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Reproductive cycle of *Marphysa sanguinea* (*Polychaeta: Eunicidae*) in a Saharan wetland: Khnifiss Lagoon (South of Morocco).

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Abstract
The Khnifiss lagoon is one of the most important wetlands along the Moroccan Atlantic coastline. Situated in a Saharan area, this ecosystem is highly diverse in terms of both fauna and flora, but has not been extensively studied. In an attempt to provide more information about the biological resources of the Khnifiss lagoon, investigations on some aspects of the biology of the annelid polychaete *Marphysa sanguinea* were undertaken from February 2005 to January 2006, and are reported here. In term of our study, the sex ratio was close to 1:1. The oocytes were classified into three classes which vary in size during the reproductive cycle. According to the mean oocytes diameter, the highest gametogenic activity occurred in August. We found that the spawning period coincided with the warm water temperatures present between May and July. The results highlighted the impact of abiotic characteristics which are unique to the Khnifiss lagoon which are influenced by the arid climate of the Sahara and by the Canary Current upwelling.

Keywords: *Marphysa sanguinea*, Khnifiss, lagoon, wetland, *Eunicidae*, reproduction

1. Introduction
Wetlands occupy 13 % of the worlds' coastline and many of these are lagoons ecosystems [1, 2]. These systems exhibit considerable morphological and climatic variation during the year and are amongst the most productive coastal environment (around 300 g C/m2/year) [3, 4] and provide a multitude of ecosystem services [5, 6]. Exploited in different ways, coastal lagoons sustain various human activities [7]. Such exploitation is therefore responsible for some of the ecological pressures encountered in these ecosystems [8]. In Morocco lagoons are represented by four complexes; the Mar Chica lagoon on the Mediterranean coast, while the Merja Zerga, Oualidia and Khnifiss lagoons are situated in the Atlantic coast. The Khnifiss lagoon presents several unique characters. Due to its geographic situation in the south of Morocco, the Khnifiss lagoon is the largest wetland in the desert bioclimatic zone [9] and plays an important role for desert fauna and flora [10, 11]. It's also an important area for the conservation of birds, especially migratory ones such as the wintering waders [12-15]. Designated as Ramsar site, the Khnifss National Park was created recently, and includes the lagoon and surrounding areas. Despite its importance, it is not well known compared o similar Northern Moroccan wetland, and is largely restricted to ornithological observations [16], sediment (9) and hydrological aspects [17, 18, 19, 20]. In order to increase our scientific knowledge of this lagoon, a research program has recently been established by our laboratory. It concerns the investigation of benthic macrofauna [21], the assessment of chemical pollution [22] and the study of the reproductive biology of some macrozoobenthos species [23]. In this work, we deal with the study of some reproductive patterns of *Marphysa sanguinea*, which is a polychaetous annelid belonging to the *Eunicida*. This species was observed in Khnifiss lagoon, which is mentioned as the known southern limit of the distribution area of this polychaete, at the boundary of the desert [24, 25]. Also, the preliminary observations on the marcozoobenthos community structure in Khnifiss, point out that *M. sanguinea* occupies a critical position in the trophic webs particularly for the avifauna, since it lives in

burrows in muddy sediment [26]. As well, *M. sanguinea* was used, in a previous study undertaken in our laboratory, as a sentinel species in the ecotoxicological assessment of Khnifiss lagoon's quality [27] and in different other ecosystems (Lake Macquarie [28]; Parramatta River [29]; Sado estuary [30]). Indeed, the reproductive strategy and the traits life cycle of *M. sanguinea* constitute an interesting reason expanding study. In fact, *M. sanguinea* is gonochoric species with an annual iteroparous reproductive strategy [31- 34]. However, scientific knowledge of life cycle and reproductive aspects of *M. sanguinea* is very scarce all over the world (35], and no published data exist concerning the Moroccan ecosystems. On account of all these considerations, we intend in this study to describe in detail the reproductive biology cycle of *M. sanguinea* from the Khnifiss lagoon.

2. Materials and Methods:

2.1. Systematic position :

Marphysa sanguinea MONTAGU 1813, is a polycheatous annelid belonging to the *Eunicidae* order. It is a polychaete of considerable size, reaching a maximum length of 40 cm. It is typical species of muddy bottoms that lives in burrows at a depth of 25–35 cm. Concerning its biogeography, the species was recorded in numerous localities often without an exhaustive description or deposition of voucher material. So, its cosmopolitan distribution was often subject to controversy. A detailed description of the species from the type locality, Devon UK was provided by Hutchings, P.A. and Karageorgopoulos and a neotype deposited in the Natural History Museum in London Nowadays, a more detailed description of Montagu neotype was validated [36]. Moreover, the identification of new species of the genus have supported the suggestion that *M. sanguinea* is a complex of different species morphological indistinct, rather than a widespread cosmopolitan [36, 37]. Hope the authors have deposited voucher specimens somewhere

2.2. Sampling site :

The sampling site (**28° N 02'165 - 12° W 14'155**) is located at the central channel of the Khnifiss lagoon in a *Zostera* bed (figure 1). The substratum is of sandy mud with a high amount of organic matter. Monthly samples were collected by excavating the sediment to a depth of 30 cm. As soon as possible, the sediment is sieved on a sieve of 1 mm vacuum mesh. Cleared of debris and washed, the specimens are fixed in Bouin fluid. At the same time temperature, salinity and pH water were recorded.

2.3. Histology of gonads :

In the laboratory, samples were transferred to another fixative volume for 48 h. A series of alcohols baths of increasing concentration was applied. Once dehydrated, the specimens were preserved in Butanol liquid. After paraffin inclusion, 5 µm thin layers were made for each animal. Mounted on slides, the slices were stained with eosin-hematoxylen as indicated in [38] and examined under the microscope with about 100 oocytes measured per individual.

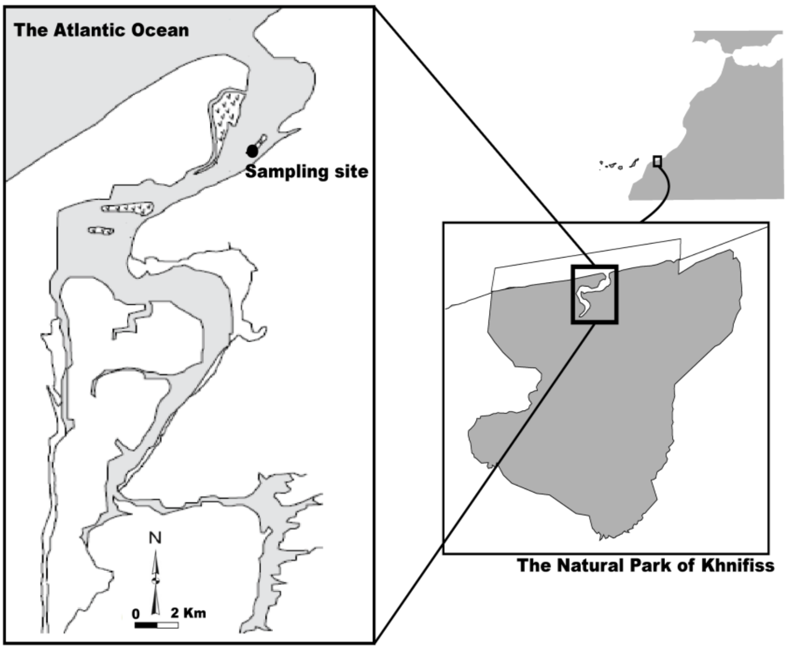

Fig.1: Location of the sampling site (●) at the Khnifiss lagoon.

3. Results:

3.1. Temporal variation of temperature, salinity and pH:

Figure 2 illustrates the temporal variation of these three parameters. The water temperature shows an annual cycle with a marked seasonal pattern with two distinct periods (Figure 2 A). A winter season from October to March with temperatures between 16.8°C and 21.5°C with an average of 19.15°C. A summer season occurs from April and September varying between 20.2 °C and 29.8°C with 24.9°C as average. In contrast salinity (Fig. 2B) does not exhibit any seasonal variation and generally is higher than in the ocean with the highest concentration of 39.1 ‰ in May 2005, while it fall down to a minimum of 36.1‰. The ph varies between 7.49 and 8.53 in December and July respectively.

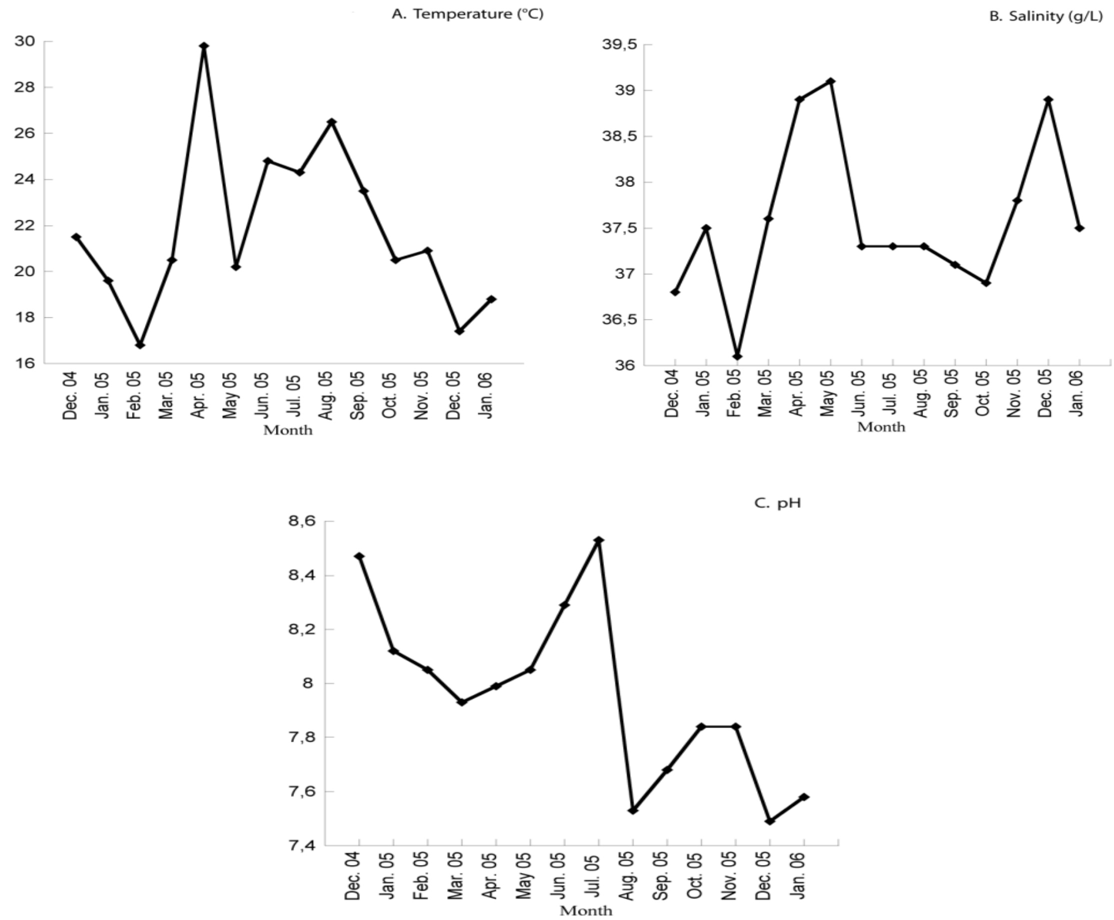

Fig. 2: Seasonal variation of some parameters of water between December 2004 and January 2006 at the Khnifiss lagoon. A: temperature, B: salinity and C: pH.

3.2. Reproductive cycle of Marphysa sanguinea:

3.2.1. Sex-ratio:

The sex ratio is calculated for the sexed animals. For the entire collected individuals, the microscopic examination was performed. The figure 3 summarizes the obtained results. At the Khnifiss lagoon, the *M. sanguinea* population is characterised by a sex ratio of 1:1.16). However, for a percentage of the population (8.61 %) the sex was not determined and they represented juveniles.

3.2.2. Temporal evolution of oocyte diameter in M. sanguinea:

The histological study allowed the gametogenic cycle to be determined Thus; the morula sperm are visible on the approach of the maturity of males individuals, whereas, for females, oogenesis occurs throughout the year, with the presence of oocytes of various sizes throughout the year. The temporal evolution of the oocytes population composition is illustrated in the figure 4. Three main oocytes classes can be distinguished: The small with a diameter oocytes less than 100 µm. the medium class including oocytes with a diameter ranged between

100 and 200 µm. the last class showed a large oocyte sizes characterised by a diameter greater than 200 µm. During the whole study period, the temporal distribution of the oocytes classes occurs as follows: From May to September, the small oocytes proportion initiates an enlargement. In fact, it represents 43 % of all observed oocytes. In this period, we take note of the minimum diameter of 36 µm in July. In contrast, large oocytes represent only 10 % and 9 % in August and September respectively. Successively, in October, the part of large oocytes starts to augment and represents, in December and January, about 60 % of the oocyte population. For this period, we notify a decrease in the abundance of all oocytes categories, and the whole oocytes population is reduced in number and in dimensional scope. From March, the large oocytes began to decrease in number, even if the largest diameter (276 µm) was observed in April.

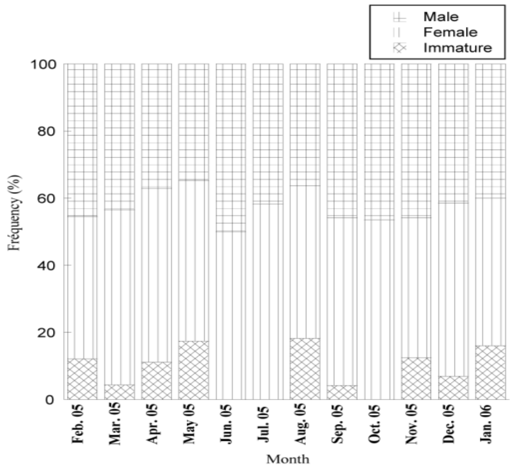

Fig. 3 : Females and males percentages of *M. sanguinea* in the Khnifiss lagoon

For the period study, the means oocyte diameter curve displays four successively periods altering a period of great oocyte's diameter and another period of tiny oocyte's diameter (Fig. 5). From February to April, the oocyte diameter is important and the values reach more than 202 µm, while the three consecutive months are characterised the oocyte volume fall down and attain in July 81 µm. The cycle is reinitiated. In August, the oocyte diameter reaches 160 µm. This tendency continues in September and October. Whilst and henceforth November, the mean oocyte diameter exhibit a diminution tendency which covers also December and January.

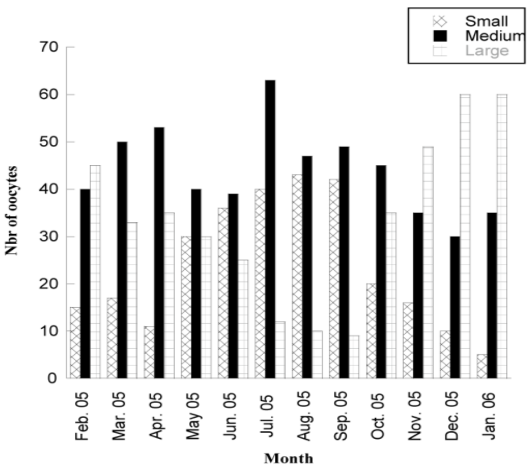

Fig. 4: Distribution of size classes of oocytes in *M. sanguinea* of Khnifiss lagoon from February 2005 to January 2006.

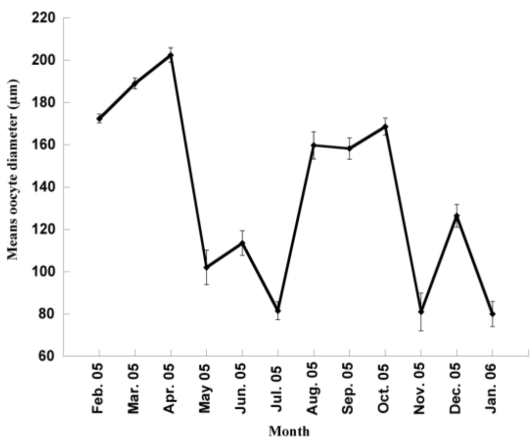

Fig. 5 : Temporal evolution of the oocyte dimater of *M. sanguine*

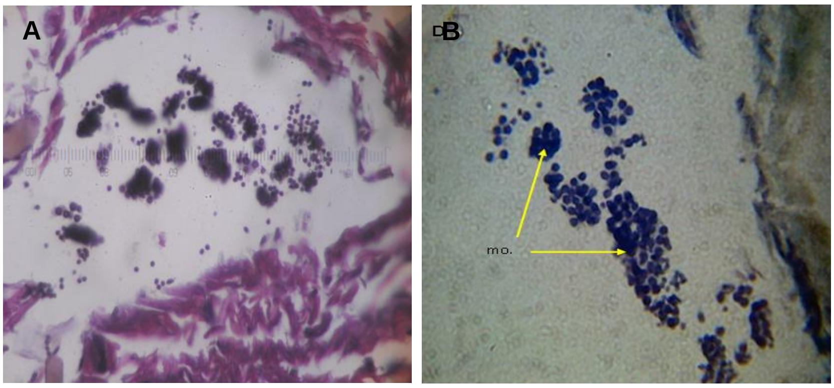

Fig. 6 : Sperm morulas (A; G× 40) (B; G× 100)

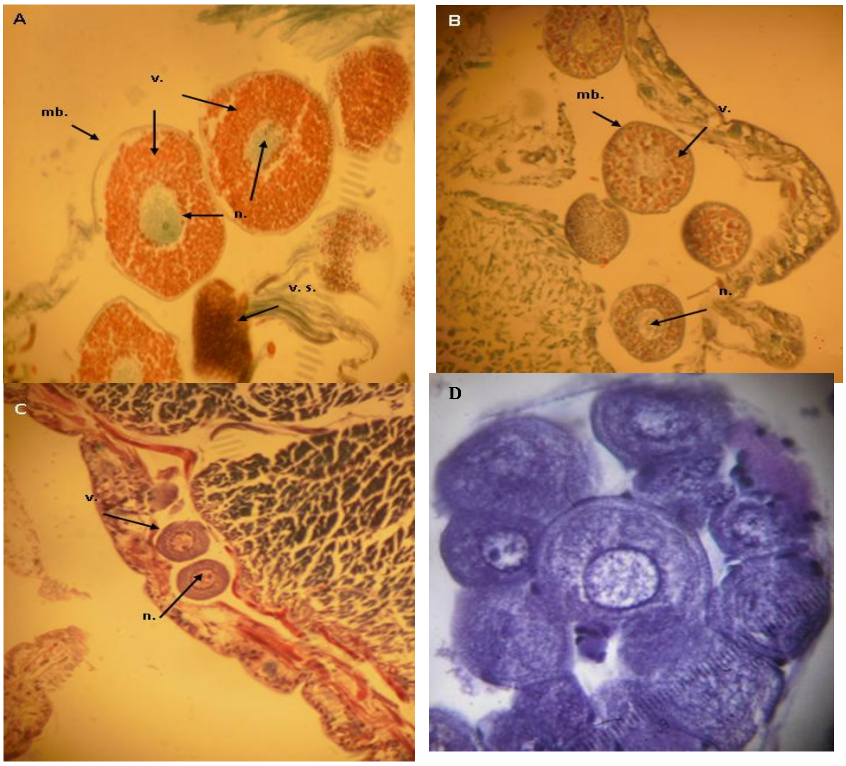

Fig. 7: The different histological stages of oogenesis in females of *M. sanguinea*. **A**. large oocyte (G× 100), **B**. medium oocyte (G × 100), **C**. small oocyte (G× 100) and D. Oocytes enveloped by a thin layer structure forming a cluster (G × 100). **mb**. Member oocyte, **mo**. Morula sperm, **n**. nuclus **v**. Vitellus and **v. s**. Blood vessel.

4. Discussion:

4.1. Water Lagoon conditions:

In studied site, the monitoring of the physicochemical parameters allowed highlighting a seasonal variation in temperature, while the warm period between April and September. Salinity and pH showed no seasonality. However, the water salinity has shown a peak of 39.1 ‰ in May. This would be the result of significant evaporation of the lagoon waters since April, which is the hottest month during the study period (29.8 ° C). The pH measuring underlines the alkaline character of this body water. The same thermal profile has been reported by [20] with warm temperatures in September and cooler temperatures in February. According to the same author, the water salinity inside the lagoon is higher than the oceanic water salinity. Also, they denote high salinity values in neap tide comparing to spring tide [20]. It's assumed that this situation is currently caused by the localization of Khnifiss lagoon in the Saharian bioclimatic floor, with negligible inputs of freshwater (The average annual rainfall is around 40 mm) [39]. Besides, the Khnifiss lagoon opens up into the Atlantic Ocean by one narrow inlet called Foum Awedri. The lagoon continues upstream beyond a huge flat of salt covered area named Sebkha Tazra [9]. Moreover, the shallowness waters of the lagoon (depths varied from 2.5

m to 8.7 m) would promote the heating of the water column and high rate of evaporation, which is the cause of elevated salinity values in the lagoon [20]. These effects are moderated by the penetration of oceanic water into the lagoon as shown by the alkalinity of the lagoonal water. Moreover, the water temperature seasonality seems to fit well with the seasonal upwelling activity in the coastal area surrounding the lagoon. Several studies point out a decrease of the water temperature and an increase in nutrients following the intense activity of upwelling [40-42].

4.2. Reproductive cycle of Marphysa sanguinea:

The Khnifiss population is characterized by an almost equal sex-ratio (1:1.16). A similar result was reported for the population of *M. sanguinea* in the lagoon of Venice [33; 34]. The sex of a small proportion of individuals has not been identified. This could be explained by the localization of the gonadal activity in the central segments of the polychaete body [34]. The monitoring of the female reproductive cycle showed three classes of oocytes according to their size: small (ø < 100 µm), medium (100 < ø <200 µm) and large (ø >200 µm). [43] states that the female sex gonad development of *M. sanguinea* can be separated into four stages: previtellogenesis corresponding to a proliferating and growing stage, vitellogenesis as maturing stage, spawning stage and resting stage. According to [32], as the female progress in sexual maturity scale, the oocytes accumulate nutriments and growth in diameter. The three oocytes classes refer to the enhancement in oocyte dimensions during the oogenesis stages: previtellogenesis, vitellogenesis and spawning [32]. *M. sanguinea* adopt an annual iteroparous reproductive strategy with an extra-ovarian oogenesis [44, 32]. In this oogenesis type, previtellogenic oocytes, the smaller oocytes, are enveloped by a thin layer of follicle cells and released, in successive manner, into the coelom (Fig. 7D) [45]. Subsequently, the oocytes are fated to achieve the vitellogenesis in the coelomic fluid (100-200µm in diameter) [45]. Once they become mature, the oocytes measure more than 200 µm and are ready for spawning [35].

Then, the following of the classes of small and large oocytes, throughout the year, enabled to survey the gametogenic activity and to locate the spawning time for this polycheate. In fact, the shift in relative abundance of small and large oocytes can be related to the gametogenic activity for the first and for the spawning period for the latter. In this way, the spawning period in Khnifiss occurs between Mars and July, while the gametogenic activity reached its maximum with high abundance of small oocytes during the May-September period. The means oocytes diameter decrease upon May as a result of progressive release of large oocyte. During this period, the Khnifiss lagoon is characterized by a warmest weather conditions. This result seems to be in accordance with the conclusions made by [35] for *M. sanguinea* population in the Venice lagoon. By comparing populations of the Khnifiss lagoon and those of Venice lagoon, the gametogenic activity meets its higher level, for both lagoons, between May and September. Whereas, the spawning period shows a slight change, since at Khnifiss, the spawning period lasts between March and July, whereas it occurs between April and May at the Venice lagoon [34; 35]. Although, the spawning period take place between July and August in the Rushan area, and the proliferating stage occurs from December to June [43]. Many authors suggest that the spawning period may change in different years because of gametogenesis and gamete release could be influenced by temperature [46, 47, 48, 49, 34]. In fact, this plasticity in modulating the reproductive cycle characteristics seems to be a response to the necessity to maximize fitness [50], which would be in part responsible of the success to colonize many different habitats [44]. [47] states that the rate development of juvenile is positively correlated to the water temperature [47]. Thus, the breeding season occurs in periods of the year where there are suitable conditions for larval development, as larvae do not tolerate low temperature [31, 47]. According to the present study the spawning period of *M. sanguinea* in Khnifiss lagoon take place within months of elevated water temperatures. Consequently, and even in a boundary of the distribution area of this polycheate and in a saharan lagoon, we find that the reproduction cycle is managed by adopting the same adaptability discussed in [35] for the population of *M. sanguinea*, in the Venice lagoon under an European climate. In Khnifiss lagoon, *M. sanguinea* shows some shift in its reproductive strategy features in order to respond to its habitat conditions, especially the water temperature within the lagoon. This highlights the significant influence of water temperature on the reproductive cycle of this species. In fact, notwithstanding the lagoon Khnifiss is located in arid desert zone; the water temperature is moderated by the influence of upwelling generated, on a seasonal basis, by the current of Canaries.

Conclusion

The study of the biology of the polycheate *M. sanguinea* has enabled a balanced a balanced sex ratio. Sapawning periods take place between May and July. The highest gametogenic activity occurred in August. The spawning period coincided with months of May, June and July which are characterized by warm water temperatures.

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