INSTM Bulletin
Marine and Freshwater Sciences

Vol. 48, 2023, 49–58

Research Article

Striped red mullet (*Mullus surmuletus*, Linnaeus, 1758) from Rafraf-Sidi Ali Mekki region (Bizerte, Tunisia): length-weight relationship and histological assessment of gonad maturation

Raouf DHAOUADI *[1], Raja BOUHALFAYA [1], Mohamed Oussama EL HAFI [2], Olfa BEN ABDALLAH BEN HADJ HAMIDA [2], Amine MOSBAH [3], Dhiaeddine TARHOUNI [4], Wassila AYARI [2] & Hechmi MISSAOUI [2]

[1]National School of Veterinary Medicine of Sidi Thabet. 2020. Ariana. Tunisia
[2]National Institute of Marine Sciences and Technology
[3]Ministry of National Defense. Directorate General of Military Health
[4]CRDA of Bizerte

***Correspondence**: dhaouadi5@yahoo.fr

Received: 12.01.2023; **Accepted**: 25.5.2023; **Published**: 02.11.2023

Abstract

The aim of this study is to investigate the gonadal changes of striped red mullet (*Mullus surmuletus* Linnaeus, 1758) in Rafraf – Sidi Ali Mekki region in Tunisia. A total of 243 specimens were collected monthly between March 2014 and February 2015. Fulton's condition factor (K) and the length-weight parameters were estimated. There was correlation between the fish total length and weight (R^2:0.75).

The K values ranged between 0.93 and 3.38. The values of b parameter ranged from 3.18. gonadosomatic and hepatosomatic indices show maximum values during April. Different stages in females and in males were observed according to gonadal development.

Keywords: *Mullus surmuletus*; length-weight relationship; histology; gonad development; Tunisia.

1. Introduction

The latest ecological studies conducted in Tunisian demersal fisheries highlight the alarming level of exploitation where most fish resources are either in the full exploitation phase or in the overexploitation phase. Among the fish stocks most threatened by overexploitation is striped red mullet, *Mullus surmuletus*, locally called "Trilia Hjar". This species is recognized for its fairly high market value and constitutes an interesting component of the demersal fishery in the North region. During the latest decades, the intensive fishing effort has significantly reduced fish stocks and hampered the ecosystem's ability for regeneration and sustainability. This threat not only jeopardizes biodiversity in the sea but also deepens, even more, the food security crises

worldwide in a time when the need for food has never been more crucial (Chérif, 2014).

In Tunisia, many publications have been interested in fish stocks and their biology (Gharbi and Ktari, 1981). Chérif et al. (2007) presented the biological parameters of red mullet from the Tunisian North coast. Better knowledge of the biology, physiology and reproduction of this species would lead to better management of the natural stock.

The aim of the present study was to investigate the weight-length relationships and to describe the histological gonad maturation of striped red mullet (*Mullus surmuletus* Linnaeus, 1758) in Rafraf – Sidi Ali Mekki region (Tunisia).

2. Materials and methods

2.1. Fish sampling

Samples of striped red mullet (*Mullus surmuletus*) were monthly collected from March 2014 to February 2015 from coasts of the Mediterranean Sea of Rafraf - Sidi Ali Mekki region situated in the North of Tunisia (37°11′N; 10°11′E).

A total of 243 *M. surmuletus* specimens (159 females and 84 males), with an average weight and length of 85.5 g (range: 40 to 180 g) and 17.4 cm (range: 11 to 24 cm) respectively, were studied.

For each fish, total length (in cm) and total weight (in g) were measured, and its sex determined by visual examination of the gonads during the reproduction season. Maturation stage was determined by examining gonad sections (stained with hematoxylin-eosin (H.E.)) under microscope at 100 x magnification.

2.2. Length-weight relationship

The length-weight relationships were expressed as follows:

$$Wt = a.(Lt)^b$$

Where *Wt*: total body weight in g; *Lt*: total body length in cm; *a*: regression intercept; *b*: slope of regression line. Growth type either isometric (*b*=3) or allometric, in this case, it can be negative allometric (*b*<3) or positive allometric (*b*>3) (Froese, 2006).

The values of the constants *a* and *b* were calculated by the logarithmic transformation of the above equation:

$$Log(Wt) = Log(a) + b.Log(Lt)$$

The Fulton's condition factor (*K*) was determined using the following formula:

$$K = 100\frac{Wt}{(Lt)^3}$$

2.3. Gonadosomatic and hepatosomatic indices

Gonad and liver from each fish were dissected and weighted. The Gonadosomatic indice (GSI) and hepatosomatic indice (HIS) were calculated as percentage (%) of relative weight of gonad and liver to total body weight, respectively.

2.4. Histology

In order to determine the maturation stage of the studied fishes, representative sections from the middle parts of the gonads were sliced and fixed in 10% formaline solution. Gonad sections were stained with hematoxylin-eosin (H.E.) and examined under microscope at 100x and 400x magnifications.

Histological sections of the gonads were examined under a light microscope to determine the stage of gonad development using Zorita et al. (2008) criteria (table1).

Table 1: Stages of gonad development in *M. surmuletus* (Zorita et al., 2008)

	Females	**Males**
Stage I	Oogonia	Only spermatogonia
Stage II	Pre-vitellogenic oocyte	Spermatogonia + spermatocytes

Stage III	Early vitellogenic oocyte	Spermatogonia + spermatocytes + spermatids
Stage IV	Vitellogenic oocyte	sperm
Stage V	Mature oocyte	Post-oviposition
Stage VI	Post-oviposition	-----

2.5. Statistical analysis

The association between length and weight variables was investigated by linear regression and the determination coefficient (R^2) was calculated.

3. Results

3.1. Sex-ratio

The total sample was represented by 159 female and 84 male individuals. The sex-ratio is dominated by females, with a percentage of 65.43%. The distribution of sex according to months is reported in the figure 1.

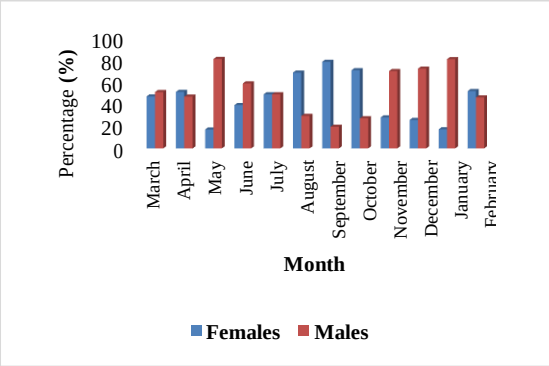

Figure1: Monthly percentage of females and males

3.2. Length-weight relationship

The total length of females ranged from 11 to 24 cm (18.12 ± 2.32 cm) and of males from 11 to 21 cm (16.02 ± 2.44 cm). The female weight ranged from 40 to 180 g, with an average of 93.22 ± 29.31 g. In males, weight ranged from 40 to 140 g, with an average of 70.84 ± 21.85g. For total sample, there was a positive correlation between total length and weight (R^2= 0.75) (*p<0.05*).

The calculated length-weight equation for the whole sample is: $Wt = 0.3(Lt)^{3.18}$ Length-weight relationships were separately estimated for females and males.

- Females: $Wt = 0.48(Lt)^{3.2}$W =0.48L, R^2=0.78, n= 159 (*p<0.05*)
- Males: $Wt = 0.21(Lt)^{3.14}$W =0.21L, R^2=0.63, n= 84 (*p<0.05*)

The Fulton's condition factor (*K*) values ranged from0.93 to 3.38, with an average of 1.63 ± 0.45. The maximum value was observed in November- December (figure 2).

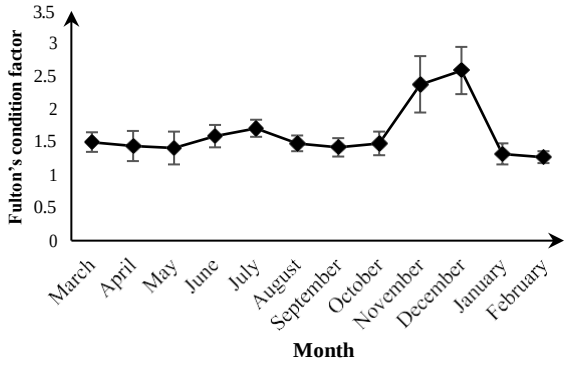

Figure 2: Monthly variation of Felton's condition factor (*K*)

3.3. Gonadosomatic and hepatosomatic indices

The gonadosomatic indice (GSI), used to determine the reproductive period, was calculated from samples taken monthly from females and males. For total sample, the GSI ranged from 0.1 to 3.8%, with an average of 0.72 ± 0.41%. The monthly GSI was an increase starting from January reaching maximum values in April (figure 3).

The hepatosomatic indice (HIS) was an average of 1.24 ± 0.63% (ranged from 0.13 to 5.08%), with a maximum value in April (figure 3).

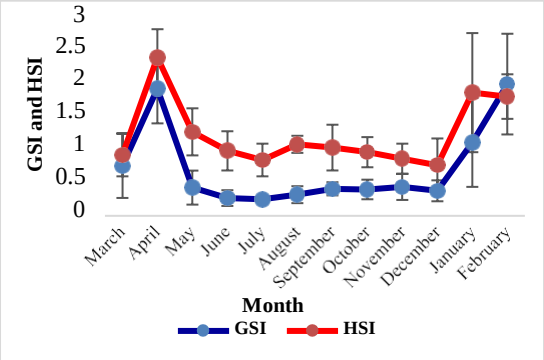

Figure 3: Monthly evolution of gonadosomatic and hepatosomatic indices of *M. surmuletus*

3.4. Histological study

3.4.1. Ovaries

A total of 134 ovaries were analyzed from March to October 2014. The majority of ovaries were in stages III and IV, with a percentage of 35.55% and 23.7% respectively (figure 4).

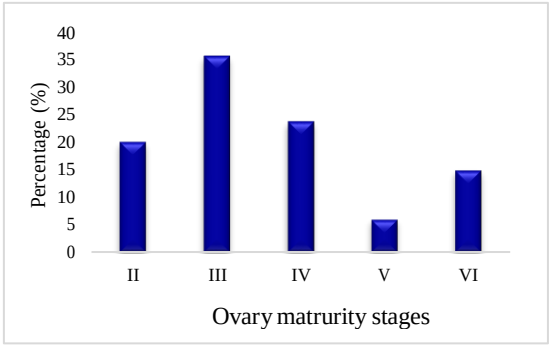

Figure 4: Ovaries maturity stages of *M. surmuletus* (period: March - October 2014)

Developing phase III was present almost during all period from March to October. Stage V, corresponding to mature oocytes, was observed during March and April (figure 5).

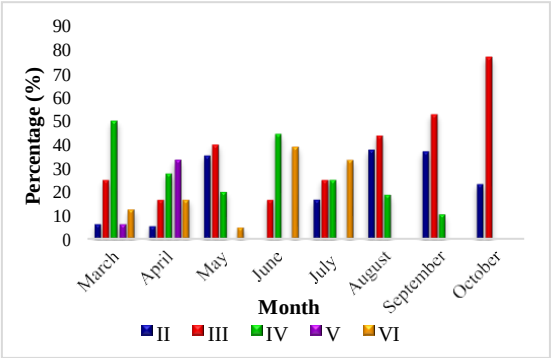

Figure 5: Monthly distribution of ovarian development stages

At low magnification (x 40), in immature female, oocytes are spherical with a central nucleus (figure 6).

In stage II or the pre-vitellogenic stage (early phase of vitellogenesis), the oocyte has a nucleus containing nucleolus. The nucleoli are round (figure 7).

Stage III corresponds to the appearance of the cortical alveoli. The oocytes contain a homogeneous cytoplasm, large nucleus and a large number of small elongated-looking peripheral nucleoli which are more numerous, and peripheral distribution in this stage. Vacuoles form in the cytoplasm and begin to arrange themselves around the nucleus (figure 8).

During vitellogenesis (stage IV), the oocyte is larger in size and oil droplets are greater and more pronounced (figure 9). At maturity (stage V), the oocytes reach the maximum size. Germinal vesicles, which are an indicator of oocyte maturation, are observed. The *zona radiata* is thickened. The nucleus membrane is dissolved and granular concentration increases. Oocyte membranes become thicker, and perivitelline space becomes wider in this stage. Yolk inclusions completely invaded the cytoplasm (figure 10). At stage V, non-hydrated oocytes and hydrated oocytes are observed (figures 11 and 12).Large hydratedoocytes and post-ovulatory follicles are observed.

Oil droplets grow in number or merge together and become larger.

Figure 13 shows post-ovulation stage. Undeveloped or under developed oocytes can be observed. There are many athreptic follicles in the
ovary.

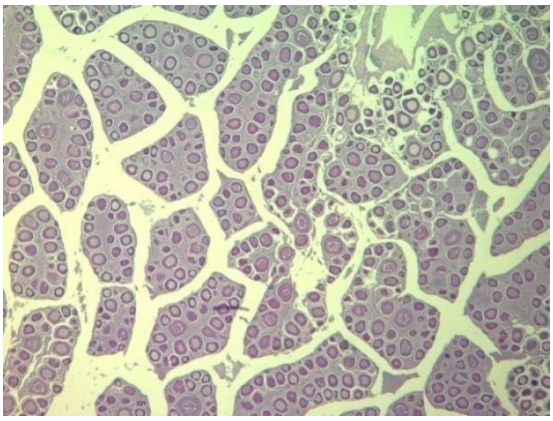

Figure 6: Histological structure of *M. surmuletus* ovary (HE x 40)

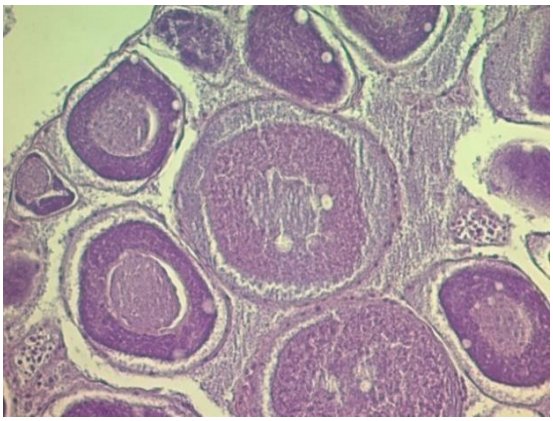

Figure 9: Stage IV of ovarian development (HE x 400)

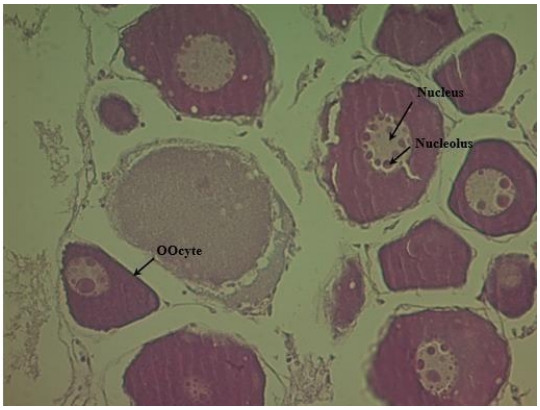

Figure 7: *M. surmuletus* ovary in stage II (HE x 400)

Figure 10: Stage V or stage of mature oocytes (HE x 100)

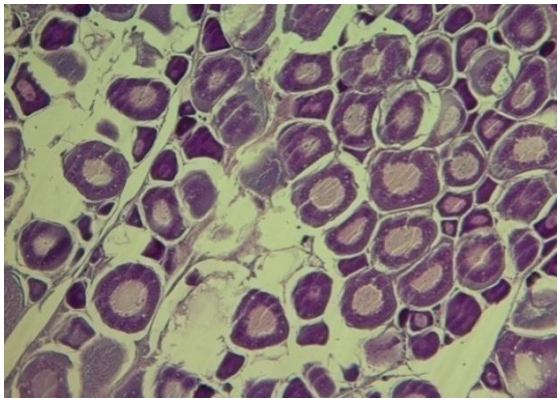

Figure 8: Histological section of *M. surmuletus* ovary at stage III of development (HE x 200)

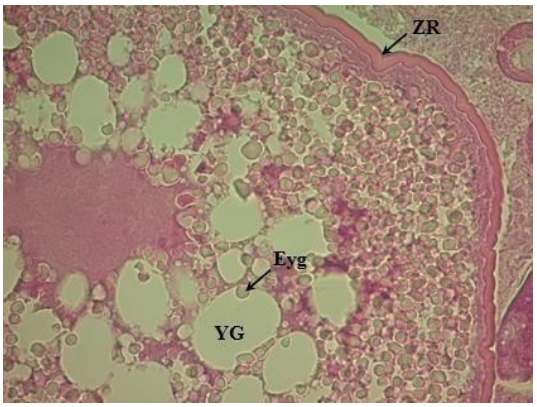

Figure 11: Unhydrated oocyte (HE x 400)
ZR: *zona radiata*; YG: Yolk globule; Eyg: Egg yolk granules

Figure 12: Hydrated oocyte (HE x 400)
ZR: *zona radiata*; Fec: Follicular epithelium cells; HF: Hydrating fluid

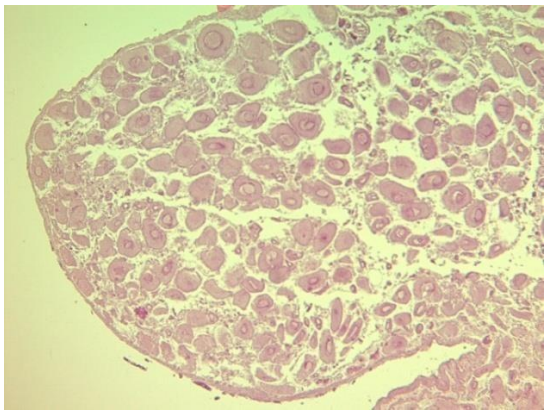

Figure 13: Post-ovulation stage (athreptic follicles) (HE x100)

3.4.2. Testes

35 testes were studied from March to April 2014. Four developing stages were observed: II, III, IV and V (figure 14).

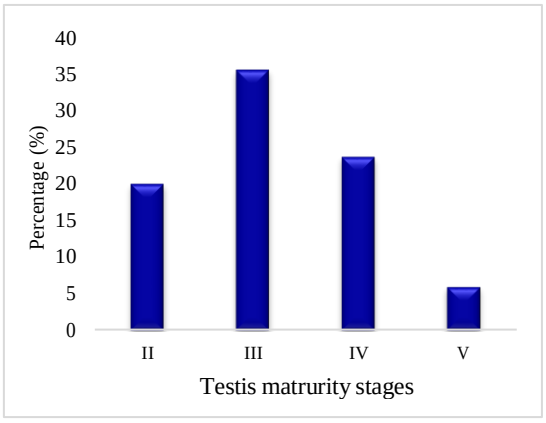

Figure 14: Testicular development stages of *M. surmuletus* (March – April 2014)

The table 2 gives the distribution of monthly testicular development stages.
In stage II, spermatogonial cells and primary spermatocyte mass are observed (figure 15).Stage III testicles show cysts containing, in addition to spermatogonia and spermatocytes, spermatids (figure 16).In stage IV, numerous spermatozoa and spermatids are observed in the testis (figure 17).Stage V testicles show a decrease in the number of spermatozoa. Thus, after spawning, there is no sperm production, and regressed male gonad tissue and hollow tubules are observed. Some individuals possess residual sperms in their tubules (figure 18).

Table 2: Distribution of monthly testicular development stages

	II	III	IV	V	Total
March	---	---	9	---	9
April	---	---	3	2	5
May	---	---	2	1	3
June	---	---	1	1	2
July	---	---	---	6	6
August	1	1	1	1	4
September	1	---	---	---	1
October	1	4	---	---	5

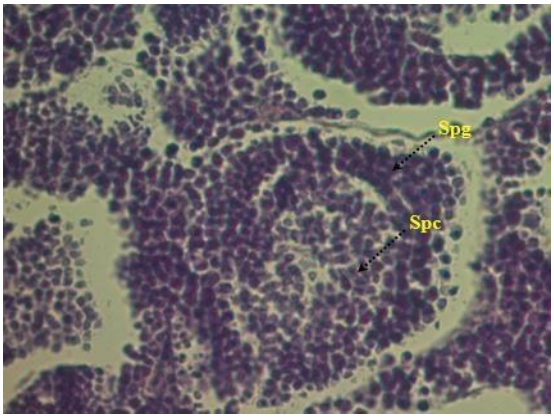

Figure 15: Histological section of testis at development stage II (HE x 400)
Spg: Spermatogonia; Spc: Spermatocyte

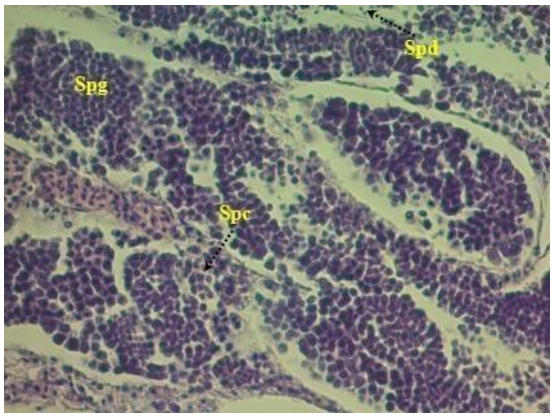

Figure16: Stage III of testicular development (HE x 400)
Spg: Spermatogonia; Spc: Spermatocyte: Spd: Spermatid

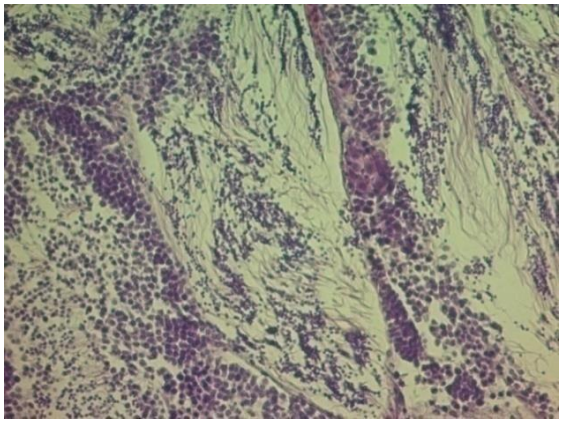

Figure 17: Stage IV of testicular development (HE x 400)

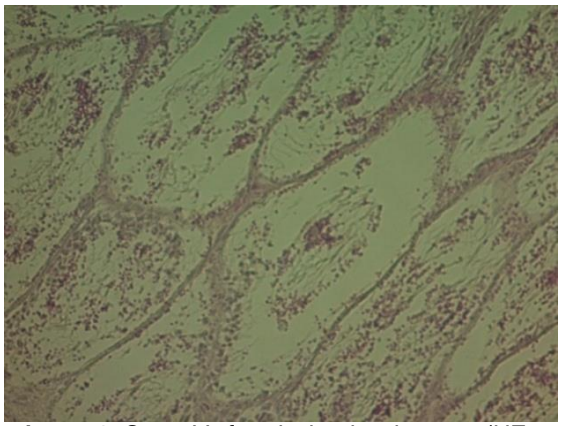

Photo 18: Stage V of testicular development (HE x 400)

4. Discussion

Striped red mullet *Mullus surmuletus*is a common demersal fish with a high commercial value. It represents one of the two principal species of the *Mullus* genus, and it constitutes one of the main target species of many demersal fisheries operating in the northern Tunisian coasts. However, studies on its exploitable potential and its management remain fragmentary and very sketchy. Indeed, very few studies have been devoted to the biology and dynamic of mullet populations (Gharbi and Ktari, 1979; Chérif, 2014).

Our results show a highly significant correlation (p<0.05) between total length and weight (R^2= 0.75). The analysis of these parameters reveals minor growth allometry for the whole sample (b= 3.18). The length-weight relation showed in the present study has similar growth patterns with those obtained in others regions. Mahé et al. (2005) reported that, at the beginning of its life, striped red mullet has almost proportional growth in length and weight (b=3.050). These results corroborate the study of Andaloro and Giarritta (1985).

Length-weight relationships were also established separately for males and females by Zarrad et al. (2000) in the Gulf of Gabes. These authors noted that the growth of females is relatively faster than that of males, but the difference is not significant. Previous studies carried out in the North-East Atlantic show that females were heavier and larger than males (N'Da, 1992; Renones et al., 1995).
The gonad development is assessed by measuring the gonadosomatic indice (GSI). For total sample, GSI varies from 0.1 to 3.8%, with an average value of 0.72%. Monthly evolution of GSI, for females and males, indicates a peak in April.

Chérif (2014) reports that the ovary maturation period is characterized by a significant and regular increase in GSI which begins in January and continues until April. This agrees with our study. Indeed, during this period, GSI increased

from 1.98 in January to reach its maximum value in April (7.32). The spawning period spreads over 3 months; it begins in April and seems to continue until July when the GSI drops to 0.85. The maximum emission of oocytes takes place in June.

In the Aegean Sea, according to Arslan and İşmen (2013), striped red mullet spawning occurs in April - May. In Algeria, on the coasts of Mostaganem, the reproduction of this species takes place in spring, with a peak of the gonadosomatic indice in April (Kherraz et al., 2014). However, in the English Channel, the spawning season for *M. surmuletus* extends from May to July (Mahé et al., 2013).

In our study, the hepatosomatic indice (HSI) ranges from 0.13 to 5.08%, with an average of 1.24 ± 0.63%. For both sexes, the HSI reaches maximum values during April. It also tends to increase in January. The HSI shows an evolution comparable to that of the GSI and indicates its remarkable reliability to be used as a parameter of reproduction for *Mullus surmuletus*.

The histological study of gonad development stages allows to identify with precision spawning period. Our work indicates that stage III of gonad development is observed from March to October. Stage V, corresponding to mature oocytes, is observed during March and April. The gonad histological structure confirms the different periods of reproduction. Thus, oocytes in the early stages of vitellogenesis (I and II) are still very small between December and February. Thereafter, the distribution of maturation stages is very heterogeneous, but the oocytes in very advanced vitellogenesis occupy the major part of the ovaries. Mature oocytes are abundant in March and April.

In males, the monitoring of testicular development stages revealed that the appearance of spermatozoa in the medulla is observed at stage IV. During March and April, stages IV and V predominate and spermatozoa are emitted into the external environment, thus coinciding with the maturity of the oocytes in the females.

The monthly variation of GSI, RHS and gonad maturity stages as well as gonad histological structure allowed to determine the spawning period between May and July; this is the case for the Mediterranean (Lalami, 1971) and the Northeast Atlantic (N'Da, 1992) areas.

5. Conclusion

In this study, five stages of gonadal development of *M. surmuletus* females was showed; while in males, testicular development was addressed in four stages. Based on the results of the gonadosomatic indice and the histological observation of the gonads, *Mullus surmuletus* spawn between May and July in Rafraf-Sidi Ali El Mekki region (Tunisia). The results obtained in this study represent important input data for stock assessment and sustainable management of this commercially important fish species.

Acknowledgements

The authors would like to thank Pr. Abdelkader Amara, Pr. Ahmed Rejeb and Yahia Khlifi (Ecole Nationale de Médecine Vétérinaire de Sidi Thabet, Tunisia) for the histopathological analysis.

Bibliographic references

1. Andaloro, F. & Giarritta, S.P. (1985). Contribution to the knowledge of the age and growth of striped mullet (*Mullus barbatus* L. 1758) and red mullet (*Mullus surmuletus* L. 1758) in the Sicilian Channel. In: GFCM. *Report of the Second Technical Consultation on Stock Assessment in the Central Mediterranean*, Mazara Del Vallo, Italy, 136: 89-92.

2. Arslan, M. & İşmen, A. (2013). Age, growth and reproduction of *Mullus surmuletus* (Linnaeus, 1758) in Saros Bay (Northern Aegean Sea). *J. Black Sea/Mediterranean Environment*, 19 (2), 217-233.
3. Chérif, M. (2014). Red mullet from the Tunisian North coast: biology, exploitation and population dynamics. [Doctoral Thesis in Agricultural Sciences. University of Carthage].
4. Chérif, M., Gharbi, H., Jarboui, O., Mrabet, R. & Missaoui, H. (2007). Le rouget de roche (*Mullus surmuletus* l. 1758) des côtes nord Tunisiennes : reproduction, sexualité et croissance. *Bull. Inst. Natn. Scien. Tech. Mer. Salammbo*, 34, 9-19. https://www.instm-bulletin.tn/index.php/bulletin/article/view/644/423
5. Gharbi, H. & Ktari, M.H. (1979). Régime alimentaire des rougets (*Mullus barbatus* Linnaeus, 1758 et *Mullus surmuletus* Linnaeus, 1758) du Golfe de Tunis. *Bull. Inst. Natn. Scient. Tech. Oceanogr.Pêche Salammbo*, 6 (1-4), 41-52. https://www.instm-bulletin.tn/index.php/bulletin/article/view/1154/781
6. Gharbi H., & Ktari, M.H. (1981). Biologie de *Mullus barbatus* Linnaeus, 1758 et *Mullus surmuletus* Linnaeus, 1758 (Poissons, Teleosteens, Mullides) des côtes tunisiennes, taille et âge de première maturité sexuelle, cycle sexuel et coefficient de condition. *Bull. Inst. Natn. Scient. Tech. Oceanogr. Pêche Salammbo*, 8, 41-51.https://www.instm-bulletin.tn/index.php/bulletin/article/view/1109/747
7. Kherraz, A., Kherraz, A., Benghali, S., Mouffok, S. & Boutiba, Z. (2014). Reproductive Biology and Growth of Red Mullet, *Mullus Surmuletus* (Linne, 1758) in Western Algeria Coasts. *Journal Academica*, 4(4), 121-129.
8. Lalami, Y. (1971). Contribution à l'étude systématique, biologique, écologique et statistique des poissons de la pêcherie d'Alger. *Pelagos, Bull. Inst, oceanogr. Algiers*, 3 (4), 1-150.
9. Mahé, K., Destombe, A., Coppin, F., Koubbi, P., Vaz, S., Le Roy, D. & Carpentier, A., (2005). Le rouget barbet de roche *Mullus surmuletus* (L. 1758) en Manche orientale et mer du Nord. *Rapport de contrat IFREMER/CRPMEM Nord-Pas-de-Calais*. https://archimer.ifremer.fr/doc/00000/2351
10. Mahé, K., Coppin F., Vaz, S. & Carpentier, A. (2013). Striped red mullet (*Mullus surmuletus*, Linnaeus, 1758) in the eastern English Channel and southern North Sea: growth and reproductive biology. *Journal of Applied Ichthyology*, 29(5), 1067-1072.https://doi.org/10.1111/jai.12266
11. N'Da, K. (1992). 12.

    Biologie du rouget de roche *Mullus surmuletus* (Poisson Mullidae) dans le nord du golfe de Gascogne: Reproducteurs, larves et juvéniles.[Thèse de Doctorat. Université Bretagne Occidentale, Brest, France].
12. Renones, O., Massuti, E. & Morales-Nin, B. (1995). Life history of the red mullet *Mullus surmuletus* from the bottom-trawel fishery off the island of Majorca (North-West Mediterranean). *Marine Biology*, 123 (3), 411-419. https://doi.org/10.1007/BF00349219
13. Zarrad, R., El Abed, A., Missaoui, H., Gharbi, H. & Ben Abdallah, L. (2000). Analyse descriptive de la pecherie du golfe de Tunis. *Bull. Inst. Natn. Sci. Tech. Mer. Salammbo*, 27, 27-34. https://www.instm-bulletin.tn/index.php/bulletin/article/view/829/557
14. Zorita, I., Ortiz-Zarragoitia, M., Apraiz, I., Cancio, I., Orbea, A., Soto, M., Marigómez, I. & Cajaraville, M.P. (2008). Assessment of biological effects of environmental pollution along

the NW Mediterranean Sea using red mullets as sentinel organisms. *Environnemental Pollution*, 153 (1), 157-168. https://doi.org/10.1016/j.envpol.2007.07.028