

Stages of Sexual Maturation in *Scomber japonicus* (Houttuyn, 1782) on The Libyan Western Coast: A Histological Study

Alkhamesa M. S. Essa¹, Mohamed A. F. Berfad², and Esam M. K. Buzaid^{2, 3, *}

¹) Zoology Department, Faculty Science, Sebratha University, Sebratha, Libya.

²) High Institute of Marine Science Technologies, Al-Khoms, Libya.

³) Department of marine sciences, Faculty of Sciences, Omar Al-Mukhtar University, Albayda, Libya.

*Corresponding author: esam.buzaid@hotmail.com

مراحل النضج الجنسي لأسماك الكوالي (*Scomber japonicus* (Houttuyn, 1782) من الساحل الغربي الليبي: دراسة نسيجية

الخامسة محمد سالم عيسى¹، محمد عياد فرج برفاد²، وعصام محمود خميس بوزيد^{2, 3, *}

¹ قسم علم الحيوان، كلية العلوم، جامعة صبراتة، صبراتة، ليبيا.

² المعهد العالي لتقنيات علوم البحار، الخمس، ليبيا.

³ قسم علوم البحار، كلية العلوم، جامعة عمر المختار، البيضاء، ليبيا.

Received: 03 October 2023; Revised: 12 December 2023; Accepted: 18 December 2023

Abstract

About 142 fish specimens of *Scomber japonicus* (Houttuyn, 1782) were collected between the coast of Tripoli (east) and Zuwara (west), from May 2018 to April 2019, to study the stages of oogenetic and spermatogenetic development, by analyzing their histological sections. The results showed that the developmental status of these gonads is Asynchronous development type; It was found that both the ovaries and testes mature gradually so that these fish can lay eggs and sperm in the form of batches during the breeding season (June - October).

Keywords: Western Coast of Libya, *Scomber japonicus*, Oogenetic, Spermatogenetic, Asynchronous, Histological, Spawning.

الملخص

تُجمعت 142 عينة من أسماك الكوالي (*Scomber japonicus*, Houttuyn, 1782) من ساحل طرابلس شرقاً إلى زوارة غرباً، بين مايو 2018 وأبريل 2019، لدراسة مراحل تطور كلا من خلايا المبايض في الإناث، وخلايا خصصي الذكور، وذلك باستخدام القطاعات النسيجية. وقد أظهرت النتائج أن الحالة التطورية لهذه المناسل غير متزامنة Asynchronous Development؛ كما وجد أن كلاً من المبايض والخصي تنضج تدريجياً، بحيث يمكن لهذه الأسماك وضع البيض والحيوانات المنوية على شكل دفعات خلال موسم التكاثر (من شهر يونيو وحتى أكتوبر).

الكلمات الدالة: الساحل الغربي لليبييا، سمكة الكوالي، الخلايا البيضية الأنثوية، الخلايا المنوية الذكرية، نسيجية، موسم التكاثر.

1. Introduction

Chub mackerels, *Scomber japonicus* (Houttuyn, 1782) are geographically distributed in moderate and warm waters of the Indian, Pacific, and Atlantic oceans, and in the Mediterranean as well (Whitehead *et al.*, 1986; and Sabour, 2018). These middle-sized fish (25–32 cm) (Golani

et al., 2006). El-Mor, (2002) mentioned that *Sardinella aurita* is the main prey of Mackerels in the east and south of the Mediterranean.

This species has become an increasingly important commercial species in the Libyan waters. However, the fishing efforts applied inversely to this species then influenced their stocks. Therefore, it is essential to understand the reproductive biology and spawning period of chub mackerel, to estimate their population dynamics comprehensively (Rinchar and Kestenmont, 2003). Furthermore, Sabour, (2018) stated that indicators of reproductive status (the size at the first maturity and spawning season) are required basically to proper assessment and management of stocks of this species.

The gonads' maturation and spawning processes of *S. japonicas* are divided by Farrago *et al.* (2022) four maturity stages of the reproductive cycle into four stages. Whereas Sabour, (2018) recorded the development stages of gonad maturation were regarded histologically into six steps. These processes are affected by the change of some limitation factors, as Soriano *et al.* (2009) mentioned; such as temperature, salinity, oxygen, and food abundance. The geographical distribution is another factor that affects the spawning season of *S. japonicus*.

Bony fish, in general, have widely-varied patterns of spawning. Take some of them (Ungaro, 2008): i) Semelparous (Monocyclic) species: which lay eggs once during their lives, and then die; ii) Total (Isochronal) Spawners: that lay their eggs once in the whole breeding season, in less than a week; iii) Hermaphroditic species: Where one individual can produce eggs and sperm at the same time, or in exchange times. Back to the Chub Mackerels: Their females lay eggs in batches at close intervals at the beginning of the season; and Partially as iv) (Heterochronal) spawners, which extend in spawning for a prolonged period that may reach a few months. Histologically, the tissue segments of gonads in this pattern are distinguished by different maturity stages in their egg cells and sperm.

Genten *et al.* (2009) stated that the histological examination of ovarian tissue commonly reveals eggs at all stages of development and degeneration. Recent researchers take histological techniques seriously as one of the modern methods to understand reproduction in fish (West, 1990; Tyler & Sumpter, 1996; and Blazer, 2002). Farrag *et al.* (2022) confirmed the spawning time of Chub Mackerel via yolky stages with a partial mode of asynchronous oocytes. The reproduction data, such as estimates of fecundity and spawning, are important topics for understanding fish biology and their population dynamics (Shalloof and Salama, 2008; Costache *et al.*, 2011; Farrag *et al.*, 2019; and Farrag *et al.*, 2022).

Even though Libya has long coast over the Mediterranean, Sasi *et al.* (2016) and Berfad *et al.* (2018) on this species. The major aim of this work is to update the reproductive knowledge on the Chub mackerel, *S. japonicus* on the western Libyan coast, with more detailed data regarding the G.S.I. and using the histological structure and reproduction pattern of males and females during the breeding season.

2. Materials and Methods

1.2. Sampling

142 different-sized specimens of *S. japonicus* were collected from the coast of Tripoli (east) to Zuwara (west), a monthly, from May 2018 to April 2019. The standard length, from the forehead to the last vertebrae of the column for each specimen, was recorded. They were annotated and their sex was determined outwardly.

2.2. Laboratory preparation for tissues

According to Zawistowski (1986); the gonad samples were prepared for the tissue sections. A sharp scalpel was used for cutting in the middle. Then fixing in 10% buffered formaldehyde solution; A dehydration process was carried out in an ascending concentration of ethyl alcohol (70; 80; 90 and 100%); and then cleared in xylol, before embedding in the paraffin-wax molds. The microtome was used to cut, in thickness ranging between 5-7 μm for each section, before loading on glassy slides and staining; using Hematoxylin and Eosin Stains. Finally, Canada balsam was applied on, and covered by slides.

2.3. Tissue microscopy

To identify the development of the gonads, the slides were placed under a binocular light microscope (10X - 40X); where four stages of development of oocytes for females, were recorded as indicated by Murua & Saborido-Rey (2003), and four stages of development of spermatogonia in male testicles, as Ungaro (2008) mentioned.

3. Results and Discussion

In this work, the standard length (SL) in males and females of *S. japonicus* ranged between 15.5-30 and 15-24.5 cm respectively. Based on the analysis of histological sections of the ovaries and testicles of *S. japonicus*, it was found that they are Asynchronous Development, thus, they mature in inconsistent periods from the beginning of the breeding season (Fig. 1). The same happens to the most of Scombrids, this indicates to the long spawning period; with laying eggs frequently (Chellappa *et al.*, 2010). Farrag *et al.* (2022) divided the reproductive cycle for males and females of *S. japonicas* into four maturity stages: i) immature; ii) maturing; iii) ripe and spawning; and iv) spent stage.

Microscopically; these analyzed histological sections showed that ovaries were found filled randomly by eggs of all sizes and developmental stages, in the spawning season between June and October, as it will be detailed below. These results differ from what was reported by Carvalho *et al.* (2002) on *S. japonicus* in the Atlantic Ocean, where these fishes go through one reproductive cycle annually, between March and July.

3.1. Microscopic characteristics of ovaries

Murua & Saborido-Rey (2003) elaborated on the stages of oogenetic development of oocytes, based on morphological changes in these cells as follows:

3.1.1. Primary Stage:

In this phase, the ovary consists of clusters of Oogonia cells, which act as a stock that produces the embryonic cells to the ovary, and appear as small granules distributed in the cytoplasm, and

the nucleus appears in the center of the cell and grows to form a perinuclear oocyte (Figure 2). The females of *S. japonicas*, with many other bony fish species, are similar in the point that they have these oocytes (Selman *et al.*, 1993; and Grier, 2000). In females of this study; it was found that they get this stage during November, and they are ranged between fishes that lay eggs for the first time or others that repeat the spawning cycle, depending on the fish size.

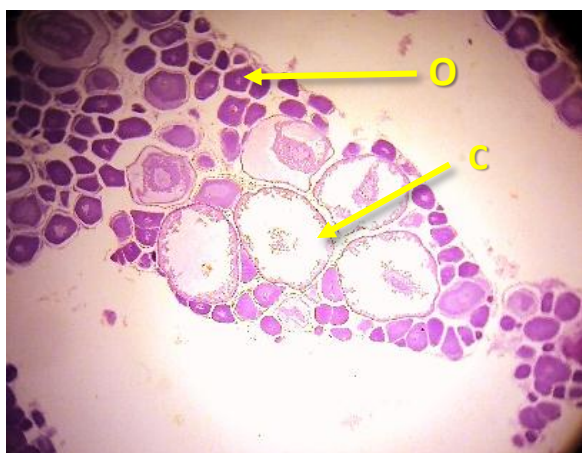


Figure 1. Different stages of oocyte development: Oogonia (O), where the oocytes appear in the initial stage, that called “Cortical Alveoli stage (CA)”, when large cells filled with fat (10X magnification).

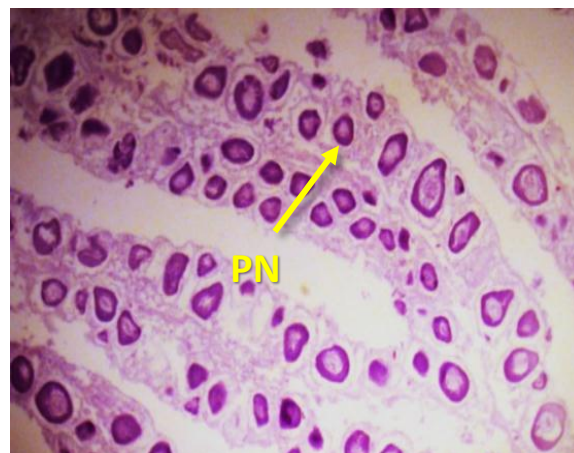


Figure 2. A cross-section of a female ovary in the primary stage; showing PNO cells (perinuclear oocytes). The nucleus appears in the middle and the nuclear membrane is thick (20X magnification).

3.1.2. Cortical Alveoli Stage:

It is characterized by an increase in the size of the ovaries due to the filling of the oocytes with fatty droplets, which accumulate in the cytoplasm of the cells (Figure 3). These oocyte cells appear in December and till the end of the spawning period. Soriano *et al.* (2009) attributed that to the fact that this type of fish lays eggs in batches during the season.

3.1.3. Vitellogenic (Yolk) Stage:

When the ovarian cells are in different stages of the formation of the yolk, and the size of their fatty droplets increases and unite together; to form a “liquefaction”, to fill all the space of the cytoplasm. As a result, the nucleus begins to migrate to the animal pole, and thus the egg becomes ready for the hormonal stimulation. In this work; it was found that females pass through this stage in May (Figure 4 a & b). Wiegand (1996) mentioned that this is recognized in females of the pelagic fishes because these eggs are filled with fatty droplets that help them to float in the water column.

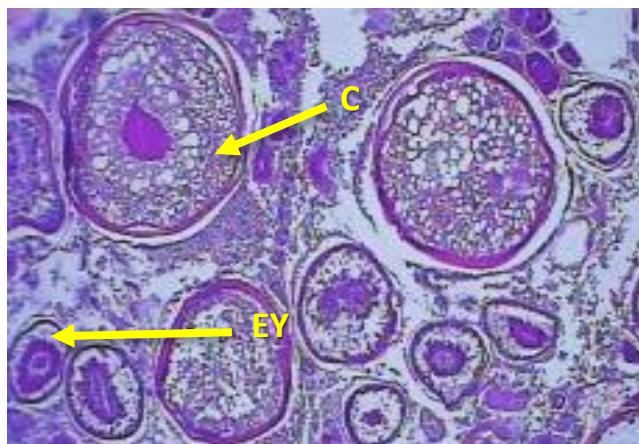


Figure 3. A cross section of a female ovary in the Cortical Alveoli Stage (CA); (EYO) Early Yolk Oocyte, where the nucleus is intermediate and the cytoplasm is filled with fatty granules and they increase in size until they reach (CA) (magnification power 20X).

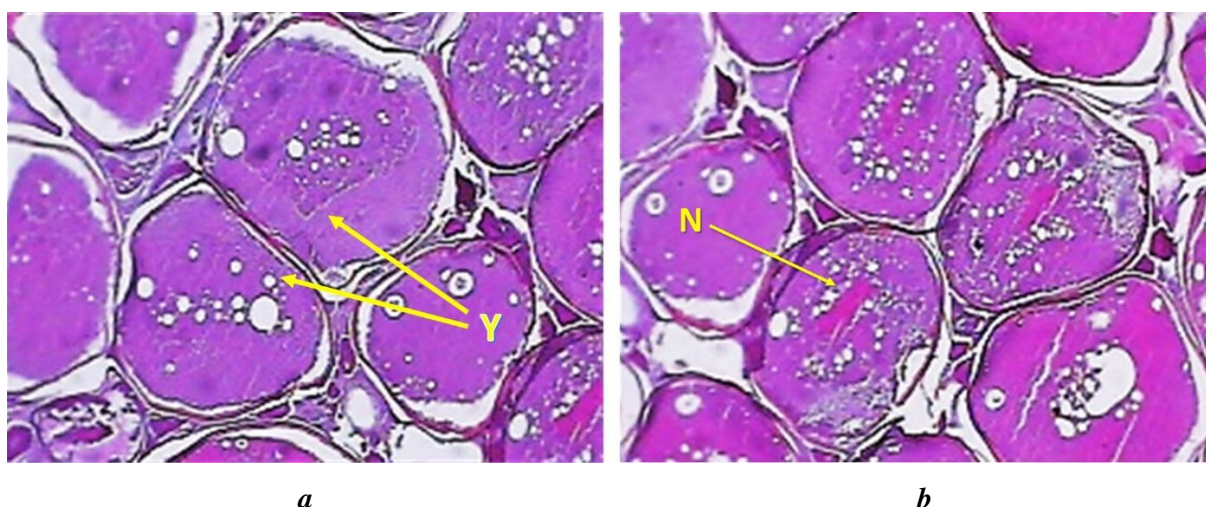


Figure 4. A cross-section of a female ovary in the Vitellogenic (Yolk) stage;
a) (N) the nucleus loses its circular shape and begins to migrate to the periphery;
b) (Y) Liquefaction process occurs in the yolk, covering all the cytoplasm and the nucleus disappearing (magnification 40X).

3.1.4. Maturation Stage:

It is recognized by swollen cells because of being filled with yolk, and absorbing amounts of water of “Hydration” by water saturation. This is resulting in the “Ripe Oocyte”, this stage is preparing before laying eggs directly, which is recorded in this work in June and till late July. At the end of the maturity stage, these cells lose their circular shape, become irregular, and turn into “empty follicles”, to indicate to emptying of all their contents from the mature eggs outside the ovary (outside the body), and the empty cells are reabsorbed by the body (Figure. 5 a & b). Later; the cells appear in the weakness stage of “Spent” in August and September.

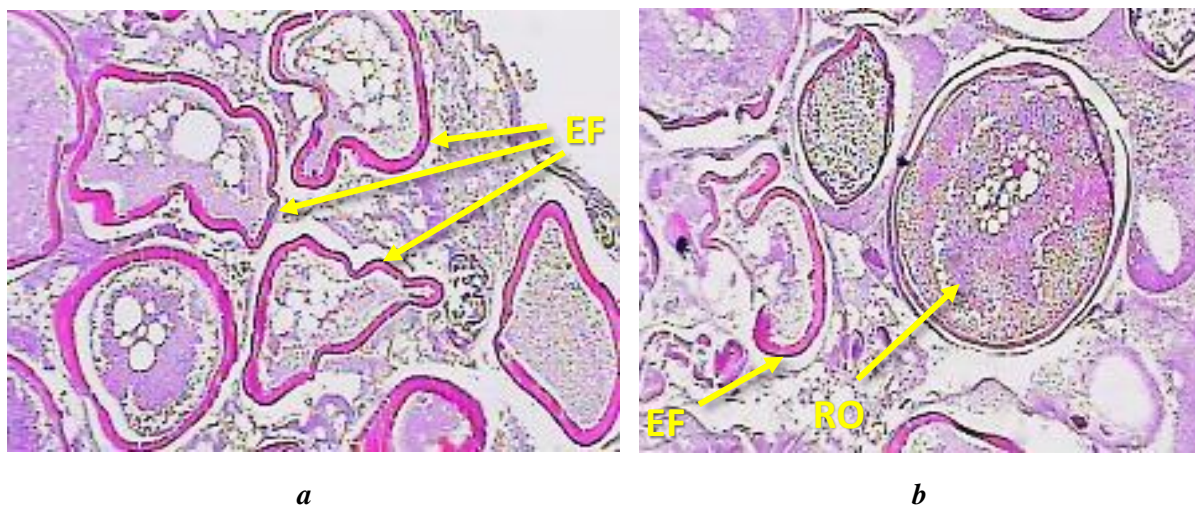


Figure 5. A cross-section of a female ovary in the Maturation Stage

a) (RO) Ripe Oocyte, which are oocytes filled with sphincters and reach their maximum size during the breeding season; *b)* Cells lose their circular shape and (EF) Empty Follicles become irregular in shape (20X magnification power).

3.2. Microscopic characteristics of Testicles

Where Ungaro (2008) named four stages for the development of spermatogonia, based on the shape-shifting in male cells as follows:

3.2.1. Spermatogonia Stage:

An initial stage of male cell development. Histologically; it appears as small fatty lumps, with no nucleus are distinguished. In this work; this stage was recognized in immature fish in December and continued even during the first months of breeding season (Figure 6).

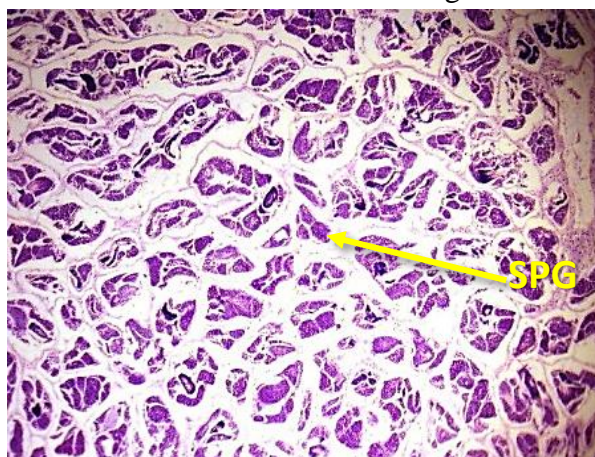


Figure 6. A cross-section of a male testis in the Spermatogonia stage: Spermatogonia (SPG) cells appear as small fatty masses (20X magnification).

3.2.2. Stage Spermatoocytes:

When the cells are shaped as small dots under the microscope, these dots are aggregations of sperm cells, formed in April, as illustrated in Figure (7).

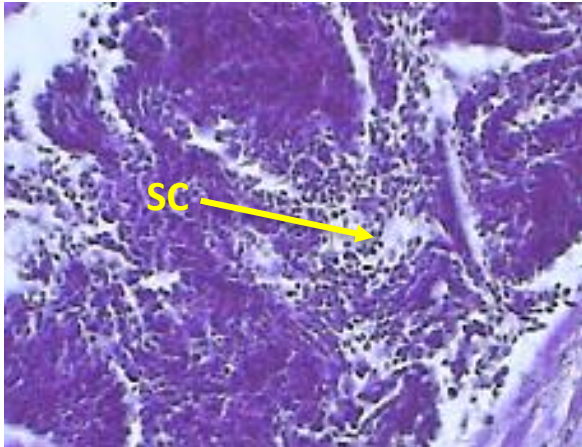


Figure 7. A cross-section of a male testicle in the Spermatocytes stage: Spermatocytes (SC) appear as clusters of spermatocytes.

3.2.3. Spermatids Stage:

The sperms begin to differentiate and develop, inside sacs surrounded by a fatty membrane. Figuratively; the cells are more regular and increasingly sized in this work in May (Figure 8).

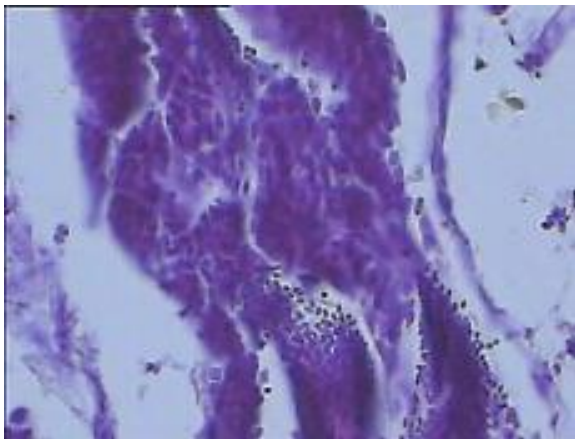


Figure 8. A cross-section of a male testicle in the Spermatids stage: the shape of the cells in this stage is more differentiated and larger in size.

3.2.4. Spermatozoa Stage:

The mature sperm at the beginning of this stage appears as an animal with a head and a long tail (Figure 9.a). In this work; they appeared in males of *S. japonicus* in early June: This indicates that the males have begun to reproduce and spend out sperm. Ending by empty lobes appear in the testicles after most of the spermatozoa have been developed. In Figure (9.b); this spend-out stage appeared at July beginning, till throughout of September, when it could be recognized by the change in the shape of the diminished cells and the lack of sperm.

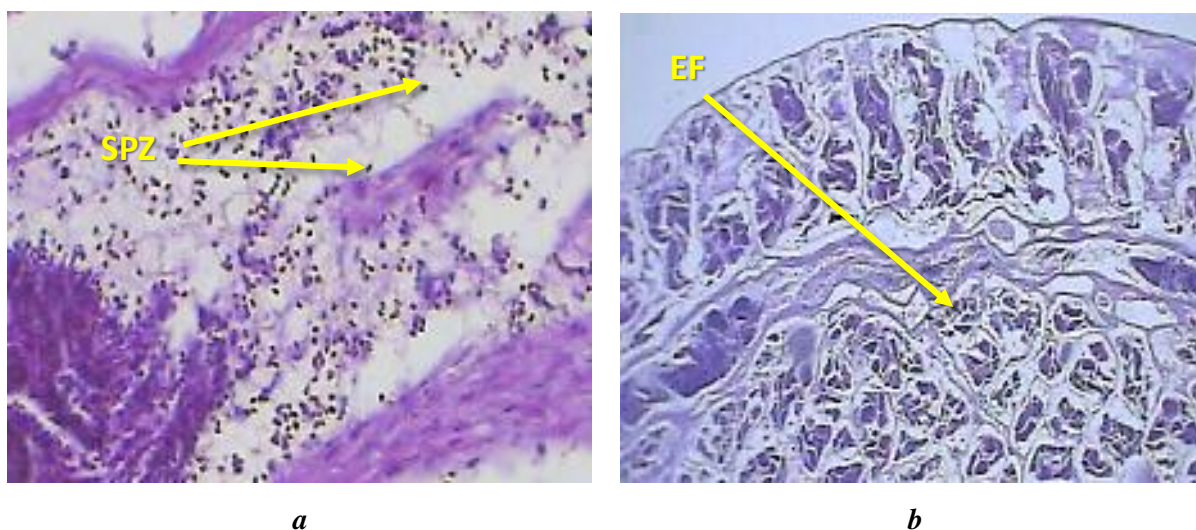


Figure 9 (a-b): A cross-section of a male testicle in the Spermatozoa stage: *a*) At the beginning of this stage, the spermatogonia (SPZ) appears clearly and consists of a head and a long tail (magnification power 20X); *b*) At the end of the Spermatozoa stage, empty lobes are formed after they have emptied their contents of sperm (10X magnification).

4. Conclusion

Histological techniques are considered as one of the modern methods to study the reproduction of various fish species, in order to recognize the season and patterns of their reproduction, then to provide the necessary information to improve the management of their stocks. In this work, looking at the histological sections of the male and female gonads of *Scomper japonicus*, it was found that both of them develop slowly; the activity begins in the breeding season in the summer months (June, July, and August), laying eggs and sperm in batches.

References

- Blazer, V. S. (2002). Histopathological assessment of gonadal tissue in wild fishes. *Fish Physiology and Biochemistry*, 26, 85-101.
- Caramantin-Soriano, H., Vega-Pérez, L. A., & Ñiquen, M. (2009). The influence of the 1992-1993 el niño on the reproductive biology of *Scomber japonicus peruanus* (Jordán & Hubb, 1925). *Brazilian Journal of Oceanography*, 57, 263-272.
- Carvalho, N., Perrotta, R. G., & Isidro, E. (2002). Age, growth and maturity in the chub mackerel (*Scomber japonicus* Houttuyn, 1782) from the Azores. *Arquipelago. Life and Marine Sciences*, 19A, 93-99.
- Chellappa, S., Lima, J. T. A. X., Araújo, A., & Chellappa, N. T. (2010). Ovarian development and spawning of Serra Spanish mackerel in coastal waters of Northeastern Brazil. *Brazilian Journal of Biology*, 70, 451-456.
- Costache, M., Oprea, D., Radu, D., & Bucur, C. (2011). Testing the reproductive potential of Nile Tilapia (*Oreochromis niloticus*) under eco technological conditions from Nucet. *Bulletin UASVM Animal Science and Biotechnologies*, 68, 118-124

- El-Mor, M. E. E. (2002). *Ecological and Biological studies on juvenile commercial fishes on Port Said Coast, Mediterranean Sea, Egypt*. PhD Thesis, Suez Canal University, Egypt.
- MS Farrag, M., AK El-Haweet, A. E., GM Osman, A., Kh A Akel, E. S., & A Moustafa, M. (2019). Reproductive behavior of the silver-stripe blaasop; *Lagocephalus scleratus* (Gmelin, 1789) from the Mediterranean coast, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, 23(3), 441-454.
- Farrag, M. M., Ismail, R. F., El-Haweet, A. A., Fattah, M. F. A., & Osman, A. G. (2022). Reproductive parameters of the chub mackerel, *Scomber japonicus* (Houttuyn, 1782) from the East Mediterranean Sea, Egypt. *Iranian Journal of Ichthyology*, 9(2), 96-110.
- Grier, H. (2000). Ovarian germinal epithelium and folliculogenesis in the common snook, *Centropomus undecimalis* (Teleostei: Centropomidae). *Journal of Morphology*, 243(3), 265-281.
- Genten, F., Terwinghe, E., & Danguy, A. (2009). *Atlas of fish histology*. CRC Press. 244 pp.
- Georgina Gluyas-Millán, M., & Quiñonez-Velázquez, C. (1997). Age, growth, and reproduction of Pacific mackerel *Scomber japonicus* in the Gulf of California. *Bulletin of Marine Science*, 61(3), 837-847.
- Golani, D., Ozturk B. and Basusta, N. (2006). *The Fishes of the Eastern Mediterranean*. Turkish Marine Research Foundation, Istanbul, Turkey. 259 pp.
- Murua, H., & Saborido-Rey, F. (2003). Female reproductive strategies of marine fish species of the North Atlantic. *J. Northw. Atl. Fish. Sci*, 3, 23-31.
- Rinchard, J., & Kestemont, P. (2003). Liver changes related to oocyte growth in roach, a single spawner fish, and in bleak and white bream, two multiple spawner fish. *International Review of Hydrobiology: A Journal Covering all Aspects of Limnology and Marine Biology*, 88(1), 68-76.
- Sabour, W. (2018). Morphological and Histological Study of the Gonads Maturation Stages in the Chub Mackerel *Scomber japonicus* (Houttuyn, 1782) in the Coastal Water of Latakia, *Syrian Journal of Agricultural Research*, 5(2), 259 – 273.
- Selman, K., Wallace, R. A., & Barr, V. (1988). Oogenesis in *Fundulus heteroclitus*. V. The relationship of yolk vesicles and cortical alveoli. *Journal of Experimental Zoology*, 246(1), 42-56.
- Shalloof, K. A. S. & Salama, H. M. (2008). Investigations on some aspects of reproductive biology in *Oreochromis niloticus* Linn. 1757 inhabited Abu-Zaabal Lake, Egypt. *Global Veterinaria*, 2, 351-359.
- Tyler, C. R., & Sumpter, J. P. (1996). Oocyte growth and development in teleosts. *Reviews in fish biology and fisheries*, 6, 287-318.
- Ungaro, N. (2008). *Field manual on macroscopic identification of maturity stages for the Mediterranean fishery resources*. MedSudMed Technical Documents (FAO/MedSudMed). Documents No. 21: pp. 34.
- West, G. (1990). Methods of assessing ovarian development in fishes: a review. *Marine and freshwater research*, 41(2), 199-222.
- Wiegand, M. D. (1996). Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in fish biology and fisheries*, 6, 259-286.



Whitehead, P.J.P; M.L. Bauchot; J.C Hureau; J. Nielsen; and E. Tortonese (1986). *Fishes of the Northeastern Atlantic and the Mediterranean*. Vol. 2. UNESCO, Paris, pp. 1274.

Zawistowski, S. (1965). *Technika histologiczna, histologia oraz podstawy histopatologii*. Państwowy Zakład Wydawnictw Lekarskich. (PZWL), Warszawa. pp. 544.