Biomonitoring of environmental pollution on the Algerian west coast using caged mussels *Mytilus galloprovincialis**

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Zoheïr M. Taleb[1,*]
Imène Benali[1]
Hamida Gherras[1]
Amina Ykhlef-Allal[1]
Benabdellah Bachir-Bouiadjra[2]
Jean-Claude Amiard[3]
Zitouni Boutiba[1]

[1] Réseau de Surveillance Environnementale (RSE),
Department of Biology,
University of Oran Es Senia,
31000 Oran, Algeria;

e-mail: mztaleb@yahoo.fr

*corresponding author

[2] Department of Ressources Halieutiques et Sciences de la Mer,
Abdelhamid Ibn Badis University of Mostaganem,
27000 Mostaganem, Algeria

[3] Service d'Ecotoxicologie, MMS (Mer, Molécules, Santé), Pôle Mer et Littoral,
University of Nantes,
44322 Nantes Cedex 3, France

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Abstract

An active biomonitoring study was carried out on the Algerian west coast using wild reference mussels (*Mytilus galloprovincialis*) sampled from the Kristel (K) site and transplanted in net cages during one month (between May and June 2007) to Oran Harbour (OH) and Mostaganem Harbour (MH), areas characterised by high levels of urban and industrial pollution. The biological response of the mussels was evaluated by their condition index and the use of a general stress biomarker (evaluation of lysosomal membrane stability: the neutral red retention time (NRRT) method), a genotoxic effects biomarker (determination of micronuclei (MN) frequency) and a neurotoxic effects biomarker (determination of the acetylcholinesterase (AChE) concentration).

Compared to the K reference specimens, OH and MH caged mussels presented a significant decrease of NRRT in lysosomal haemocytes (56.45 ± 26.48 min and 67.25 ± 22.77 min, respectively) (78 ± 16.97 min for K mussels), an MN frequency respectively 7.3 and 9 times higher in the haemocytes and the gill cells of the OH caged mussels, and 7.2 and 6.4 times higher in the two tissues of the MH caged mussels. Significant inhibition of AChE activity was noted in the gills (16.93 ± 3.1 nmol min^{-1} mg prot^{-1}) and the digestive gland (7.69 ± 1.79 nmol min^{-1} mg prot^{-1}) of the OH mussels, but only in the gills (23.21 ± 5.94 nmol min^{-1} mg prot^{-1}) of the MH mussels, compared to the organs of the K control specimens (35.9 ± 6.4 nmol min^{-1} mg prot^{-1} in the gills and 11.17 ± 0.49 nmol min^{-1} mg prot^{-1} in the digestive gland).

This study reflects the interest in such in situ biomonitoring assays and the utility of these biomarkers for assessing the effects of pollution in the Algerian coastal marine environment.

1. Introduction

Preserving the quality of coastal areas has become an international priority. Public sensitivity, along with the concerns of marine professionals and maritime authorities have persuaded many countries to set up biomonitoring networks covering the whole of their territory. These are intended to assess the quality of the marine environment and thereafter to help work out an environmental policy based on concrete measures and reliable statistics (UNEP 1997, Viarengo et al. 2000a,b, OSPAR 2004, ICES 2007, UNEP/MAP/MED POL 2007, Viarengo et al. 2008).

Water quality is a key parameter in the health of the marine environment. Traditionally, water quality monitoring would be undertaken at the chemical level: excessive amounts (above established thresholds) of single chemicals or mixtures of toxic pollutants would be sought but no information given about the biological responses of organisms. In the last three decades, however, recommended biomarkers in aquatic organisms have become a major tool for environmental quality evaluation and risk assessment (Viarengo et al. 1990, Depledge 1994, Lowe et al. 1995, Livingstone

et al. 2000, Lionetto et al. 2003, Regoli et al. 2004, Amiard et al. 2006). The term 'biomarker' has been adopted in the context of environmental monitoring and in the process has expanded widely, beyond its original constraints, to encompass almost any response that may be indicative of a biological effect. Biomarkers are defined in a recent Natural England report as biochemical, cellular, physiological or behavioural variations in the tissue or body fluids or at the level of the whole organism that provide evidence of exposure to chemical pollutants and may also indicate a toxic effect (Long et al. 2004).

In the Algerian western coastal zone, domestic and industrial untreated wastewater effluents represent a major source of chemical contamination for the aquatic environment (Sogreah Ingenierie 1998, Boutiba et al. 2003, Taleb 2007). This change in water composition is bound to have deleterious effects – cellular, metabolic and DNA changes – not only on the organisms inhabiting these areas, but also on human health (Boutiba et al. 2003, Taleb et al. 2007a,b).

In accordance with the second phase of the first regional marine pollution biomonitoring project, developed by our laboratory on the Algerian west coast (Taleb et al. 2007a,b) to predict the general environmental impact of the pollutants contained in municipal effluent discharges, a set of recommended biomarkers in marine mussels was investigated (UNEP 1997, UNEP/RAMOGE 1999, Bocquené & Galgani 2004, ICES 2004). The study was based on the determination of lysosomal membrane stability using the neutral red retention time (NRRT) method (general stress), the evaluation of micronucleus (MN) frequency (genotoxic effects), and the determination of acetylcholinesterase (AChE) activity (presence of organophosphorus compounds, carbamates and some heavy metals) in mussels *Mytilus galloprovincialis*.

Two studies have shown that lysosomal changes could represent nearly 80% of the variations of both integrated cellular and physiological responses in the stressed state (Allen & Moore 2004, Moore et al. 2004); such changes would therefore be ideal as a prognostic reference measurement for general mussel health. The MN assay is one of the most promising techniques for identifying genetic alterations in environmental animals and an alternative estimate of exposure to genotoxins (Burgeot et al. 1996, Bolognesi et al. 2004, Taleb et al. 2007a,b, Villela et al. 2007, Koukouzika & Dimitriadis 2008, Siu et al. 2008). AChE (AChE EC 3.1.1.7) activity was also taken into consideration because its inhibition has been used in some coastal biomonitoring programmes as a biomarker of organophosphate and carbamate pesticide exposure in bivalves (Galgani & Bocquené 1989, Radenac et al. 1998, Binelli et al. 2006).

The Molluscs, in particular the *Mytilus* bivalves, are commonly used as bio-integrators of marine pollution as they have the ability to accumulate and concentrate such pollutants as heavy metals and hydrocarbons (Besada et al. 2002, Mourgaud et al. 2002, Boutiba et al. 2003, Roméo et al. 2005). However, any assessment of the chemical contamination levels as well as the biological effects on the Algerian west coast is confined to the availability of wild mussels in the polluted monitoring areas (Taleb 2007). The caged bivalve approach is an alternative method for monitoring environmental effects as it has certain advantages, including a known exposure period, the selection of monitoring stations that are independent of the presence of natural populations and their distance from the coast, measurements optimised by the use of homogeneous samples (size and age) in comparison to the indigenous population, and the influence of adaptive phenomena (Mourgaud et al. 2002, Roméo et al. 2003, Bolognesi et al. 2004, Regoli et al. 2004, Nigro et al. 2006).

The aim of the current paper was to evaluate the effects of exposure to wastewater discharge in Oran and Mostaganem harbours by measuring a battery of biomarkers in mussels transplanted to these impacted areas (hereafter referred to as OH mussels, MH mussels) and comparing their values with those in mussels from the Kristel reference site (controls). The results of the present study are expected to provide a useful database for the assessment of marine pollution and the application of active biomonitoring on the Algerian west coast. This is especially important in view of the substantial commercial and recreational fishing interests based in the area and the development of the tourist sector there.

2. Material and methods

2.1. Sampling area and experimental design

Native mussels (approximately 100 individuals; 5 ± 0.5 cm shell length) from the Kristel (K) reference site sampled on 21 May 2007 were placed in cages (33.5×53.5 cm) of polyethylene netting and transplanted to 5–10 m water depth in Oran Harbour (OH) and Mostaganem Harbour (MH) (Figure 1). These coastal areas suffer from high levels of urban and industrial pollution, since they are continually exposed to discharges of untreated wastewater and consequently to contamination by heavy metals, polyaromatic hydrocarbons and organochlorine compounds (Taleb 1997, Taleb et al. 1997, Sogreah Ingenierie 1998, Taleb & Boutiba 1999, Boutiba et al. 2003, Kies 2005, Bouragba-Benazza et al. 2006).

After a field exposure at the study stations of one month's duration, the cages were collected; dead animals were counted and discarded.

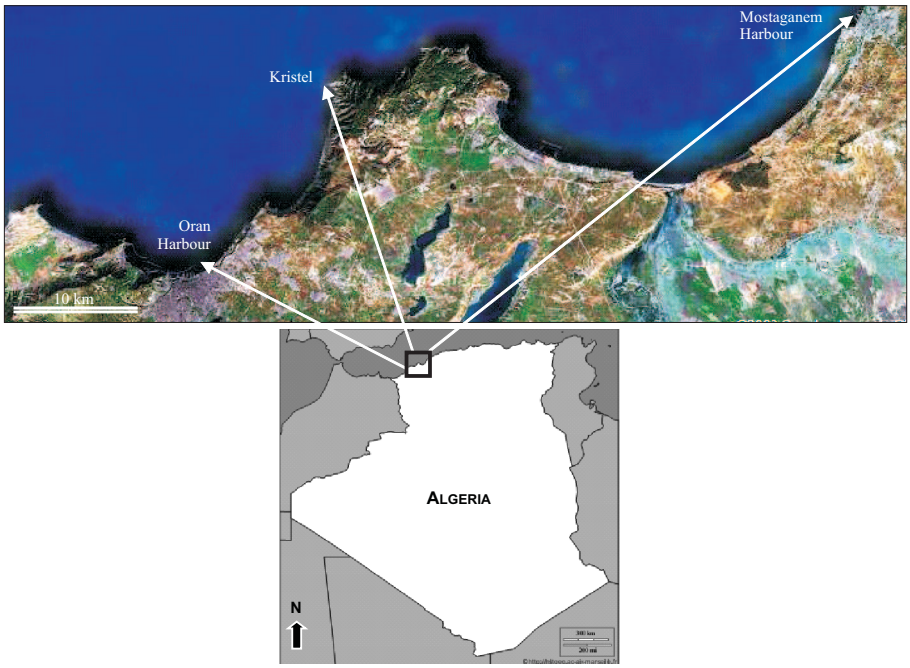

Figure 1. Map showing the mussel sampling sites (adapted from Google maps, 2008)

Table 1. Sampling site characteristics: Kristel (K), Oran Harbour (OH) and Mostaganem Harbour (MH)

Site	Area – Position	Sampling date*	Temp. [°C]	Salinity [PSU]	Dissolved O_2 [mg dm^{-3}]	pH	Turbidity [NTU]
K	Bay of Oran 35°52′32.44′′N 0°29′22.76′′W	21.05.2007	22.9	39	8.10	8.23	1.04
OH	Bay of Oran 35°43′02.99′′N 0°37′55.85′′W	19.06.2007	24.5	38.5	6.70	7.99	1.40
MH	Gulf of Arzew 35°55′59.48′′N 0°04′21.10′′E	19.06.2007	25.5	39	5.90	8.06	2.08

*Physical parameter data were obtained only for these sampling dates.

The surviving mussels were transported in cold, aerated water collected from the sampling sites to the laboratory, where they were maintained in flow-through raceway systems of seawater at ambient temperature and

salinity for at least 2 days prior to experimental use. Depuration of mussels facilitates the removal of any residual sediment in the soft tissues or body cavity.

Some physico-chemical parameters were measured in situ at all the sampling sites: dissolved oxygen and temperature (33ri/set WTW oximeter), salinity (20/21/201bp handheld refractometer), turbidity (Turb 35OIR) and pH (288820 Hanna pHmeter). The sampling stations were geo-referenced with a Garmin GPS 12 Navigator (Table 1).

2.2. Body condition index

The condition index (CI) was measured on ten animals from each cage, using the ratio of the weight of soft tissue to the total weight (shell + soft tissues + palleal liquid) of the mussel, multiplied by 100 (Amiard et al. 1998).

2.3. Biomarker analyses

2.3.1. Evaluation of NRRT in haemolymph

The analytical method was performed according to Lowe et al. (1992, 1995) and the procedure proposed by UNEP/RAMOGE (1999), with slight modifications. Haemolymph was withdrawn from the posterior adductor muscle of ten mussels in physiological saline so as to obtain a 1:1 v/v suspension of cell/physiological saline. The suspension obtained from each mussel was spread on Poly-L-Lysine (1/10) prepared slides and transferred to a lightproof humidity chamber for 15 min to allow the cells to attach. After incubation, 40 μl of neutral red (NR) working solution were dropped onto each slide (20 mg of NR powder were dissolved in 1 ml of di-methyl-sulphoxide and 5 μl of the stock solution were transferred in 995 μl of physiological saline). After 15 min, the slides were quickly examined at 400× magnification and the images digitalised using computer-enhanced automatic image analysis. The system included a charged coupled device (CCD) Sony colour camera mounted on a Zeiss light microscope. Image software (Pinnacle Studio, v. 8) electronically captured the microscopic images displayed on a television screen (Sony Trinitron) and stored them on a personal computer. Where there was evidence of dye loss from the lysosomes to the cytosol in at least 50% of the cells examined (granular haemocytes), the time following the NR probe application represented the NRRT for the mussel.

2.3.2. MN in haemolymph and gill tissue

The micronuclei frequency was determined according to the procedure proposed by UNEP/RAMOGE (1999). Haemolymph was withdrawn from the posterior adductor muscle of five mussels in physiological saline so as to obtain a 1:1 v/v suspension of cell/physiological saline. Suspensions were spread on slides, transferred to a lightproof humidity chamber, and allowed to attach. Cells were then fixed in methanol:acetic acid (3:1), stained with 3% Giemsa and mounted in Eukitt. Gill cells were isolated by enzymatic digestion with a solution of Dispase I (Neutral protease, Boehringer Mannheim, Germany). The cellular suspension obtained by filtration was centrifuged, and aliquots of the resuspended pellet were fixed in methanol:acetic acid (3:1) overnight, spread on slides, stained with 3% Giemsa and mounted in Eukitt. The stained slides were analysed under the same Zeiss light microscope at a final magnification of 1000× under oil immersion. The scoring of slides involved examining more than 1000 agranular haemocytes and epithelial-like gill cells. The criteria used for identifying micronuclei are given in UNEP/RAMOGE (1999).

2.3.3. Determination of AChE activity

Gills, digestive gland and tissue homogenates of five mussels were ground in Tris buffer (0.1 M, pH 7.5) and centrifuged at 9000 g for 20 min at 4°C. Aliquots of the supernatant (S9 fraction) were frozen at −80°C until analysis. The S9 containing the cytosolic proteins was removed and used to determine AChE activity. Protein concentrations were determined according to the Bradford (1976) method using bovine serum albumin (BSA) as standard. AChE activity was determined using the Ellman et al. (1961) method. Acetylthiocholine was hydrolysed by AChE, producing thiocholine and acetic acid. The released thiocholine reacts with 5,5'-dithio-bis-2-nitrobenzoate (DTNB) to produce 5-thio-2-nitrobenzoate (TNB), a yellow compound which absorbs at 412 nm. For this purpose, 50 μl of the stock solution containing AChE fractions (S9) was added to a reaction mixture containing 850 μl Tris 100 mM pH 7.5 and 50 μl of 1.875 mM DTNB (Sigma-Aldrich). After pre-incubation, the reaction was started by the addition of 50 μl of 8.25 mM acetylthiocholine (Sigma-Aldrich). AChE activity was determined by kinetic measurement for 30 min at 20°C using an Anthelie Advanced Junior n°285 spectrophotometer. Results were expressed as nmoles thiocholine produced per min and per mg protein.

2.4. Statistical analysis

The data are presented as the mean ± standard error of the mean. The significant variations of the CI and the biomarkers recorded at each site were tested by one-way ANOVA. The data were tested for normality and homogeneity of variance using the Kolmogorov-Smirnoff and Levene's tests, respectively, prior to applying post-hoc comparisons. For homogeneous data, post-hoc comparisons were made using the HSD Tukey test (CI and AChE) and the Newman-Keuls test (NRRT). For MN data exhibiting heterogeneity of variance, the non-parametric Mann-Whitney U-test was performed.

Statistical analyses were carried out using Microsoft STATISTICA (v. 6.0) statistical software and the significance level for all statistical tests was set at $p < 0.05$.

3. Results

3.1. Physical parameters

Comparison of the various physical parameters measured at the three sampling sites of *Mytilus galloprovincialis* (Table 1) showed the salinity and pH values to be homogeneous. However, the MH translocation site had the lowest dissolved oxygen concentration but the highest turbidity of the three sites. Likewise, the dissolved oxygen concentration at the OH transplantation site compared to that at the K reference station was indicative of possible organic pollution. Nevertheless, the sea temperature gradients at the three stations were normal for the sampling period, with a slight increase at the two harbour sites of Oran and Mostaganem.

3.2. Physiological status

The mortality of mussels estimated in the two nets after the transplantation period in OH and MH was relatively low (Table 2). However, the CI of the OH caged mussels was significantly ($p < 0.05$) higher than that of the MH mussel population (Table 2).

3.3. Lysosomal alterations

The digitalised microscopic images of the various preparations of haemolymph samples of the two transplanted mussel populations and those of the reference site revealed dye leakage from the lysosomes to the cytosol in

Table 2. Physiological status of Kristel (K) wild mussels and Oran Harbour (OH) and Mostaganem Harbour (MH) caged mussels

Site	Mortality (%)	Condition index (CI) (mean ± SD, n = 10)
K	–	27.79 ± 3.67^{ab}
OH	2.5	30.74 ± 3.44^{a}
MH	8.57	25.17 ± 2.98^{b}

*No significant difference was found between CI values with the same superscript (Tukey test – $p > 0.05$).

more than 50% of the caged mussel haemocytes (48 ± 28.85 min in OH and 56.25 ± 25.62 min in MH), whereas this lysosomal membrane destabilisation of haemocytes was obvious starting from 75 ± 21.21 min in 50% of the K reference mussels.

The K mussels translocated to OH and MH exhibited a general deterioration of the lysosomal system. A significant ($p < 0.05$) decrease in NRRT was noted in the lysosomal haemocytes of the OH and MH caged mussels (56.45 ± 26.48 min and 67.25 ± 22.77 min, respectively) compared to the controls from Kristel (78 ± 16.97 min) (Figure 2).

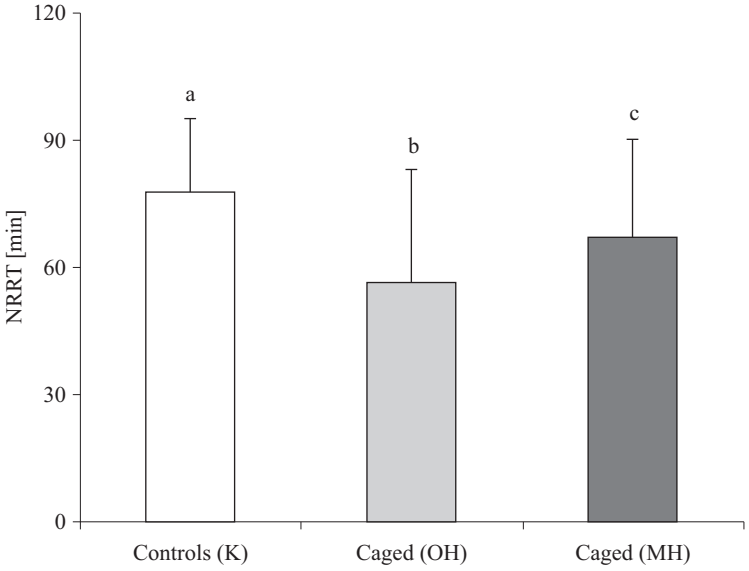

Figure 2. Neutral red retention times [min] in haemocytes of *Mytilus galloprovincialis* from Kristel (K), and transplanted to Oran Harbour (OH) and Mostaganem Harbour (MH). The different letters indicate significant differences (Newman-Keuls test) between means (n = 10): p (a/b) < 0.01; p (a/c) < 0.05

3.4. Genotoxicity

The MN frequencies noted in the haemolymph of the OH and MH mussels varied from 5.5 to 9.44‰ and from 5.11 to 9.89‰, respectively. These values are significantly ($p < 0.05$) higher than those recorded in the haemolymph of the K control mussels, which varied from 1.6 to 2.47‰ (Figure 3). The same applies to the gill cells, where the Mann-Whitney U-test indicated a significant increase ($p < 0.01$) in MN frequency, which varied from 5.67 to 11.78‰ in the OH mussels and from 4.46 to 6.27‰ in the MH mussels, whereas haemolymph MN induction was between 0 and 1.18‰ in the K reference mussels (Figure 3). However, there was no significant difference between the *M. galloprovincialis* populations transplanted in the two polluted harbour sites (Figure 3).

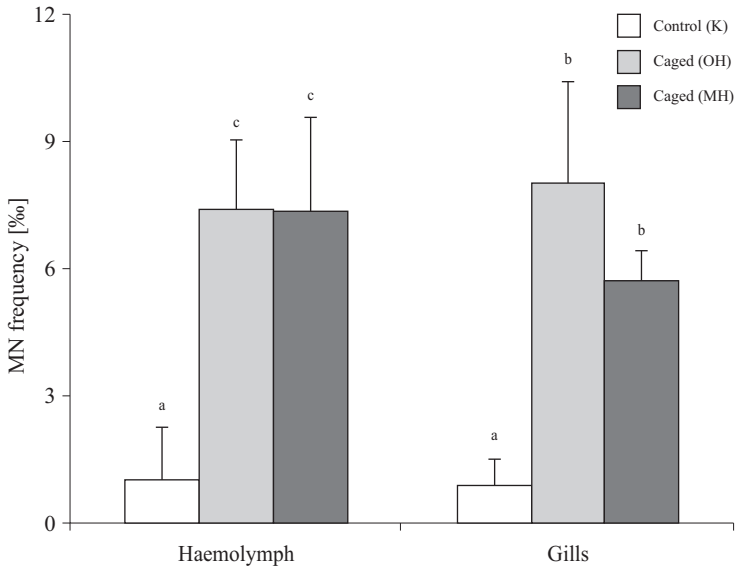

Figure 3. MN frequency [‰] in the gills and the haemolymph of mussels from Kristel (K), and transplanted to Oran Harbour (OH) and Mostaganem Harbour (MH). The different letters indicate significant differences (Mann-Whitney test) between means ($n = 5$): $p\,(a/b) < 0.01$; $p\,(a/c) < 0.05$

In comparison with the K controls, the OH mussels exhibited an MN frequency 7.3 and 9 times higher in the haemocytes and the gill cells, respectively. Similarly, in the MH mussels, the MN frequency was 7.2 and 6.4 times higher in the respective tissues than in the K mussels.

3.5. AChE activity

Analysis of AChE in the gills, digestive gland and homogenate of the K reference mussels and in the corresponding organs/preparations of the OH and MH mussels revealed the highest AChE concentration in the gills of the native K mussels (43.61 nmol min^{-1} mg $prot^{-1}$), and the lowest concentration in the digestive gland of the same population after its transplantation to the OH site (5.52 nmol min^{-1} mg $prot^{-1}$). AChE activity varied from 9.89 to 43.61 nmol min^{-1} mg $prot^{-1}$ in the gills, from 5.69 to 11.55 nmol min^{-1} mg $prot^{-1}$ in the digestive gland, and from 5.53 to 15.62 nmol min^{-1} mg $prot^{-1}$ in the homogenate. In comparison with the K controls, AChE activity was significantly inhibited in both gills and digestive glands of the OH mussels (Tukey test – $p < 0.05$) (Figure 4) but only in the gills of the MH mussels (Tukey – $p < 0.01$).

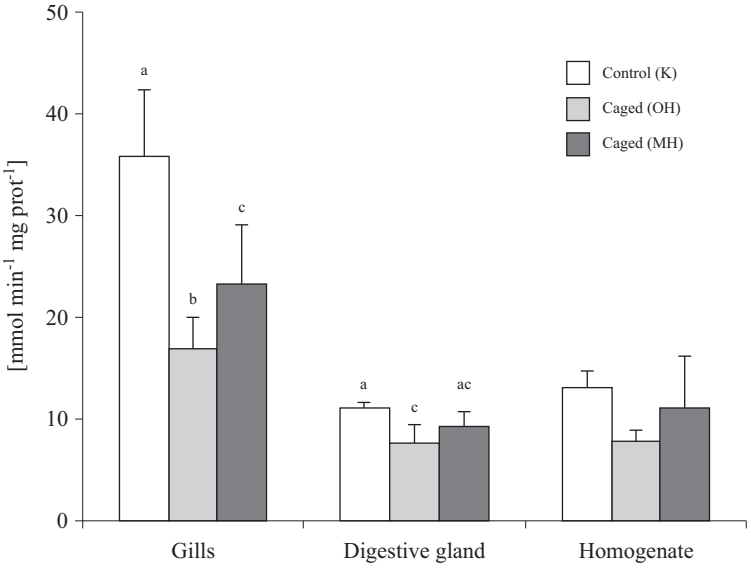

Figure 4. AChE activity [nmol min^{-1} mg $prot^{-1}$] in *Mytilus galloprovincialis* tissues from Kristel (K), and transplanted to Oran Harbour (OH) and Mostaganem Harbour (MH). The different letters indicate significant differences (Tukey test) between means ($n = 5$): $p\,(a/b) < 0.01$; $p\,(a/c) < 0.05$

4. Discussion

4.1. Physical parameters and physiological status

The low mortality of mussels at the two harbour sites to which they were transplanted (Table 2) provides further confirmation of the eco-

biological status of these bivalves and of their usefulness in marine pollution biomonitoring programmes due to their resistance to stressors in impacted areas. Nevertheless, the mortality of some specimens, especially in MH, was probably caused by the variation of certain physical factors, in particular the sea temperature (c. 23°C), which was as high as c. 26°C in MH, and also the decrease in dissolved oxygen from 8.1 mg dm^{-3} at K to 5.9 and 6.7 mg dm^{-3} in MH and OH, respectively (Table 1). Nevertheless, Andral & Alzieu (2002) observed that the mortality of mussels during transplantation campaigns was due mainly to the stress associated with the calibration and sampling operations.

The higher CI of the OH caged mussels compared to the other mussel populations (Table 2) can be explained by the trophicity of the OH site (Bouragba-Benazza et al. 2006, Taleb et al. 2006) and the chronic exposure of these mussels to the organic matter in the urban sewage discharged into Oran Harbour (Boutiba et al. 2003, Bouragba-Benazza et al. 2006, Taleb et al. 2007b). On the other hand, translocation does not seem to have significantly changed the somatic weight of the mussels (Table 2) after this short period (one month) of caging at the two harbour sites.

The mussels have a very great adaptive capacity as regards the food they consume. They are able to adapt to a great variety of organic-rich particles present in the seston (Arifin & Bendell-Young 2001). *Mytilus* mussels seem to possess compensatory mechanisms controlling particle transport at the level of the ventral groove cilia, enabling the animal to adapt to modifications in the food supply even in an oligotrophic area (Richoux & Thompson 2001).

4.2. Lysosomal membrane stability

Changing membrane stability in the lysosomes, significant subcellular elements producing enzymes in bivalves, could be the first detectable indicator of a stress response (Harding et al. 2004).

It has been reported that morpho-functional alteration of the lysosomes is an obvious consequence of chemical pollution (Moore 1982). Indeed, they play an important role in the cell compartmentalisation of heavy metals and organic xenobiotics, affected as they are by the toxicity of these substances (Lowe & Moore 1979, Winston & Di Giulio 1991). Lysosomal integrity was significantly correlated with tissue levels of PAHs in the Mediterranean mussel *Mytilus galloprovincialis* and brown mussel *Perna perna* (Francioni et al. 2007, Pereira et al. 2007). Moreover, a good correlation was reported between the reduction in antioxidant capabilities, the time of neutral red retention and the changes in DNA integrity in marine organisms (Camus et al. 2002, Regoli et al. 2004). This has made

lysosomal membrane destabilisation a practicable biomarker of cellular stress in mussels (Moore 1982, Viarengo et al. 1987, Harding et al. 2004, Koukouzika & Dimitriadis 2005, 2008, Taleb et al. 2007a,b) and a coastal biomonitoring tool recommended by international organisations such as WHO-FAO (GESAMP 1980) and UNEP (UNEP 1997, UNEP/RAMOGE 1999).

In the present study, a general change in the lysosomes was manifested in the mussels transplanted to the two impacted sites (OH and MH) by a higher NRRT in lysosome haemocytes than in the K reference specimens (Figure 2). The results of this study confirm earlier findings of short lysosomal membrane labilisation periods in OH mussels, exposed to a mixture of domestic and industrial wastewaters (Taleb et al. 2003, Taleb et al. 2007a,b). The impact of urban and industrial coastal activities on the quality of the coastal waters at Mostaganem (Benghali et al. 2007) and in adjacent areas (Bouragba-Benazza et al. 2006, Taleb et al. 2006) may also have contributed to the greater destabilisation of lysosomes in MH caged mussels. In contrast, the mussels from the Kristel reference site, which were in good physiological condition, were more easily able to cope with natural stressors and to preserve the integrity of their lysosomal membranes.

4.3. Genotoxicity

Despite the occasionally high variability in inter-individual MN frequencies recorded in this study (Figure 3), the response of this genotoxicity biomarker revealed a good gradient classification of genotoxic impact, confirming the sensitivity and usefulness of this test for coastal biomonitoring. MN induction in haemolymph and gill cells in the two caged populations was more than seven and six times stronger, respectively, than in the control specimens. These results reflect genetic deterioration, which implies the accumulation of organic and inorganic pollutants with clastrogenic and mutagenic activity (Kalpaxis et al. 2004). This is therefore an endorsement of our passive biomonitoring data on exposure to genotoxic agents in this area (Taleb et al. 2007a,b), the potential effect of heavy metal bioaccumulation in *M. galloprovincialis* from OH (Boutiba et al. 2003), and the ability of such metals to induce a dose-related increase in MN frequency in mussels (Bolognesi et al. 1999).

In the present study, gill cells displayed a greater sensitivity than haemocytes in caged mussels as compared to the controls; Bolognesi et al. (2004) observed the same. Being the filter feeding apparatus and respiratory organ, the gills represent the first biological target of the cytogenetic effects induced by persistent contaminants such as PAHs and heavy metals (Bolognesi et al. 1999, Bolognesi et al. 2004) and as such are ideal target

organs for biomonitoring studies (Bolognesi et al. 2004, Koukouzika & Dimitriadis 2005, 2008, Taleb et al. 2007a,b).

The use of transplanted mussels as a biological model in genotoxic studies for the same period of exposure is the recommended method for avoiding the effects of adaptive mechanisms and inter-individual variability (Bolognesi et al. 2004, Nigro et al. 2006). Other physiological and physical factors, such as the age of the mussels and the water temperature, can influence MN induction in these bivalves (Brunetti et al. 1992). It was found that simple comparisons between MN induction in mussels sampled from polluted and reference sites could influence the use of the MN test in in situ biomonitoring (Izquierdo et al. 2003). Nevertheless, it has been found (Baršienė et al. 2003) that MN frequency is significantly induced by the levels of pollutants. Our results corroborate this observation: there was a significant increase in MN frequency in the OH and MH mussels compared to the K reference specimens. This can be related to the environmental quality of the study sites, since the genetic variability factor of the *M. galloprovincialis* populations was reduced.

4.4. AChE activity

The absence of a correlation between the variation in AChE activity and the size of mussels reported in the literature (McHenery et al. 1997, Lau & Wong 2003, Lionetto et al. 2003) enabled us to avoid size selective operations and consequently to handle and stress the mussels during the caging preparations.

Roméo et al. (2003) noted higher AChE concentrations in the gills than in the digestive gland, and several studies have suggested that the biological response of the gills is more sensitive than that of other tissues, e.g. digestive gland, muscle, mantle and homogenate of *M. galloprovincialis* (Najimi et al. 1997, Valbonesi et al. 2003, Lau et al. 2004, Damiens et al. 2007). Our results are in agreement with the literature data, clearly highlighting the greater AChE activity in the gills than in the digestive gland and homogenate, as well as its significant sensitivity following the exposure of the mussels to a polluted environment. A proportional response of AChE activity was observed in *M. galloprovincialis* in an aquatic environment polluted with a variety of heavy metals and detergents (Guilhermino et al. 1998) and pesticides (Lionetto et al. 2003). The significant decrease in this biomarker in the OH mussels, particularly in the gills, suggests a response induced by anti-cholinesterase contaminants: we know that this harbour site is continuously exposed to the discharges of untreated urban sewage and industrial effluents and consequently to several pollutants such as heavy metals (Boutiba et al. 2003, Bouragba-Benazza et al. 2006). A correlation

was reported between heavy metal pollution and decreases in AChE activity in mussels from industrialised areas and harbour sectors (Bocquené et al. 1997, Najimi et al. 1997, Lionetto et al. 2003, Lau et al. 2004, Magni et al. 2006). Likewise, Devi & Fingerman (1995), Amiard-Triquet et al. (1998) and Dellali et al. (2001) noted that a reduction in AChE activity may be an indication of metal contamination.

Despite the agricultural activities in the Mostaganem area and thus the possible chronic application of pesticides and their potentially toxic effect on mussels *M. galloprovincialis*, the less marked activity of AChE in the MH caged mussels than in the OH transplants was unexpected. Thus, the degree of inhibition of AChE activity in MH specimens was probably related to the anthropogenic effects of urban and industrial coastal pollution.

5. Conclusions

The biomarkers analysed in this study clearly differentiated the contaminated areas (Oran and Mostaganem Harbours) from the Kristel reference site, confirming their usefulness as early warning tools for the detection of anthropogenic environmental disturbances.

Focusing as it does on coastal pollution biomonitoring on the Algerian west coast, this case study could serve as a regional pilot project to demonstrate the utility of such a biomonitoring bioassay strategy and the potential roles of biomarkers for assessing environmental stress levels. From this perspective, we are planning to use an integrated analysis of chemicals and biomarkers to evaluate the possible development of a stress syndrome in caged mussels exposed to a field metal pollution gradient on the Algerian west coast.

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