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Resources**

Field study of metal concentrations and biomarker responses in the neogastropod, *Murex trunculus*, from Bizerta Lagoon (Tunisia)

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Abstract – This study aims to evaluate the health status of Bizerta lagoon using a sentinel species, the Neogastropod *Murex trunculus*. Trace metal concentrations (Cd, Cu and Zn) in the foot and digestive gland, and biomarkers such as acetylcholinesterase (AChE), catalase (CAT) and glutathione transferase (GST) activities in the digestive gland, were determined in samples collected at four sites at different periods of the year. One site, in the North of the lagoon, is influenced by urban and industrial waste waters. Another, to the south, is located at the outfall of Ichkeul Lake. A third site is one that may be impacted by eutrophication processes, and the last, in the center of lagoon, is considered as less polluted. Temperature and salinity were simultaneously measured during the mollusc samplings. They varied greatly, from 13.5 °C and 30 psu in February to much higher values in summer-autumn: 30.7 °C and 37 psu. Metal concentrations were always higher in the digestive gland than in the foot. In the digestive gland, the lowest Cd and Cu concentrations were found in the center of the lagoon (mean ± standard deviation: $9.35 ± 0.78$ μg Cd g^{-1} and $84.5 ± 15.3$ μg Cu g^{-1}) and no significant Zn variations were noted ($682 ± 38$ μg g^{-1}) between sites. Metal concentrations in both types of tissue were higher in winter than in summer. AChE varied significantly among sites with high activities for individuals in the center of lagoon ($15.4 ± 0.3$ nmol min^{-1}mg^{-1} protein), whereas GST were low ($12.2 ± 0.8$ nmol min^{-1}mg^{-1} protein); CAT activities were low in this site $8.7 ± 0.5$ μmol min^{-1}mg^{-1} protein. Season also had an influence on biomarkers, with low AChE in winter and rather low CAT and GST in summer. Principal component analysis showed that the center of lagoon can be considered as a reference site, whereas high chemical and biochemical responses were found in *M. trunculus* from other sites; particularly at the site which may be subject to eutrophication.

Key words: Trace metals / Copper / Cadmium / Zinc / Biomarkers / *Murex trunculus* / Mediterranean Sea

Résumé – Étude *in situ* de concentrations en métaux-traces et réponses de biomarqueurs chez le néogastéropode, *Murex trunculus*, de la lagune de Bizerte (Tunisie). Cette étude a pour but d'évaluer l'état de la lagune de Bizerte en utilisant une espèce sentinelle, le néogastéropode, *Murex trunculus*. Dans ce but, les concentrations en métaux-traces (Cd, Cu et Zn) du pied et de la glande digestive, ainsi que des biomarqueurs, tels que les activités de l'acétylcholinestérase (AChE), catalase (CAT), glutathion transférase (GST) dans la glande digestive, sont déterminés dans des échantillons prélevés en quatre sites, à différentes périodes de l'année. Au nord de la lagune, un site est influencé par des eaux usées urbaines et industrielles, au sud un site est localisé à la sortie du lac Ichkeul, un troisième site peut être soumis à des processus d'eutrophisation, et enfin celui du centre est considéré comme moins pollué. La température et la salinité sont mesurées pendant la collecte des mollusques. Elles varient beaucoup de 13,5 °C et 30 psu en février à des valeurs bien plus élevées en été-automne : 30,7 °C et 37 psu. Les concentrations en métaux sont toujours plus fortes dans la glande digestive que dans le pied. Dans la glande digestive, les plus faibles concentrations de Cd et Cu sont trouvées au centre de la lagune (moyenne ± écart-type : $9,35 ± 0,78$ μg Cd g^{-1} et $84,5 ± 15,3$ μg Cu g^{-1}) et il n'y a pas de variations significatives du zinc ($682 ± 38$ μg g^{-1}) en fonction du lieu de prélèvement. Les concentrations métalliques dans les deux tissus sont plus élevées en hiver qu'en été. AChE varie significativement en fonction des sites avec de fortes activités au centre de la lagune ($15,4 ± 0,3$ nmol min^{-1}mg^{-1} protéine) tandis que CAT et GST sont faibles (respectivement $8,7 ± 0,5$ μmol min^{-1}mg^{-1} protéine et $12,2 ± 0,8$ nmol min^{-1}mg^{-1} protéine). La saison a aussi

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une influence sur les biomarqueurs avec AChE faible en hiver et CAT et GST plutôt faibles en été. Une analyse en composantes principales montre que le centre de la lagune peut être considéré comme un site de référence, tandis que des réponses chimiques et biochimiques élevées sont trouvées dans *M. trunculus* prélevé en d'autres sites et en particulier au site où des phénomènes d'eutrophisation peuvent survenir.

1 Introduction

Bizerta lagoon (Fig. 1), situated in northern Tunisia (between 37°08' N, 9°46 E and 37°16 N, 9°56 E), occupies an area of 15×10^6 m^2 and represents an economically important body of water host to a variety of fishing and aquaculture activities. With its location between Lake Ichkeul and the Mediterranean, the lagoon environment is characterized by a great variability in ambient conditions. Like most Mediterranean lagoons, this variability particularly concerns hydrological parameters such as salinity, temperature and dissolved oxygen levels. In addition, this body of water is subject to eutrophication phenomena during the summer period (Dellali et al. 2001). The natural fragility of the lagoon is increased by anthropic disturbances of different types. Domestic and/or industrial waste is released by neighboring towns: 400 000 inhabitants have been recorded in the Bizerta district and 400 industrial units (Trabelsi and Driss 2005) are present along the shores of the lagoon (ANPE 1989; El Menif et al. 2006). Waste of agricultural origin may reach the lagoon as a result of leaching from cultivated land, which covers an area of 172×10^6 m^2 mainly devoted to cereal crops (ANPE 1989). Furthermore, even though this aspect is not addressed in the present paper, one must bear in mind that imposex has recently been reported in *Hexaplex (Murex) trunculus* collected from Bizerta lagoon and channel (El Menif et al. 2006; Lahbib et al. 2008). These authors report that rates of occurrence and degree of imposex were higher in the channel (probably due to the use of antifouling paints, containing organotin tributylin , TBT, on boats) than in the lagoon itself. It appears important that the Bizerta lagoon should be the subject of a monitoring program.

Roméo et al. (2006) studied the biomarker responses of *Murex trunculus*, exposed to selected pollutant metals (cadmium and copper) and organic compounds (carbofuran and lindane) under laboratory conditions. These results suggested that *M. trunculus* could be an interesting sentinel species for monitoring pollution. Furthermore, these neogastropods are abundant in the Bizerta lagoon. This species is found both in relatively clean and polluted areas, and are easy to find and sample. They are predatory animals feeding on bivalves (including clams and mussels) and are also consumed by man.

The first objective of the present work was to determine concentrations of metals (cadmium, copper and zinc) in *Murex trunculus* specimens. *M. trunculus* samples were collected from four sites differently influenced by metal pollution (Chouba et al. 2002). Several papers have been written concerning their ability to concentrate cadmium (Bouquegneau et al. 1988; Dallinger et al. 1989), but also copper and mercury (Catsiki and Arnoux 1987). Our study was conducted using different sampling sites. Almost an annual cycle (from December to October) was taken into consideration since reproductive cycle is known to influence metal uptake (Boyden and Philips 1981).

The second purpose of the work was to evaluate selected biomarkers in the chosen sentinel species. The use of biomarkers measured at the molecular or cellular level has been proposed as a sensitive "early warning" tool for biological effect measurement in environmental quality assessment (McCarthy and Shugart 1990). The selected biomarkers should indicate that the organism has been exposed to pollutants, and/or the magnitude of an organism's response to a pollutant (Cajaraville et al. 2000). Three biomarkers (acetylcholinesterase AChE activity, catalase CAT activity and glutathione transferase GST activity) were evaluated in *Murex trunculus* collected from the Bizerta lagoon. The choice of these markers has already been described elsewhere (Roméo et al. 2006). Briefly, glutathione transferase (GST EC 2.5.1.18) plays a role in detoxification of organic compounds and metals (Power and Sheehan 1996; Khessiba et al. 2001). Catalase (CAT EC 1.11.1.6.) is a major antioxidant enzyme (Roméo et al. 2000); and acetylcholinesterase (AChE EC 3.1.1.7.) is an enzyme involved in the synaptic transmission of nerve impulses that may be inhibited by some neurotoxic compounds targeted to cause this mode of toxicity (Galgani and Bocquené 1991). Like metals, biomarker parameters were determined as a function of sampling site and season.

2 Materials and methods

2.1 Sample collection

Murex trunculus (Neogastropoda, Muricidae) were collected from four sites of Bizerta lagoon (Fig. 1). Menzel Abderrahmen (MA), sampling area 37°13'43 N, 9°51'46 E, is located near a town of 10 000 inhabitants, surrounded by industrial units. The sampling site receives a constant influx of non-treated wastewater. It is probable that a vast array of pollutants arrive with this waste, particularly metals (Dellali et al. 2001). Menzel Jemil (MJ), 37°13'04 N, 9°54'46 E, is situated in the aquacultural (mussels and clams) part of the lagoon, an area that is subject to eutrophication in summer (Dellali et al. 2001). Menzel Bourguiba (MB), 37°08'35 N, 9°49'07 E, is influenced by the waters from Ichkeul lake. Lastly, the site R, 37°11'33 N, 9°52'04 E, is in the middle of the lagoon and considered as being far from pollution sources.

The mean depth of Bizerta lagoon reaches 7 m with a maximum value at 15 m (Harzallah 2003). Consequently, the mean sampling depth was between 1.5 and 2 m in MA, MJ and MB, whereas it ranged between 8 m and 9 m in the center of the lagoon. None of the sampling areas ever dries up in summer.

Fifteen individuals from each site were collected monthly from December 2003 to July 2004, and another sampling was performed in October 2004. For metal analysis, samples ($n = 5$ in each case) were depurated for 48 h to eliminate gut contents, and tissues were then dissected. Digestive gland and foot were
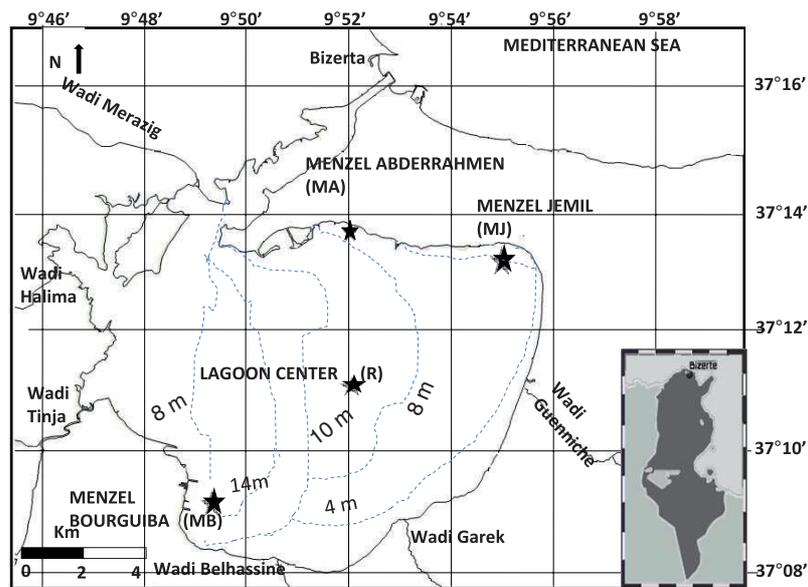
Fig. 1. Sampling locations in Bizerta lagoon (Tunisia): MA: Menzel Abderrahmen, MB: Menzel Bourguiba, MJ: Menzel Jemil and R (reference station).

isolated and dried at 50 °C to constant weight. For biomarker determinations, samples ($n = 10$) were not depurated before dissection of the digestive gland, which was immediately homogenized.

Temperature and salinity were measured simultaneously during the mollusc samplings, using a field thermometer (CG867) and a field salinometer (WTW LF 196).

2.2 Metal analyses

Dried samples (ratio wet to dry weight, approx. 5) were taken to the Laboratoire de Nice, France. Samples were prepared under a laminar flow hood and digested (duplicate digestion in each case) with Suprapur nitric acid 65% Merck in a microwave oven. Cd, Cu and Zn analyses were carried out directly on the digested solution, using an atomic absorption spectrophotometer equipped with an air-acetylene flame (Cu, Zn), or with a graphite furnace (Cd). Deuterium background correction was used when necessary. The analytical procedure was checked using a standard reference material (lobster hepatopancreas TORT-1 provided by CNR, Canada). The results with the standard (not shown) demonstrated that the metal analyses agreed well with the certified values.

2.3 Biochemical analysis

Digestive glands were homogenized in a Tris buffer (Tris 50mM, NaCl 150 mM, pH 7.4, 0.1% antiprotease cocktail, 1 mM DTT [dithiothreitol]) in a 1/5 w/v (weight/volume) ratio, using a Potter-Elvehjem glass homogenizer fitted with a teflon pestle. Homogenates were then centrifuged for 25 min at $9000 \times g$. All procedures were carried out at 0–4 °C. Aliquots of the supernatant (S9 fraction) were frozen at –80 °C until

analysis. All enzyme assays and total protein levels were determined on S9 fractions at 20 °C. Total proteins were determined according to Bradford (1976). Acetylcholinesterase activity was determined using the method of Ellman et al. (1961) adapted to a microplate reader by Galgani and Bocquené (1991). The pH conditions of enzymatic reaction (DTNB and the substrate acetylthiocholine) were chosen as described in Scaps and Borot (2000). Catalase activities were assayed as described in Claiborne (1985). The variations of absorbance at 240 nm, caused by the dismutation of hydrogen peroxide, were measured as a function of time ($\varepsilon = 40$ M^{-1}cm^{-1}). GST activities were measured by spectrophotometry at 340 nm, following conjugation of the acceptor substrate 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (Habig et al. 1974).

2.4 Statistical analysis

Statistical analyses were carried out using the software Statbox. Data were tested for homogeneity of variance and for normal distribution. Two-way ANOVAs were used in the analysis of data, revealing some statistical differences in metal content and biomarker response according to sampling site or date. Post-hoc comparisons between locations (or seasons) were made using the Scheffé's test to determine which values differed significantly. Principal component analysis was performed to discriminate the different sites using the entire dataset.

3 Results

3.1 Environmental conditions

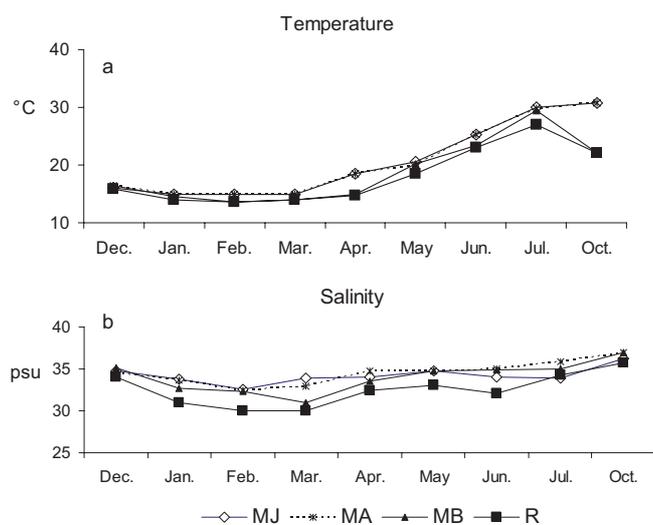*Murex trunculus* is a very common neogastropod in the Bizerta lagoon, dwelling in rocky and muddy bottoms where it

Fig. 2. Temperature (°C) and salinity (psu) at the four sites in Bizerta lagoon from December 2003 to October 2004.

finds optimal living conditions all year round (abiotic parameters and food availability). The total weight of animals (flesh + shell) tended to be higher at the R site (24.3 ± 4.9 g) compared with MB (21.9 ± 5.3 g), MJ (21.8 ± 4.0 g) and MA (19.4 ± 2.0 g). The Bizerta lagoon is a changing environment due to its low depth and restricted hydrodynamics in summer. Temperature variations are shown in Figure 2, low values are observed in December, January, February (minimum at R: 13.5 °C and MB 13.6 °C) and March, whereas higher levels are found in June, July and October (maximum at MJ and MA: 30.7 °C). MJ and MA on the one hand and MB and R on the other, present almost the same fluctuations over the year. Salinity (Fig. 2) shows low values in winter months January, February and March (minimum at R: 30.0 psu in this period), whereas higher levels are noted in May, June, July and October (maximum at MA: 37.0 psu). At R, salinity was generally lower than at the other three sites.

3.2 Distribution of metals in two tissues of *Murex trunculus*

Data were analysed together (5 individuals × 9 dates × 4 sites; $n = 180$). Two-way ANOVAs were performed with sampling site and date as factors, which had significant effects in all cases except for the variation of copper as a function of sampling site in the foot of *Murex trunculus* and levels of zinc in both tissues. Significance of post-hoc comparisons are shown in Table 1.

Variations among sampling sites are presented for foot and digestive gland, respectively (Fig. 3). In foot, the lowest cadmium concentration was recorded at R (0.25 ± 0.03 µg Cd g^{-1}) compared with the other sites. For copper and zinc, variations were not significant in this tissue (mean values of 56.3 ± 2.2 µg Cu g^{-1} and 451 ± 28 µg Zn g^{-1}). In digestive gland, the lowest cadmium concentration was found at R (9.35 ± 0.78 µg Cd g^{-1}) and the highest at MJ (15.91 ± 1.61 µg Cd g^{-1}); the lowest copper concentration was recorded at R (84.5 ± 15.3 µg Cu g^{-1}).
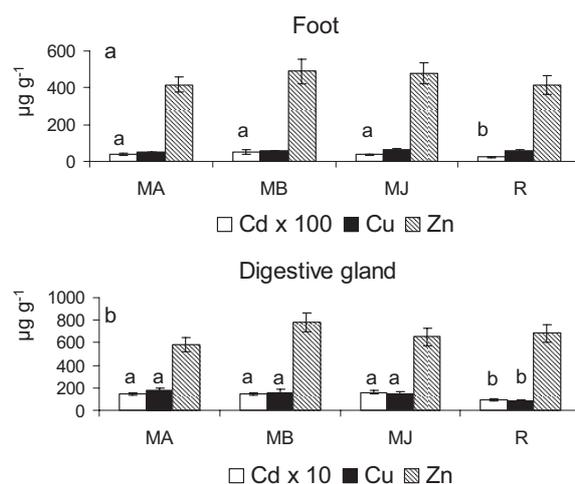

Fig. 3. Trace-metal concentrations (Cd, Cu and Zn) in the foot and digestive gland of *Murex trunculus* collected at four stations (MA, MB, MJ and R) in Bizerta lagoon. Concentrations (in µg g^{-1} dry weight); for cadmium, concentrations are multiplied by 100 (foot) and 10 (digestive gland). For each metal, bars with the same superscript are not significantly different ($p > 0.05$, post-hoc comparison after significant ANOVA).

A mean zinc concentration of 682 ± 38 µg Zn g^{-1} was found in the digestive gland.

Variations in metals (in foot and digestive gland) are presented as a function of sampling date (Table 1). In foot, the lowest concentrations of cadmium and zinc were noted in June and the highest in October, whereas the lowest Cu concentrations were found in February and the highest in March. In digestive gland, cadmium concentrations ranged from a maximum in May to a minimum in June. The lowest copper concentration in digestive gland was recorded in April whereas the highest was found in December. Zinc concentrations decrease from March to July with a peak in May, the highest value being recorded in October.

3.3 Biochemical responses

Data were grouped together ($n = 10$ individuals × 9 dates × 4 sites; $n = 360$). Two-way ANOVAs were performed with sampling site and date as factors; which were highly significant ($p < 0.0001$). Significance of post-hoc comparisons are shown (Fig. 4 and Table 2).

Figure 4 shows the variations of biomarkers among sampling sites in the digestive gland of *Murex trunculus*. The highest AChE activity (in nmol min^{-1}mg^{-1}protein) was found at R (15.4 ± 0.3) followed by those at MA and MJ (not significantly different between these 2 sites : 9.0 ± 0.5, and 9.4 ± 0.4, respectively), and finally those at MB (5.2 ± 0.4). CAT activity (in µmol min^{-1}mg^{-1} protein) was higher at MA and MJ (24.9 ± 2.7 and 23.6 ± 2.0, respectively) compared with MB and R sites (17.9 ± 1.4 and 8.7 ± 0.5, respectively). GST activity (in nmol min^{-1}mg^{-1} protein) was significantly higher at MJ (21.7 ± 0.6) than at MA and MB (14.8 ± 0.7 and 12.9 ± 0.7, respectively) and at R (12.2 ± 0.8).

Table 1. Mean values ± 1 SD of metal concentrations in the foot and digestive gland of *Murex trunculus* collected from the Bizerta lagoon on the nine sampling dates from December 2003 to October 2004. Concentrations with the same superscripts are not significantly different ($p > 0.05$, Scheffé's test post-hoc comparison after significant ANOVA).

| Sampling dates	Cadmium (μg g^{-1} dry weight)		Copper (μg g^{-1} dry weight)		Zinc (μg g^{-1} dry weight)	
	Foot	Digestive gland	Foot	Digestive gland	Foot	Digestive gland
December	0.27 ± 0.03 [abc]	11.41 ± 1.46 [c]	54.4± 6.0 [a]	241.4 ± 52.3 [a]	622 ± 89[ab]	663 ± 58[cd]
January	0.48 ± 0.07[cd]	11.62 ± 1.62[c]	54.9 ± 6.0[ab]	235.5 ± 40.6 [a]	506 ± 63[abc]	936 ± 124[b]
February	0.32 ± 0.04 [bc]	12.63 ± 2.28[c]	41.6 ± 3.5[b]	124.3 ± 30.1 [b]	672 ± 88[a]	714 ± 108[bc]
March	0.69 ± 0.10 [de]	18.49 ± 2.54[ab]	64.7 ± 7.4 [a]	153.1 ± 39.5 [ab]	465 ± 113[bc]	440 ± 40[de]
April	0.29 ± 0.04 [abc]	11.45 ± 1.70[c]	54.4 ± 6.0 [ab]	57.0 ± 16.0 [b]	333 ± 42[cd]	319 ± 33[e]
May	0.19 ± 0.03[ab]	19.84 ± 2.21[a]	45. 8 ± 5.0 [b]	147.3 ± 21.7 [ab]	198 ± 21[d]	702 ± 97[bc]
June	0.08 ± 0.01[a]	10.65 ± 1.18[c]	64.5 ± 6.4 [a]	102.0 ± 30.8 [b]	180 ± 28[d]	379 ± 38[e]
July	0.43 ± 0.07 [bcd]	12.49 ± 2.58[bc]	59.6 ± 7.7 [ab]	159.4 ± 63.5 [ab]	309 ± 59[cd]	568 ± 121[cde]
October	0.80 ± 0.17[e]	15.49 ± 1.41[abc]	55.1 ± 6.5 [ab]	61.6 ± 16.5[b]	677 ± 85[a]	1232 ± 102[a]
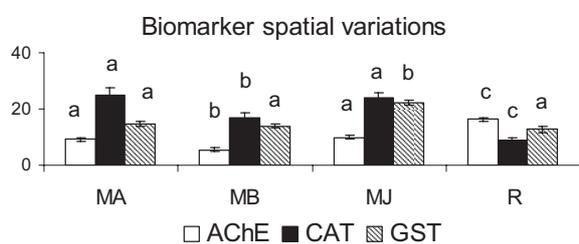
Fig. 4. Biomarkers: AChE and GST activities (nmol min^{-1}mg^{-1} protein) and CAT activities (μmol min^{-1}mg^{-1} protein) in the digestive gland of *Murex trunculus* as a function of sampling site (MA, MB, MJ and R) in Bizerta lagoon.

Table 2. Mean values ± 1 SD of biomarker AChE and GST activities (in nmol min^{-1} mg^{-1} protein) and CAT activities (in μmol min^{-1} mg^{-1} protein) in the digestive gland of *Murex trunculus* collected from the Bizerta lagoon according to the nine sampling dates from December 2003 to October 2004. Activities with the same superscripts are not significantly different ($p > 0.05$ Scheffé's test post-hoc comparison after significant ANOVA).

Sampling dates	AChE Acetyl-cholinesterase (nmol min^{-1} mg^{-1} protein)	CAT Catalase (μmol min^{-1} mg^{-1} protein)	GST Glutahione transferase (nmol min^{-1} mg^{-1} protein)
December	13.2 ± 1.2 [a]	11.1 ± 0.5 [a]	29.1 ± 1.0 [a]
January	7.3 ± 0.9 [b]	10.5 ± 0.7 [a]	15.7 ± 0.9 [b]
February	7.3 ± 0.9 [b]	11.1 ± 0.8 [a]	11.7 ± 0.9 [c]
March	8.5 ± 0.8 [b]	22.4 ± 1.9 [b]	16.7 ± 1.0 [b]
April	9.3 ± 0.7 [ab]	34.9 ± 2.2 [c]	12.9 ± 0.9 [bc]
May	11.4 ± 0.8 [ab]	34.0 ± 3.9 [c]	15.9 ± 0.7 [b]
June	9.4 ± 0.8 [ab]	4.7 ± 0.4 [d]	11.8 ± 1.1 [c]
July	7.6 ± 0.5 [b]	6.3 ± 0.5 [d]	10.7 ± 0.8[c]
October	13.5 ± 0.9 [a]	24.5 ± 2.0 [b]	14.0 ± 0.7 [b]

Seasonal variations in the biomarkers are presented in Table 2. Low AChE activity was observed in January, February and March compared with that in October. Seasonal variations of CAT activity were particularly significant, with very low values in June and July compared with those of April and
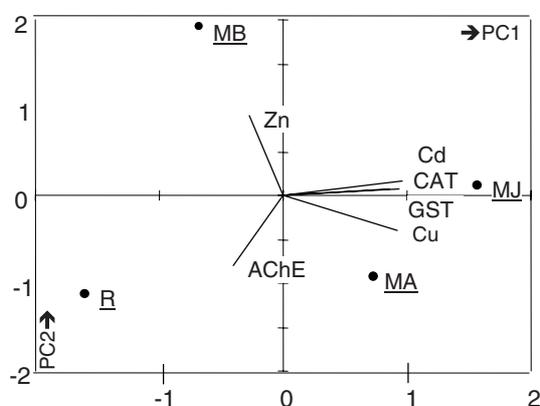

Fig. 5. Principal component analysis (= 180) including all calculated correlation coefficients (AChE, CAT and GST activities merged together; Cu, Cd and Zn concentrations) in *Murex trunculus* at the four stations (MA, MB, MJ and R) in Bizerta lagoon, their statistical weight in the PCA is underlined.

May. GST activities were low in June and July and elevated in December.

Principal component analysis (PCA) was performed with individual sampling sites, grouping the dates. Six variables were taken into consideration: Cd, Cu and Zn concentrations and AChE, CAT and GST activities measured in the digestive gland. In the original correlation matrix of all parameters, cadmium concentrations were correlated with those of copper and zinc ($r = 0.37$ and $r = 0.27$, respectively, $p < 0.01$). Furthermore, CAT activity was correlated with cadmium concentration ($r = 0.32$, $p < 0.01$). The results of the PCA are presented in Figure 5 as the correlations of variables with the principal component axes (PC1 and PC2). Sites MA, MB, MJ and R are placed according to their "statistical" weight. The first two axes represent 60% and 28% of the total variance, i.e. 88% cumulated variance. Copper and cadmium together with CAT and GST activities (correlation coefficients $r = 0.92$, 0.96, 0.94, 0.85, $n = 180$, $p < 0.01$, respectively) correlate with the first axis PC1. The second axis represents zinc in the positive part ($r = 0.91$) and AChE in the negative part ($r = -0.79$).

4 Discussion

Metal concentrations were always found to be higher in the digestive gland than in the foot of *Murex trunculus*. Copper is accumulated to a level in the digestive gland that was 4-fold the level found in the foot, and cadmium accumulated from about 50 to 100-fold more. Zinc was slightly higher in the digestive gland than in the foot. The digestive gland constitutes the accumulation and detoxification site of metals in gastropods (Bebianno and Langston 1998).

Seasonal variations in the foot and digestive gland of *Murex trunculus* show that zinc and cadmium concentrations tended to decrease from March to July in both tissues with a peak in May in the digestive gland (Table 1). These variations could be linked to the gastropod reproductive cycle. This cycle was investigated in the Muricidae (purple dye Murex) *Bolinus brandaris* collected from the Western Mediterranean by Ramón and Amor (2002), and in *M. trunculus* under laboratory conditions (Vasconcelos et al. 2004); the authors reported two reproductive peaks (April and June-July), with the first stage of gametogenesis beginning in November. Maximum ripening and spawning are attained at the end of June and during July. The high zinc concentrations in *M. trunculus* from the Bizerta lagoon may correspond to gametogenesis, and lower concentrations to spawning (April and June-July) since this metal is incorporated in the spawn (Julshamn and Braekkan 1976; Roméo 1987). Bouquegneau et al. (1988) reported that cadmium concentrations were very high in the digestive gland. The variations they observed had no link with the reproductive cycle, and a notable drop in cadmium concentrations coincided with the construction of a growth ring; cadmium may therefore play a role in controlling the shell formation. Dallinger et al. (1989) found that in response to elevated cadmium concentrations in the environment, *M. trunculus* accumulates high levels of cadmium and metallothionein-like proteins. Unfortunately we could not determine metallothioneins in the present work. Copper, which did not show significant variation as a function of season in the present work, may be stored in neogastropods (*Murex Bolinus brandaris*) as copper sulphide (Bouquegneau and Martoja 1982).

High Cd and Cu concentrations in *Murex trunculus* from sites along the shore of Bizerta lagoon may be due to higher anthropogenic influence in these areas compared with the center of the lagoon (site R).

Many authors have examined variations of biomarker enzyme activities in marine invertebrate organisms as a function of water temperature and salinity (Damiens et al. 2004; Pfeifer et al. 2005; Cailleaud et al. 2007). In clams, *Ruditapes decussatus* from the Bizerta lagoon, Dellali et al. (2001, 2004) reported a negative relationship between temperature and AChE activity. In oyster larvae, a decrease of AChE activity was reported as salinity increased (Damiens et al. 2004). Bocquené et al. (1997) emphasized that AChE activity is not directly linked to age, sex or reproductive period, and that among significant environmental parameters, attention must be paid to the temperature of the medium as well as to the temperature of measurement (tested in the present study). The variations in AChE activity that we observed with water temperature and salinity cannot easily be explained (Table 2), as this was low either at low temperature and salinity

(January, February, March) or at higher temperature and salinity (October). The weight of animals may have an influence on AChE activity measured in *Murex trunculus* since AChE was shown to be negatively correlated with the weight of individuals (Burgeot et al. 1996). This could partly explain the high AChE activity found in R, where *M. trunculus* presented the highest weight. In clams *R. decussatus* and mussels *Mytilus galloprovincialis* from the Bizerta lagoon, Dellali et al. (2001, 2004) reported that catalase activity is low from September to February, increases in March, April and May, and is particularly high during the summer months (June to September); the augmentation was attributed to microbial stress, particularly the considerable elevation in faecal flora and the presence of Vibrionacea. In the present work, the high antioxidant enzyme activity of catalase observed in April-May in *M. trunculus* may be due to microbial pollution. However, in summer, when water temperature in the Bizerta lagoon reaches very high levels (~30 °C), oxidative stress caused by microbes may be too high in *M. trunculus* and lead to CAT inhibition in June-July. Few data exist on the influence of salinity on CAT activity in invertebrate organisms. Low GST activity was noted in *M. trunculus* from January to July compared with October and December in the present study (Table 2). Power and Sheehan (1996) reported that gill GST activity in the mussel *Mytilus edulis* peaks in the winter months probably due to anaerobic metabolism during this period. Bebianno et al. (2007) found higher GST activity in gills of Mediterranean mussels at low salinity. The high GST activity found in our samples collected in December may due to copper taken up by *M. trunculus* rather than salinity or temperature. High GST activity in mussels associated with high copper concentrations in tissues was reported as resulting from copper effect on glutathione (Canesi et al. 1999).

Differences in biomarker responses are more significant among sampling sites than among seasons. AChE is generally inhibited by organophosphorous and carbamate insecticide compounds (Galgani and Bocquené 1991). The inhibition of AChE activity by heavy metals, detergents and algal toxins (Lehtonen et al. 2003) has also been acknowledged. AChE activity in *Murex trunculus* may be decreased by the presence of heavy metals along the shores of Bizerta lagoon. CAT and GST activities show higher values along the shore of the lagoon than in its center. Catalase is an anti-oxidant enzyme and GST activity may also take part in anti-oxidant defense (Cossu et al. 1997). Copper is known to stimulate the peroxidation of membrane lipids and stimulate catalase induction, and in some cases GST induction as mentioned above. Although cadmium is a non-redox metal, it is known to enhance the intracellular formation of reactive oxygen species (ROS) and to promote cellular oxidative stress (Stohs et al. 2000).

Principal component analysis demonstrated that the neogastropods from MJ and to a lesser extent MA have elevated copper and cadmium concentrations and high biomarker responses (CAT and GST). Animals from MB presented different characteristics with a high zinc concentration and low AChE activity. *Murex trunculus* from R, which we considered as our reference site, presented relatively low metal concentrations and low biomarker responses (low CAT and GST and high AChE). Biotic (weight of animals) and abiotic parameters

(temperature and salinity) were slightly different at this site, compared with the others .

The present work shows the possibility of using metal concentrations (cadmium and zinc) and biomarker levels in the Neogastropod *Murex trunculus* to biomonitor the health status of the semi-enclosed area constituting the Bizerta lagoon. Metal concentrations and biomarker levels in *Murex trunculus*, collected in four sites over one year, vary as a function of season and sampling site with high responses noted particularly in MJ (Menzel Jemil) where bivalves are grown in aquaculture. This means that biomonitoring, through pollutant concentration determination and biomarker measurements, should be performed regularly in this area.

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