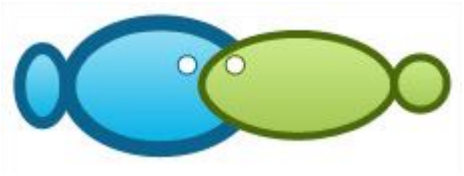

Study of reproduction of sardine, *Sardina pilchardus* in the North of Atlantic Moroccan area

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Abstract. This work aims to analyze some aspects of biology of *Sardina pilchardus* sampled during a period between November 2014 and October 2015 in Casablanca area. The study of reproduction showed that the sex ratio is in favor of females. The females are dominant in all class sizes. The sardines sampled during the period of study present different stage of sexual maturity. This implies that there is a difference in the maturation period and emission of gametes between individuals of the population. We also noted a difference in the percentage of maturation with the seasons. The monthly evolution of the gonad index (RGS) aims to determine spawning periods for a full annual cycle. Sardine reproduces throughout the year and with a major peak in winter. The condition coefficient k evolves in the same way for both sexes. The sizes at first sexual maturity (L_{50}) are 19.8 cm and 17.6 cm for males and females respectively.

Key Words: Atlantic, *Sardina pilchardus*, sex ratio, size at first sexual maturity (L_{50}).

Introduction. With a coastline of 3500 km and an important diversity of its marine wealth, Morocco has a real fishing potential concentrated mainly in central and south Atlantic. Small pelagic fish, mainly sardines, anchovies, mackerel, horse mackerel and sardinella represent quantitatively the main resources exploited and almost 80% of total catches (Amenzoui 2010).

Sardine is the most important species in terms of catch and biomass. Morocco is the world's leading producer of high quality sardine and leader in the international market for canned sardines. In 2013 the production is dominated by the landings of sardine (61%), with nearly 704 800 tones (INRH 2015).

The socio-economic importance of marine fisheries requires managers to strengthen biological studies needed to assess these resources in order to allow fishermen to make the most of natural stands on one hand, and to safeguard stocks by appropriate regulatory measures on the other hand.

In recent years, efforts have focused on the adoption of strategies to ensure sustainable exploitation of Moroccan resources (INRH 2015). The variability of the species in short life requires continuous monitoring of the levels of biomass, catch levels and the quality of recruitment. A knowledge of species biology through scientific studies used to go the way of the definition of relevant biological indicators able to describe the status of the monitored populations.

Within this framework, our work is trying to achieve a biological study of sardine, *Sardina pilchardus* to better understand the different phases of its sexual cycle, the gonad index, condition factor K and size at first sexual maturity.

Material and Method. During the period between November 2014 and October 2015, 284 sardines were sampled in the Casablanca area (Figure 1). The sampling method is that described by Belvèze (1984). The samples were carried out monthly. For each individual, the total length (measured to the centimeter below 0.5), the total weight and gonad weight are weighed tenth of gram, sex and sexual maturity are determined.

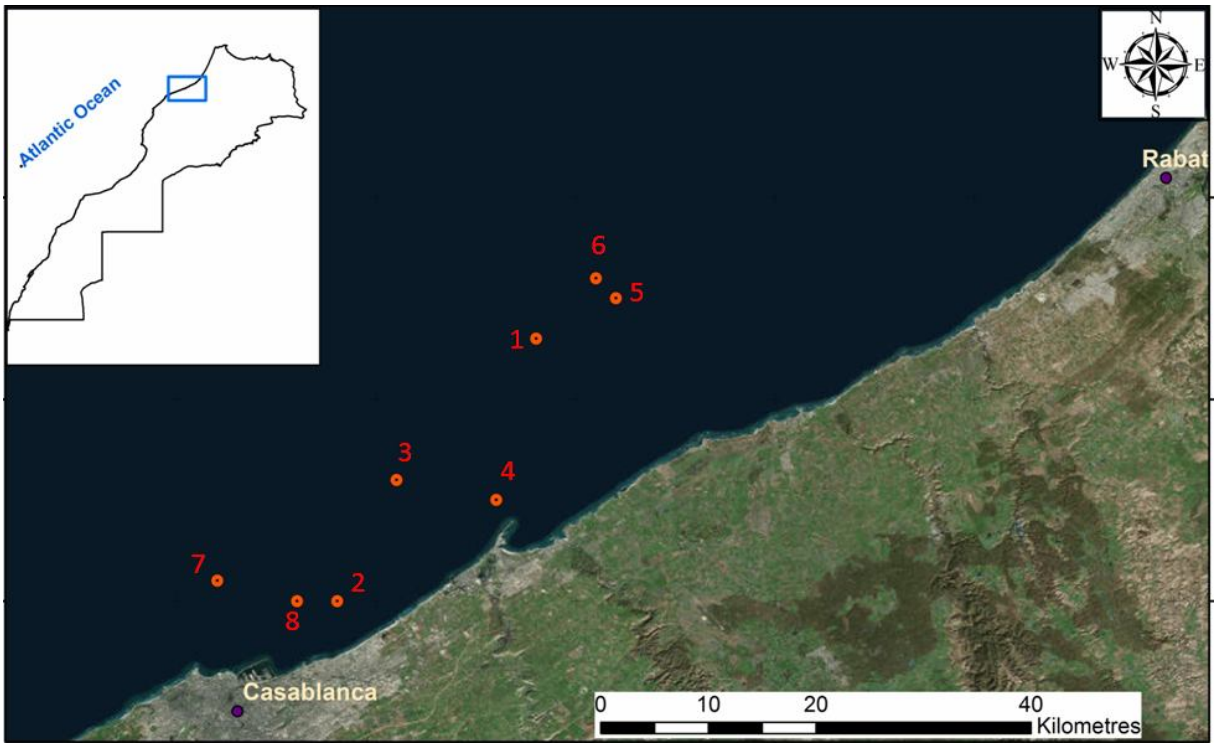

Figure 1. Sampling stations of *Sardina pilchardus* in the Casablanca Moroccan area.

Reproduction study

Sex ratio. It is the proportion of individuals of each gender of a given population. This index helps determine the overall structure of the population in males and females, and also control its variation with the seasons. The χ^2 test was used to end look if the difference in proportions of identified gender is significant. This test consists of comparing the observed values with the theoretical values equality of the workforce:

$$\chi^2_{obs} = \Sigma (N_{obs} - N_{cal})/N_{cal}$$

where: N_{obs}: the actual observed sex in the sample;

N cal: the theoretical number calculated sex in the sample ((effective male + effective female)/2).

The alternative hypothesis proposes that there is a significant difference between the gender proportions. If χobs < χμ; 0.05, the main hypothesis is accepted. If χobs > χ μ; 0.05, the main hypothesis is rejected.

Sexual maturity. The macroscopic sexual maturity includes five stages (Tables 1 and 2) (FAO 1978).

The size at first sexual maturity. This refers to the length at which 50% of individuals in a population are sexually mature. This definition was adopted in this study. The determination of the L_{50} was made by grouping the individuals sampled during the main breeding season, by sex and size class. Then, the proportion of mature individuals of each size class was calculated. The sexual maturity threshold is set at stage III which is the beginning of the gonad development phase (FAO 1978).

The logistic model, sigmoid symmetrical type is selected for graphing (Pope et al 1983):

$$P = 1/(1+e\text{-}(a + b * L)) \quad (1)$$

where: P: mature proportion by size class;

L: total length;

a: intercept;
b: slope.

The parameters a and b are obtained, after logarithmic transformation of the expression (1) by the least squares method (Sokal & Rohlf 1994):

$$-\text{Ln}((100 - p)/p) = a + b * L \quad (2)$$

The representation of maturity is realized by considering all pairs of values with the exception of those which have a ratio: P = 0 and P = 1.

$$L_{50} = -a/b$$

Table 1

Stages of macroscopic ovarian development (FAO 1978)

Stages of maturity	*Oogenesis*	*Features ovarian*
Stage I	Immature	Ovary thin, translucent pink and invisible oocytes
Stage II	Immature or resting	Ovary little bulky, pinkish color, intense vascularization in fish in sexual rest, less intense in immature development and invisible oocytes
Stage III	Early maturation	Ovary midsize, pale pink to light orange and some oocytes are sometimes visible
Stage IV	Pre-spawning and spawning or pre-issuance and issuance	Ovary very bulky, occupying the entire abdominal screen, highly vascularized, the ovary wall is very thin and transparent, the large size hyaline oocytes are clearly visible and are expelled to the lesser pressure on the abdomen
Stage V	Post-spawning or post-show	Flange ovary highly vascularized, its red color, the ovary wall became very thick

Table 2

Stages of macroscopic testicular development (FAO 1978)

Stages of maturity	*Spermatogenesis*	*Features testicles*
Stage I	Immature	Testis small, translucent and very thin
Stage II	Immature or resting	White testicle, more or less symmetrical
Stage III	Early maturation	Wider testicles, white color and no liquid flows, if we make an incision
Stage IV	Emission of sperm	Very big and soft testicles, and sperm flows through pressure on the fish's belly
Stage V	Post-spawning or post-show	Testis large, very flabby, highly vascularized and pressure on the sale does not release more sperm

Gonad index. Variations in the gonad are almost estimated with respect to the parametres such as the length of the body, the total body weight or the somatic weight (Kartas & Quignard 1984). The expression used in this study is the gonad index, abbreviated RGS, equals both gonad weight divided by the total weight of the body and expresses gonad weight as a percentage of body weight (Bougis 1952):

$$\text{RGS} = \text{gonad weight} * 100/\text{total body weight}$$

Condition factor. This factor is suitable for comparing the fish of the same species status in the seasons or the different locations or different sexes, if the parameter b deviates too much from the value 3, the condition factor will vary depending on the size directly as if it's greater than 3 and inversely if it's lower. Condition factor therefore reflects the ecological and physiological conditions (Belvèze 1984):

$$K = (\text{total body weight}/aL^b) * 10^n \quad \text{(Fréon 1979)}$$

Results and Discussion

Sex ratio. The proportions of males and females sampled monthly in period between November 2014 and October 2015 are shown in Table 3, the comparison of proportions of males and females by the χ^2 test has established rules corresponding decision.

Table 3

Comparison of the proportions of males and females and decision rule

Number of males (%)	*Number of females (%)*	χ^2_{Obs}	χ^2 (1; 0.05)	*Rule decision*
29%	71%	49.02	3.84	There is a significant difference between males and females

In 284 sampled sardines, 201 were females (71%) and 83 were males (29%). There is a significant difference between the two sexes (χ^2 test).

Sex ratio depending on size class. The evolution of sex ratio depending on size showed that size class between 11 and 13 cm shows a complete lack of males. In all size class, the curve shows a dominance of females with a gradual increase depending on size (Figure 2).

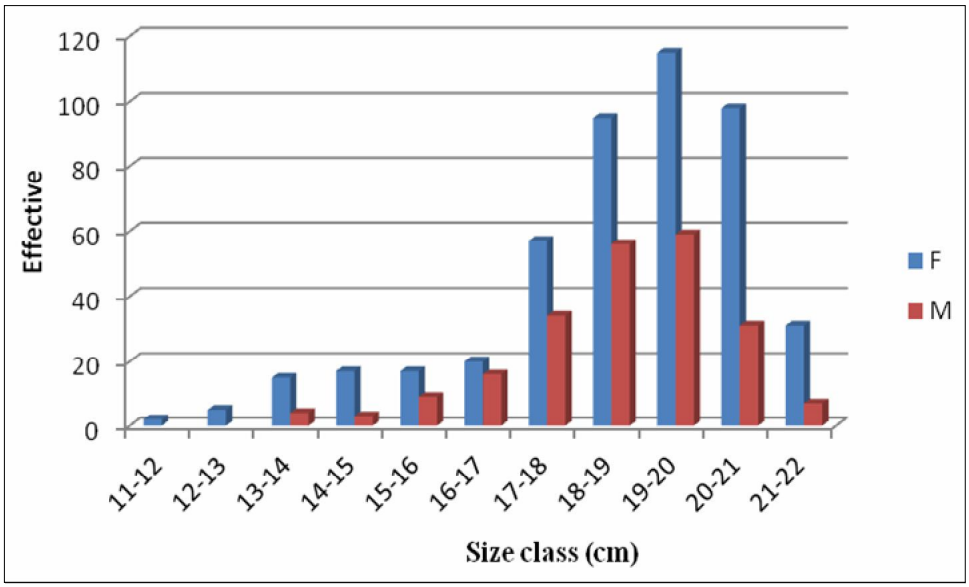

Figure 2. The evolution of sex ratio depending on class size.

Sexual maturity. The percentage of seasonal variation of sexual maturity stages shows that males and females are at different stages throughout the year, but their percentages vary. Sardines in post-spawning or post-issuance are poorly represented in our samples. The immature or early maturation are represented throughout the year with high percentage in summer. The percentages of reproductive sardines are observed in winter. After this season, most individuals have come to breed and fall in periods of sexual rest (stages I and II) (Figure 3).

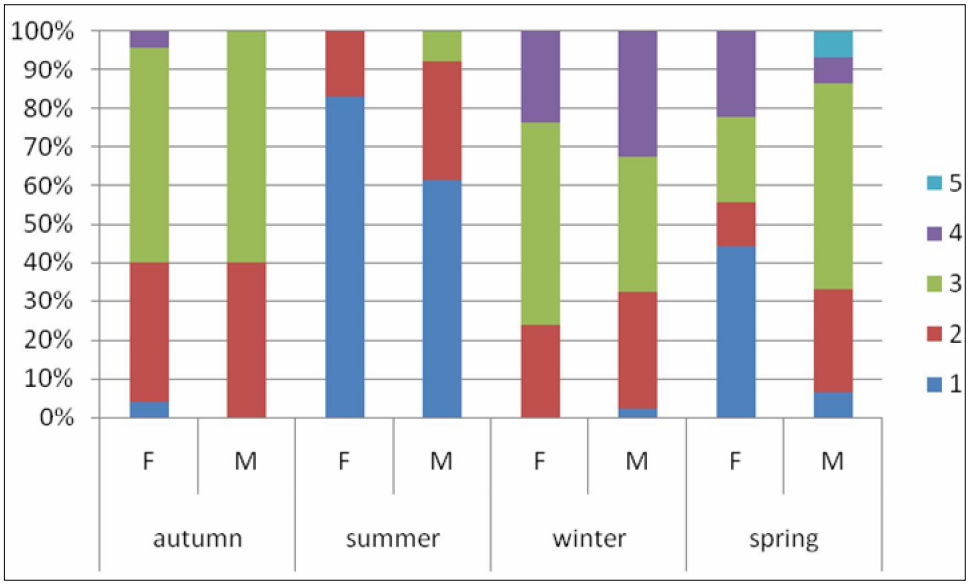

Figure 3. Evolution of seasonal frequency of sexual maturity stages for sardines sampling in Casablanca area, period between November 2014 and October 2015.

Size at first sexual maturity. The corresponding average sizes L_{50} developed for the study period between November 2014 and October 2015, are at 17.6 cm and 19.8 cm for males and females respectively. Females reach its sexual maturity at a larger size than males (Figure 4).

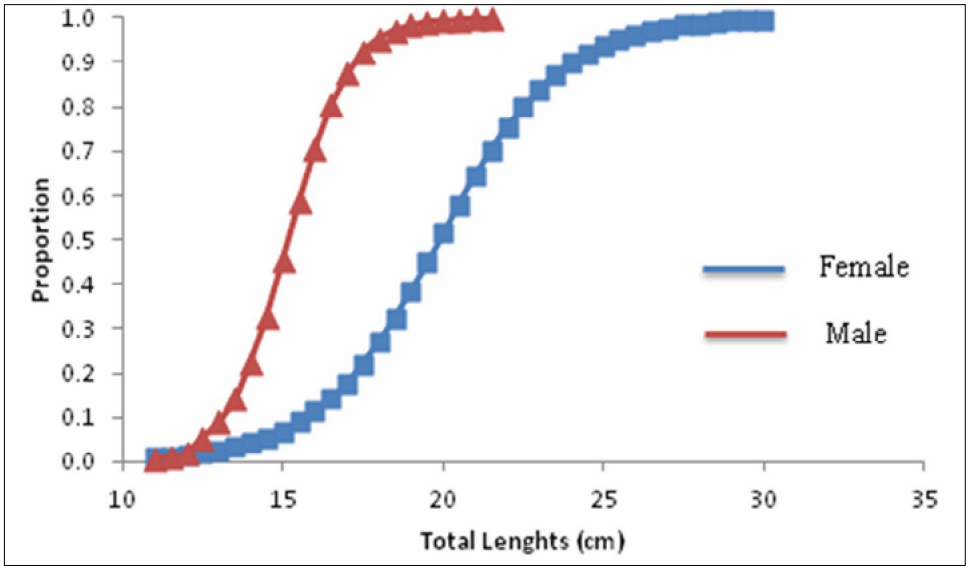

Figure 4. The size at first sexual maturity of sardine sampling in Casablanca area, period between November 2014 and October 2015.

Gonad index. For males, the average RGS is high in February. It became minimal in July. A similar evolution of RGS average was observed in females while the RGS became minimum in August. We observed that the males had an average of RGS higher than those of females. In the region of Casablanca, sardines can reproduce in the period between November and May and have a maximum period of sexual activity between November and February (Figure 5).

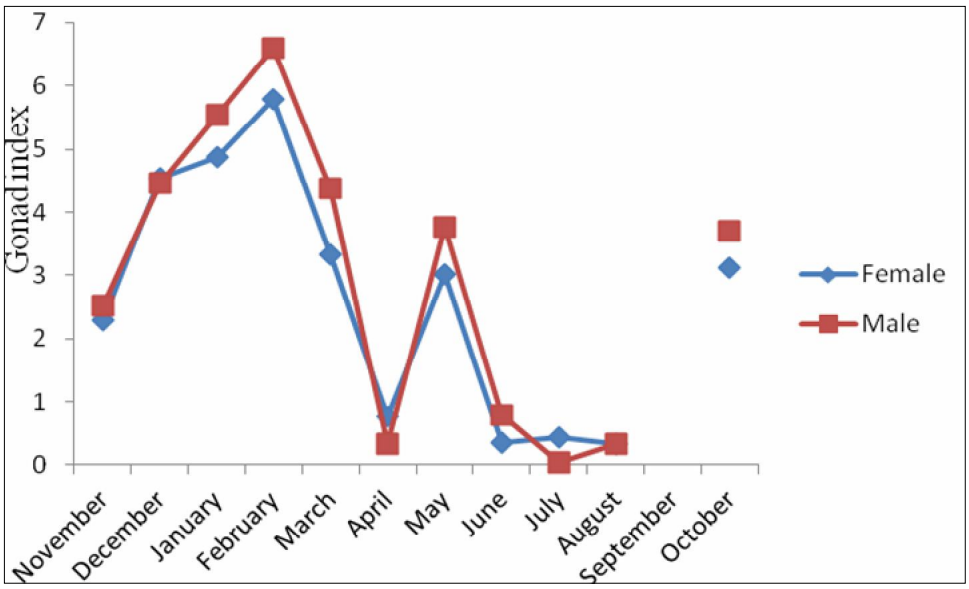

Figure 5. Monthly evolution of gonad index (RGS) of sardine sampling in Casablanca area, period between November 2014 and October 2015.

Condition factor k. A graph of monthly averages condition factor shows that it has a somewhat similar pattern in both sexes and interannual variability. In November, the condition factor reaches a higher value comparing the other months and it is minimal in May for the both sexes (Figure 6).

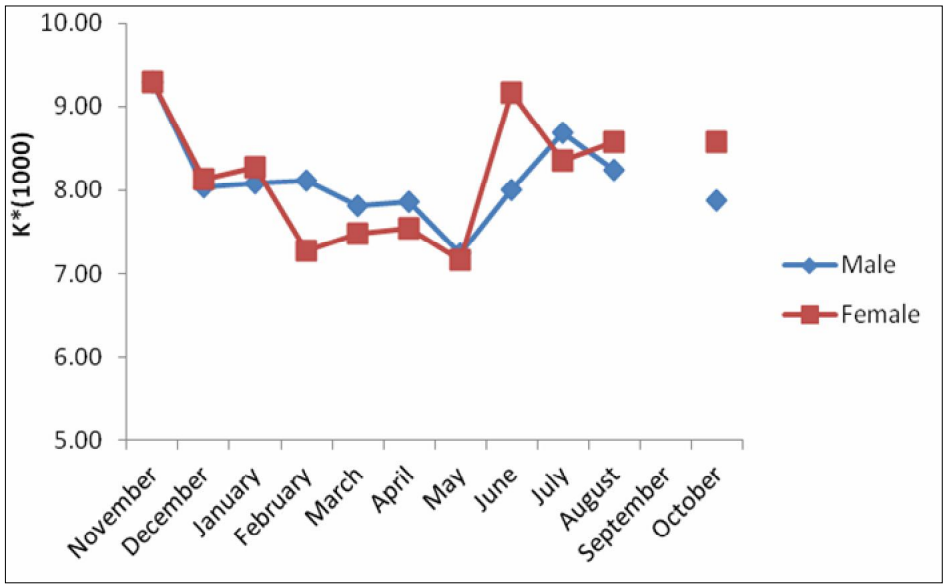

Figure 6. Monthly evolution of average condition factor K of sardine sampling in Casablanca area, period between November 2014 and October 2015.

Discussion. In population dynamics studies, it is always necessary to know the sex ratio. The analysis of the sex ratio changes as we take into account the entire population or size classes separately. Moreover, the sex ratio changes with time.

Moroccan sardine is gonochoric and without sexual dimorphism. The overall sex ratio in Casablanca area is in favor of females with a significant predominance (71%). The variation of sex ratio depending on class size shows a total lack of males in the initial classes (11-13 cm), while females are present in all size class.

The variation of the sex ratio depending on the size indicates a high gender composition in all size class. The dependence of the sex ratio in function on the size of fish was also mentioned by Lee (1961), Bouchereau (1981), Kartas (1981), Belvèze

(1984), Perez et al (1985), Abad & Giraldez (1992), Amenzoui et al (2006) and Khemiri (2006). This biological characteristic seems to be a general rule in Clupeiformes as it was observed in other species such as the flat sardinella, *Sardinella maderensis* (Boely 1979); round sardinella, *Sardinella aurita* (Baali et al 2015; Gaamour 1999); Peru anchovy, *Engraulis mordax* (Bakun & Parrish 1982); *Engraulis encrasicolus* in the Bay of Cadiz (Khemiri 2006). The strong femininity for large class sizes can be the result of several factors, the most plausible would be greater durability, faster growth of females, and significant vulnerability to fishing gear or different migratory movements from those of males. The dominance of females in the small class sizes might be explained by an earlier development of the ovaries.

The size at first sexual maturity is 17.6 cm for males and 19.8 cm for females during our studying period. Amenzoui et al (2006) found that the size at first sexual maturity is 15.8 cm in the southern of Atlantic Moroccan area.

Generally, in Moroccan coast, regardless of the region, sardines can breed for the first time during its first year of life. In its range, sardine can reach its first sexual maturity at the length between 8 and 20 cm corresponding to the individual's age groups between 0 to 2 years (Tables 4 and 5).

Table 4

Size at first sexual maturity *Sardina pilchardus* in different regions of the Mediterranean (L: total length)

Mediterranean	*Males (L_{50} cm)*	*Females (L_{50} cm)*	*References*
Lion Gulf	13.8	14	Lee (1961)
Castellion	11.7	11.3	Andreu et al (1950)
Tunisian coast (north)	11.4	11.4	Khemiri (2006)
Tunisian coast (south)	11.1	11.1	Khemiri (2006)

Table 5

Size at first sexual maturity *Sardina pilchardus* in different regions of the Atlantic Ocean (L: total length)

Atlantic Ocean	*Males (L50 cm)*	*Females (L50 cm)*	*References*
Agadir area	14.5	14.7	Amenzoui et al (2006)
	14.1	14.4	Amenzoui (2010)
Laayoune area	15.8	15.8	Amenzoui et al (2006)
	15.4	15.9	Amenzoui (2010)
Northern France	16.8	16.8	Silva et al (2004)
Casablanca area	17.6	19.8	Present study

The authors determined the spawning period of sardines in Moroccan Atlantic waters based on the abundance of sardine eggs and larvae (Furnestin 1959; Ettahiri 1996; Ettahiri et al 2003; Berraho 2007), the distribution over time of the macroscopic sexual maturity stages (Belvèze 1984) or even on the combined study of different stages of sexual maturity (Amenzoui et al 2005; Amenzoui et al 2006). Fluctuating stages of maturation during the period November 2014 and October 2015 show a breakdown of the maturation stages according to the seasons and interannual variation percentage (Tables 6 and 7).

Table 6

Reproduction periods of *Sardina pilchardus* in different areas of the Atlantic Ocean

Atlantic Ocean	*J*	*F*	*M*	*A*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*	*References*
Moroccan Atlantic coast	x	x	x	x	x	x	x	x	x	x	x	x	Furnestin (1959)
Golf of Gascogne	x	x	x	x	x	x							L'Herrou (1971)
Galice	x	x	x	x	x								Perez et al (1985)
Portugal	x	x	x	x	x					x	x	x	Ré et al (1990)
Manche			x	x	x	x	x	x	x	x			Haynes & Nichols (1994)
Bay of Douarnenez		x	x	x	x	x	x						Duff (1997)
Iles Canaries	x	x	x										Mendez-Villamil Mata et al (1997)
Portugal	x	x	x	x	x					x	x	x	Zwolinski et al (2001)
Safi area	x	x	x	x	x								Amenzoui (2010)
Agadir area	x	x	x	x	x	x	x						Amenzoui (2010)
Laayoune area	x	x	x	x	x	x	x	x	x	x	x	x	Amenzoui (2010)
Casablanca area	x	x	x		x						x	x	Present study

Table 7

Reproduction periods of *Sardina pilchardus* in different areas of the Mediterranean

Mediterranean Sea	*J*	*F*	*M*	*A*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*	*References*
Lion Golf	x	x	x	x	x	x	x		x	x	x	x	Chavance (1980)
Bay of Algiers	x	x	x									x	Bouchereau (1981)
Algerian coast	x	x	x									x	Djabali & Mouhoub (1989)
Bay of Oran	x	x	x							x	x	x	Tomasini et al (1989)
Malaga coast	x	x	x							x	x	x	Abad & Giraldez (1992)
Greek coast	x	x	x							x	x	x	Voulgaridou & Stergiou (2003)
Greek coast	x	x	x									x	Ganias et al (2004)
Tunisian coast	x	x	x							x	x	x	Khemiri (2006)
Adriatic Sea (Croatia)	x	x	x	x						x	x	x	Sinovčić et al (2008)

The combined study of RGS and sexual maturity stages showed that at the Casablanca area sardine can reproduce on half of the year with a maximum reproduction period between November and February. This result is confirmed by the ichthyoplankton studies (Furnestin 1959; Ettahiri 1996; Ettahiri et al 2003): sardine eggs are collected throughout the year along the Atlantic coast of Morocco, the density is highest in winter and became low in summer. However, there are inter-variations of the sexual cycle of the sardine and which are due to environmental conditions especially temperature (Abad & Giraldez 1992; Ettahiri 1996; Ettahiri et al 2003).

The condition factor is a compensatory trait that reflects the seasonal accumulation and depletion of energy so it can provide a reliable indication of the total annual production (Winters & Wheeler 1994). Similarly, Ware & Tanasichuk (1989), and Cardinale & Arrhenius (2000) report that the condition factor is the only reliable growth index for pelagic fish. In the summer season the trophic conditions are favorable as sardines feed heavily while accumulating reserves. This is confirmed by experimental study of sardines and that showed that ovarian development for the winter and spring spawning is strongly conditioned by the accumulation of reserves in summer (Tsuruta 1987).

In autumn, season of the gonad maturation phase, the decrease of condition factor K can be explained by the fact that reserves are invested in the development of

sexual products and gonad development increasingly compresses the digestive tract of the fish (Lahaye 1980).

In winter, zooplankton is less abundant (Somoue 2004), fish eat little and the issuance of eggs helps to accentuate the reduction of its mass. This weight loss stops with the recovery of food and condition improves in the spring with a greater or lesser time lag between years.

Conclusions. This study is made by samples Sardina pilchardus exploited in the Casablanca area during the period November 2014 to October 2015. The sex ratio in favor of females. Its evolution depending on the size showed that females are dominant in all size classes sizes at first maturity (L50) are 19.8 and 17.6 cm in females and males respectively. The monthly evolution of the gonad index (RGS) to determine spawning periods for a full annual cycle. Sardina pilchardus reproduces throughout the year and with a major peak in winter. The condition factor k evolves in the same way for both sexes.

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