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Histological Characteristic of the Gonads of Sardine *Sardina Pilchardus* (Walb, 1792) in the Coast East of Algeria (Gulf of Annaba)

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Abstract

To illustrate the different kinetics of oogenesis and spawning strategies, reproduction in the Sardine (*Sardina pilchardus*, Walbaum 1792) was discussed. The histological samples, realized over the 3 periods of sexual cycle: oogenesis, egg and rest, were analyzed for 2217 specimens caught in the period from November 2015 to October 2016 using a microscopic scale of oocyte development with 6 stages. The histological results allowed us to determine the size of first sexual maturity is 12 cm, acquired during the third year of life of the sardine. Monthly monitoring of the evolution of macroscopic and microscopic stages of sexual maturity, allowed us to suggest that the waters of the coastal eastern Algeria, sardines spawn from December to March. Sexual rest is observed in the 2 sexes in May, during the summer and part of fall. Spawning and sperm emission ending in April. Microscopic observations show six stages of maturity in females and four males. Earlier maturation, oocytes come together in one lot, come into vitellogenesis and evolve synchronously to mature simultaneously. Separated from each other by the gap, they are issued in a fractional short laying. Ongoing recruitment of pre-vitellogenic oocytes determined new ways of oogenesis and lying, there are as many as egg laying lots placed in vitellogenesis. This type of distribution allows multimodal oocyte laying series with lots of small eggs released during a long period of time, post-spawn is difficult to identify. These results illustrate the complexity of multiple clutches.

Keywords: Sardine, Mediterranean, reproduction, spermatogenesis, oogenesis

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Introduction

The sardine was selected as priority species in the stock assessment studies in the Mediterranean by the Scientific Advisory Committee (SAC) of the General Fisheries Commission for the Mediterranean (GFCM) concerning fisheries management (FAO - GFCM 2009). The Commission of European Communities (CE, 2001) estimates that knowledge of stock status is a prerequisite for the definition of management measures, often very demanding, especially when it comes to stock small pelagic like anchovies or sardines. Each year, young fish from breeding reached a sufficient size to join the group of individuals exploitable on a fishery. Sardine is a short-lived species for which recruitment age specimens consisting of 1 (one year) constitutes 70% of the biomass (ICES, 2006); environmental conditions play a major role in the success of recruitment (Brander, 2005).

However, below a critical threshold, the spawning biomass becomes a limiting factor and the risk of bad hires estate increase. Therefore, the protection of juveniles is essential, given that the recruitment is highly dependent on the abundance of spawners. The latter is the result of all the processes that take place between the breeding season adult sardines, breeding or spawning biomass (BR) and the arrival of young fish or recruits on the fishing grounds.

It is necessary to understand what happens to organic scale between these 2 steps for understanding the reproductive strategy of sardines and optimize inventory management. Since the 90s the working group of the International Council of Marine Exploration (ICES, 2005) to assess the reserves by cohort analysis, develops and monitors the study programs on species of commercial interest. In terms of economic value, the sardine is, for much of the European fleets, the majority share in tonnage; it would have increased by 50-70% over the last ten years (ICES, 2006).

In Algeria, the pelagic fishery is dominated by landed *S. pilchardus* which represents 58% of total captured (Ministry of Fisheries and Fish Resources - MPRH, 2008). Small pelagic species concerned are mainly: *Sardina pilchardus*, *Sardinella aurita*, *Engraulis eucrasicolus*, and Atlantic horse mackerel and *Scomber scombrus* representative tonnage landed 80% of fisheries. We discuss in this study, analysis of the different phases of the evolution of the reproductive cycle of *S. pilchardus*. For a better understanding of the operating state of its stock and to collect data necessary for the implementation of a plan of management, we proceeded to determine its size at first maturity, its maturation ovarian, her eggs and his recovery phase or sexual rest (Bedairia Djebar, 2009), (Bedairia, 2011). The results obtained are indispensable data for evaluating the optimum age at first capture, estimating the size of a stock and its renewal potential.

Materials and methods

From November 2015 to October 2016, we followed the weekly changing stages of sexual maturity in individual's sins 2217 in the Algerian East coastal waters (Fig.1) for LT size classes ranging from 7.5 to 20 cm. A detailed distinction between the stages of maturity has been performed by histological examination.

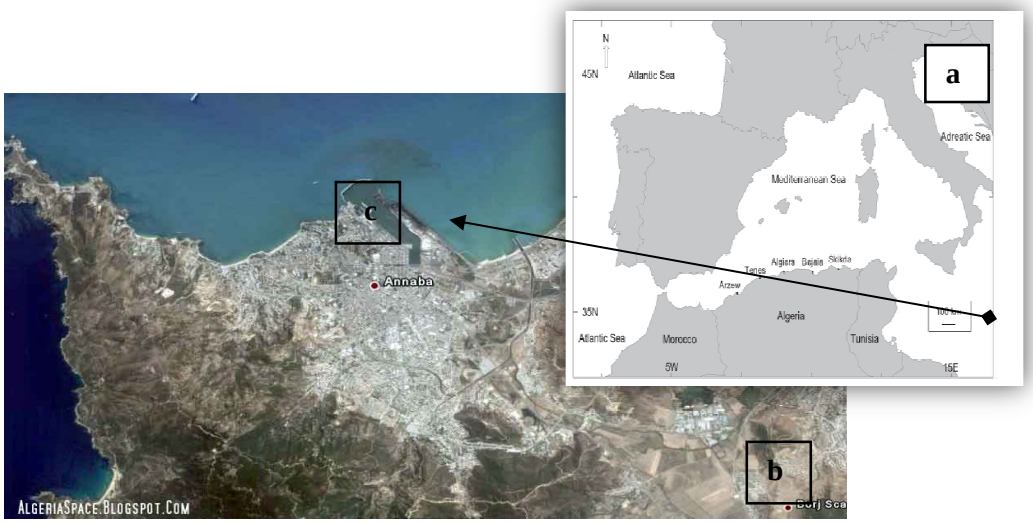

Fig.1: Satellite Map showing the Gulf of Annaba (study area). a: overall situation, b: magnifying glass showing the boundary of the study area, c: fishing port of Annaba.

Sex determination and dynamics of the sexual cycle study of the sexual cycle was conducted from data collected during a biological cycle of November 2015 to October 2016. We considered, as reported by West (1990) that the review gross sexual development based on external characteristics of the gonads, was an imprecise indicator of reproductive condition and do not determine the degree of maturity of the ovaries and testes. To avoid this ambiguity we have strengthened the macroscopic analysis by the study of microscopic characteristics.

Microscopic analysis Histological study was conducted on straight gonads on the back, a section of 0.5 cm thickness is taken from the middle part of the female gonads and male sardines captured between November 2015 and March 2016 in various stages of development. After fixing the aqueous Bouin (Bouin, 1897) for three days, the pieces are dehydrated ethanol increasing degree and cloudy toluene and embedded in paraffin to be cut to 7 microns and stained with Masson's trichrome and according Martoja Martoja - Pierson (1967) before microscopic observation.

Digital acquisition photomicrographs and the measures of the oocyte diameter were obtained using the image processing software Motic Images Plus 2.0. ML.

Results and discussions

Results of microscopic observations of the stages of sexual maturity

Oogenesis. Ovarian cycle consists of six stages (St. I to St. VI), this is a reflection of oogenesis, spawning or degeneration of a part of oocytes from the ovary. Oocyte growth can be decomposed into two main phases: the pre-vitellogenesis followed vitellogenesis. The different criteria used to define these stages include oocyte size, homogeneity, the quantity, and distribution of inclusion bodies in the cytoplasm, such as the yolk blood cells. The ovaries are organized into slices. Stage I, to immaturity or sexual inactivity is followed by stage II, pre-vitellogenesis or early maturation and the III corresponding to vitellogenesis or maturation, stage IV, laying the V, partial or post-spawning fractionated and finally the stage VI or sexual rest and recovery cycle.

The immature ovary (St. I). In periods of sexual rest, in ovarian immature individuals, we encounter a cluster of three types of cells:

- **Oocyte pre-perinuclear (opa):** small cells and not yet differentiated nuclei (Fig 2 a.).

- **The perinuclear oocyte (op) (Fig 2 a.)** Or primary oocyte, small cell 20 to 25 microns carrying a large eccentric nucleus (N) and a dense and homogeneous cytoplasm, rounded outline without any regular training yolk granules. The oocyte perinuclear resulting from the mitotic division of the germ cell, which is transformed into pre-vitellogenic oocyte.

- **The oocyte pre-vitellogenic (opv) primary (Fig. 2 b) and secondary (Fig. 3 a)** are larger with a bigger core than the perinuclear oocyte containing one or more nucleoli (Nu) and a homogeneous staining cytoplasm containing granules of carbohydrate nature (+ reaction with Schiff's reagent). The structure of the nuclear envelope becomes irregular it presents cul-de-sacs that houses the nucleoli, become fewer and less visible; this is the beginning of vitellogenesis.

Ovary early vitellogenic (St. II). In early vitellogenesis (Figs 2 b and 3 a), the ovary contains opa, op and opv being transformed into ovotide or oocyte vitellogenic primary larger. At this stage, the primary oocytes vitellogenic volume doubles filling the follicular lumen, their sizes vary between 100 and 150 microns. Rounded and bulky, their cytoplasm surrounded by cytoplasmic membranes is then invaded by lipid vacuoles. During vitellogenesis, the yolk sacs accumulate and organize into piping on the outskirts of the vitellogenic primary oocyte before gradually occupy the cytoplasm. And follicular cell membranes are well differentiated by the external library, cord follicular flattened cells and finally the establishment of the zona radiata.

Ovary advanced vitellogenesis (St. III). Vitellogenic Advanced (Fig. 3 a), the mature oocytes ovarian fill light and are ready to be expelled. Mature gonads then have a typically dense appearance since all grouped follicles are filled with big eggs. These follicles are surrounded by oocytes at all stages of pre-vitellogenesis and vitellogenesis. We also note the presence of many colored inclusions phloxin, gradually merging into blood cells and pushing the yolk vesicles in the periphery of the oocyte. Arguably the stage of vitellogenesis Advanced is characterized by the presence of two varieties of oocytes: secondary and tertiary.

- **The secondary oocyte:** virtually all of its cytoplasm is occupied by two types of granules yolk: fine granules size distributed homogeneously in the cytoplasm and larger which constitute a ring around the envelope nuclear (Fig. 3 a).

- **Tertiary egg:** the egg at the end of secondary vitellogenesis, hydrates and becomes hyaline (Oh) (Fig 2b.), Inclusions yolk invade the cytoplasm while the core when it is pushed to the invisible periphery. The oocyte hyaline is characterized by the presence of clear enclaves corresponding to the start of the liquefaction of the yolk. It is hardly met in histological sections because it is brittle and breaks in contact with fixatives.

Ovary egg (St. IV). End vitellogenic (Fig. 3 b) the oocyte has reached its mature size is expelled from its follicle. At this time, the nucleus has disappeared and the presence of empty follicles is irrefutable evidence of spawning. The connective tissue, upon which mature follicles become loose and the follicles are emptied gradually as laying proceeds.

At the end of vitellogenesis, the cells fuse to form yolk platelets, are hyaline oocytes. At this stage, we find mature oocytes in some follicles while others are totally empty. Partly spawned by females are distinguished from others by the simultaneous presence in their ovaries, oocytes of vitellogenic representing the five stages of development and post-ovulatory follicles (fpo).

Throughout the period of laying smaller eggs vitellogenic alongside larger, which when mature, are issued in batches, spawning is called fractional. Ovarian follicular atresia partial and post-lay (St. V). At the end of the laying period (Fig. 3c), the vitellogenic oocytes are still present in the ovary, they do not complete their maturation and degenerate.

The appearance of fpo, oocytes expelled their nuclei and kept their zona radiata and some cytoplasmic inclusions are witnesses of an earlier spawning, interfollicular space and follicular light are generally devoid of mature oocytes which gives a gonad flaccid appearance that observed macroscopically stage V.

Oocytes atresia pre-ovulatory and post-ovulatory follicles are also encountered. The follicular atresia, indicates the end of the nesting season and the start of the recovery phase, it first affects the oocytes to the most advanced stages and hyaline. Oocytes in early atresia are easily recognizable during periods of pre-oviposition, oviposition and / or post-spawning. In degeneration, they lose their arrangement then gradually disappear, their only remaining flat and corrugated follicular envelope.

Stage follicular atresia. Post-ovulatory follicles have a wide and irregular light, an envelope formed of a single layer of follicular cells and a theca prismatic conjunctiva (Fig. 3 c). This phase begins with sardine Algerian East coast in May. A reduced amount of non vitellogenic expelled before laying eggs come into atresia. It can happen in the end of season, when environmental conditions are unfavorable, a whole lot of oocytes in vitellogenesis becomes atretic. Generally four key events associated with the oocyte atresia: shrinkage and distortion of the follicle, disorganization and liquefaction of the yolk, the follicular cell hypertrophy and the ingestion and digestion of the yolk by follicular cells.

Ovary sexual rest (St. VI). At the beginning of the recovery period, it remains in the ovaries that oocytes (opv) associated in rare residual oocytes degenerating and some (fpo). The sexual rest is the phase that resembles the immature stage I in which the ovaries are reorganizing histologically in ovarian strips. Oocytes which are based on the epithelium of the lamina are no longer juxtaposed as in immature ovary.

We observe a subsequent reconstitution of ovarian strips and the establishment of a new stock of oocytes (opv) by multiplication and evolution of oogonia (opa) and small oocytes (op) drafted in immature follicles. At this stage, pre-vitellogenic oocytes in the early growth of the cytoplasm let appear a perinuclear structure of oocyte and pre-vitellogenic slow growth. Their central enlarged nuclei (N) contain numerous nucleoli arranged at the periphery, they are devoid of yolk granules and nuclear envelope is irregular. Sometimes the nucleus contains nucleoli between the folds of the nuclear envelope with yolk sacs in peripheral edging. At this stage it is difficult to distinguish macroscopically male female sex is then classified as indeterminate.

Spermatogenesis. In male sardines, spermatogenesis takes place inside cysts, is a succession of meiotic divisions. To assess testicular development we have identified four stages of maturity (I St. in St. IV):

- Stage I or stage of development of spermatogonia and Sertoli cells
- Maturation stage II or stage I and II spermatocytes and spermatids,
- Stage III spermiogenesis with appearance of sperm.
- Stage IV post-issuance and sexual rest.

These stages of sexual development, succeed over time and differ in testicular structures (Fig. 4).

Testis development (St. I). The immature testis (Figs 4a and b), organized in tubular structure, presents a set of seminiferous tubules, it is surrounded by a membrane against which Sertoli cells are plated. Leydig cells to endocrine function are located in the interstitial space between two tubules. Each seminiferous tubule contains spermatogonia rounded with a large central core lining the blade basale.

A this point, the testicle is not yet able to produce sperm, it is organized into cysts within which all the sex sells at the same stage of differentiation. In second phase division (Fig. 4 b), the latter have a lot of haploid chromosomes, they undergo meiotic divisions to give spermatids which will differentiate into spermatozoa. Thus, germ cells at various stages of maturation may be present in the lumens of the tubules.

Testis maturation (St. II). Testicular this well organized cysts containing all stages of spermatogenesis (Figs 4 c, d and e). Spermatogonial cysts to become large due to increased mitotic activity of germ cells within it. In the connective tissue juxtaposed to the envelope of the cyst and from the increase in spermatogonia, born spermatocytes undergoing meiotic divisions to give spermatids which size is distinctly lower. Sperm from the last phase of spermatogenesis, are distinguished from other germ cells by the presence of a long flagellum.

Testicle emission (St. III). The presence of empty cysts sperm is a sure sign of testicular broadcast. At this stage, the centripetal spermiogenesis is in the cyst spermatids (Fig. 4 f). Spermatogenesis is characterized by the disappearance of stages of multiplication and training spermatogonia spermatocytes.

Testicular post-issuance and sexual rest (St. IV). Spermatic cysts (Figs 4 g and h) were emptied of their sperm, is the stage of post-issuance or sexual rest; their structure is disorganized, their diameter shrinks and the interstitial connective tissue that persist residual germ cells. The illustration of the successive stages of gonadal development in *S. pilchardus* of the Algerian East coast has allowed us to identify the different periods of the sexual cycle, which is frequently linked to the evolution of biotic and abiotic factors.

Discussion

Understanding the causes of fluctuations in fish stocks is one of the main topics of study of research in fisheries. Pelagic species make up the largest share of global marine catch, representing approximately 26% (22.5 10^6 tonnes) of the total catch. In the Mediterranean, they represent 50% of the total catch. 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.

This histological study, we describe the main evolutionary phases macroscopic of reproduction in *S. pilchardus* the East of Algeria. 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 as called Pinto and Andreu (1957) microscopic. 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 spermatogenesis.

The results of the macroscopic approach allowed us to limit the breeding season between December and March, to determine the size of first sexual maturity of 12 cm and group sexual maturity five stages. These results are consistent with those obtained by Mouhoub in 1986 when work on the biology of sardine fished in the coastal Algerian, where he shows that five maturity stages are sufficient to establish the size at first maturity. They are also comparable to those reported by Kartas (1981) *S. pilchardus* caught in the Mediterranean.

This author states that the size at first maturity of mature individuals of Tunisian coast is 12.3 cm for females and 11.8 cm for males. The Algiers coast Mouhoub (1986) estimated the size at first maturity at 12.9 cm for females and 11.9 cm for males. Bouchereau (1981) the 12.3 cm for both sexes sampled in the Bay of Oran. Finally, (Amenzoui *et al.,* 2001) estimated the size at first maturity of sardine Laayoune region (Morocco) to 16.3 cm and 17.5 cm respectively for males and females.

Our findings are comparable with those obtained in the Atlantic by Rodriguez-Roda (1970) in Cadiz in Spain which sets the size at first maturity at 10.5 cm for males and 11.5 cm for females sardines and those estimated by Larrañeta (1976) indicating that the sardine Castellon reach their size at first maturity to 11, 7 cm and 11.3 cm respectively in males and females. Detailed analysis of these results shows that Mediterranean sardines acquire their size at first maturity rather than those of the Atlantic. Abed *et al.,* (1993), changes in the size at first maturity may be due to the different strategies developed by fish in different environments to better adapt to environmental conditions.

Microscopic analysis has 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. Thus, we have combined observations from macroscopic and microscopic stages of maturity to know the status of individual sexual maturity, also known as gonadal maturity profile involving five stages. Bouchereau (1981) was inspired by the scale of sexual maturity ordered into seven stages, established for herring (Wood, 1930) to study the sardine fishery on the west coast of the Algerian coast. This scale has been slightly modified by Fontana in 1969 for the study of sexual maturity of *S. aurita* in the Pointe-Noire region of the Congo.

This maturity scale used in the Atlantic, along the African coast, is different from the one advocated in the present study that spanned six stages for females and four males. This divergence of results is explained by the fact that oogenesis *S. pilchardus* of the Algerian East coast has more than one group of oocytes that changes to the laying. However, several distinct groups of oocytes are highlighted, allowing assuming that the spawning of these fish is split during the same season, ovaries simultaneously with the histological characteristics of post-spawning, pre-vitellogenesis and vitellogenesis.

The simultaneous presence of oocytes at different stages of development and post-ovulatory follicles (fpo) classifies sardine Algerian East coast among Mediterranean species nesting sériée: during the spawning period of several successive batches are oocytes issued, the fpo testify. After partial spawn, it goes through a loop which is repeated n-1 times the number (n) of sets of clutches. This loop system ends with the increased frequency of atretic oocytes, this is the last series of nesting announced by a decrease in pre-vitellogenic oocytes (opv) vitellogenic primary oocytes (OVP) and presence some fpo. Post-ovulatory follicles have a wide and irregular light and a membrane formed of a single layer of follicular prismatic cells and a theca conjunctiva.

Spermatogenesis is usually divided into two stages: maturation and spermiogenesis, based on nuclear and cytoplasmic features (Nagahama, 1983). This subdivision was not selected in this study, microscopic analysis of spermatogenesis in male sardines Algerian East coast allowed us to describe four evolutionary stages: development (spermatogonia and Sertoli cells), maturation (spermatocytes and II and spermatids), emission or spermiogenesis (sperm) and post-issuance or sexual rest.

To better understand the life cycle of the sardine, it seems we need to deepen our biological knowledge of its reproductive strategy across the Algerian coast. Better understanding also requires consideration of the behavior and individual strategies, essential data for inventory management. Finally, we can simulate jointly developing cohorts and growth across the individual by combining the effects of environmental factors on the demography and growth of sardine. This need is even greater in Algeria for about a decade, stocks of sardines fisheries decline and are prone to strong management measures, including areas corresponding to critical habitat, spawning area or zone nursery, which instead of being protected, are often exploited.

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