissue fat content and mesenteric fat scale) seemed to be suitable to be used to project their contribution to sardine reproduction after we found differences linked to sex, as occurred with the GSI parameter for active spawners, which was higher in females. In addition, reproductive cycle by sex showed a more advanced gonad maturation in females, although this became similar during the active reproductive season. Current and future threats to the stock status of sardines should foster to continuously monitor those determining indicators of health status and reproductive potential to manage the resource effectively. It is suggested to include environmental variables (i.e., SST, chlorophyll-a, and OPFish) in condition and reproductive studies, and to analyse their implications in sardine stocks over time.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/fishes7030105/s1>, Figure S1: Example of the available portion of productivity for the fish (OPFish; %) in months of the year 2020 in the study area (Gulf of Trieste).

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Institutional Review Board Statement: Ethical review and approval were not necessary since the fish used in this study were dead and obtained from the fishing sector destined for human consumption.

Data Availability Statement: The biological data presented in this study are available on request from the corresponding author. The data are not publicly available due to the fact that authors continue performing further analysis with the inclusion of new variables. Freely accessible environmental data can be found in the references mentioned along the manuscript.

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