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The study of the Histology of Sardine Sardina pilchardus (Walbaum, 1792) in the Moroccan North Atlantic

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ABSTRACT: In the North of Atlantic Moroccan coast, sardine *Sardina pilchardus* breeding occurs all year round, due to the existence of the upwelling phenomenon which shows very marked seasonal variations, with some periods being much more favorable than others. Thus, spawning of sardines is maximum in winter, the season of minimum upwelling and minimum zooplankton production. The spawning is low in summer, which coincides with the season of maximum upwelling and maximum zooplankton production. However, temperature seems to be the essential factor in triggering spawning either by stimulating physiological mechanisms or by trophic enrichment of the medium.

KEYWORDS: Atlantic Moroccan, Sardina pilchardus, upwelling.

I. INTRODUCTION

The fishing sector is among the most dynamic sectors in Morocco; it represents 2.5% of Moroccan Gross Domestic Product (GDP) and provides employment for more than 450000 people [1]. This is due to the geographical situation of Morocco with 3500 km of coast, which the Atlantic facade occupies more than 85% [1]. In this area most of the major fishing ports are distributed. The small pelagic belong to the principal resources of Moroccan littoral whose sardine occupies the first rank and represents more than 86% of the total weight of landings [2].

In recent years, efforts have focused on the adoption of strategies for ensure a sustainable exploitation of Moroccan resources. The variable nature of this short-lived species requires continuous monitoring of biomass levels, levels of catches and the quality of recruitment. Knowledge of the biology of species due to scientific studies can go in the direction of the definition of indicators relevant biological data describing the status of the populations monitored. The evolution of parameters such as first maturity and fertility declined by age or size may provide information on population responses to stress conditions imposed by the environment or fishing.

The study of the biology of species is of paramount importance in the monitoring of exploited populations for a reasoned management of the stocks.



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II. MATERIALS AND METHODS

Years 2015 and 2016, we followed the monthly changing stages of sexual maturity in 221 individuals in the Nord of Atlantic Morocco. The total length class was ranging from 9.5 to 24 cm. A detailed distinction between the stages of maturity has been performed by histological examination (Fig 1).



Fig 1. Sampling stations of Sardina pilchardus in the north of the Moroccan Atlantic coast.

After dissection of the fish, a part is taken from the middle of the female gonads of various stages of development. After fixation in the aqueous Bouin [3], the pieces are dehydrated in ethanol increasing degree and cloudy toluene. After that they were embedded in paraffin to be cut between 5 and 7 microns and stained with Masson's trichrome before microscopic observation [4].

III. RESULTS

1. Fertility and the body mass relationship

Fertility increases progressively with female body mass, the function that links the two factors in 2015 and 2016 respectively is expressed by the following equations: $F = 675 (BM)^{0.811}$ and $F = 333.2 (BM)^{0.425}$ (Fig 2) Where F is the fertility and BM is the body mass.

Among individuals weighing less than 60 grams, the number of oocytes fluctuates between 14000 and 20000 oocytes. While for those weighing more than 60 grams, the number of oocytes increases gradually until it reaches about 26000 oocytes for the both years (Fig 2).



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The average values of fertility were respectively 19116 (\pm 267) and 17298 (\pm 223) oocytes by spawning event and mature female for mean body mass of 62.25 (\pm 0.3) and 53.5 (\pm 0.5) grams for 2014 and 2015 respectively. While, the relative fertility shows the values of 311 (\pm 18) and 382 (\pm 22) oocytes per gram of female for 2015 and 2016 respectively. We noticed that the correlation between the number of oocytes and the total mass of the fish was high and positive (Fig 2).





Fig 2. Body mass and number of oocytes relationship for the sardine in 2015 (A) and 2016 (B)



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2. Fertility and total length relationship

The average values of the number of oocytes in 2015 and 2016 respectively were 19116 (\pm 267) and 17298 (\pm 223) oocytes for the average total length of 19.72 and 18.11 cm. The relative fertility were 965 (\pm 43) and 936 (\pm 32) oocytes per gram of mature female respectively. Note that large individuals are more fertile than small individuals and the relationship between fertility and the total length shows that the fertility increases with length. The correlation between the number of oocytes and the total length of the fish was high and positive (Fig 4).

The equation linking the two factors in 2014 is as follows: $F = 0.623 (LT)^{3.461}$ (Fig 4). The equation linking the two factors in 2015 is as follows: $F = 218.5 (LT)^{1.507}$ (Fig 5). Where F is fertility and LT is the total length.

For individuals whose size is between 18.5 and 20 cm the number of oocytes does not exceed 22000 oocytes, while for individuals of 20.5 cm and over, the number of oocytes exceed 22000 oocytes (Fig 4).



Fig 4. Total length and number of oocytes relationship for the sardine in 2015 (A) and 2016 (B)



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3. Fertility and age relationship

The study of the relationship between the number of oocytes and age in sardines is intended to establish the effect of senility on the species studied. This relation has a good correlation.

In 2015, we note that individuals of age 1 are absent in our samples. The individuals of the age 2 years have an average fertility and are limited between 12 and 18 thousand oocytes. On the other hand, the oldest individuals (3 years and over) have a higher fertility and exceed 20 miles to reach values that are close to 26,000 oocytes. In 2016, we observe that all individuals have average fertility and are between 6 thousand and 25 thousand oocytes (Fig 5).





Fig 5. Age and number of oocytes relationship for the sardine in 2015 (A) and 2016 (B)



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4. Histology of gonads

The ovarian cycle takes place in five stages, this is a reflection of oogenesis, oviposition or degeneration of a part of ovarian oocytes.

The evolution of oocytes can be broken down into two stages: pre-vitellogenesis and vitellogenesis. The different criteria used to determine these phases include the size of the oocytes, the homogeneity, the quantity and the distribution of the lipid and protein inclusion bodies in the cytoplasm constituting the enclaves or yolk globules. The ovaries are organized in slices.

Fable 1: different st	tages of maturation
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Stages of maturity	Oogenesis	Features ovarian	
Stage I	Immature	 Perinuclear oocyte or primary oocyte, small cell 20 to 25 microns bearing a large eccentric nucleus (N) and a dense and homogeneous cytoplasm, rounded contour without regular formation of granules of yellow. The perinuclear oocyte resulting from the mitotic division of the germ cell, which is transformed into the pre-vitellogenic oocyte. The pre-vitellogenic oocyte is larger with a nucleus larger than the perinuclear oocyte containing one or more nucleoli and a cytoplasm of homogeneous color. The structure of the nuclear envelope becomes irregular, it presents cul-de-sac which houses the nucleoli, become less and less visible; this is the beginning of vitellogenesis. 	
Stage II	Immature or resting	The ovary contains the perinuclear oocyte and the pre-vitellogenic oocyte that is being transformed into a larger vitellogenic primary oocyte or oocyte. At this stage, the vitellogenic volume of the primary oocytes doubles fill the follicular lumen, their sizes vary between 100 and 150 microns. Their cytoplasm surrounded by cytoplasmic membranes is then invaded by lipid vacuoles and the follicular cell membranes are well differentiated by the external library, follicular cells flattened cord and finally the establishment of the radiata area (Fig 8).	
Stage III	Early maturation	mature ovarian oocytes fill the lumen and are ready to be expelled. The mature gonads then have a typically dense appearance since all the grouped follicles are filled with large eggs.	
Stage IV	Pre-spawning and spawning or preissuance and issuance	the oocyte has reached its mature size is expelled from its follicle. At this point, the nucleus has disappeared and the presence of empty follicles is a good proof of spawning. The connective tissue, on which the mature follicles are detached and the follicles are emptied progressively as and when the pose (Fig 9).	
Stage V	Post-spawning or post-show	The ovaries contain atretic oocytes as well as previtellogenic oocytes which can begin their evolution during the next sexual cycle. The spaces released by the degeneration of vitellogenic oocytes are recolonized by these resting precontogenogenic oocytes containing a large central nucleus with numerous nucleoli. Their cytoplasm contains some vitelline vesicles in peripheral border (Fig 10).	



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Fig 8. Histological features of ovaries. Oocytes at different stages of maturity. Atr: atretic oocytes, Fpo: post-ovulatory follicles



Fig 9. Histological features of the ovaries. n: nucleolus; Gl: lipid vacuole; N: nucleus; Cy: cytoplasm; Gj: yellow globule



Fig 10. Histological features of the ovaries. n: nucleolus; Cy: cytoplasm; N: nucleus; AC: cortical alveolus

IV. DISCUSSION

Understanding the causes of variations in fish stocks is one of the main topics of study of research in fisheries. Pelagic species make up the largest share of Atlantic Moroccan marine catch, representing approximately 850 000 tons of the total catch in the year 2015 [1]. These species are an essential element in marine ecosystems. Their high biomass at intermediate levels of the food chain gives them an important ecological role as a link between the planktonic production and trophic level of fish-eating or as main prey for several pelagic and demersal fish.



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This histological study, describe the main evolutionary phases macroscopic of reproduction in *Sardina pilchardus* in the Nord of Atlantic Morocco. Analysis coastline has allowed us to approach sexual maturity from the observation of the external condition of the gonads: size, color, consistency, volume, shape, vascularization and visibility of eggs [5]. The analysis allowed us to study oogenesis through the process comprising all the changes undergone by the primordial germ cell to become an egg ready to be fertilized with the yolk, its primary envelope and cortical granules. It also enabled us to address the ovogenesis.

The fertility results presented in this study estimates the reproductive potential of *Sardina pilchardus* in the North of Atlantic Morocco. *S. pilchardus* is a spawning breeder, with several spawns spread all year round and characterized by indeterminate fertility. Relationships between absolute fertility and total length, body mass, and age were observed showing a good correlation. In our study area, the average value of fertility in 2015 and 2016 was respectively 19116 (\pm 267); 17298 (\pm 223) oocytes by act of laying. While, relative fertility shows a value of 311 (\pm 18) 382 (\pm 22) oocytes per gram of female.

Mass is basically the best physiological reference. When the sardine in good condition is able to provide a lot of eggs [6]. In fact, improvements in body mass do not always result in a corresponding increase in the number of eggs, and the reverse may occur. Knowing that the mass plays a very important role in the storage of fat and the respective importance of the energy value required for egg production according to the habitat [7, 8, 9].

Fertility changes positively with the size and body mass of the fish. These results are consistent with those reported in sardine, *Sardina pilchardus* in other areas of the Atlantic Ocean [10, 11, 12] and in the Mediterranean Sea [13, 14]. The same results have been observed for other teleosts [15, 16, 17, 18, 19, 20, 21, 22].

Total length is also not an absolute reference since the fecundity of sardines of a given size depends, inter alia, on mass and age [23].

The variation in average relative fertility is strongly related to the environmental environment condition such as the amount of food available:

- The degree of predator pressure,
- The intensity of fishing.
- It can be attributed to genetic factors because of reproductive isolation since there is very little information on the genetic control of fertility [24].

In the table above this represents the variation of the fecundity of the sardine with some geographical situations (Table 2).

Regions	Fecundity (eggs)	Relative fertility (eggs /	Authors
		g)	
Region of Algiers	10000 à 75000	744 à 1537	[25]
Portugal	30227	427	[26]
Laayoune area	23150	346	[27]
LEA	7 382	325,37	[28]
North Atlantic Morocco	19116	311	Present work
(2015)			
North Atlantic Morocco	17298	382	Present work
(2016)			

Table 2. Fecundity of *S. pilchardus* sardine in some geographical areas



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V. CONCLUSION

We have implemented an automatic text detection technique from an image for In painting. Our algorithm successfully detects the text region from the image which consists of mixed text-picture-graphic regions. We have applied our algorithm on many images and found that it successfully detect the text region.

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