Growth parameters, age and first maturity of *Sardina pilchardus* from the Moroccan Mediterranean coastline

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Abstract. Understanding the factors influencing fish growth is essential for estimating the exploited population's productivity, stability, and resilience. For *Sardina pilchardus* from the Moroccan Mediterranean, age, growth parameters and condition were estimated from 360 specimens during the period of December 2020 to October 2021. All samples were collected from purse seine catches of three ports of the Moroccan Mediterranean: El Houceima, Beni-Enser, and Ras el Ma. The age was determined using the Bhattacharya method which showed a dominant frequency of the 4-years length class. The growth parameters were estimated as $L\infty$=20.48 cm, k=0.44 $year^{-1}$ and t_0=-2.33 year for combined sexes. The total length of sardine samples ranged from 9.5 cm to 19.3 cm showing a negative allometry with an allometric coefficient b<3. The highest condition factor value was recorded in the summer while the lowest value was registered in winter. On the other hand, the highest hepatosomatic index was noticed in the winter season corresponding to the spawning period of *S. pilchardus*.
Key Words: age, condition factor, growth parameters, mediterranean sea.

Introduction. The European sardine, *Sardina pilchardus* (Walbaum 1792), is a pelagic species of the Clupeidae family. Like other small fish, it forms large schools that move in unison, as a defense mechanism against predators. It is one of the most important commercially exploited species in the Mediterranean, where it is caught by purse seine fishing with artificial lighting and pelagic trawling (Stambler 2014). The Moroccan waters are considered among the fishiest worldwide and the fishing sector in the country is the main engine of the economy with catches reaching more than one million tons, a Gross domestic product of 2 to 3% and nearly 700.000 direct and indirect jobs generated (Lakhnigue et al 2019). Morocco, with its double Atlantic and Mediterranean façade and a coastline that extends to 3,500 km of high marine biodiversity, has a large stock of halieutic resources and is considered both the leading producer and exporter of sardines worldwide (FAO 2016). In the north of the country, sardine fishing practiced by purse seiners is one of the most profitable activities contributing to the economy of the region given the high yield in terms of catches. In fact, sardine landings represent approximately 70% of all small pelagic catches (Lakhnigue et al 2019). Nutritionally, the sardine is an oily fish high in long-chain omega 3 fatty acids, endowed with excellent concentrations in vitamins including A and D. It is also a good source of protein and minerals such as calcium, iron and phosphorus necessary for health and nutritional balance (Benguendouz 2018; Šimat et al 2020; Tacon & Metian 2013). All these elements are important for the prevention of inflammatory and cardiovascular diseases. The aim of this study is to provide knowledge of the biology of *S. pilchardus* from the Moroccan Mediterranean by evaluating some aspects such as age composition, growth parameters, length-weight relationship and condition. This work provides information for better understanding the stock composition, since this species is, economically, very important and constitutes one of the main pelagic fish landed by fisheries. In addition, these results could contribute to the stock assessment and management of fisheries in the region.

Material and Method

Description of the study sites. Sardine sampling was carried out at the level of three ports in the Mediterranean Sea that extends over a coastline of 512 km (El Houceima Sea from the eastern region of the Moroccan Mediterranean, Beni-Enser Sea, and Ras el Ma Sea, Figure 1). Specimens were studied over a period of one year from December 2020 until October 2021 in three targeted sites according to a factorial schema 4×3, respectively for the season and area. The studied specimens' size ranged from 9.5 to 19.3 cm. The collected individuals were immediately stored in a cooler to the Laboratory of Agricultural Production Improvement, Biotechnology and Environment in Oujda's Faculty of Sciences (Figure 1).

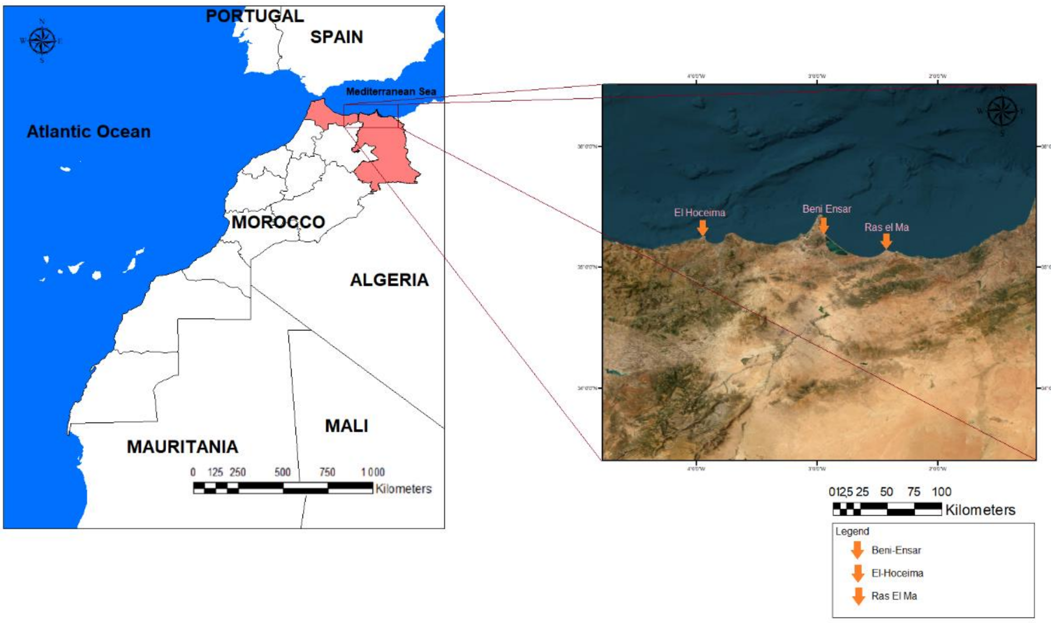

Figure 1. The geographical site of the three studied zones on the Mediterranean coast of northeast Morocco.

Biometric study

Fish measurements and weighing. For each specimen, at the laboratory, the total length (TL, cm) was measured using an ichthyometer, while the total body weight (TW, g) the eviscerated weight, and the liver weight were measured using a 3-decimal precision balance.

Fish dissection. The fish dissection was carried out using stainless steel scalpels. The gonads and the viscera were gently removed and separated. Based on the gonads' color and morphology, sex identification was determined by macroscopic observation.

Data processing. The data were organized by season and gender to determine parameters such as the condition factor, sex ratio, the length-weight relationship, aging and growth estimation, and length at first maturity.

Condition factor. Fulton's condition factor (K) was calculated according to the following equation (Simon et al 2013):

$$k = \left(\frac{TW}{TL^3}\right) \times 100$$

Where:
TW - total weight (g);
TL - total length (cm).

Hepatosomatic index. The hepatosomatic index was calculated according to Pardoe et al (2008) and Nunes et al (2011) by liver weight and body weight ratio using the formula below:

$$HSI = \left(\frac{LW}{GW}\right) \times 100$$

Where:
LW - liver weight in grams;
GW - gutted weight in grams.

Sex ratio. The sex ratio was determined with the formula:

$$SR = M/F$$

Where:
M - number of males;
F - number of females.

Length-weight relationship. The relationship between fish size and weight was determined according to Ricker (1975) using the following equation:

$$TW = aTL^b$$

Where:
TW - total weight in grams;
TL - total length in cm;
a - constant;
b - coefficient of allometry.
The degree of association between variables was calculated by the determination coefficient (R^2).

Aging and growth estimation. In population dynamics, the Von Bertalanffy (1938) model is used to mathematically express linear growth. This model takes into account both the age of recruitment and the age at first capture, according to the equation (Daget & Le Guen 1975):

$$L_t = L_\infty \left(1 - e^{-k(t-t0)}\right)$$

Where:
Lt - length of fish at time t (in year);
L∞ - the asymptotic length that a fish would expect at the theoretical infinite age;
K - coefficient representing the metabolism of fish. It is the slope of adjustment between size and instantaneous increase in size;
t_0 - the theoretical age at which the fish would be zero in size (the curve cuts the abscissa axis), but at birth the larvae or juveniles already have a length that is not zero.

The logarithmic form of the previous equation, using the theoretical age at length zero, t_0, longevity t_{max} and the growth performance index Φ, is:

$$Log(-t0) = -0.3922 - 0.2752 Log\, L\infty - 1.038 LogK \text{ (Pauly 1979)}$$

$$tmax = t0 + 2.996/K \text{ (Pauly 1983)}$$

$$\Phi = logK + 2 \log L\infty \text{ (Pauly \& Munro 1984)}$$

Age was determined using FISAT II by applying the Bhattacharya's method (1967), depending on the length frequency data (Gayanilo & Sparre 2005).

Length at first maturity. Length at first maturity L_{50} is the length at which 50% of the fish have reached their maturity. It was estimated using the expression

$$L_{50} = (2\ L_{\infty})/3$$

Statistical analysis. Experimental results were presented as means±standard deviation of triplicate determinations. The statistical analyses were achieved using the software SPSS version 20 and considering the allometric coefficient b as a source of variation. The means were compared using the t-test and the significance was declared at $p<0.05$.

Results

Sex ratio. In total, 360 specimens of *S. pilchardus* were analyzed over a period of one year from December 2020 until October 2021. The results show that the studied population is composed of 78.52% of males and 21.47% of females.

Length frequency distribution. Figure 2 shows the length frequency distribution for the assessed fish species. We have noticed that the class size of 13 to 14 cm showed the highest frequency distribution, followed by the length of 17 to 18 and that of 11 to 12 cm. The highest length recorded throughout the study period was 19.3 cm. TL of males ranged from 9.5 to 19.1 cm, and TW from 7.99 to 59.36 g. For females, the TL ranged from 13 to 19.3 cm and TW from 25.68 to 54.04 g.

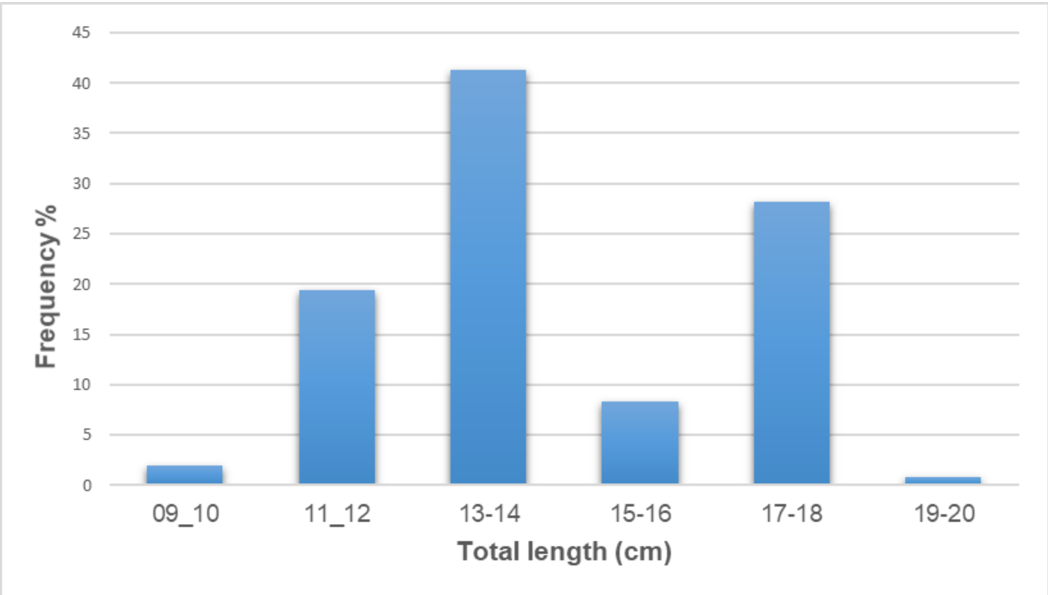

Figure 2. Length-frequency distribution of *Sardina pilchardus* from the Mediterranean coastal area of Morocco.

Length weight relationship. The relationship between total length (cm) and total weight (g) values of all samples is shown in Figure 3. A high determination coefficient (R^2) was obtained between TL and TW, 0.93 in pooled sexes, 0.76 in males, and 0.92 in females respectively (Figure 3). In addition, growth showed a negative allometric pattern in the combined sexes as well as the males and females as the value of b was different from the isometric "3". The t test allows us to conclude that at a significance level of 5%, b is different from the value 3 and therefore the growth is negative allometric.

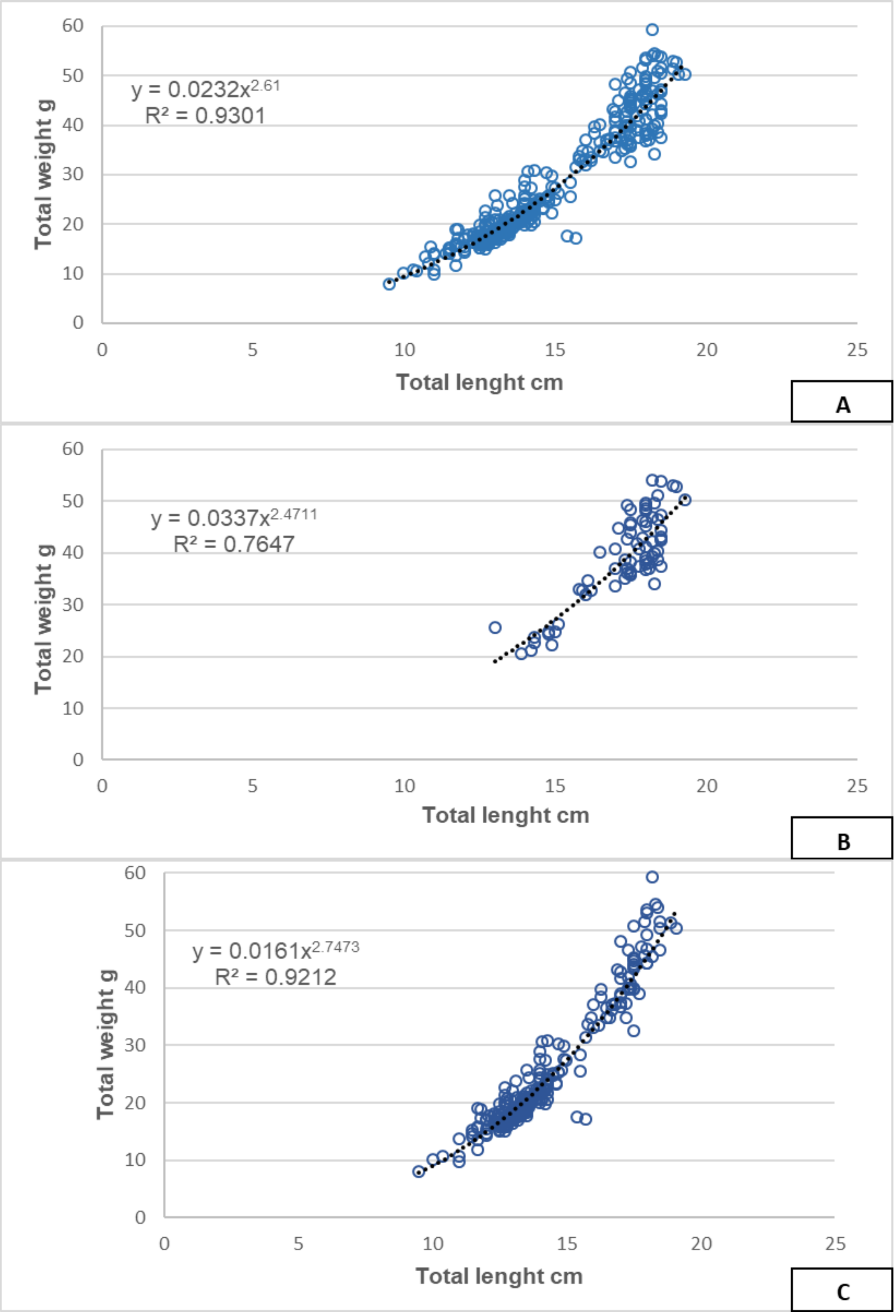

Figure 3. Length-weight relationship of *Sardina pilchardus* from the Mediterranean coastal area of Morocco (A: combined sexes, B: females, C: males).

Aging and growth estimation. The length frequency data of *S. pilchardus* was analyzed by Bhattacharya (1967) method using FiSAT II software for combined sexes. Four modal length classes (year classes) were determined (Table 1, Figure 4). These modal length classes for *S. pilchardus* were 11.66, 13.41, 16.11, and 17.78 cm with a separation index greater than 1.5 (Figure 4). Table 2 summarizes the results of aging. Ages 2 and 4 were the most present with 54.2% and 29.27%, respectively. The minimum catch was observed in 3-year-old individuals (5.42%).

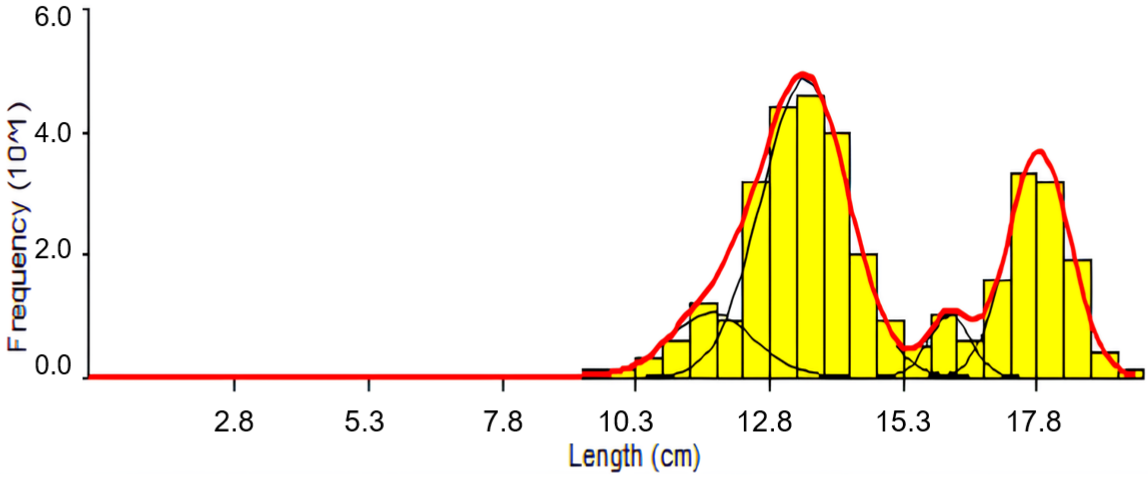

Figure 4. Length frequency of *Sardina pilchardus* using Bhattacharya's method.

Table 1
Mean length, standard deviation (SD) and separation index (S.I.) for each age group of *Sardina pilchardus* obtained by Bhattacharya's method

Age (Years)	*Mean length (cm)*	*SD*	*Population (Individuals)*		*S.I.*
			N	*%*	
1	11.66	0.780	41.00	11.11	N.A.
2	13.41	0.820	200.00	54.2	2.190
3	16.11	0.390	20.00	5.42	4.460
4	17.78	0.580	108.00	29.27	3.440

The growth parameters were obtained using the ELEFAN I method and the results showed that L_∞ for pooled sex was 20.48 cm, and the value of annual growth rate k was 0.44 year^{-1}, while t_0 was calculated at -2.33 year. The parameters of Von Bertalanffy's model aided in constructing the absolute linear growth. The maximum age (t_{max}) for *S. pilchardus* was found to be 4.47 years and the growth performance index (φ') was estimated at 2.26 (Table 2).

Table 2
The von Bertalanffy growth parameters calculated for *Sardina pilchardus* from the Mediterranean coastal area of Morocco

	L_∞ (cm)	*K (year^{-1})*	*t_0 (year)*	*φ'*	*t_{max} (year)*
Combined sexes	20.48	0.44	-2.33	2.26	4.47

Length at first maturity. The length at first maturity is defined by the length for which 50% of individuals in a population are sexually mature during the breeding period. The sexual maturity threshold is fixed at stage III which corresponds to the beginning of the gonad development phase (Mounir et al 2020). The calculation of L_{50} was based on data for both sexes combined. The L_{50} obtained was 13.65 cm. According to Table 2, the age at first maturity is 2 years.

Condition factor. This factor is suitable for comparing the condition of fish of the same species in different seasons or places or for different sexes. The condition factor reflects therefore the ecological and physiological conditions (Belveze 1984). The highest condition factor value was recorded in the summer while the lowest value was registered in winter, Figure 5.

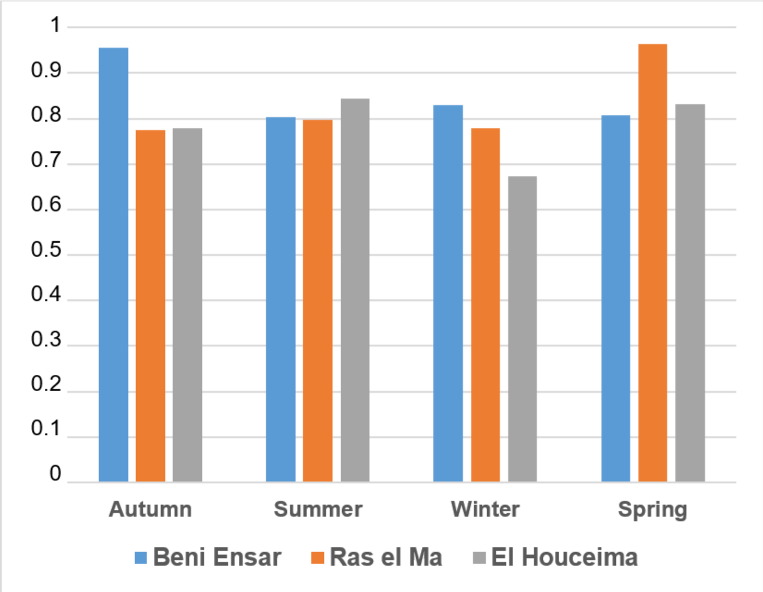

Figure 5. Condition factor of *Sardina pilchardus* from the Moroccan Mediterranean coastline according to the area and season.

Hepatosomatic index. The highest hepatosomatic index was noticed in the winter season which corresponds to the spawning period of *S. pilchardus* (Figure 6).

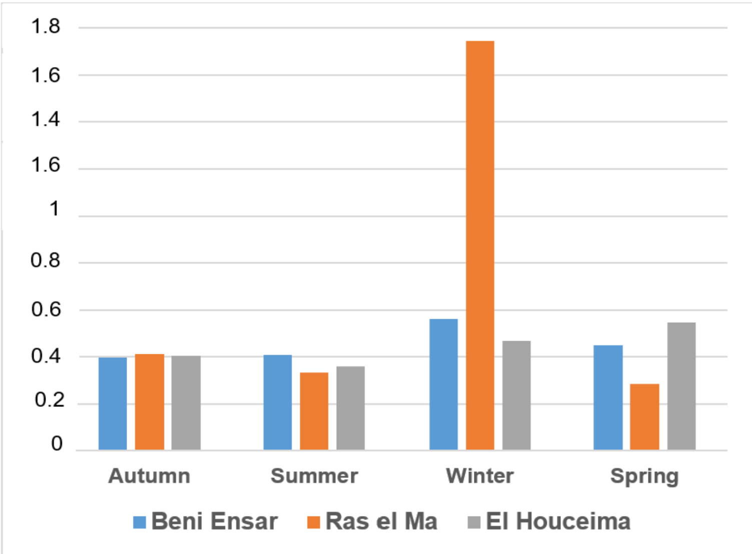

Figure 6: Hepatosomatic index of *Sardina pilchardus* from the Moroccan Mediterranean coastline according to the area and season.

Discussion. The numerical distribution of the sexes or sex ratio is an important biological index because the proportion of males and females can affect the reproductive success of the species. As seen, the population consisted of 78.52% of males and 21.47% of females. The sex ratio was higher than 1 and was skewed in favor of males. Similar results have been reported in the Senegalese coasts (Fréon & Stéquert 1979) and Aegean Sea (Cinahgir 1996), while females predominate in the Moroccan Atlantic coast (Abderrazik et al 2018; Amenzoui et al 2006). This dominance could be explained by several factors such as environmental factors and the non-identical spatial distribution of the two sexes. The sex ratio provides information on the gender balance within a population. It was calculated from individuals of known sex, because in *S. pilchardus*, there is no sexual dimorphism. Although the sex ratio in most species is close to 1, this may vary from one species to another differing from one population to another of the same species, and may also vary year after year within the same population (Nikolsky & Birkett 1963). In our study, the sex ratio is much higher than 1. This could be due to pollution exposure, and environmental factors such as climate change. In fact, high-temperature exposure can influence the genetic sex of the individual. At present, there are more than twenty species of fish for which temperature has a proven effect on sex, and this includes species without distinct sex chromosomes such as bass and zebrafish which are so-called polygenic species (Geffroy et al 2021). Temperature can also determine the sex of certain species (we speak of Temperature Sex Determination, TSD), without major effects of genetic background. Thus, in the same way, that turtles or crocodiles respond to temperature, certain species of fish can adapt their sex to the environment. In more than 95% of cases, the warmer the water temperature, the more masculinized individuals are (Geffroy & Wedekind 2020). Other factors, such as the density of individuals and the food quantity and quality, can also influence. In fact, in many species, abundant food (a low-density population) favors the predominance of females. On the other hand, poor food (a high population density) increases the rate of masculinity. The pH of water can also affect the sex of fish. Furthermore, the significant dominance of males could be explained as well by the fact that adult females in intense reproductive periods are found in depths that are not covered by commercial purse seiners (Amenzoui & Baali 2018). There is an allometric relationship between fish size and weight in the majority of species (Keznine et al 2020). This relationship is influenced by the availability of food, the development of the gonads, and reproduction (Mounir et al 2020). It is frequent in fishing biology to estimate the modifications that growth can cause to the morphology of the species. It also makes it possible to follow, depending on the size, the changes in sex, and the maturity of the gonads. In this study, a total of 360 specimens of *S. pilchardus* were studied. The Sardine size of captured fish ranged between 9.5 and 19.3 cm. The difference in sizes is due to age, sex, gonadal development, and gear selectivity. Depending on the species the allometric coefficient b can be between 2.5 and 4 (Daget & Le Guen 1975). If b is equal to 3, the growth is isometric: the gains in weight and length are proportional. If b is different from 3, the growth is allometric (minor if b is inferior to 3, major if b is superior to 3 (Znari & Mounir 2021). In the current study, the exponent b of the length-weight relationship in each sex (b=2.471 for females, b=2.747 for males) showed that weight increased with length following a negative allometry pattern. The b values in our study were found to be close to the Edremit Bay in Turkey (Erdoğan et al 2010) and to the Adriatic and Ionian Seas (Mustać et al 2020; Petrakis & Stergiou 1995; Koutrakis & Tsikliras 2003; Mustac & Sinovcic 2007). However, they were different from the results found in other studies with positive allometry in Tunisian waters (Khemiri 2006) and the Adriatic Sea (Mustać et al 2020). The b values differ depending on sex, age, food availability, and seasons. In addition, the b growth exponent can be affected by environmental conditions, growth increment, and hydrological environmental conditions (Sinovcic 2000). The age range during the study period didn't exceed 4 years. However, in the Mediterranean Sea, age ranges have displayed higher ages reaching up even 8 years (Tsikliras & Koutrakis 2013). In the oceanic waters, sardines have reached ages of up to 13 years and have shown greater lengths than in the Mediterranean with total lengths of 24 to 27 cm (Silva et al 2008). Yet, in recent years, a contraction of the age classes was observed in individuals not older than 4 years in sardine stocks across the Mediterranean Sea with a decline in landings and overexploitation of this resource (FAO 2016). A comparison with the available data for this species showed similar results in the Algerian eastern coast (Dahel et al 2016). However, lower results were reported by different authors (Basilone et al 2023; Erdoğan et al 2010). In return, higher results have been registered in the Moroccan west Mediterranean (Keznine et al 2020). According to Ricker (1975) and Tıraşın (1993), this difference could be due to the environmental factors, fishing pressure and differences in the pelagic habitat.

Growth parameters (Von Bertalanffy's parameters) of sardine in combined sexes were L_∞=20.48 cm, k=0.44 year^{-1}, t_0=-2.33 year. These results are comparable to some, higher or lower than others and this could be explained by the difference in sampling regions and periods, the difference in the numbers of sampled fish, or in the used reading methods such as otolithometry or scalimetry (Keznine et al 2020), as shown in Table 3. This may be explained by the strategy and the period of sampling, the size of the largest fish individual (L_{max}), the sample size and the biological features. Thus, we can assume that the sampling carried out in this study was well representative given that we have a wide range of lengths. Besides, L_∞ =20.48 cm estimated in this study is realistic since the largest specimen sampled is 19.3 cm.

Table 3

Comparison of growth parameters of sardine with the literature

Region	*L∞*	*K*	*T0*	*Age*	*References*
The current study	20.48	0.44	-2.33	1-4	The current study
Adriatic Sea	20.5	0.46	-0.5	1-8	Sinovčić et al (2008)
Sicily Italy,	18	0.459	-1.99	0-3	Basilone et al (2023)
Adriatic Sea	19.71	0.286	-1.82	1-5	Mustać et al (2020)
Aegean Sea	15.23	0.47	-1.21	1 3	Erdoğan et al (2010)
Moroccan Mediterranean	20.21	0.47	-1.93	1-6	Keznine et al (2020)
Atlantic Ocean (Agadir region)	22.640	0.33	-	1-3	Abderrazik et al (2018)

L_∞-Asymptotic length that a fish would expect at the theoretical infinite age; K-Condition factor; t_0-The theoretical age at which the fish would be zero in size.

The estimated growth performance index of *S. pilchardus* in this study is Φ=2.26. This value is in concordance with the literature on Atlantic and Mediterranean coasts (Silva et al 2008; Erdoğan et al 2010) where there were reported slightly lower values in the Aegean Sea. According to Boudjadi & Rachedi (2021), variations in the growth performance index could be due to the methodological approaches adopted for the calculation, sampling data, period of sampling, sample size, as well as the size of the largest individual, food, and changes in the environment. Fulton's condition factor (CF) is used to determine a fish's physiological state, including its reproductive capacity and sexual maturity, as well as the environmental influence (degree of nutrition) on the species (Costa 2019; González-Kother et al 2020). It indicates the changes in food reserves, the general fish condition and its well-being. CF is an indicator of the physiological state of the fish, it reflects the seasonal accumulation and depletion of energy (Gou et al 2023). Its seasonal monitoring makes it possible to deduce a strategy of energy intake. In fisheries ecology, it is used to assess the quality of habitats. If they are of good quality, they allow optimal growth and survival (Gibson 1994). In fact, environmental conditions, in particular temperature, as well as the quality and quantity of food directly influence growth (Mugwanya et al 2022). Our findings in CF were reasonable given that the highest values were observed in summer and this would be due to the food availability resulting from high planktonic production in this season (Amenzoui 2010). Indeed, the sardines feed intensively in summer and store these energy reserves which will be progressively granted to maturation and to the gamete reproduction in adults. It is during the period of sexual rest, during the spring, summer and autumn seasons, that the sardine feeds the most and increases its somatic weight. Several studies in the Mediterranean and Adriatic Sea (Caballero-Huertas et al 2022a; Frigola-Tepe et al 2022) and in the Atlantic Ocean (Caballero-Huertas et al 2022b) have shown that the evolution of the CF varies in the opposite direction to the gonadosomatic index. Sardine therefore uses muscle lipid reserves to develop gametes. In sardines, the storage of lipids takes place in the muscles, inside or between the muscle fibers (Caballero-Huertas et al 2022a). On the other hand, it is minimal in winter when the zooplankton is less abundant; the fish therefore eat little and the emission of gametes contributes to weight loss. This period corresponds to the reproduction period of the Moroccan Mediterranean sardines (Keznine et al 2020).

The seasonal evolution of the hepatosomatic index shows a maximum value in winter (reproduction period of the Moroccan Mediterranean sardine) and remains low during the other seasons (period of sexual rest), which shows that the liver does not intervene in the transfer of lipid reserves necessary for vitellogenesis (Amenzoui 2010). These results confirm the classification of sardines among oily fish characterized by lipid accumulation in the muscles and by a deposit of mesenteric and subcutaneous fat (Amenzoui 2010).

On the Mediterranean coast, the size at first sexual maturity was estimated as 13.65 cm for the combined sexes. According to the Figure 2, this is the most exploited size range and that could pose a threat to the sardine population in the Moroccan Mediterranean. Several studies carried out on size at the first maturity of sardines show the differences in maturation from one region to another. On the Mediterranean coast, similar values were reported by Keznine et al (2020) with an L_{50} of 13.29 cm for combined sexes. On the Atlantic coast in Agadir region, similar results were recorded as well with an L_{50} of 13.13 cm for both sexes. In Laayoune Region, Amenzoui et al (2006) reported higher values with an L_{50} of 15.8 cm for both sexes (23-27), while on the Tunisian coasts, lower values were registered by Khemiri (2006), with an L_{50} of 11.3 cm for both sexes. These differences in maturity length within the same species in different regions could be explained by genetic factors. Changes in the environment and food availability can also be an influencing factor (Neves et al 2023; Véron et al 2020).

Conclusions. The current study provides information about growth parameters, age at first maturity and condition of *S. pilchardus*. This species has an important commercial value and represents one of the main fish products on both the Mediterranean and Atlantic coasts. The age was determined using the Bhattacharya method which showed a dominant frequency of the 4-years length class. The growth parameters of the Von Bertalanffy equation that characterize sardines from the Moroccan Mediterranean region as well as those related to the length-weight relationship are respectively, for combined sexes, $L\infty$=20.48 cm, k=0.44 $year^{-1}$ and t0=-2.33 year; a=0.0161 and b=2.74. This information is important not just for improving our understanding of *S. pilchardus* biology, but also has practical implications for a better stock status assessment. However, more research is needed to get a more thorough knowledge of this species. These future studies should cover a larger range of factors, including reproductive behaviors and ecological interactions. We can refine our understanding and increase our efforts to protect *S. pilchardus* by diving further into its biology for the benefit of both marine ecosystems and sustainable fisheries management.

Conflicts of interest. The authors declare no conflicts of interest

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