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Histological Followed the Sexual Cycle of the Mediterranean Sardine *Sardina Pilchardus* (Walbaum 1792) fished in Gulf AnnabaNortheast of Algeria.

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Abstract:*The study of reproduction of Sardina pilchardus was performed on the basis of samples coming from commercial landings at the port of Annaba, eastern Algeria (Mediterranean Sea) during the period from March 2012 to February 2013.The report somatic and gonadal condition factor (K) are negatively correlated. K showed high values during the sexual resting phase and low values during the breeding season. The average size of first maturity (L50) was achieved in 15.0 ± 0.31 cm and 17.5 ± 0.35 cm respectively in males and females.Changes in condition factor (K) and the sexual cycle were related to the upwelling*.
Keywords: *Gulf of Annaba, Sardina pilchardus, spawning, size at first sexual maturity*

I. Introduction

The small pelagic fish are an important fishing potential along the Mediterranean. They consist of sardines, mackerel, anchovies and sardines. Sardine fisheries one of the main components of the Algerian fisheries, Sardine (Sardina pilchardus Walbaum, 1792) is a clupeid who people the Gulf of Annaba, it is limited by virtual reference alignments, and it extends over 40 km, from Cape Guard in the West to Cape Rosa East. Regeneration of small pelagic has contributed to the increase in fishable stock. The exploitation rate is approximately ± 56% (Bedairia, et al. 2007).

Our study aims to monitor sexual maturity by the macroscopic and microscopic gonad followed *Sardina pilchardus* during an investigation and determination of the size at first sexual maturity year.

II. Materials and Methods

2.1. Presentation of the study area

Gulf Annaba is located in the east of Algeria between 2 provinces "Annaba and El Tarf (Fig.1) lengthening of Cape Guard in West (7 ° 16 ' ' E) to Cape Rosa in East (8 ° 15 ' ' N), it extends over 40 km with a maximum depth of 65m.

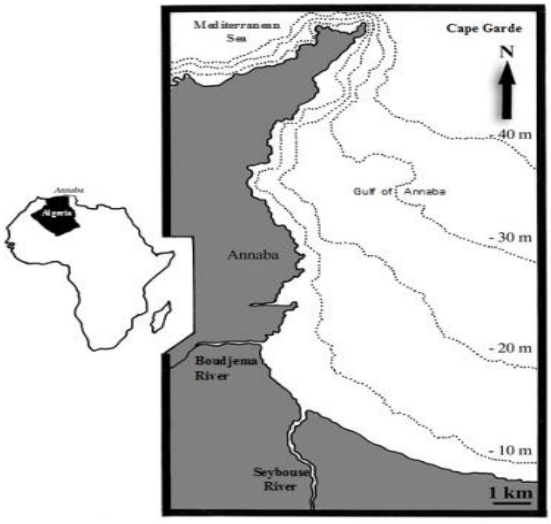


Fig. 1: Location of the study area at the level of Annaba gulf. (Frehi et al, 2003).

2.2 Material

2.2.1 Biological Material

Of freshly caught individuals of the sardine (Sardina pilchardus) caught by fishing vessels called sardine boat attached to the port of Annaba, Algeria.

2.2.2 Laboratory Material:

2.2.2.1 Formorphometry:

- Ichtyo meter - Precision balance-Binocular-Tape measure-Bouin or 10% formalin

2.2.2.2 For the microscopic followed of sexual maturity

-Tube -label-Baume -The immersion oil-Dyes (hematoxylin + eosin)-Microtome
-Paraffin - Microscope slides-lamellae -Optical microscope equipped with digital camera

2.3 Methods

2.3.1 Morphometric method

2.3.1.1 Methods of Sampling and levy

A total of 540 sardines sampled at random for morphometric study; of which 250 were female sex, and 290 male sex, while 72 individuals were analyzed served in monitoring of reproduction parameters.Studied the sardines collected from landings made between June 2012 and May 2013 at the fishing port in the Gulf of Annaba (Fig.1). The sampling frequency is weekly and depending on the availability of fish.

At each sampling campaign (Fig.2), a sample comprising six individuals per size class person measured between 7.5 and 19.5 cm were divided into 12 classes with an interval length of 1 cm. For each individual, the total length (TL) is measured, the total weight (PT) gutted weight (PE) and gonad weights (PG) are weighed to the tenth of a gram, sex and sexual maturity were macroscopically identified.

2.3.1.2 Tracking Methods of the evolution of sexual maturity.

The scale of sexual maturity is macroscopic (Belveze, 1984) and includes the seven stages of sexual maturity known in male fish and females:

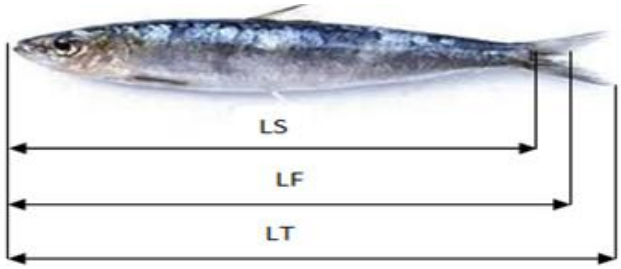


Fig.2: Measurements benchmark made in Sardina pilchardus
(Lt = total length, Lf = fork length, Ls = standard length).

2.3.2 Study macroscopic of gonads:

2.3.2.1Examen macroscopic of the gonads

Extracted and weighed, are the subject of an observation of their general appearance using a binocular microscope (Zeiss), in order to determine the morphology and classify macroscopic gonad stages of sexual maturity.The gross examination of the gonads allow you to define six stages:

Step A: The testes and ovaries are very thin and small close in a few millimeters wide. They are transparent or light pink color and arranged in a V whose apex is located at the rear end of the body cavity, the oocytes and spermatogonia are not visible.

Step B: The ovary changes from pale pink to dark pink then light orange. Some oocytes may be visible through the ovarian membrane at the end of Step B, but remains ovarian ferme.la gonad does not exceed the front quarter of the abdominal cavity.

Step C: It is from this point that we can easily distinguish with the naked eye, between the male and female gonads staining is variable light orange or dark yellow. The ovary becomes larger less firm testes are roses, the surface becomes grainy and oocytes are slightly visible to the naked eye.

Step D: It's puberty; the gonads are well developed and generally occupy a large part of the abdominal cavity. The ovarian membrane is very thin (Fig. 3). The eggs are transparent and clearly visible.After spawning, the ovary is flaccid, highly vascularized. It is usually red and salmon pink color at the end of the stage, especially in the posterior part. Seen through the membrane distended ovarian oocytes of about 450 p characterizing the early

Step E: The gonads fill most of the body cavity. The slightest pressure on the abdomen was drained milt in the males and eggs in females. There remain some transparent eggs but they will degenerate and instead remain hyaline areas.

Step F: This stage follows the laying empty gonads become flaccid. After this stage,thereturn to first stage of maturation.After the last egg, the ovary is exhausted, completely collapse. It has the appearance of an empty bag and has a red coloration due to a very high vascularization.There are also brown spots' representing areas of necrosis in the process.

Again, you can meet some residual transparent eggs. It should be added, on the macroscopic characters, that there is a general trend ofappearance and color of the ovary during maturation but these characters are not always present alone rigorous criteria for recognition.

The fish are sometimes red-blood gonads might be tempted to attribute to point F. In fact the measurement of oocytes shows that this is a stage B or C. These hemorrhages were due to trauma caused when catching fish. Moreover, in Step C the oocytes are not always very visible through the ovarian membrane (Fontana, 1969).

To define the breeding season, we followed the evolution of monthly gonad index

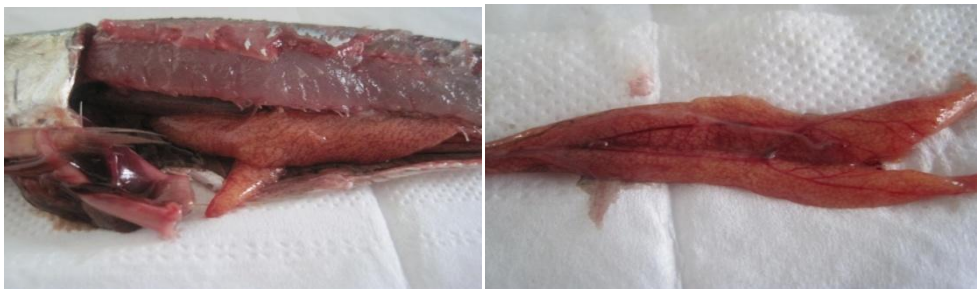


Fig. 3: Femalegonads*Sardina pilchardus* (Saoudi.h et *al*, 2012)

Fig. 4: Male gonads *Sardina pilchardus* (Saoudi.h et *al*, 2012)

2.3.2.2 Method of microscopic examination gonads:

Microscopic examination is performed following the histology of all collected gonads. These fish must be fresh.

Table 2: The different stages of sexual maturity in female *Sardina pilchardus* fished in the Gulf of Annaba (by FONTANA, 1969).

Stages		**In females of *Sardina pilchardus***
I	Immature and resting	In immature ovarian oocytes polyhedral shape are arranged regularly along the ovarian lamellae. Oocytes characteristics of stage A oocytes represent the general stock, reserve
II	Resting	The same as (immature)
III	Maturing	Is characterized above all by the beginning of vitellogenesis.
IV	Prespawning	Oocytes are round. It can occur a start of water absorption by the oocytes.
V	spawning	Mature eggs. A histological section the different cellular organelles. Follicles have broken out following the sudden absorption water ova. The rest of the ovary is occupied by the general oocytes stock,
VI	Post spawning	We can still see at the beginning stage of this residual mature ova in the posterior part of the ovary and in ovarian canal. Oocytes of the most advanced stock correspond to those of early stage III
VII	End of the spawning	The ovary after the last spawning appears clearly disorganized and all maturing oocytes showed signs of necrosis. All these cells will degenerate and be reabsorbed; only perpetuate oocytes of general stock.

Table 3: The different stages of sexual maturity in the male Sardina pilchardus fished in the Gulf of Annaba (by A. FONTANA, 1969).

Stages		**In male of *Sardina pilchardus***
I	Immature et repos	White or slightly translucent and very fine gonad in the form of knife blade
II	In process of maturity	White gonad; no liquid flows if there is an incision.
III	Prespawning	Gonad flaccid and white - white liquid elapses whenan incision ispracticed.
IV	Spawning	Gonad big and soft. Sperm flowing any pressure exerted on the abdomen
V	Post spawning	Gonad flaccid and having a very fine vasculature particularly in the posterior portion. At the end of spawning, gonad very flabby, and highly vascularized exhausted.

2.3.3 Method of monitoring the evolution of sexual maturity

2.3.3.1 The scale of sexual maturityis microscopic (Belveze, 1984) and includes the seven Cinque and sexual maturity stages known in males and females, respectively (Table 1 and 2):

2.3.3.2 The spawning periodof sardine is determined using two approaches: a qualitative approach based on the monitoring of monthly fluctuations in the percentage of different stages of gonad development, and a quantitative approach based on the monitoring of monthly changes the gonad index RGS (Lahaye, 1980) and condition factor K (Idrissi, 1985):

$$\boldsymbol{RGS = \frac{G}{W} \times 100}$$

with G is the gonad weight and
W the total weight of the fish.

$$\mathbf{K= \frac{W}{L^3} \times 100}$$

with W the total weight
L the total length of the fish.

2.3.3.3 The size at first maturity (L_{50})is defined as the fork lengths (Lf_{50}) at which 50% of individuals are mature (La Roche et al, 1983).

In this study, the total length was used. The percentage of mature individuals in each length class was calculated by setting the threshold of maturity from stage III, which is the beginning of the development phase of the gonads. The logistic model, type symmetrical sigmoid is chosen for the graphical representation (Pope et al, 1983):

$$\mathbf{P = \frac{100}{1+e^{-(a+b*l)}}} \qquad \mathbf{(1)}$$

with p percentage of mature individuals by size class (L).
The parameters a and b are obtained by logarithmic transformation of the expression (1)
This gives the equation of a straight (2) having the form:

$$\mathbf{-ln\left(\frac{100-p}{p}\right) = a+b*L} \qquad (2)$$

2.3.4 Histological Method:

The ovaries and testes sample sardine were analyzed histologically in the histopathology laboratory "NIHA" located in the province of Annaba, East of Algeria.

A section of the central part of the right lobe of the gonads has been selected, sectioned at 5 µ, included in aqueous Bouin in a small volume, due to its rapid penetration into the tissues of fish and colored with Hematoxylin and Eosin (McDonough CJ et al, 2005).

Gonads (females, males) that were fixed beforehand are included in paraffin blocks. The transverse sections are formed by using a microtome

"Microm" Nucleo cytoplasmic observation was performed using an optical microscope equipped with a digital camera (Zeiss ®).

III. Results

3.1 Results of the morphometric method:

Table 4: Results of measurements performed on the sardine during the months of the study

Months	**Lt (cm) Average**	**Ls (cm) Average**	**Lf (cm) Average**	**Pt(g) Average**	**PG (g) Average**	**PE (g) Average**
June 2012	13.24	11.20	10.68	21.45	0.70	19.91
July 2012	12.94	11.65	10.60	19.24	0.22	19.05
August 2012	12.44	11.91	11.34	20.10	0.27	19.21
September 2012	11.76	10.28	9.76	14.11	0.64	13.52
October 2012	12.26	10.31	10.45	14.48	0.76	13.16

November 2012	13.30	11.41	10.70	17.37	1.13	16.09
December 2012	16.27	14.73	14.95	28.86	1.60	24.88
January 20123	13.08	11.18	11.70	16.78	0.74	13.81
Februry 2013	16.25	14.46	14.13	28.54	1.60	25.38
March 2013	14.4	13.15	12.68	24.92	0.91	24.14
April 2013	12.08	10.75	10.38	16.68	0.36	15.50
May 2013	11.93	10.5	9.93	14.88	0.29	15.54

The results of measurements performed during the 12-month study shows changeshomogenous in different lengths average minimum and maximum values correspond to the months of September and December of 2012 (Table 4).

Table 5: Annual Followed analysis of sexual maturity in 72 sardines reserved for the study of reproduction

Months	**No monthly total individuals**	**Maturity stages**													
		I		II		III		V		IV		IIV		IIIV	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
June 2012	6	3		1			1	1							
July 2012	6		4		1	1									
August 2012	6	2		1	2		1								
September 2012	6	1		1		1	1	1		1					
October 2012	6		1			2	2	1							
November 2012	6					1		1	2	1					
December 2012	6									1	2	1	2		
January 20123	6		1				1		1				1	2	
Februry 2013	6										1		1	3	1
March 2013	6			1		1	1	1		1			1		
April 2013	6	4	1	1											
May 2013	6	1	2			1		1							

Analysis of Table 5 clearly shows the presence of an annual sexual activity in *Sardina pilchardus* fished in the Gulf of Annaba however a sexual repo is registered and corresponds to the month of July-August 2012, it is important to note that in our study considers mature individuals from the macroscopic stage III on the scale of sexual maturity.

3.2 Monthly variation in the percentage of stages of sexual maturity:

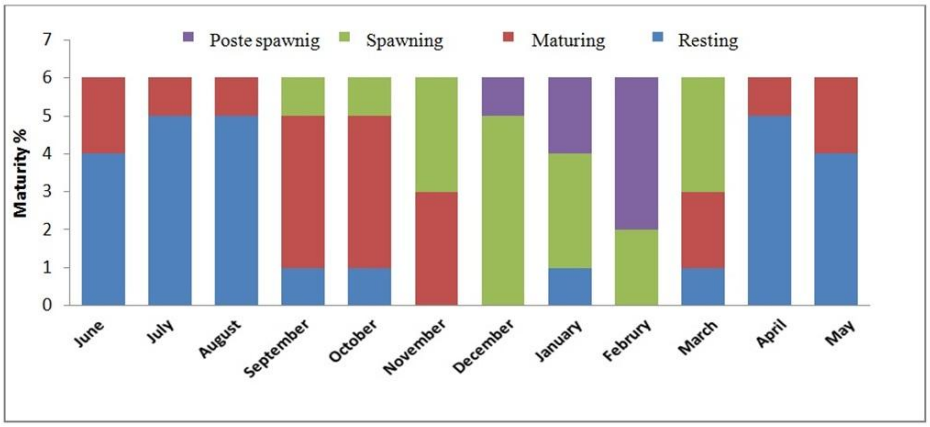


Fig. 5: Annual change in the percentage of sexual maturity in *Sardina pilchardus*

The monthly variation in the percentage of stages of sexual maturity shows that males and females are at different stages of sexual maturity throughout the year but their percentages are variable (Fig. 5). Sardines early maturation (stage III) and post spawning or post-issuance are poorly represented in our samples (Fig. 5). Mature individuals may be encountered throughout the year, but it is autumn that the percentage is higher. Lowest percentages of sardine reproduction were observed in summer. During this season, most sardines have finished reproducing and enter into sexual rest period (stages I and II). However, some fish may happen again in the summer.

These four categories include:

1- The rest category means the stage I & II
2- Category means maturation stages III & IV
3- Category means reproduction stages V & VI
4- Category means post spawning stage VII

3.3 Results of the Method of Monitoring the Evolution of Sexual Maturity
3.3.1 Monthly Variation of Gonadosomatic Report (GSR) During a Year of Study (2012-2013).

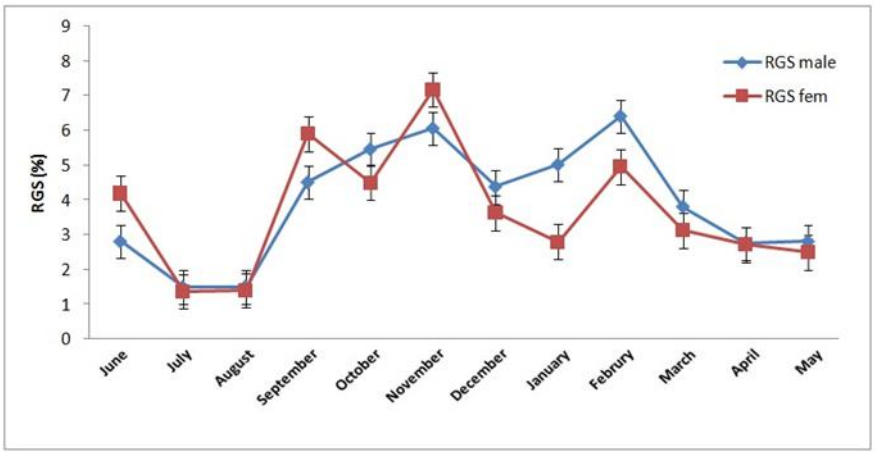


Fig. 6: Evolution of the monthly variation in the gonadossomaticindex, RGS in Sardina pilchardus from June 2012 to May 2013

In males, the average RGS is maximum in February 2013, and it becomes minimal in August 2012 From June 2012 to May 2013, a similar evolution of the average RGS was observed in females (Fig. 6). However, in females, we observed that the average RGS have higher values than those of males.
In the region of Annaba, sardines can breed between September and June and have a period of high sexual activity between November and February.

3.3.2 Monthly Variation of Condition Factor (K) during a year of study (2012-2013).

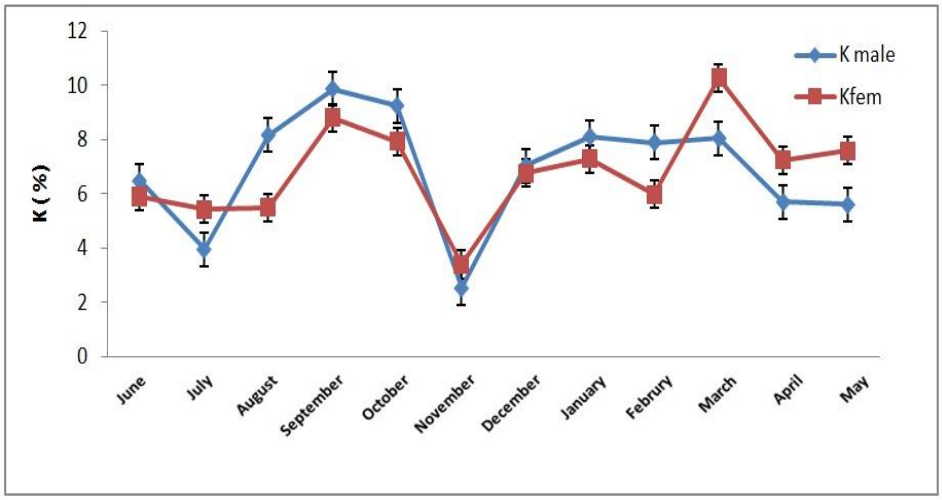


Fig. 7: Evolution of monthly variation in condition factor K medium in *Sardina pilchardus* from June 2012 to May 2013

The graphical representation of monthly average shows that K has a similar trend in both sexes and variability between monthly (*Fig. 7*). From June 2012 to May 2013, K was reached in a lower value than other me. Males, K is minimum in April 2013 July and November 2012 and is maximum in August, September and October 2012. Condition factor K goes through a period of growth in the spring and a longer period of decline between September and November and December and February.

3.3.3 Size at First Sexual Maturity L50:

In sardines, the proportion of mature individuals was calculated during the period of peak spawning was defined by previous analyzes, between June 2012 and May 2013. Thus, the class size at first sexual maturity average both sexes combined was 13.5 cm (Fig. 8).

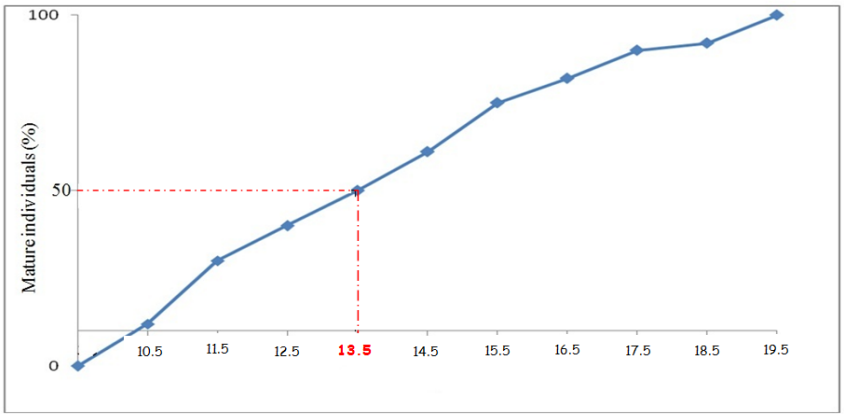


Fig. 8: Change in the percentage of mature individuals compared to the size of *Sardina pilchardus* fished in the Gulf of Annaba by applying the logistic model

3.3 Results of the histological method

3.3.1 in the female

Reproductive Cycle:

Relying on the histological observations of gonad development and monthly fluctuation in RGS, the Sardine ovaries go through five successive stages. The frequency distribution of ovary developmental stage isshown in Fig. 9.

1) Early Growing Stage:

Species in the early growing stage were first observed in April. The number of oocytes in the peri-nucleolusstage vary between 100 µm in diameter in the ovaries increased gradually, and oocytes in the oil droplet stageranging from 100 to 150 µm in diameter show in the ovarian lamellae (Fig. 9a).

2) Growing Stage:

The ovary accumulated oocytes in the yolk globule phase with a granular yolk globule ranging from 150 to300 µm in diameter happen during the beginning of September, including those in the oil-droplet stage (Fig.9b).

3) Mature Stage:

The GSI reached the top in October and December. Individuals had mature oocytes stages ranging from 300to 480 µm in diameter with a big oil-droplet (Fig.9c).

4) Spawning Stage:

There was individual variation during migration time, and fish migrate offshore between November andJanuary to spawn, so no colonies were found during the spawning stage.

5) Resting and Recovery Stage:

There was a rapid decrease in GS in November. The ovaries contained primarily immature oocytes and afew ovulatory follicles. Individuals in this stage showed up from November to March (Fig. 9d).

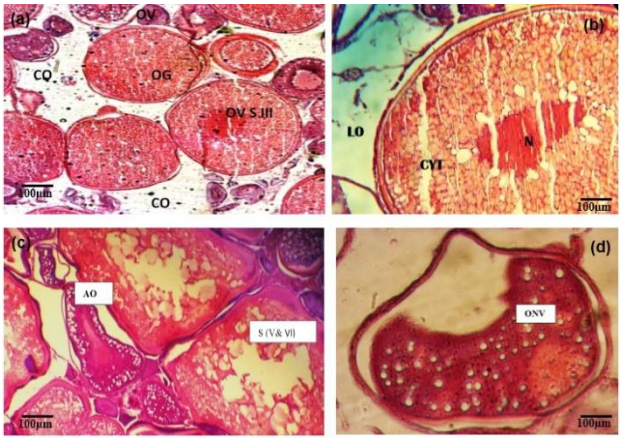


Fig. 9: Maturation stage of the ovaries of *Sardina pilchardus* collected from gulf of Annaba.

(a) Pre- vitellogenic yolk stage**, (b,c)mature in the late vitellogenic yolk stage, (d) after spawning with : CO:** Ovarian Cavity**, OV:** Oocyte vitellogenic**, OG:** Oogonia**, OVS** (G x 100) **III:** Stage III oocytes **(G x 100),** Onset of maturation of the ovary (stage III), **CYT**: cytoplasm, **N**: nucleus, **AO**: **Start of oocyte atresia**
(G x 150).**ONV**: Oocyte non vitellogenic
(GX 400) Stage 7 **post spawning**

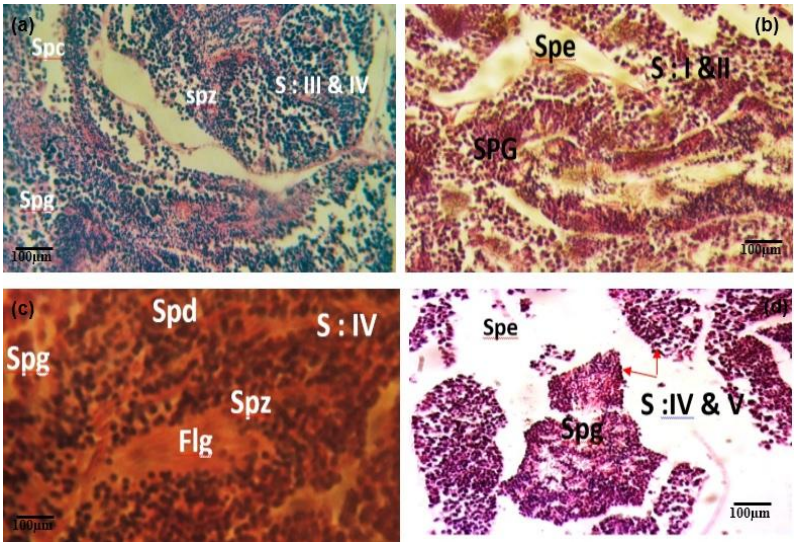


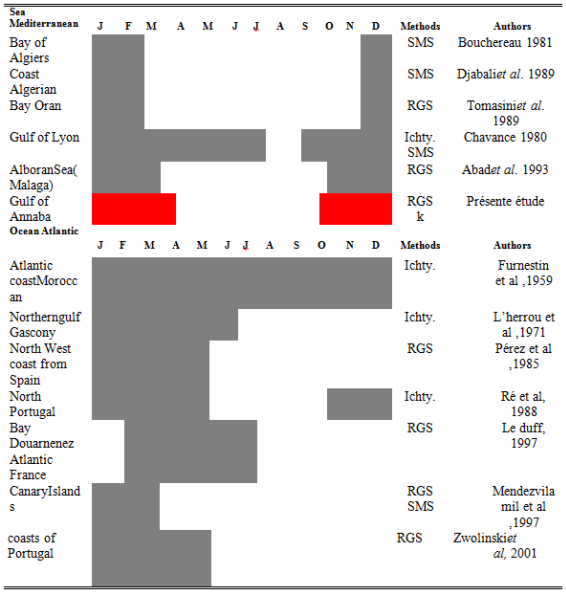
Fig. 10: Maturation of testes of *Sardina pilchardus* from gulf of Annaba.
Spg: spermatogonia, **Spz**: Sperm, **Spd**: spermatids, **Flg**: Flagellum sperm, S: IV Stage of sexual maturity (G x 60), **Spe**: Sperm

Concerning the evolution of sexual maturity male gonad, there is a growing trend with peaks during the months of maturities from August to February which coincides with the spawning period of the same Fig. 10 clearly shows the dominance of the spermatid stage.

IV. Discussion

Morphometric parameters Lt, Ls, Lf, Pt, Pe, Pg, and thz stages of sexual maturity recorded during 12 months of sampling for the species *Sardina pilchardus* sin in Annaba golf clearly show variations on all the parameters studied.
These variations are due to the sampling that was (single and independent) strategy taken randomly

Table 6: Period of reproduction of Sardina pilchardus its distribution (SMS: study result Sexual maturity stages, Ichty: study result ichthyoplankton, RGS: study result report gonadal somatic K: result study condition factor).



The combined study of RGS and sexual maturity stages showed that the level of the Annaba region, sardine can reproduce throughout the year with a period of maximum reproduction between November and February. This result is shown by studies ichtyo plankton (Furneistin 1959, Ettahiri 1996, Ettahirietal 2003): Sardine eggs are collected throughout the year along the Mediterranean and Atlantic coast, their density is highest in winter and becomes weak in summer. However, there are inter monthly variations of the sexual cycle of the sardine and which are due to environmental conditions of the particular middle in the temperature (Abadet al. 1993 Ettahiri 1996 Ettahiri et al, 2003).

The work done by some authors in the Mediterranean Sea and the Atlantic Ocean on the breeding periods of Sardina pilchardus (Table 6) indicate the existence of a variable breeding season by region.

Our results for the condition factor K and RGS vary inversely. It is a common feature of Sardina pilchardus Mediterranean Sea (Kartas 1984, Tomasini et al. 1989, 1993 Abadet al.1993) and Atlantic Ocean (Pérez et al. 1985 Zwolinskiet al. 2001).

The evolution of K shows that males and females have a similar strategy in the use of energy intake during gonad maturation and spawning. The maximum value of K corresponds to the month preceding the start of reproduction (October). This implies an accumulation of reserves in the sardine before the breeding season and then transfer the energy to the production of gametes in accordance with the work Abadet al. 1993 Freon, al. 1997 andZwolinskiet al. 2001.

It is summer season when trophic conditions are favorable (Somaoue, 2004) sardines feed heavily while accumulating reserves. This is confirmed by an experimental study in Tunisia on sardine and showed that ovarian development for laying winter and spring is strongly influenced by the accumulation of reserves in summer (Tsuruta, 1987).

In autumn, maturing gonads, decreased K can be explained by the fact that reserves are invested in the development of sexual products and gonad development compresses more the digestive tract of fish (Lahaye, 1980).

Changes in condition factor observed in the Mediterranean are related to the upwelling indices (Pérez et al. 1985). Changes in upwelling periods affect spawning periods for certain species of clupeid *Sardina aurata* (Roy et al. 1991).

The optimum temperature for reproduction of sardine Algerian between 16 and 17 ° C in winter and 18 in summer and 19.5 (Ettahiri 1996,Ettahiriet al. 2003). Throughout our study period, temperatures ranged between 11.3 and 28.9 ° C and it is likely these favorable thermal factors that cause spawning spread throughout the year. Reproduction of sardines would be in the main dependence of temperature rise but the food rich environment could play a significant role. Indeed, the availability of food for adults can influence sexual maturation. Poor trophic conditions do not allow the folds accumulate reserves; its yolk is then inhibited (Horwood et al. 1989).

Table 7: Size of first maturity of Sardina pilchardus in various sectors of the Mediterranean and the Atlantic Ocean

MediterraneanSea	**Males**	**Females**	**Authors**
Gulf of Lyon	13.8	14	Lee 1961
Castellon	11.7	11.3	Larraneta 1976
Bay Oran	11.1	11.1	Bouchereau 1981
AlboranSea	12.8	12.5	Abad*et al*, 1993
Gulf of Annaba (Algérie)	13.5	13.5	Présente étude
Gadiz	10.5	11.5	Rodriguez-Roda, 1970
Canaryislands	15	15.2	Mendez-Vilamil*et al*, 1997
laayouneRegion (Morocco)	16.3	17.5	Khadija Amenzoui et al ,2001

The size at first maturity varies depending on the year and the area in question (Table 7). This variability between monthly is normally due to the temporal variability of the date of onset of laying (early or late depending on the month spawning) and corresponding annual recruitment (Abadet al. 1993). L_{50} may also vary according to sex except sardines Oran bay, and the Canary Islands: the L50 is the same for both sexes (Table7).Our results (from 2012-2013) are close to those obtained with GOLF LION.

V. Conclusion and Recommendations

The presence, density, variety, prey quality (species, size, nutritional quality) and the physical and chemical conditions of the environment would influence heavily the requirements of the fish and their ability to grow, reproduce and survive.This study allowed us to identify the reproductive cycle of *Sardina pilchardus* fished in the Gulf of Annaba. Compared to the same species sampled in different geographical regions. This study discusses the role of salinity in implementing of maturity. At the end of this study, other work should be initiated on several species is the use of the method of molecular biology

To reach this objective, rational management of these resources requires consideration of human activity, fishing, as ecosystem component. Knowing that it is very difficult to envisage a dynamic spatial-balancing option of fishing effort for small pelagic a result of their geographic distribution highly variable, the tendency is to propose management measures to limit catches of younger fish and to promote the return of big fish.In fact the big fish are more interesting and commercially more efficient at reproduction and consequently the renewal of the stock.

In the region of Annaba, the reproduction of the sardine occurs all year, but the existence of the upwelling phenomenon that shows well defined seasonal variations, some periods are much more favorable than others. Thus, spawning sardine is maximum in winter, the minimum upwelling season and a minimum of zooplankton production. It is low in summer, maximum seasonal upwelling and maximum production zooplankton .The temperature appears to be key factor in triggering spawning or by stimulation of the physiological mechanisms either by mid-trophic enrichmentThe spawning *Sardina pilchardus* is maximum in winter, minimum upwelling season and a minimum of zooplankton production in this case the season plays a major role in triggering spawning either by stimulation of the physiological mechanisms either trophic enrichment of the environment

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