



Spatial variability of linear growth of *Sardina pilchardus* (Walbaum, 1792) from the Moroccan Atlantic coast by using otolithometry

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ABSTRACT

The present work provides a comparative study of the linear growth of sardine, *Sardina pilchardus*, in the Moroccan Atlantic coast. Sardines with medium and large sizes were the most exploited. Thus, the fishing effort is geared towards adults; high mortality rates are mostly recorded among young adults in the age groups 2+ and 3+ years. This study was conducted to answer the problematic state of the spatial variability of the sardines of the Moroccan coast by detecting the readings of age from the otoliths. Results showed that sardine has a significant linear growth during its first year of life, then growth speed decreases with the increase of age. With respect to their linear growth rate, the specimens under study can be separated into three zones from north to south; namely, 'Larache' 'Safi' and 'Dakhla'. Von Bertalanffy's equations were presented for each zone. In conclusion, this study remarkably showed the existence of a spatial variability of the linear growth of sardines of the Moroccan littoral. It divides the Moroccan coast into three zones; from north to south.

INTRODUCTION

Sardina pilchardus (Walbaum, 1792) is one of the most important species in terms of catches and biomass in Morocco (Amenzoui, 2010). The total catches in the year 2015 was 850 000 tons (MAFRDWF, 2018). Thus, Morocco is ranked the first world producer of the sardine of superior quality and a leader in the international market of the canned sardine. This pelagic species can play a structural role in the dynamics of the marine ecosystem.

The dynamic population models used in the management of fishery resources and the biological studies of fish require age data to determine the composition, based on the age of the catch and the growth rates (**Ricker, 1973**). The age determination is traditionally carried out from the seasonal growth marks formed in the calcified tissues “scales, bones, fin rays [and] otoliths” (**Williams & Bedford, 1974**). Given the correlation between fish size and bony structure size, it is possible to determine the growth rates by measuring the distances between successive growth structures. The seasonal variations of growth can be studied as a function of the environmental conditions as well as the involved genetic stock (**Campana, 1987; Carlander, 1987**).

Since the 1970s, with the development of a relatively cheap computer equipment, the implementation of semi-automatic measuring programs and age determination based on the otoliths (**Mason, 1974**) or growth rings (circuli) in scales were both initiated (**Troadec & Prouzet, 1986; Gandelin & Laval, 1987; Gunn et al., 2008**). The discovery that growth structures were formed with a daily periodicity in the otoliths (**Pannella, 1971**) exponentially increased the use of the daily growth increments in the study of growth variations (**Gutiérrez & Morales-Nin, 1986; Cermeño et al., 2003**), and recruitment (**Marin et al., 2003**). In addition, the relationship of environmental parameters and survival (**Campana et al., 2001**) was also considered. Evidently, the problems of age determination increased when growth structures with an annual periodicity was substituted with daily structures. Another characteristic of the otoliths is that their shape and relative size are specific to each species (**Gaemers, 1976; Nolf, 1989; Lombarte & Morales-Nin, 1995; Assis, 2003**), implying a genetic regulation in their formation. Consequently, the morphological study of otoliths can be very useful in the classification of species and the determination of phylogenetic lines (**Lombarte & Castellón, 1991; Lombarte et al., 2018**).

Statistical methods still depend on the analysis of morphometric distributions for regularly sampled populations (**Daget & Le Guen, 1975; Do Chi, 1977; Vasconcelos et al., 2018**). Statistical analysis requires a Gaussian distribution of the measured variables for individuals born at the same time and large numbers. Therefore, it is particularly sensitive to the high variability of the corresponding values.

The methods of estimating individual age are numerous since fish growth is continuous, and the rate of metabolism of these poikilothermal vertebrates is a function of biotic and abiotic environmental conditions (**Vitale et al., 2019**). The calcified parts of the fish behave like recorders of the variations of the growth, theoretically potential to account for the "curriculum vitae" of the animal at any time. Applications of age studies require an accuracy in the baseline data (for instance, in establishing growth curves). In age studies, the researches will be satisfied with otolithometry as age estimation technique in sardines, the knowledge of the rhythms of appearance of the growth marks on the mineralized pieces is a necessary condition for their interpretation in terms of age,

and what is termed "validation" is still too often dismissed by ichthyologists (**Beamish & Farane, 1983**).

One of the most remarkable advances in fish age estimation studies from the 1970s is probably the discovery of the free daily growth zones on otoliths (**Pannella, 1971; Beamish & Farlane, 1985**). Some applications are still subject to many criticisms, such as the use of backscatter techniques or the calculation of growth rates (**Moreau, 1987; Francis, 1990**). Another way of a current research on skeletochronology concerns is the comparisons between the growth marks of several calcified pieces (**Hunter *et al.*, 2018**).

Because of the development of computer tools, image analysis techniques have opened new perspectives for reading the calcified parts. Digital image processing was initially viewed as a tool for reading assistance (**Mason, 1974; Mc Gowan, 1987; Dutta *et al.*, 2016**), but more specialized fisheries monitoring laboratories have considered using it as a tool of automatic reading.

Hence, the interest of this work was to test the homogeneity in terms of growth of the Moroccan sardine for the evaluation and the adoption of an adequate strategy for the rational management and the sustainable use of sardine fisheries. It is therefore interested in highlighting the differences between the linear growth of sardine as a function of space, while studying the causes and consequences of this spatial variability.

In order to better implement a fisheries management strategy, it is important to properly identify management units since the lack of knowledge of population structure complicates and reduces the quality of management plans. It is within this framework that this work's objective is to examine the structure of sardine population and highlight the existing spatial variability in the linear growth of sardine of Moroccan coastline.

MATERIALS AND METHODS

1. Sampling

Sampling of sardines from commercial purse seine catches was carried out at the main Moroccan Atlantic ports: Larache, Safi and Dakhla (Fig. 1) in April 2018. Thus, fisheries monitoring concerned 2500 specimens collected of *S. pilchardus*, taking into account all size frequencies. This sampling covered almost the entire sardine size, ranging from 8 to 22.5 cm. After their extraction, the pairs of otoliths of each specimen were placed in listed small tube. In this study, researchers retained 2235 right otoliths with no crystallization or damage (Table 1).

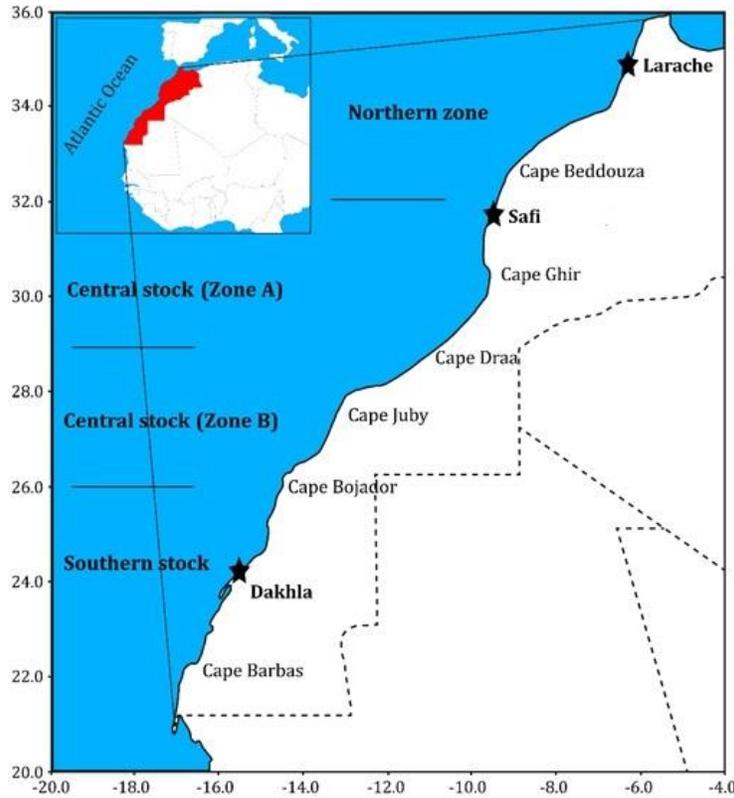


Fig. 1. Locations of the sampling sites and delimitation of areas of the three stocks of *S. pilchardus* of Atlantic Moroccan

Table 1. Number of observed and valid otoliths of *S. pilchardus* collected from different Moroccan Atlantic ports

Port	Observed otoliths	Valid otoliths
Larache	450	415
Safi	1360	1300
Dakhla	572	520
Total	2382	2235

2. Preparation and reading otoliths

Selected otoliths were mounted in inclusion in the "EUKITT" polyester resin on black plates and hollowed with numbered cells. The reading of sardine age was based on binocular observation of growth streaks of whole otoliths mounted on black platelets. For each sampled individual the age, the edge (opaque or hyaline), the outer diameter of the otoliths, and the corresponding distances for each ring (A_t) were noted. A number of 2235 otoliths were observed to carry out this study. In order to make the best possible estimate of age and reduce possible otolith analysis errors, three readings were taken by three different observers. The age adopted is the one that shows a better agreement between different readings.

3. Linear growth

In population dynamics, the model of **Von Bertalanffy (1938)** was chosen to express mathematically the linear growth. This model adjusts both from the age of recruitment and the age at first capture. In addition, it is the easiest to integrate into production equations (**Daget & Le Guen, 1975**). Its expression is as follows:

$$L_t = L_\infty \{1 - e^{-k(t-t_0)}\}$$

Where:

L_t: Length of fish at time (in year).

L_∞: 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. In the derivative, K sets the mode of decreasing growth rate as size increases.

T₀: 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 test used to compare the growth parameters of the same species of the same stock or different stocks are the phi-prime test (Ø') (**Pauly & Munro, 1984**) designated by the following expression:

$$\text{Ø}' = \log_{10} k + 2 \log_{10} L_\infty$$

K is expressed on an annual basis and L_∞ in cm. This test is based on finding of **Pauly (1979)** that phi-prime values are very similar within neighboring taxa and have narrow normal distributions (**Sparre & Venema, 1996**).

4. Statistical analysis

The statistical software XLSTAT was used to perform all calculations. Analysis of covariance (ANCOVA) was used to compare the linear growth in different areas. The ANCOVA is performed when some of the explanatory variables are discontinuous, while others are continuous. The model is composed of both factors and regressors. Researchers investigated if the relations between the variable to be explained (size) and the continuous explanatory variables (age) remain the same for all the modalities of the discrete explanatory variables (port).

A two-way ANCOVA was considered; the dependent variable is the size and it varies according to age and port that form the explanatory variables.

RESULTS

1. Location of the first ring

The sardine has a very long growth and weight in its first year of life for it reaches 90% of its size during this year. Hence, it is crucial to determine the location of the first ring that reflects the influence of environmental conditions on sardine growth.

The current results revealed that the measurements of the locations of the first ring (A1) were 1.1, 1.19 and 1.41 mm for the specimens of Larrache, Safi and Dakhla, respectively (Table 2). Differences between the distances at the first ring were determined; Dakhla is distinguished by a distance A1 higher than those of Larache and Safi ports.

Table 2. Range (minimum and maximum) and Mean \pm standard deviation of the first ring (A1, mm) in otoliths of *S. pilchardus* from the Moroccan Atlantic coast

Locality	N	Mean \pm SD	Minimum	Maximum
Larache	415	1.10 \pm 0.14	0.76	1.46
Safi	1300	1.19 \pm 0.3	0.94	1.51
Dakhla	520	1.41 \pm 0.17	1.08	1.87

2. Waist-age keys

Otolith reading and annual mark counting allowed each size class to obtain a pair of data, number of size-marks that correspond to an age-length pair, in addition to the age-length keys (age is expressed in years and total length in mm). The latter were determined for each port, taking their average date of birth on the first day of January.

2.1. Larache

The youngest sardines sampled in the port of Larache belong to age group 0⁺. The oldest are 4⁺ years old. The samples were mainly individuals aged 2⁺ and 3⁺, representing 43.37% and 29.40% of the total number of Larache's specimens. It was found that the growth rate of female specimens is higher than that of male specimens at age 3⁺ and 4⁺ years (Table 3).

2.2. Safi

The individuals caught in the port of Safi were from 0⁺ to 6⁺ years of age. The majority are in both classes; 2⁺ (36.38%) and 3⁺ (20.31%) years. Young specimens belonging to age classes 0⁺ and 1⁺ make up 29.92% of all individuals. While, the older ones fit the age classes of 4⁺, 5⁺ and 6⁺ years, representing 13.39% of the total number (Table 4).

2.3. Dakhla

The key to height-age shows that at the Dakhla level, the youngest and small-sized individuals belong to age class 0⁺, while the larger individuals are part of the 5⁺ and 6⁺ year-old classes. Thus, it was observed that individuals aged 2⁺ and 3⁺ are the most

abundant with 43.34 and 27.95% of the total number of individuals, respectively. While, the older ones that fit in age classes of 4+, 5+ and 6+ years are represented by 12.95% of the total (Table 5).

2.4. Key size-age in different port's samples

In total, 2382 pairs of otoliths from *S. pilchardus* have been examined. The counting of the rings, detecting the growth judgments on the otoliths allowed estimating the fish age for males and females separately. The key size-age shows that the age group 2 is the most dominant in males and females, respectively with: 48.51%, 165.6 mm and 36.67%, 169.7mm, in Larrache, which is similar for males in Safi 43.87% with 189.5 mm in the average size. However, the age group 3+ was dominant for females: 30.12% and 208.6 mm in the average size. On the other hand, the age group 2+ was the most dominant in males and females in Dakhla fishing area and represent about 43%, and the average size is 185mm for males in the age group 2+. Comparatively, the average size for females in the same group is 205.2 mm (Tables 3, 4 and 5).

Table 3. Size- age key for specimens of *S. pilchardus* collected from Larache port in Moroccan Atlantic coast

Sex	Age (years)	Fish number		Fish length at time (L_t) in mm		
		N	%	Mean \pm SD*	minimum	maximum
Males	0+	7	2.98	154.7 \pm 2.5	145.8	170
	1+	14	5.96	161.1 \pm 4.1	170	185
	2+	114	48.51	165.6 \pm 6.7	175	190
	3+	76	32.34	176.2 \pm 4.9	190	200
	4+	24	10.21	177.5 \pm 5.2	170.6	200
Total		235	100	169.3 \pm 4.3	145.8	200
Females	0+	2	1.11	154.1 \pm 4.5	142.5	169.5
	1+	47	26.11	161.6 \pm 6.3	154.5	180
	2+	66	36.67	169.7 \pm 7.1	166	193
	3+	46	25.56	181.7 \pm 7.7	176	195.4
	4+	19	10.56	194.8 \pm 3.1	190	205
Total		180	100	174 \pm 5.8	142.5	205
Whole	0+	9	2.17	154.3 \pm 3.2	142.5	170
	1+	61	14.70	161.2 \pm 4.8	154.5	185
	2+	180	43.37	167.5 \pm 6.9	166	193
	3+	122	29.40	179.1 \pm 6.3	176	200
	4+	43	10.36	187.6 \pm 4.1	170.6	205
Total		415	100	171.6 \pm 4.8	142.5	205

*SD : Standard deviation

Table 4. Size- age key for specimens of *S. pilchardus* collected from Safi port in Moroccan Atlantic coast

Sex	Age (years)	Fish number		Fish length at time (L_t) in mm		
		N	%	Mean \pm SD*	minimum	maximum
Males	0+	109	17.14	159.7 \pm 5.5	125	164
	1+	106	16.67	173.9 \pm 7.3	150	185
	2+	279	43.87	189.5 \pm 7.5	170	190
	3+	64	10.06	189.6 \pm 7.7	182	215
	4+	58	9.12	224.6 \pm 4.1	210	230
	5+	20	3.14	226.7 \pm 7.0	215	234
Total		636	100	192.3\pm5.8	125	234
Females	0+	82	12.35	159.7 \pm 5.5	130	162.5
	1+	92	13.86	169.3 \pm 7.3	150	180
	2+	194	29.21	191.7 \pm 7.5	170	198
	3+	200	30.12	208.6 \pm 7.7	190	215
	4+	76	11.45	221.5 \pm 4.1	200	230
	5+	12	1.81	227.2 \pm 7.0	215	236
Total		664	100	199.5\pm6.2	130	239
Whole	0+	191	14.69	159.7 \pm 5.5	125	164
	1+	198	15.23	170.2 \pm 5.7	150	185
	2+	473	36.38	190.6 \pm 6.4	170	198
	3+	264	20.31	199.8 \pm 5.2	190	215
	4+	134	10.31	221.9 \pm 7.3	200	230
	5+	32	2.46	226.5 \pm 5.0	215	236
Total		1300	100	196.8\pm5.5	125	239

*SD : Standard deviation

Table 5. Size- age key for specimens of *S. pilchardus* collected from Dakhla port in Moroccan Atlantic coast

Sex	Age (years)	Fish number		Fish length at time (L_t) in mm		
		N	%	Mean \pm SD*	minimum	Maximum
Males	0+	11	4.30	158.5 \pm 2.5	125	164
	1+	29	11.33	182.5 \pm 4.3	150	185
	2+	110	42.97	185 \pm 7.4	170	190
	3+	80	31.25	213 \pm 6.7	190	215
	4+	18	7.03	227.3 \pm 4.1	210	230
	5+	5	1.95	230.1 \pm 6.6	215	230
Total		256	100	206.8\pm5.9	125	250
Females	0+	2	0.72	158.5 \pm 3.5	120	162.5
	1+	42	15.16	180.3 \pm 2.1	150	180
	2+	121	43.68	205.2 \pm 8.1	170	193
	3+	69	24.91	222.1 \pm 6.4	200	228
	4+	26	9.39	226.6 \pm 4.1	210	230
	5+	12	4.33	235.1 \pm 6.3	215	245
Total		277	100	216.2\pm4.6	120	265
Whole	0+	13	2.44	158.5 \pm 2	120	164
	1+	71	13.32	181.2 \pm 3.1	150	185
	2+	231	43.34	192.3 \pm 6.3	170	193
	3+	149	27.95	217.6 \pm 4.2	190	228
	4+	44	8.26	226.3 \pm 5.4	210	230
	5+	17	3.19	232.7 \pm 6.5	215	245
Total		533	100	209.2\pm5.1	120	265

3. Linear growth in samples of different ports

The results of this study revealed a strong heterogeneity of the linear growth in sardines along the Moroccan coastline. Segregation was confirmed by an ANCOVA test, explaining the total variance with high significance ($p < 0.0001$). This differentiation allowed the researchers to divide the Moroccan sardine stock into three zones (Table 6).

Table 6. Statistical analysis (F and p values) of linear growth of *S. pilchardus* collected from Larache port in Moroccan Atlantic coast

Group	F	p
Larache/Safi	27.91	0.00001
Safi/Dakhla	39.63	0.00001
Dakhla/Larache	53.41	0.00001

3.1. Larache

The von Bertalanffy equation, expressing the linear growth as a function of time at Larache (Fig. 2) is presented in the following form:

$$L_t = 195.15 (1 - e^{-0.18(t+7.37)})$$

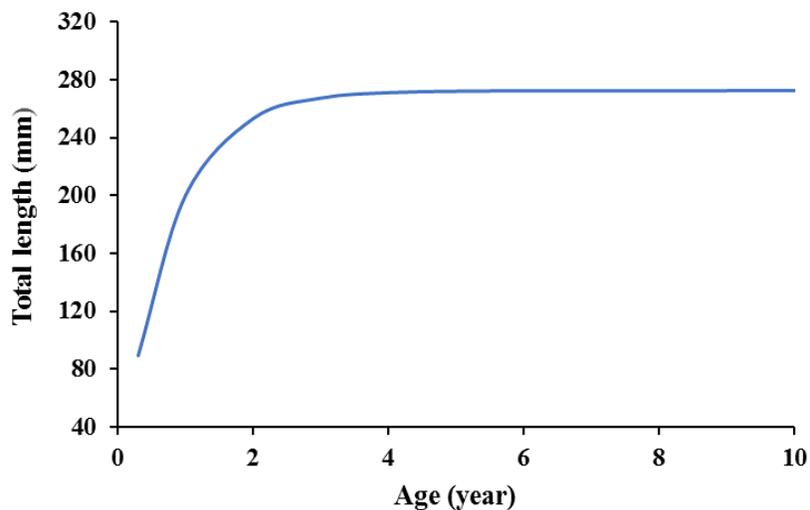


Fig. 2. A histogram showing the growth curve of *S. pilchardus* in Larache

3.2. Safi

The von Bertalanffy equation, expressing the linear growth as a function of time at Safi (Fig. 3) is illustrated in the following form:

$$L_t = 256.56 (1 - e^{-0.21(t+3.98)})$$

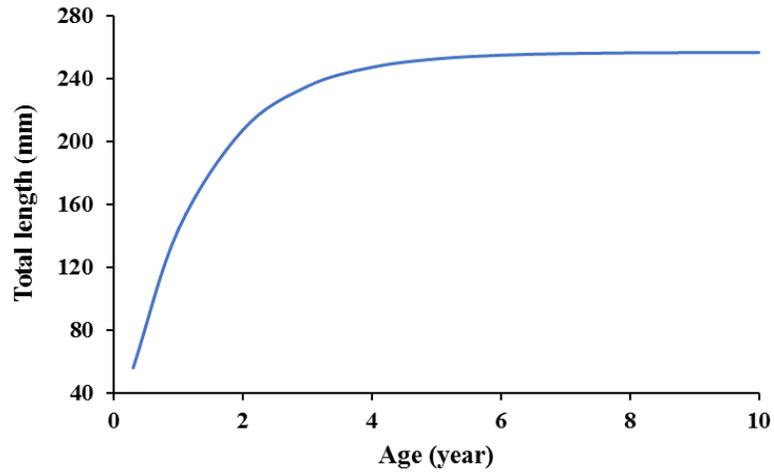


Fig. 3. Growth curve of *S. pilchardus* in Safi

3.3. Dakhla

The von Bertalanffy equation, expressing the linear growth as a function of time at Dakhla (Fig. 4) is shown in the following form:

$$L_t = 272.05 (1 - e^{-0.3(t+2.13)}).$$

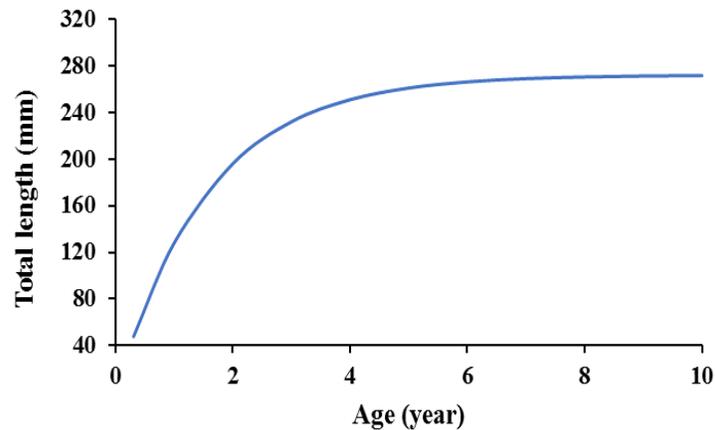


Fig. 4. A histogram showing the growth curve of *S. pilchardus* in Dakhla

DISCUSSION

The results of the current study showed the presence of three zones, according to the linear growth rate of *S. pilchardus* from north to south; 'Larache' 'Safi' and 'Dakhla'. This difference can be related to the environmental and hydrological characteristics of the Moroccan coast, notably the presence of coastal upwelling, recurring hydrodynamic structures such as the Cape-Ghir filament and the presence of certain gires linked to the topography of the coast (El Mghazli *et al.*, 2020; Znari & Mounir, 2021). Since the distribution and sardine abundance are highly influenced by hydro-climatic conditions, concurrent to all pelagic fish (Abdellaoui *et al.*, 2017), it responds quickly to its

environment. These conditions can influence the linear growth of sardine, creating the differences found between these three areas.

Sardines have a way of life similar to a type r demographic strategy, as for most small pelagic. Species with this type of population strategy have a delicate balance: a small variation in environmental conditions can lead to high variability in biology and growth (**Whittaker & Goodman, 1979**).

Sardine of the Moroccan continental shelf is one of the pelagic species subservient to upwellings of the Canary current. It adopts a strategy in upwelling ecosystems that is to take advantage of high productivity coupled with the enrichment of coastal waters to invest in growth. Since upwelling does not show up similarly along the Moroccan coast, it causes the spatial variability of linear growth of sardines. Upwelling is a major factor in the biology of sardines; it divides the Moroccan Atlantic coast into three different stocks; northern, central and southern.

The northern stock; namely, the Larache samples differ from the others by a relatively slow linear growth that may mainly be attributed to the lack of upwelling. The latter is considered as a promoter of primary and secondary production that condition the linear growth of sardines. Additionally, **Scherbitch *et al.* (1997)** reported that the northern sardines have a low growth rate compared to the southern ones. The central one, stretching from Safi to Boujador, is subject to a summer seasonal upwelling homogeneous throughout the area.

The linear growth is closely related to primary production. The northern zone still has low levels of chlorophyll (average $0.2 \mu\text{g} / \text{m}^3$) compared to the central and southern zones, where the average levels are of the order of $0.6 \mu\text{g} / \text{m}^3$ (**El Mghazli *et al.*, 2020**), relatively low growth of sardines is recorded in the northern zone.

Comparison of average lengths by age group has shown that there may be different populations of sardines in the Atlantic. Differences between these populations could be explained by changes in the environment, which would result from different degrees of salinity, temperature and food. Moreover, these differences could be related to the different adaptations of sardines to their environment (**Checkley Jr *et al.*, 2017; Mounir *et al.*, 2021**). Environmental conditions, especially the temperature factor and the quality and quantity of food directly influence fish growth. Good quality habitats allow optimal growth and the survival of the present species. Nevertheless, these differences could result from the genotype-phenotype relationship. Thus, individuals with high growth probably have a particular genotypic structure (**Atarhouch *et al.*, 2006; Chlaïda *et al.*, 2009**).

Sardines living in areas of Moroccan upwelling, where primary production is intense, grow much faster than sardines living in the Mediterranean Sea, where food is less abundant. The differences in the average length by age group observed between the other areas and the Mediterranean could be explained by the differences between the

Mediterranean Sea and the Atlantic Ocean including the temperature of the water, the degree of salinity, and the pronounced oligotrophy of the Mediterranean and the isolation of the breeders (**Stanichny *et al.*, 2005**).

Notably, the life cycle parameters of many marine fish stocks vary in response to changes taking place in environmental conditions (**Nye *et al.*, 2009**). These fluctuations can also be due to fishing. Fish from a poorly exploited stock, where biomass is important and highly intraspecific food competition, has a slower growth rate than those from an overexploited stock where biomass has fallen to a very low level. The combination of mortality and food productivity are factors that are rarely stable, thus result in fluctuations in growth within the same stock that may be large enough to prevent the definitive use of a curve or age key- length. Therefore, these fluctuations can affect fish physiology as well as the abundance and density of their food source (**Mounir *et al.*, 2021**).

The comparative study of growth between the different zones revealed that the linear growth favors sardines from the Dakhla area. They could probably have benefited from the favorable conditions for their growth (temperature, trophic resources during their recruitment). Other processes could occur and cause differences in growth between areas, such as density-dependence, size-dependent mortality (**Sinclair *et al.*, 2002**) and genetic characteristic (**Barnett-Johnson *et al.*, 2010**).

The biological parameters analyzed in this study vary between regions because of their plasticity to the effects of changing environmental conditions. These differences between areas are probably maintained by the combination of biotic and abiotic processes of the environment and the high pressure of fishing in the Moroccan marine ecosystem.

Increased fishing can influence spatial trends in sardine growth parameters (**Gaggiotti & Vetter, 1999**). Remarkably, a high fishing pressure can cause the decrease in the biomass, the decrease of the maximal size, decrease of the growth and a decline in the maturity rate within fish stocks.

CONCLUSION

This study clearly shows the existence of a spatial variability of the linear growth of sardines of the Moroccan littoral. It divides the Moroccan coast into three zones; from north to south; namely, Zone 1: Larch, Zone 2: Safi and Zone 3: Dakhla. Complementary studies including biological and seasonal parameters for each zone separately are needed in order to ensure better management for the sustainable exploitation of the sardine fisheries.

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