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# Changes in Biochemical Composition of the Gonads of *Donax trunculus* L. (Mollusca, Bivalvia) from the Gulf of Annaba (Algeria) in Relation to Reproductive Events and Pollution

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**Abstract**

Seasonal changes in biochemical composition of the bivalve *Donax trunculus* L. (Mollusca, Bivalvia) were studied during eight months from January to August 2009 in the gulf of Annaba. The samples were collected monthly at two sites: El Battah, a site far from any source of pollution, and Sidi Salem, a site located near human and industrial wastes. The amounts of carbohydrate, lipid, and protein were determined in gonad-visceral mass. The monthly amounts of the different components in gonads varied from 33.68 to 118.09 for carbohydrates, 6.68 to 44.45 for lipids and 3.56 to 36.72 µg/mg of wet tissue for proteins, respectively. A three-way ANOVA indicated significant effects of time, sex, and treatment. Lowest values of components were observed during the spawning phase. There was a significant difference in amounts of these gonad components between the two sites. Globally, the lowest values of carbohydrate and proteins, and the highest values of lipids were recorded in individuals sampled at Sidi Salem.



**Keywords**: Gulf of Annaba, *Donax trunculus*, Carbohydrates, Proteins, Lipids, Reproduction, Pollution.

## 1. Introduction

The continuous anthropogenic pressure exerted on the marine environment constitutes a real environmental problem (Lavado *et al.*, 2006; Sanchez *et al.*, 2007). The gulf of Annaba is the most important touristic and economic coastal zone located in east of Algeria. It is continuously affected by various contaminants from urban, agricultural, harbour and industrial activities (Semadi and Deruelle, 1993; Abdenour *et al.*, 2000). Bivalves play an important role in the ecosystem equilibrium and constitute an important economic endpoint. They are important representatives of the primary consumers in limnic systems and, therefore, an important link in the aquatic food chain. *Donax trunculus* L. (Mollusca, Bivalvia) is widely distributed along the Mediterranean coast and frequently used in marine pollution studies (Gaspar *et al.*, 1999; Moukrim *et al.*, 2004; Usero *et al.*, 2005; Beldi *et al.*, 2006; Sifi *et al.*, 2007). In addition, this species exists in higher densities in the sand beaches of the gulf of Annaba in Algeria (Degiovanni and Moueza, 1972) and is consumed by the local population (Beldi *et al.*, 2006). In Algeria, the growth, population dynamic and reproductive cycle of *D. trunculus* from Algiers bay (Mouëza, 1972; Mouëza and Frenkiel-Renault, 1973) and Annaba bay (Beldi, 2007) were examined. In addition, *D. trunculus* is used for many years ago as a bioindicator species in monitoring programs for the assessment the impact of pollution on marine organisms in the gulf of Annaba (Sifi *et al.*, 2007).

It has been reported that biochemical indicators provide more accurate information about gonad performance and environmental stress (Smolders *et al.*, 2005). Several studies on seasonal variation in biochemical composition of bivalves have been carried out in relation to reproduction (Giese, 1969; Ansell *et al.*, 1980; Zandee *et al.*, 1980; Baber and Blake, 1981; Polak *et al.*, 1987; Pazos *et al.*, 1997**;** Ojea *et al.*, 2004). For the gulf of Annaba, no corresponding data have been found in the literature. Therefore, the present study was undertaken in order to evaluate monthly variations of the main gonad components (carbohydrates, lipids, proteins) in the edible mollusk *D. trunculus* sampled at two sites in the gulf of Annaba. The observed changes are discussed in relation to reproductive events as well as to pollution since contamination status was different in the two studied sites (Beldi *et al.*, 2006; Sifi *et al.*, 2007).

## 2. Materials and Methods

### *2.1. Sampling sites*

The gulf of Annaba is located in the East of Algeria. It is limited by the Rosa Cap (8° 15 ' E and 36° 38 ' N) in the East, and by the Garde Cap (7° 16 ' E and 36° 68 ' N) in the West. El Battah site (36° 50 ' N - 8° 50 ' E) is located

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about 30 km to the East of Annaba, far from any source of pollution and expected as a relatively clean site. Sidi Salem site (36° 50 ' N - 7° 47 ' E) is situated about 1 km to the East of Annaba city and considered affected by urban, harbor and industrial wastes (Fig. 1).

*2.2. Samples collection*

*D. trunculus* of standardized shell size (length: 27 ± 1 mm) were collected monthly from January to August 2009, from the two selected sites in the gulf of Annaba. Sex was analyzed by macroscopic inspection according to the color of the gonad: violet in the females, yellowish-orange in the males (Gaspar *et al.*, 1999). Samples were taken from the same part of the gonad. Thus, the middle part of gonad was dissected and stored at –20°C until analysis.

*2.3. Analytical methods*

Protein, carbohydrate and lipid were extracted from the same gonad sample following the procedure of Shibko *et al.* (1966). In brief, each sample of gonad (weight: 37-45 mg) was individually homogenized in 1 ml of trichloroacetic acid (20%) and then centrifuged (5,000 g for 10 min). The supernatant was used for the carbohydrates determination as described by Duchateau and Florkin (1959) using anthrone as reagent and glucose as standard, while the pellet added with a mixture of ether and chloroform (1V/1V) was subjected to a second centrifugation (5.000 g for 10 min). The resulted supernatant was used to quantify the lipids based on the vanillin method of Goldsworthy *et al.* (1972), Finally, protein concentration was determined in resulting pellet using the Bradford (1976) assay with blue brilliant of coomassie (G 250, Merck) as reagent and bovine serum albumin (Sigma) as standard.

*2.4. Statistical analysis*

The normality of data was verified using the Kolmogorov-Smirnov test, and the homogeneity of variances was checked by Levene's test. Data were expressed as Mean ± Standard Deviation (M ± SD). Comparison of mean values was estimated by Student's t-test. The effects of time, sampling sites and sex were tested by a three-way analysis of variance (ANOVA). The relation between the gonad components was also examined. All statistical analyses were performed using MINITAB Software (Version 14, PA State College, USA) and $p< 0.05$ was considered to be a statistically significant difference.

## 3. Results

*3.1. Changes in carbohydrate contents*

Monthly variation of the carbohydrate amount is shown in figure 2. Changes in carbohydrate amounts showed a decrease in March followed by two peaks; the first one occurred in April, and the second in July for females and August for males, respectively. The monthly amounts of carbohydrate in gonad ranged from 33 (March) to 119 (August) µg/mg tissue. The amounts of carbohydrates were higher in the female gonad than in the male gonad (Fig. 2). The differences between males and females are significant in January, February, March, April, May, June, July and August for El Battah ($p< 0.01$), and in January, February, March, May, June, July and August for Sidi Salem ($p< 0.001$), respectively. In addition, greater values were also recorded in El Battah compared to Sidi Salem. Significant differences ($p< 0.001$) between the two studied sites were observed in January, March, April, May, June, July and August for males, and January, February, March, April, May, June, July and August for females. This was confirmed by statistical analysis. Indeed, three-way ANOVA indicated significant effects ($p< 0.001$) of both time ($F= 887.95$; $df= 7, 90$), sex ($F= 978.14$; $df= 1, 90$) and site ($F= 22.67$; $df= 1, 90$).

*3.2. Changes in lipid contents*

Biochemical data related to lipid amounts are summarized in figure 3. Variations in lipid levels presented two decreases: in spring (March for males; April for females) and at the beginning of summer (June for males; July for females). The values varied from 6.68 µg/mg tissue in March to 48.45 µg/mg tissue in August. Data subjected to three-way ANOVA revealed significant effects ($p< 0.001$) of both time ($F= 2184.77$; $df= 7, 116$), sex ($F= 4379.45$; $df= 1, 116$) and site ($F= 2776.85$; $df= 1, 116$). The highest values were observed in females in the two sites (Fig. 3). Significant differences between males and females are recorded for all months in El Battah ($p< 0.01$) and Sidi Salem ($p< 0.001$). Moreover, values from Sidi Salem were significantly ($p< 0.01$) higher as compared to those of El Battah (Males: in January, March, February, April, May, July and August; Females: in January, February, May and July).

*3.3. Changes in protein contents*

Results from protein analysis are presented in figure 4. Changes in the protein amounts during the reproductive period showed that the lowest values were recorded in spring (April). The highest values were observed in summer (June-August). A three way ANOVA revealed significant effects ($p< 0.001$) of both time ($F= 1078.04$; $df= 7, 115$), sex ($F= 286.76$; $df= 1, 115$) and site ($F= 1971.70$; $df= 1, 115$). Amounts of protein were significantly higher in females than males in January, February, March, June and July for El Battah ($p< 0.01$), and in February, March and July for Sidi Salem ($p< 0.001$) (Fig. 4). The differences between the two sites are significant in both males and females for all months ($p< 0.001$). Statistical analyses on the relation between the gonad components were also analyzed (Table 1). We observed significant ($p< 0.05$) correlations between the different components except at El Battah between carbohydrates-proteins in males ($R= -0.004$; $p= 0.985$) and lipids-proteins in females ($R= -0.139$; 0.528), and at Sidi Salem only between carbohydrates-proteins in females ($R= 0.357$; $p= 0.087$).

## 4. Discussion

In Algiers Bay, the sexual cycle of *D. trunculus* includes a sexual rest (November - January) and a reproductive period (February-September) (Mouëza and Frenkiel-Renault,1973).

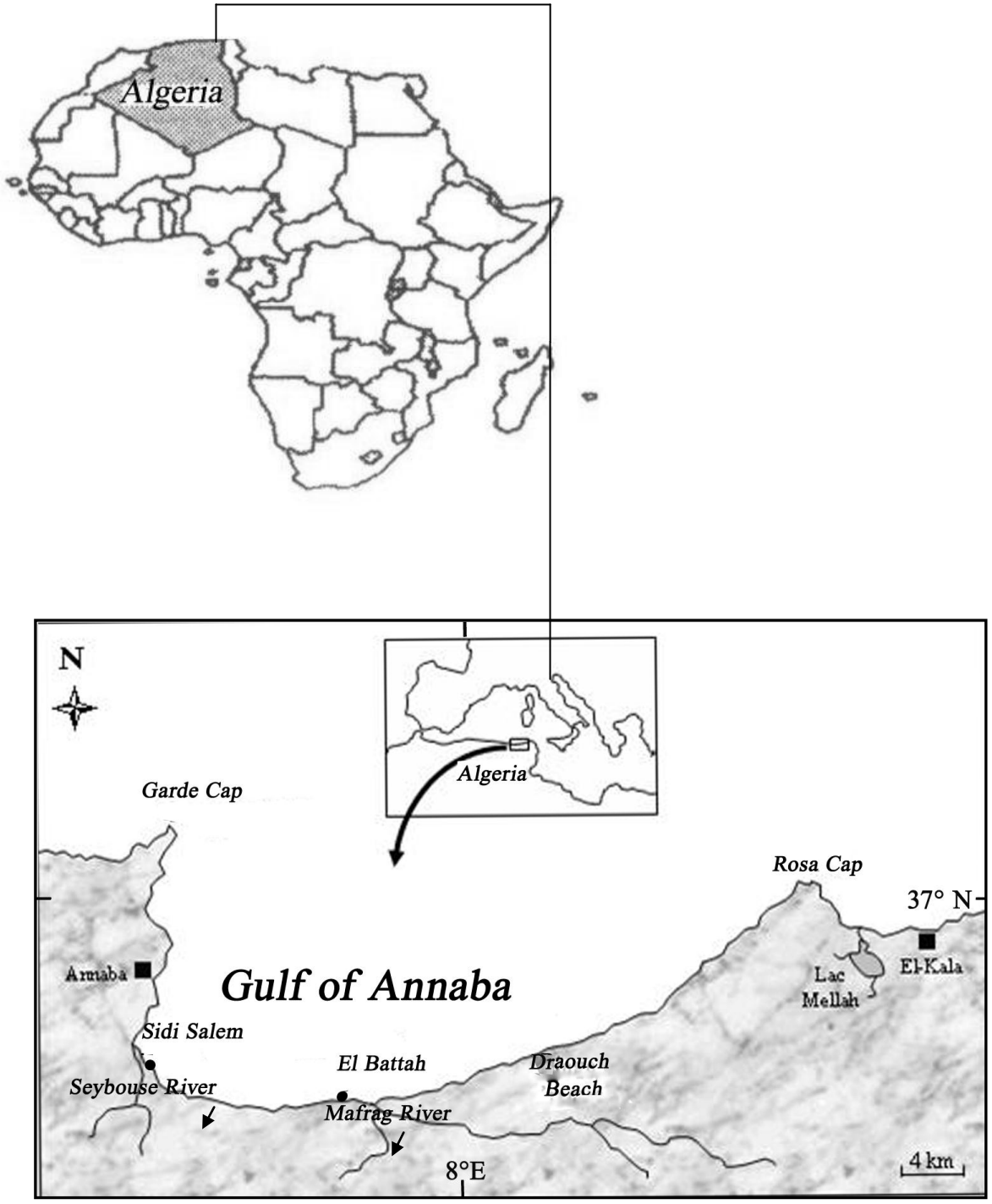


Figure 1. Location of the studied area in Algeria and of the two sampling sites in the gulf of Annaba.

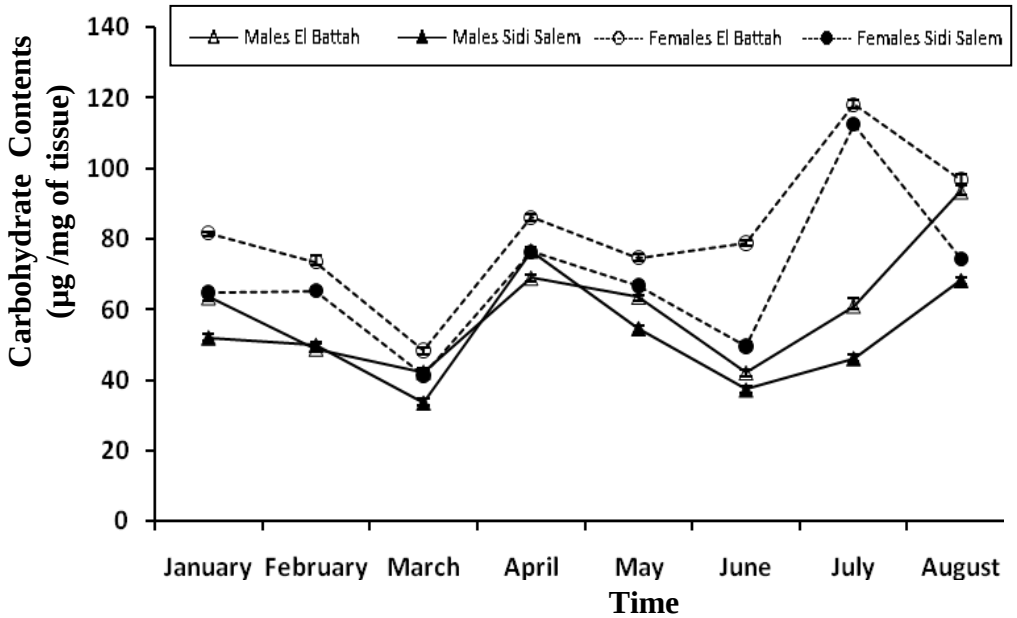


Figure 2. Carbohydrate contents (µg/mg of tissue) in male and female gonads of *Donax trunculus* collected during the reproductive period at two sites (El Battah and Sidi Salem) in the gulf of Annaba in 2009 (mean ± SD; n= 4-6).

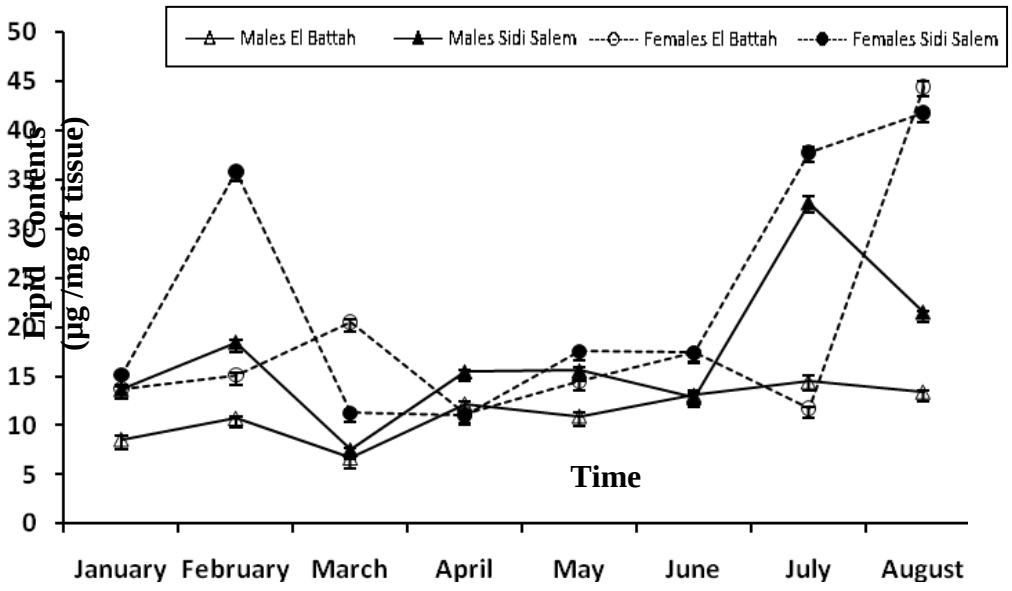


Figure 3. Lipid contents (µg/mg of tissue) in male and female gonads of *Donax trunculus* collected during the reproductive period at two sites (El Battah and Sidi Salem) in the gulf of Annaba in 2009 (mean ± SD; n= 4-6).

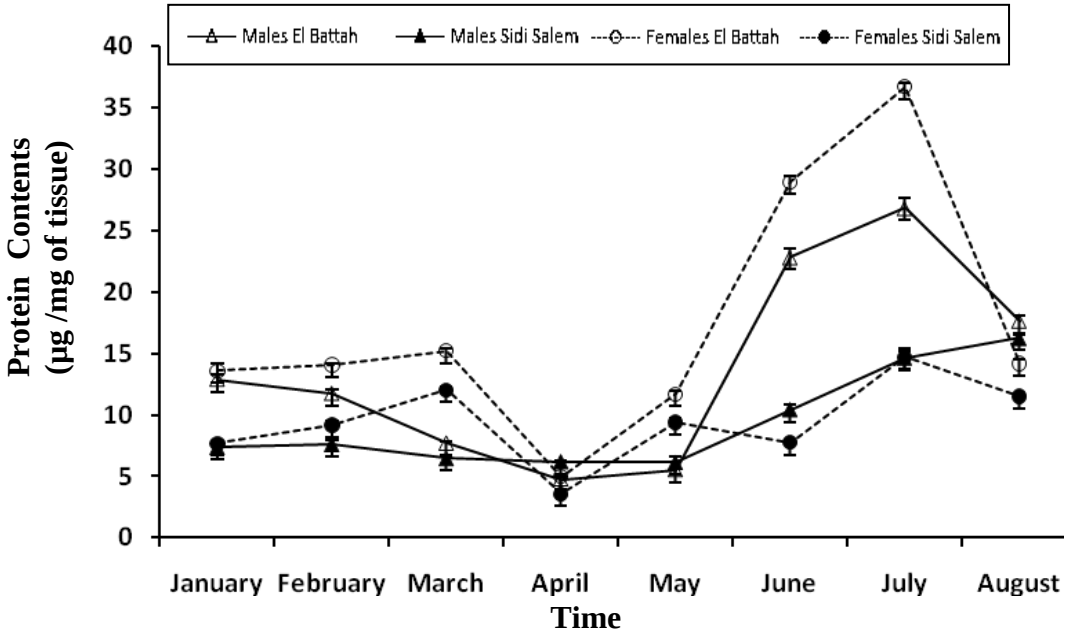


Figure 4. Protein contents (µg/mg of tissue) in male and female gonads of *Donax trunculus* collected during the reproductive period at two sites (El Battah and Sidi Salem) in the gulf of Annaba in 2009 (mean ± SD; n= 4-6).

Table 1. Relation between the different gonad components of *Donax trunculus* collected at El Battah (EB) and Sidi Salem (SS) in the gulf of Annaba (R: coefficient of correlation; *p*: significance level).

| Sites | Relation | Male gonads | | Female gonads | |
|---|---|---|---|---|---|
| | | R | *P* | R | *P* |
| EB | Carbohydrates-Lipids | 0.430 | 0.036 | 0.427 | 0.038 |
| | Carbohydrates-Proteins | -0.004 | 0.985 | -0.568 | 0.004 |
| | Lipids-Proteins | 0.651 | 0.001 | -0.139 | 0.518 |
| SS | Carbohydrates-Lipids | 0.697 | 0.000 | 0.547 | 0.006 |
| | Carbohydrates-Proteins | 0.701 | 0.000 | 0.357 | 0.087 |
| | Lipids-Proteins | 0.798 | 0.000 | 0.600 | 0.002 |

Gonadal development begins in February and maturity is achieved in spring. -Spawning and gametogenesis occur in summer (Mouëza and Frenkiel-Renault, 1973). Concerning the gulf of Annaba, the reproductive period is observed from January to August, the resting period occurs from September to December and two periods of recruitment are observed: the first in spring and the second in autumn Beldi (2007).

The biochemical analysis shows that carbohydrates are the main component of gonad. The changes in biochemical composition of gonads are related to the reproductive cycle of *D. trunculus* in the gulf of Annaba confirming previous studies showing an elevation in protein and lipid amounts during the gametogenesis, and a decrease of these components related to spawning phase, while a decrease in carbohydrate essentially glycogen during the reproductive period (Ojea *et al.*, 2004; Dridi *et al.*, 2006; Mladinea *et al.*, 2007; Joaquim *et al.*, 2008). The changes in carbohydrates and lipids recorded in the present study are in accordance with previous observations made in the same species in the Algiers bay (Ansell *et al.*, 1980) or in other mollusc species (Zandee *et al.*, 1980; Polak *et al.*, 1987; Pazos *et al.*, 1997). Moreover, our results indicated higher amounts of biochemical components in females of *D. trunculus*. This could be explained by a difference in the energy demand between male and female gametes (Beninger *et al.*, 2003). In addition, triacylglycerols and acylglycerols were the principal constituents of the oocytes (Napolitano *et al.*, 1992) and spermatozoids do not store lipid reserves (Soudant *et al.*, 1996). Lastly, male bivalves produce small spermatozoa with few energy reserves by comparison with females which elaborate vitellin for developing oocytes (Beninger and Le Pennec, 1997).

The lowest values in carbohydrates and proteins, and the highest values in lipids recorded in individuals sampled at Sidi Salem are probably related to the level of exposition of this site. Indeed, this site is affected by several pollutants such as heavy metals released from

factories and port activities. This is supported by our previous findings since Beldi *et al.* (2006) reported significant amounts of cadmium in tissues of *D. trunculus* collected from Sidi Salem in comparison with individuals from El Battah. More recently, Sifi *et al.* (2007), in a biomonitoring study on the gulf of Annaba, observed a significant effect on AChE and GST activities in *D. trunculus* collected from Sidi Salem as compared to individuals from El Battah.

It has been shown that Cd caused a decrease in the storage of glycogen in *Mya arenaria* (Gauthier-Clerc *et al.*, 2002), while Viarengo (1989) found that this metal inhibited oxidative phosphorylation and reduced protein synthesis. This supported our observations concerning the reduction of biochemical amounts in *D. trunculus* from Sidi Salem. Indeed, Sidi Salem was more exposed to metallic pollution as compared to El Battah (Beldi *et al.*, 2006).

## 5. Conclusion

Our present study on the main components in gonads of *Donax trunculus* during the reproductive period revealed changes correlated to the reproductive events in the gulf of Annaba. Moreover, there are differences between sex and site. The difference between the two sites was related to their level of exposition to pollution.

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