Reproduction cycle of *Donax trunculus* (Mollusca, Bivalvia) in the gulf of Annaba (Northeast Algeria)

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**ABSTRACT**

This study is interested in knowing the cycle of oogenesis of the bivalve *Donax trunculus* colonizing Shallow waters of Sidi Salem and Echatt beaches in the Gulf of Annaba, during 2012. The histological analysis of gonads was complemented by changes in the condition index and associated with the temperature and salinity measured monthly. Thermal extremes varied from 15.02 (February) to 29.04 °C (July). The haline variations are lower and range between 37.07 (July) and 38.04 psu (September). The condition index fluctuates between 0.20 (July and August) and 0.32 (April). The spatial differences of this index are in favor of Sidi Salem site where individuals have higher biomass compared to those of Echatt. This would be in relation to trophic factor of this bivalve. Indeed, this site is close to the discharge of a chemical fertilizers complex which fertilizes the waters of the beach. During the period of sexual activity; fluctuations in the sex ratio at both sites generally show a predominance of males. The study of oogenesis through histological analyzes in *D. trunculus* reveals that the reproduction begins in December (stage I) with a first emit of gametes in March (stage III). This sexual activity continues until September, when we witness the highest rate of emission of gametes into the surrounding environment. Moreover, monitoring of oogenesis through microscopic observations shows a lag in the oocyte development between the two sites. Thus it detects a delay of the evolution of stage III during the month of March in the ovaries of individuals taken from the Sidi Salem site. This phenomenon could be explained by the exposure of the site to different sources of pollution from the port and other effluents carrying sewage and industrial waste in the region.

**KEYWORDS:** Bivalvia, Donax trunculus, Reproduction, Oogenesis, Gulf of Annaba.

**INTRODUCTION**

*Donax trunculus* is a strictly intertidal bivalve that colonizes sandy beaches [55] of the Atlantic-Mediterranean coasts [2]. Its geographical distribution extends from the French Atlantic coast [43] to the coasts of Senegal [37]; it is widespread in the Mediterranean [8] and Black Sea [37]. This species inhabits the turbulent environments on beaches exposed to the rhythms of the tides or under intense wave action [20]. In these environments, *D. trunculus* is able to reach very high densities [40,84] Moreover, this Donacidae has a tolerance to changes in physical and chemical environmental factors; at the condition that these changes are not too brutal [53].

The studies of ecology, biology and population dynamics of *D. trunculus* are several either at the Mediterranean level [59,17,2,3,27,64,69,25,26] or Atlantic [3,43,6,8,42,9]. But those who treat reproduction are
few; in the Mediterranean [60,5,31,83,15] and in the Atlantic [7,40]. *D. trunculus* is very abundant in the Gulf of Annaba [11] and is the subject of artisanal exploitation in the region. Despite its abundance and its use, no research on the reproductive cycle was performed [46] which dealt with the biochemistry of the gonads of this bivalve). Other works in the Gulf of Annaba were interested to use this mollusk species as bioindicator of pollution [12,77,75,33,1,44,79,10,76]. The ecological and economic importance of *D. trunculus* and lack of information about the trigger period of reproduction and the arrival of young recruits motivated us to undertake this investigation. Moreover, the study of the sexual cycle in organisms is of great importance for population dynamics to better knowledge and understanding of specificity and biogeographical distribution. In the case of this bivalve used for commercial purposes, the study of the sexual cycle could enable improved performance by determining the period during which the Edge shell may be harvested.

This work treats the oogenesis of *D. trunculus* and its reproductive cycle in the Gulf of Annaba; to better understand the biology and dynamics of this bivalve in its natural environment and to preserve the natural harvested stock.

**MATERIALS AND METHODS**

**Study zone:**

The Gulf of Annaba is located at the north-east of Algeria between two capes; Western Cape de Garde (7°16’ E - 36°68’ N) and Cape Rosa to the east (8°15’ E - 36°38’ N), distant of about 40 km. The maximum depth does not exceed 65 m. The Gulf receives fresh water through two rivers; Mafragh (South) and Seybouse (Southwest) (Fig. 1).

![Geographical position of the Gulf of Annaba and location of the sampling sites](image)

**Fig. 1:** Geographical position of the Gulf of Annaba and location of the sampling sites (S.S: Sidi Salem, Ech.: Echatt).

**Measurements of temperature and salinity:**

The temperature and salinity were measured by a thermo-salinometer type "WTW Cond.197i" thermal accuracy and respective haline 0.01°C and 0.01 psu. Measurements were performed monthly and parallel to the sampling of the bivalve *Donax trunculus* in the two study sites.

**Sampling of Edge shell:**

Sampling was done manually with a large rake commonly called "Cope". It is dragged for a distance of 5 m by the angler which travels backwards at depths varying between 1 and 1.5 m. Individuals are thus recovered in a bag made of a net of 5 mm mesh size; in order to collect individuals of different sizes.

**Condition index:**

In an environmental monitoring study, condition index (C.I) assesses the general condition of the animal, its bioenergetic status and reproductive status [68]. It was measured for 50 individuals per site during the study period after passage in the oven at 60°C for 48 h. The condition index (C.I) was calculated according to the equation of Beninger [14]:
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\[ C.I = \frac{\text{dry flesh weight}}{\text{weight of the shell}} \times 100 \]

Its monitoring allows to know the stages of gametogenesis and periods of gamete emissions.

**Sex ratio:**

The distinction between sexes is only possible during the period of gametogenesis. Indeed, the differentiation between sexes is based on the color of the gonads. Those of male have a viscous appearance, whitish to orange, while the ovaries in females are dark bluish. Thus, the two valves are opened and carried to the macroscopic observations for determining the sex of individuals.

The relative frequency of the sexes (males, females and immatures) is calculated and expressed as a percentage. Gender percentages were determined during the months of sexual activity (i.e., when the sex of individuals is recognizable).

**Oogenesis study:**

**Collection and preservation of gonads:**

After collecting the samples, mature female individuals are selected (> 10 mm) freshly collected. To take the female gonad, both valves are opened, then using a micro-scissor, ovarian are dissected. Collected gonads are preserved in formalin solution 10%, for further use in the histological study.

**Histological techniques:**

The steps of histological preparation of oogenesis *D. trunculus* are those described by Martoja and Martoja [54], using an automated apparatus "Fabric Tech. 11". The thinning is done by immersion in 3 baths of xylene [47,21]. This is followed by soak and inclusion in both paraffin baths to 60°C. Semi thin sections (2-3 microns) are achieved through a microtome from the blocks obtained. These sections were mounted on slides held in a small drop of gelatin, and placed on a hotplate at 60°C to remove the fold. They are then dewaxed to hydrate the tissue; they are subject to hematoxylin-eosin baths.

**Scale adopted for the study of oogenesis:**

As in most bivalves, in *D. trunculus*, we identified four stages of the cycle of reproduction. These stages have been identified and characterized based on morphological and cytological criteria described by several authors: Mouneyrac et al. [62] in the semelidae *Scrobicularia plana*, Delgado and Pérez-Camacho [30] in the veneridae *Ruditapes decussatus*, Gaspar and Monteiro [39] in the pharidae *Ensis siliqua* and the veneridae *Venus striatula* and Gaspar et al. [40], Tlili [82] in the Donacidae *Donax trunculus*. These stages are as follows: stage 0 (sexual rest), stage I (Resumption of genital activity), stage II (Gametogenesis) and stage III (genital maturity).

**Results:**

**Temperature and salinity:**

Thermal water variations in both sample sites are important (Fig. 2), where the thermal extremes passes from 15.02 (February, Echatt) to 29.04°C (July, Echatt), an amplitude of 14.02°C. The inter-station thermal differences are generally low and hardly exceed 2.56°C (June). Generally temperatures of Sidi Salem waters are slightly higher compared to those observed in Echatt (Fig. 2).

![Fig. 2: Spatio-temporal variations of temperature in the surveyed stations during 2012.](image-url)

The salinity fluctuations (Fig. 3) are less pronounced in comparison with those of the temperature. Indeed, haline variations range between 35.71 (April, Echatt) and 38.05 psu (September Sidi Salem), an annual amplitude of 2.34 psu.
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Fig. 3: Spatio-temporal variations in salinity at the surveyed stations during 2012.

Condition index:
Monthly monitoring of the condition index (C.I) in *Donax trunculus* taken from the two selected sites, reveals significant spatio-temporal fluctuations (Fig. 4). The most important values of this index are in spring and autumn with a peak of 8.40 (April, Echatt). Furthermore, the lowest values are recorded during the summer season with a minimum of 4.98 (Jul, Echatt). In the beach of Sidi Salem, condition index goes from 5.66 (June) to 8.28 (February). Overall, the values of this index show a slight increase for the individuals collected from the beach of Sidi Salem; while reflecting slightly higher biomass compared to those of individuals collected in Echatt.

Fig. 4: Monthly changes of condition index (C.I) in *Donax trunculus* fished in Echatt and Sidi Salem during 2012.

Sex ratio:
At *Donax trunculus*, sexual differentiation is possible only during the period of sexual activity. Therefore, the sex ratio was calculated during this period which lasts from February to October, where sex is macroscopically identifiable.

Overall, the relative frequencies show no difference between males and females in both study sites (Fig. 5). However, in the range of Echatt, there was a slight dominance of males in February (56.64%) in July (56.50%) and August (54.17%). While in the Sidi Salem beach, males dominate especially in August (61.82%) in September (54.31%) and October (69.58%). Immature individuals are identified during the period which runs from March to October. Their presence remains low (0.33 to 3.03%), except in July in Echatt (9%) and June at the beach of Sidi Salem (20%).
Fig. 5: Relative Frequency of sexes in both surveyed stations during 2012. (A: Echatt, B: Sidi Salem, M: male, F: female, I: undifferentiated).

**oogenesis study:**

Histological examination of the female gonad *Donax trunculus* at both sites, allowed the identification of four stages of gametogenic development: (1) sexual rest, (2) recovery of genital activity, (3) gametogenesis (4) genital maturity. Morphological and cytological features of differentiation between these stages are:

Stage 0: sexual rest, oocytes are very small. There is a gonadal tissue mainly composed of a connective tissue (Fig. 6). This phase is characterized by the accumulation of reserves and adipogranules vesicular cells. The cloak is so homogeneous and transparent. Therefore, it is impossible to identify the sex of *D. trunculus* at this stage.

Stage I: Resumption of genital activity, Gonies multiply hence the increased volume of oocytes at the expense of connective tissue. Sex of Edge shell can then be identified. oogonia can be observed on the wall and some vitellogenic oocytes (Fig. 7). We can also see an appearance of follicles dispersed in connective tissue and having oocytes maturation and glued to the wall. At this point, the mean oocyte diameter is about 79 microns.
Fig. 6: Stage 0 "sexual rest" appearance of a reduced size acini within which we distinguish oogonia. A: Ovary in October; B: Ovary in November; UC: undifferentiated cell; OG: oogonia. Scale bar: 87 µm.

Fig. 7: Stage I: "Resuming sexual activity." A: Ovary in December; B: ovary in January; C & D: Biometrics; OA: adhered oocyte; OG: oogonia; VO: vitélogénétique oocyte; CT: conjunctival tissue. Scale bar: 87 µm.

Stage II: Gametogenesis, we can observe on the wall of the pedunculate oocytes during detachment and mature oocytes that acquire a polygonal shape; because of their overcrowding in the light of gonadal tubules. During this phase, the mean oocyte diameter is about 120 microns. Connective tissue has shrunk in favor of developing follicles (Fig. 8). At this point, individuals are easily excitable and release their gametes under the action of external stimuli.
Stage III: Genital maturity, at this stage oocytes are partially or completely emptied of their contents. Sometimes there is the presence of residual oocytes with an average diameter of about 122 microns, not issued at the end of the cycle (Fig. 9). Acini have a torn appearance and will be resorbed by the body. Gonadal tissue will be invaded again by the connective tissue. It is the restoration of the gonad (the gametic renewal takes place). During this stage the germ activity stops completely. Important phenomena occurs in the cloak. The follicles degenerate and crumble. Amoebocytes attack gametes not laid. The animal is again at the stage of sexual rest.
Fig. 9: Stage III: Maturation of oocytes and gamete emission. A: ovary in March (GX100) B: ovary in April (GX400) C & D: Biometrics (GX400). RO: residual oocyte. Scale bar: 87 µm.

Discussion:

In this study the temperature readings performed at the beach of Sidi Salem and Echatt in a cycle [35], reveal a certain similarity between the two sites where the temperature ranges between 15.02°C (February) and 29.04°C (July) with an amplitude of 14.02°C. The comparison between the average temperatures of the two sites shows that the waters of Sidi Salem are slightly warmer than 21.76°C to 21.28°C Echatt, probably due to water discharge used to cool the complex engines of (Fertial). These results are similar to the work done in the bay of Annaba, where the amplitudes are close to 15°C [12,49,75,45]. Temperature is one of the major factors that control reproduction of bivalve molluscs, it is considered the main factor affecting gonadal development in marine bivalves. The food availability and the temperature are principal factors that affect the gametogenesis of marine bivalves in general [29,26] and of Donacidae in particular [32,48]. For *D.trunculus* Ruiz-Azcon *et al.* [71] show that at a temperature of 20°C the larvae have a large percentage of survival. Among other molluscs numerous studies show that the temperature can affect the hatching of eggs of molluscs and their rate of sexual maturity [63,15,23]. According to Serdar *et al.* [73], Gamete maturation and spawning in bivalves can continue throughout the year, so the rest period will be reduced and may even be absent whenever trigger factors (i.e, food availability and temperature) are appropriate for gametogenesis. In oysters *Crassostrea gigas* Lubert [52] and Chavez-Villalba *et al.* [23] indicate a trigger of gametogenesis from a temperature (8-9°C) in the Channel and 10°C in the Bay of Veys; with an induced emission of gametes by a threshold value (> 19-20°C). Indeed, the Edge shell in the Gulf of Annaba starts the emission of gametes into the surrounding environment from the month of March-April; parallel to the beginning of the warming of waters at the end of the winter and early spring.
The salinity of shallow waters is influenced by seasonal temperature variation; it depends on the evaporation, and arrivals of fresh water whose flow rate can vary significantly depending on rainfall. In this study the monthly salinity fluctuations do not show remarkable variation between the two study sites with average salinity of 37.08 psu for the site of Sidi Salem and 37.05 psu for the site of Echatt. The salinity varies in the range of 35.71 (April, Echatt) and 38.05 psu (September, Sidi Salem). These results are consistent with other work in the Gulf of Annaba, the saline variations oscillate between 35 and 38 psu [12,38,75,45], and close the work of Ounissi et al. [65], who report salinities between 32 and 37 psu. In the Gulf of Annaba, the saline variations do not seem to influence either the trigger or the conduct of reproduction in Donax trunculus since the differences of this ecological factor are relatively low during a cycle, taking into account the wet season and the dry season. For D. trunculus, sexual cycle may be punctuated by seasonal climatic factors. Reproduction would be triggered by sudden variations in temperature and/or salinity [53].

The condition index is one of the indices that affect the cycle of reproduction in marine organisms in general and bivalve molluscs in particular. The study of this index can provide additional information on this process [16,17]. Monthly monitoring of this index shows significant seasonal variations with an increase in spring and autumn and a decrease in summer. These variations are due to several parameters including the status of breeding and nutrition [18,78,66]. Indeed, the condition index is an indicator of the physiological state of bivalves. The decrease in index values condition which begins in May coincides with the emission of gametes, hence the importance of the gametes mass in the whole mass of individuals. Indeed, the issue of gametes causes weight loss inducing lower values of the CI. On the other hand, the increase of this index which begins in the fall may can be caused by a maturation or redevelopment of the ovary. Histological examination of the gonads allowed us to confirm this. Several authors have observed similar results [5,56,83]. In the Mediterranean, the condition index of Donax has strong values from February to June [2], reflecting the expansion of the period of reproduction. According to Roméo et al. [70], the condition index is associated with the water temperature and correspond to filling ratio of the shell by soft tissue and by that, informs on the nutritional and physiological status of the animal. The change in the condition index may also be caused by trophic conditions that are influenced by environmental conditions including pollution [28,57,35].

The study of the sex ratio in the population of Donax trunculus in the two study sites during the period of sexual activity (February to October), allowed us to reveal differences over time and depending on location where the strongest female domination is in April for the site of Echatt but shifts from one to two months in the Sidi Salem site. The rest of the cycle is characterized by a slight predominance of males. Indeed, after laying the percentage of females decreased, and there has been an increase in male individuals that extends through the winter. These results are in agreement with those of Gaspar [40] in Portugal, Deval [31] in the Turkish coast of the Marmara Sea in the eastern Mediterranean, from Tlili et al. [83] in Tunisia. Several works on D. trunculus showed a slight predominance of males compared to females during the rest period and the early gametogenesis [40,50,19]. The decrease in females after laying could be explained as differential mortality due to the effort of reproduction [40]. However, Badino and Marchionni [5] in Italy report some balance and fairness between the sexes.

The study of the sexual cycle of Donax trunculus in the Gulf of Annaba by histological analysis of female gonads during the period which runs from January to December, allowed us to see that the gonadal development starts from December in both sites. This period coincides with the cooling water (17.22°C in Sidi Salem and Echatt 16.63°C). This demonstrates the importance of abiotic factors in the reproduction of marine invertebrates in general [67], and in bivalve molluscs in particular [34,36,72]. According to Mouëza [61], low temperatures triggers gonad maturation and the emission of gametes is undertaken when it increases until a proximate degree of (20°C). Furthermore, the author reports that the temperature is not the only factor triggering the reproduction or the only limiting factor. Moreover, this factor acts differently between adult individuals and young ones, because in juveniles (from 8 to 16 mm), maturity intervene later than in adults [61].

In both study sites, the emission of gametes begins in March and continues through September with two major spawning periods: an emission peak of gamete in spring and the other in summer. This period coincides with the sharp decline in index condition values. These results agree with those reported by Tlili et al. [83] in the Gulf of Tunis. They are also close to those of Mouëza and frenkiel-Renault [58] and Ansell et al. [3] also note that two main shift laying periods; one in autumn and one in winter. However, we note that this physiological activity is closely related to the location where this species lives. Moreover, in the same species in Spain [81] and in Turkey [31] notes a single spawning period during the summer season. These differences can be explained by changes in environmental conditions that affect the frequency and duration time of gametogenesis. Furthermore, it should be noted that we have identified a delay (about a month) of stage III at the Sidi Salem site. This time lag in this site near Seybouse river is induced by the negative effects of the surrounding water pollutants and contaminants impacting consequently on the reproductive physiology of this bivalve. This same finding was reported by Tlili [82], which noted a delay in stage III at a polluted site compared to that which is less exposed to pollution. The negative influence of pollutants on the physiology of reproduction in marine bivalves, has already been reported in several studies [80,13,41,74].
Conclusion:

The monitoring of the temperature and salinity in the two selected sites in the Gulf of Annaba during a cycle (2012), shows that the thermal fluctuations of the water of the study area are important and under the direct influence of changes in the atmospheric temperature. Overall, the beach of Sidi Salem more sheltered and under the influence of cool waters of the engines of the complex Fertial, is characterized by relatively warmer waters compared to waters of the beach of Echatt more exposed (roughest) to dominant winds of Northwest sector.

However, warming waters in the late winter and early spring helps the first trigger (March) of gametes emission in the surrounding environment. The haline variations are less important, with annual amplitudes not exceeding 1.26 psu in the range of Echatt and 1.97 psu in Sidi Salem. These small variations do not allow us the highlight of this parameter in the course of reproduction for the bivalve *Donax trunculus* in the Gulf of Annaba.

The study of the condition index of *D. trunculus* showed that Sidi Salem site individuals possess a higher body weight compared to those of Echatt with decreasing values of this index during the period of release of the gametes in both sites of the study. Indeed, the weakest condition index values coincide with the release of the gametes in the spring and summer, hence the importance of the gametes mass in the body mass of individuals for this bivalve Donacidae.

For *Donax trunculus*, sexual differentiation is possible only during the period of sexual activity. In general, both sexes are equitably distributed and the differences between sexes across a cycle are low: <10%, exceptionally 22.81% (Echatt, July) and 38.16% (Sidi Salem, October). Overall, the immature individuals are poorly represented in the two sites. Histological study of oogenesis of *D. trunculus*, allowed us to locate the breeding season which runs from February to October. This study also allowed us the timing and conditions of gonadal maturation and their emission into the environment. Furthermore, this analysis allowed us to detect a slight delay in the development of oogenesis in both sites. Indeed, there is a delay of stage III, for individuals taken from the beach of Sidi Salem. This could be caused by negative effects of various pollutants from discharges from Wadi Seybouse (industrial pollution) and port (wastewater and hydrocarbons) on the reproductive physiology of this bivalve.

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