

**Monitoring the biological
effects of pollution on
the Algerian west coast
using mussels *Mytilus
galloprovincialis***

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Abstract

The Algerian west coast is the prime recipient of several forms of pollution; hence, the necessity for an impact assessment of this coastal pollution using a suite of recommended marine biomarkers, including lysosomal membrane stability in living cells by the Neutral Red Retention Time (NRRT) method, the evaluation of micronucleus (MN) frequency, and the determination of acetylcholinesterase (AChE) activity in mussels *Mytilus galloprovincialis*, sampled from the large, polluted Oran Harbour (OH) and the Maârouf (Mrf) marine mussel farm between July 2005 and April 2006. The difference in the variations of the annual physical parameters between OH and Mrf corresponds to the influence of the domestic and industrial sewage discharged by the city of Oran. The biological data of the mussels (condition index, protein content) recorded at both sites were related to their natural reproductive cycle. This indicated that intrinsic variation between the sites due to different mussel development phases was minimal. The variation in the AChE activity of some organs of OH and Mrf mussels, with minimal inhibition

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in July and a higher NRRT recorded in the granular haemocytes in the Mrf than in the OH mussels during the autumn and spring, depends on the quality of the biotope and on generic stress factors. Moreover, the variation in MN frequency, in general reflecting a non-significant seasonal and spatial genotoxic effect of the contamination at the two sampling sites, requires further investigations regarding biotic and abiotic variations.

1. Introduction

Biomonitoring has become one of the ways of predicting changes in the global environment. Many scientific programmes in different Mediterranean countries are taking this approach to the biological effects of contaminants with the aim of promoting a common and integrated strategy of using marine biomarkers in recommended sentinel species (UNEP/FAO/IOC 1993, UNEP 1997, Viarengo et al. 1997, UNEP/RAMOGÉ 1999, Cajaraville et al. 2000, Viarengo et al. 2000a,b, ICES 2004). Biomarkers, for example, mussels *Mytilus* spp., are early warning biological tools able to detect pre-pathological changes or disturbances as responses to environmental pollutants at the cellular and organism levels (Moore 1985, Amiard et al. 1986, Viarengo et al. 1990, Lionetto et al. 2003, Regoli et al. 2004, Gravato et al. 2005).

The increase in the human population (more than 40% of Algeria's population inhabits the littoral zone), industrial development in western Algeria and the absence of urban and/or industrial sewage treatment plants have turned the coastal marine environment into a prime recipient of several forms of pollution. Our research laboratory's assessment of marine pollution by contaminants bioaccumulated in marine species (e.g. mussels, sea urchins, crustaceans, fish and cetaceans) from several sites on the Algerian west coast during the last ten years has revealed high concentrations of heavy metals (Hg, Cd, Pb, Zn, Cu, Mn, Ni, Mg), organochlorine compounds (PCB and chlorinated pesticides) and polyaromatic hydrocarbons (chrysene, phenanthrene) (Taleb 1997, Taleb et al. 1997, Taleb & Boutiba 1999, 2007, Boutiba et al. 2003). This merely underlines the crucial importance of evaluating the impacts of the pollution gradients in this coastal area of Algeria.

In accordance with the current national priority environmental policy (National Action Plan for the Environment and Sustainable Development, Algerian Ministry of the Environment (PNAE-DD 2002)), the first regional marine biomonitoring project 'Use of biomarkers for the assessment of marine pollution impacts in the western Algerian coastal area' was recently developed by our laboratory. This project introduced certain recommended marine biomarkers (UNEP 1997, UNEP/RAMOGÉ 1999, Bocquené & Galgani 2004): determination of lysosomal membrane stability

in living cells by the Neutral Red Retention Time (NRRT) method (general stress), evaluation of micronucleus (MN) frequency (genotoxic effects), and determination of acetylcholinesterase (AChE) activity (presence of organophosphorus compounds, carbamates and some heavy metals) in mussels *Mytilus galloprovincialis*, the most frequently used sentinel organism in Mediterranean marine environmental biomonitoring programmes. As filter feeders, these animals have the capacity to accumulate organic and inorganic xenobiotics present in their environment (Jernelov 1996, Boutiba et al. 2003, Taleb & Boutiba 2007).

Lysosomes are subcellular organelles containing hydrolytic enzymes capable of processing damaged or redundant cellular components. They are also able to accumulate and detoxify a wide range of toxic metals and organic pollutants, capable of damaging cells (Moore 1985, Viarengo et al. 1987). However, the uptake of toxic compounds can affect lysosomal membrane integrity, which may cause lysosomal contents to leak into the cytoplasm. Changes to the permeability of the lysosomal membrane caused by several environmental pollutants can be monitored *in vitro* by using the NRRT assay (Lowe & Pipe 1994, Lowe et al. 1995b, Ringwood et al. 1998, Dailianis et al. 2003, Harding et al. 2004, Koukouzika & Dimitriadis 2005). In an unstressed state, lysosomes will accumulate and retain the cationic neutral red dye for an extended period of time. However, following a stressor, the destabilized lysosomes will coalesce to form larger lysosomal structures and the neutral red dye will leak into the cytosol of the cell across damaged membranes (Moore 1980, Lowe et al. 1995a). The NRR in mussel haemocytes is one of the most widely recommended biomarkers in marine biomonitoring programmes (UNEP 1997, UNEP/RAMOGGE 1999).

An MN is formed during the metaphase/anaphase transition of mitosis. It may arise from an intact lagging chromosome (a eugenic event leading to chromosome loss) or from an acentric chromosome fragment detaching from a chromosome after breakage (a clastogenic event) that does not integrate into the daughter nuclei. The MN test has been used in different aquatic organisms (Hose 1985, Burgeot et al. 1995, Hagger et al. 2002, Banni et al. 2003), widely so in the gills and haemocytes of bivalve molluscs (Brunetti et al. 1988, Scarpato et al. 1990, Wrisberg et al. 1992, Burgeot et al. 1996, Bolognesi et al. 1996, Venier et al. 1997, Bolognesi et al. 1999, Dailianis et al. 2003, Koukouzika & Dimitriadis 2005). Some studies on micronuclei in mussels have focused on evaluating other nuclear abnormalities, like binucleated cells, and eight-shaped, fragmented nuclei or nuclear buds (Venier et al. 1997, Baršienė et al. 2003, Dailianis et al. 2003).

The role of AChE (EC 3.1.1.7) in cholinergic transmission is to control the nerve impulse by reducing the concentration of acetylcholine (ACh) at

the synaptic junctions by a catalytic reaction of ACh hydrolysis; muscular tetany and death are thus avoided. Nevertheless, AChE inhibition leads to severe physiological weakening in marine organisms (McHenery et al. 1997, Ozmen et al. 1998). In this field, several studies have emphasized AChE inhibition in bivalves such as *M. galloprovincialis* as a biomarker in species exposed to organophosphates, carbamates and some heavy metals (Bocquené et al. 1990, Galgani et al. 1992, Bocquené et al. 1997, Najimi et al. 1997, Narbonne et al. 1999, Dellali et al. 2001, Dailianis et al. 2003).

2. Material and methods

2.1. Sampling sites

Mussels (*M. galloprovincialis*) were collected from the large Oran Harbour (OH; Oran Bay, Algerian west coast), into which 90 million m³ of untreated wastewaters are discharged annually by the Oran metropolis and many industrial units. High levels of heavy metals, polyaromatic hydrocarbons and bacterial density were recorded in the tissues of *M. galloprovincialis* at this site (Boutiba et al. 2003). The second sampling site was a marine mussel farm located in the rural area of Maârouf (Mrf) in the

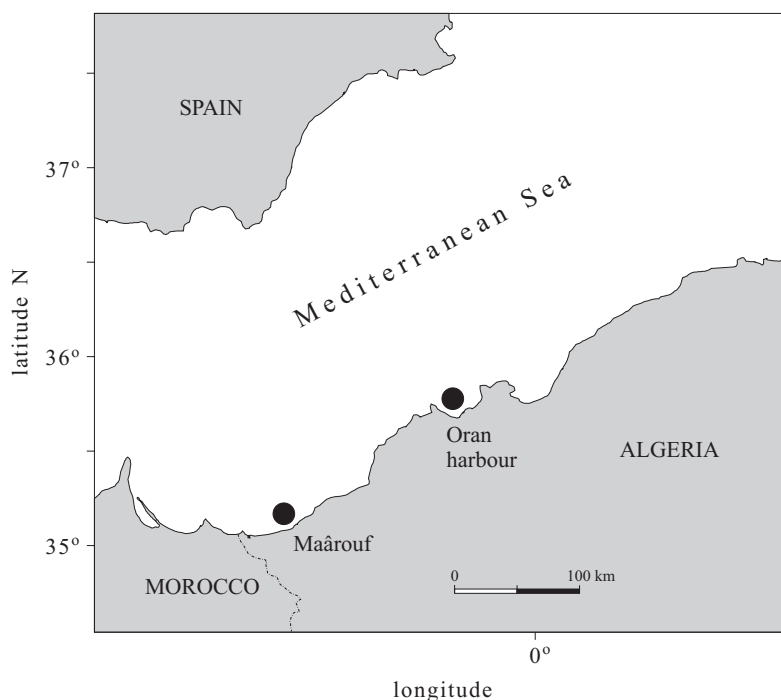


Figure 1. Sampling sites located along the Algerian west coast

extreme north-west of Algeria, approximately 200 km distant from the first site (Figure 1).

To assess the seasonal pattern of naturally (physical and biological) induced stress responses, seawater temperature, salinity, pH and dissolved oxygen were monitored in situ. At the laboratory the food supply (chlorophyll *a*) (Lorenzen 1967), the protein content (Bradford 1976) in some organs and the mussel condition index were calculated as follows: $CI = \text{tissue wt (g)}/\text{shell length (mm)} \times 100$ (Kagley et al. 2003). The sampling sites were geo-referenced with Garmin GPS 12 (Table 1).

Table 1. Data from the Oran Harbour (OH) and Maârouf (Mrf) sampling sites

Sampling site	Sampling date	Position	Temp. [°C]	Salinity [PSU]	Turbidity [NTU]	Dissolved O ₂ [mg dm ⁻³]	Chl <i>a</i> [mg m ⁻³]
OH	July 2005		27.3	34.5	4.5	3.6	2.60
	October 2005	35°42'663"N	22.4	36.5	2.5	3.9	0.51
	January 2006	00°39'320"W	13.0	37.5	1.7	4.4	0.57
	April 2006		24.0	37.0	1.5	6.8	1.88
Mrf	November 2005	35°04'249"N	20.7	39.5	1.5	6.3	0.62
	March 2006	02°03'790"W	21.7	38.5	1.0	6.2	1.05

2.2. Mussel collection and handling

In each season, both male and female mussels were sampled at random from mussel stocks and shipped in coolers with ice packs 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.

2.3. Evaluation of NRRT in haemolymph

The analytical method was performed according to Lowe et al. (1992) and Lowe et al. (1995b), 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 μ l of NR working solution were dropped onto each slide. At the end of 15 min, the slides were quickly examined at 400x magnification and the images digitalized using computer-enhanced automatic image

analysis. The system included a charged couple 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.

2.4. MN in the haemolymph and gill tissue

The micronuclei frequency was determined according to the procedure proposed by UNEP/RAMOGÉ (1999). 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. 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 analyzed under the same Zeiss light microscope at a final magnification of 1000x 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/RAMOGÉ (1999).

2.5. Determination of AChE activity

Haemolymph was collected from the posterior adductor muscles of five mussels with a sterilized syringe and placed in Eppendorf tubes. Digestive gland, haemolymph, gills and mantle/gonad complex were ground in Tris buffer (0.1M, pH 7.5). The haemolymph samples and tissue homogenates obtained were 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 propose, 50 µl of the stock

solution containing AChE fractions (S9) was added to a reaction mixture containing 850 μ l Tris 100 mM pH 7.5 and 50 μ l of 1.875 mM DTNB (Sigma-Aldrich). After pre-incubation, the reaction was started by the addition of 50 μ l 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.

2.6. Statistical analysis

Statistical analysis of the data – condition index; protein content; haemocyte NRR; MN frequency of haemocytes and gill cells; AChE activity of haemolymph, digestive gland, gills and mantle/gonad complex – were based on Duncan's test for multiple comparison and Student's *t*-test between pairs of mean values using Microsoft STATISTICA (v. 6.0) statistical software. The significance level for all statistical tests was set at $p < 0.05$.

3. Results and discussion

3.1. Physical parameters and chlorophyll *a*

The annual physical parameters in Oran Harbour (OH) summarized in Table 1 reflect the influence of the untreated domestic and industrial wastewater released by the city of Oran, mainly in July (when the salinity and dissolved oxygen were lower and the temperature and turbidity were higher). However, at the Maârrouf (Mrf) station, all the physical parameters remained relatively constant during the two months of sampling, except Chl *a*, whose concentrations were appropriate to the seasonal phytoplankton bloom.

3.2. Biological parameters

The lengths of the mussels collected did not vary markedly during the sampling period, ranging from 50 to 82 mm in the natural mussel population (OH) and from 55 to 84 mm in the mussel farm population (Mrf) (Table 2). However, there was an increase in the somatic weight of the OH mussels during July and April compared to the other months (October and January) and of those from Mrf between November and March (Table 2). The condition index reflects this seasonal somatic weight change (Table 2).

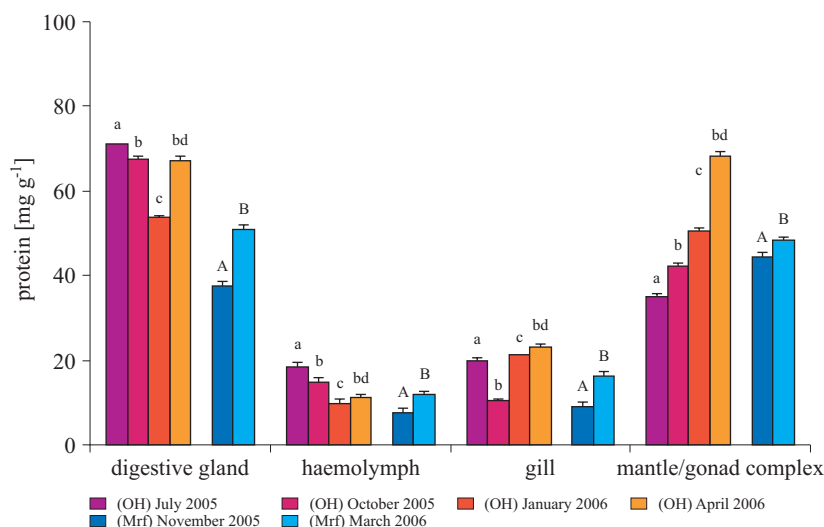
The protein level in the digestive glands, haemolymph, gills and mantle/gonad complex of the OH mussels varied significantly during the sampling periods, except in the digestive gland between April and October and in the haemolymph between April and January.

Table 2. Shell length [mm], tissue weight complex [g] and condition index (CI) of mussels, *Mytilus galloprovincialis* (values are means \pm SD, n = 10 per month, per site) sampled from Oran Harbour (OH) and Maârrouf (Mrf)

Sampling site	Sampling date	Shell length [mm]	Somatic weight [g]	Condition index (CI)
OH	July 2005	66.70 \pm 6.00	7.16 \pm 2.17	26.38 \pm 7.06 ^{cb}
	October 2005	64.90 \pm 9.80	6.33 \pm 3.20	24.20 \pm 10.88 ^b
	January 2006	69.80 \pm 5.40	6.24 \pm 1.76	13.76 \pm 6.79 ^a
	April 2006	68.20 \pm 5.50	7.30 \pm 2.34	19.95 \pm 7.52 ^{abc}
Mrf	November 2005	65.20 \pm 6.20	4.06 \pm 1.16	8.63 \pm 2.20 ^A
	March 2006	74.80 \pm 6.00	8.58 \pm 1.90	22.41 \pm 7.89 ^B

CI data with the same superscript (small letters in OH and capitals in Mrf) indicate they did not differ significantly ($p > 0.05$) after Duncan's test for multiple comparison in different seasons at OH and Student's *t*-test at Mrf.

The protein content of OH mussels peaked in the digestive gland and the haemolymph in July (summer) and in the gills and the mantle/gonad complex in April (spring) (Fig. 4, see p. 554). In the Mrf mussel culture population, the protein concentration in all the tissues was statistically ($p < 0.05$) higher in March (spring) than in November (Figure 2).

**Figure 2.** Seasonal variation in the protein content [mg g⁻¹] in *Mytilus galloprovincialis* tissues from Oran Harbour (OH) and Maârrouf (Mrf) (mean \pm SD, n = 5). Statistical significance is based on Duncan's test for multiple comparison in different seasons at OH and Student's *t*-test at Mrf. Data with the same superscript (small letters in OH and capitals in Mrf) indicate they did not differ significantly ($p > 0.05$)

This annual seasonal variation in the protein concentration at both sites was related to their natural reproductive cycle. Protein concentrations usually decrease just after the period of spawning and increase in the resting stage.

The annual seasonal variation in the protein contents which, generally speaking, reach their highest level during April and July, reflects the periods of rest (development) and spawning. The protein level is thus a general indicator reflecting the resting-spawning cycle (Lee 1986, Mohan & Kalyani 1989); indeed, proteins are accumulated and stored during the rest period in preparation for the spawning phase, and will decrease after spawning. We may therefore presume, according to the recorded biological data of the OH mussels, that the spawning periods are October and January, corresponding to autumn and winter.

3.3. Evaluation of NRRT

The NRR times recorded in the granular haemocytes of OH and Mrf mussels showed that the seasonal variation of this biomarker did not follow a clear pattern. The labilization time of the haemocyte lysosomal membrane in the OH mussels was significantly ($p < 0.05$) less during July (45.33 ± 30.04 min) than in October (50.75 ± 27.77 min) and January (52.13 ± 26.70 min), whereas no significant differences ($p < 0.05$) were noted at this site, or even between the NRRT of the two sampling periods of the Mrf mussels, for the other sampling periods.

During autumn and spring, NRRT were statistically higher ($p < 0.05$) in the Mrf than in the OH mussels (Figure 3).

The results of the NRRT evaluation, indicating the general stress in relation to the seasonal and environmental conditions in mussels collected from an aquaculture farm (Harding et al. 2004), reflect a seasonal variation model of membrane lysosomal destabilization, which is correlated with the reproductive cycle of the species and consequently with its condition index. Nevertheless, we noticed in our OH samples that for the two periods of spawning (October and January), NRR times are significantly higher than those noted during July, which is the post-spawning period with the highest condition index (Table 2).

The NRRT variations in OH mussels compared to those of Mrf showed that lysosomal membrane stability is quite dependent on the quality of the biotope and the levels of chemical contamination. The data of Boutiba et al. (2003) and Taleb & Boutiba (2007) reflect the significant extent of chemical stress caused in particular by heavy metals in *M. galloprovincialis* in Oran Harbour. This general stress on the mussel populations of Oran Bay was

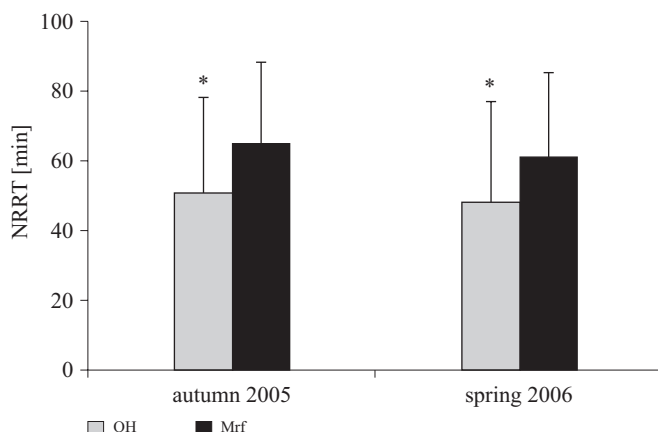


Figure 3. Neutral red retention times [min] in *Mytilus galloprovincialis* haemocytes from Oran Harbour (OH) and Maârouf (Mrf) expressed as mean \pm SD from ten mussels. Statistical significance is based on Student's *t*-test between pairs of mean values. * indicates statistical significance at $p < 0.05$

reported following low temporal values of the lysosomal membrane stability (Taleb et al. 2003).

A reduction in the lysosomal membrane stability associated with the exposure of mussels (*M. galloprovincialis*), clams (*Slaps philippinarum*) and oysters (*Crassostrea gigas*, *Crassostrea virginica*) to pollutants such as heavy metals and polyaromatic hydrocarbons has been reported (Moore 1991, Lowe et al. 1995b, Ringwood et al. 1998, Lowe & Fossato 2000, Viarengo et al. 2000c, Woo-Geon & Sang-Man 2005).

The level of mussel contamination is proportional to the degree of pollution of the surrounding waters, in particular by polyaromatic hydrocarbons (Nott & Moore 1987), which tend to be bioaccumulated in some organelles such as lysosomes, inducing continual destabilization of the lysosomal membrane (Woo-Geon & Sang-Man 2005).

The results of many investigations indicate that the physicochemical factors of the biotope, such as temperature variations, hypoxia and hyposalinity (Moore et al. 1979, Hauton et al. 1998), can affect the integrity of haemocyte lysosomes. Other studies have affirmed that the summer increase in the water temperature reduces the stability of the lysosomal membrane (Tremblay et al. 1998), which could also explain the significantly lowest NRRT in July in the mussels from Oran Bay, where we noted a water temperature of 27.3°C and a low oxygen saturation (Table 1).

The lysosomal membrane destabilization times, ranging between 40 and 56.25 min for OH mussels and between 56.25 and 75 min for Mrf specimens, are comparable with those noted in the same species from the northern

coasts of Greece (Dailianis et al. 2003), which do not exceed 50 min in populations exposed to anthropogenic pollution and 65 min in a culture population (Koukouzika & Dimitriadis 2005). There is therefore a clear link between the levels of stress to which the mussel populations used in our study are exposed and the quality of their biotope.

3.4. Determination of the MN frequency

The results of the MN test applied to the haemolymph and gill cells of OH *M. galloprovincialis*, indicated an annual frequency varying respectively from 0.99 to 3.3‰ and from 1 to 4.75‰, with genotoxicity peaking in January; in the Mrf mussels, MN varied from 0.99 to 1.67‰ in the haemocytes and from 0.76 to 1‰ in gill cells. Nevertheless, Duncan's test did not reveal any significant difference ($p < 0.05$) in the annual seasonal variation of the MN frequency in these tissues of OH mussels. The same applies to the Mrf mussels between the two periods of sampling.

In addition, comparison of the MN induction noted in the OH and Mrf mussels during the autumn and spring revealed a significant difference ($p < 0.05$) only in the haemocytes.

The experimental results indicated a higher MN frequency in the gill cells as compared to the haemocytes in the OH mussels. The same result was reported by Bolognesi et al. (1999), Baršienė et al. (2003) and Dailianis et al. (2003). Nevertheless, the agranulocyte haemocytes and the gill cells remain suitable for genotoxic evaluation in *M. galloprovincialis* (Venier et al. 1997).

The appearance of MN is obvious after the mussels' exposure to chemical contaminants such as heavy metals and organic pollutants (Mersh & Beauvais 1997, Venier et al. 1997, Bolognesi et al. 1999). However, the MN frequency found in the present study varied significantly within the same experimental groups of mussels (Figure 4). Such internal individual variability, as displayed here by the OH *M. galloprovincialis*, has been reported from elsewhere in the same species (Koukouzika & Dimitriadis 2005) and in other bivalves (Wrisberg et al. 1992, Mersh & Beauvais 1997). Moreover, the variation in MN frequency in general reflects a non-significant seasonal and spatial genotoxic effect of the contamination at the two sampling sites. It seems, in contrast to the pollution gradient, that other factors, such as high cell death rates (Brunetti et al. 1988) and deterioration of cell division (Wrisberg et al. 1992), can have a great influence on MN formation. It has even been mentioned that MN induction in the gill cells of *M. galloprovincialis* can decrease following long exposure to a polluted environment (Scarpato et al. 1990).

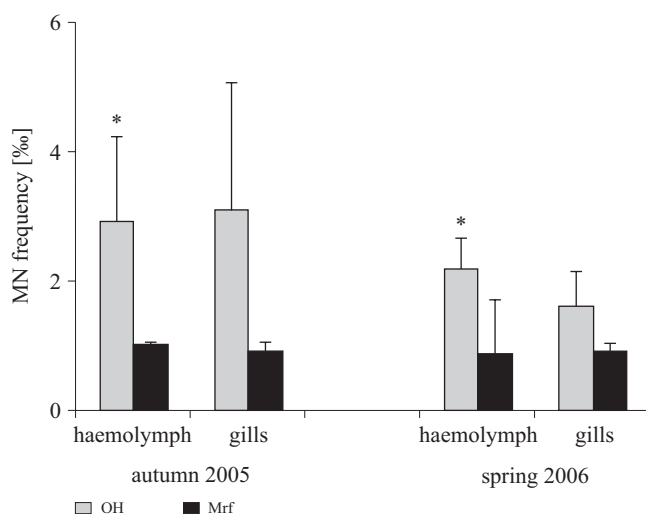


Figure 4. MN frequency [%] in the gills and the haemolymph of mussels from Oran Harbour (OH) and Maârouf (Mrf), expressed as mean values \pm SD from five mussels. Statistical significance is based on Student's *t*-test between pairs of mean values. * indicates statistical significance at $p < 0.05$

It appears, therefore, that use of the MN test to evaluate genotoxic stress will require further investigations related to biotic variations (age, sex, development cycle) and environmental parameters (Dolcetti & Venier 2002, Koukouzika & Dimitriadis 2005) before it can be routinely used in biomonitoring programmes of marine pollution.

3.5. Determination of AChE activity

As far as the AChE activity levels measured in the digestive glands of the OH mussels are concerned, we noted a maximum level in July ($52.17 \pm 0.13 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$) and a minimum in April ($32.88 \pm 0.51 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$). Moreover, statistical analysis revealed a significant ($p < 0.05$) annual seasonal variation in AChE.

In contrast, the profile of this same seasonal difference of AChE activity in the gills and the mantle/gonad complex was the opposite of that of the digestive gland, with large concentrations found in October (gills: $395.8 \pm 13.54 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$, mantle/gonad complex: $53.42 \pm 1.22 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$) and minimum values in July (gills: $154.91 \pm 4.51 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$, mantle/gonad complex: $34.38 \pm 0.95 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$).

In the haemolymph, AChE activity reached a maximum in October ($1083.3 \pm 69.94 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$) and a minimum in July ($556.75 \pm 29.04 \text{ nmol min}^{-1} \text{ mg prot}^{-1}$). Compared with the other tissues,

the activity of this enzyme in the haemolymph exhibited significant ($p < 0.05$) seasonal differences only between October and the other sampling months.

For the Mrf mussels, a significantly ($p < 0.05$) higher AChE activity was detected in November as compared to March only in the haemolymph, gills and mantle/gonad complex; this is the reverse of what takes place in the digestive gland.

Geographical comparison of AChE activity in all tissues of OH and Mrf *M. galloprovincialis* during the autumn (October for OH and November for Mrf) and the spring (April for OH and March for Mrf) showed that AChE concentrations are significantly ($p < 0.05$) lower in the digestive gland and the mantle/gonad complex in OH mussels in both seasons; this is the reverse of the situation in the haemolymph and the gills (Figure 5).

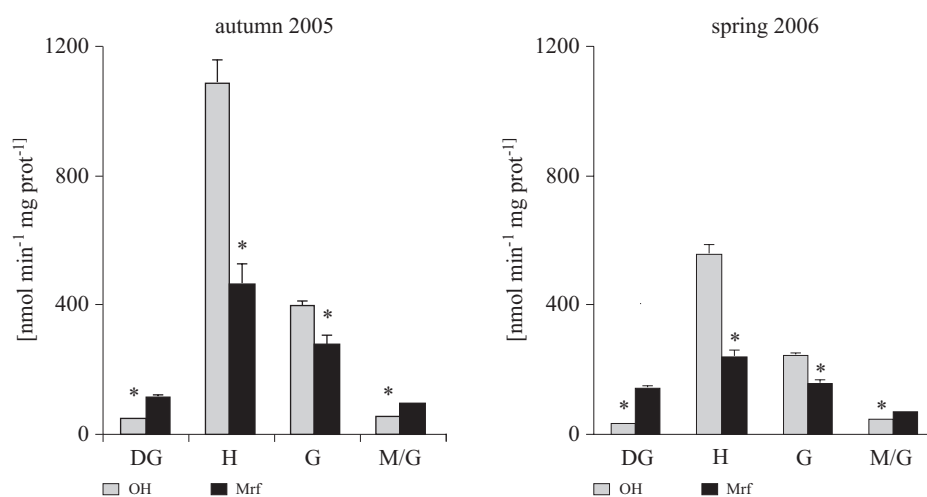


Figure 5. AChE activity [$\text{nmol min}^{-1} \text{mg prot}^{-1}$] in *Mytilus galloprovincialis* tissues (DG: digestive gland; H: haemolymph; G: gills; M/G: mantle/gonad complex) from Oran Harbour (OH) and Maârouf (Mrf) (mean \pm SD, $n = 5$). Statistical significance is based on Student's t -test between pairs of mean values. * indicates statistical significance at $p < 0.05$. Significant differences are indicated by * $p < 0.05$

In the various tissues of OH and Mrf *M. galloprovincialis* the enzyme is distributed, in decreasing order of concentration, in the haemolymph, the gills, the digestive gland and the mantle/gonad complex. The same was observed in this bivalve off the northern Greek coasts (Dailianis et al. 2003). Nevertheless, Najimi et al. (1997), noted that the highest enzymatic activity was in the digestive gland and the gills compared with the mantle/gonad complex and the haemolymph of *M. galloprovincialis*,

while Ozretic & Krajinovic-Ozretic (1992) noted that the hepatopancreas was the richest source of esterase in this species.

Likewise, in the mussel *Perna perna* the highest AChE concentrations were measured in the digestive gland and the haemolymph, and the lowest in the gills, whereas in *Mytilus edulis*, the highest activity was in the gills (Bocquené et al. 1990). The role of this enzyme can be related to its distribution in the various tissues (Dailianis et al. 2003).

Our results relating to the seasonal variations of AChE activity in *M. galloprovincialis* are in agreement with those cited by Najimi et al. (1997), Dellali et al. (2001) and Dailianis et al. (2003).

The high AChE activity in the haemolymph compared to other tissues probably indicates that the role of this enzyme in molluscs may not be directly related to nervous impulse transmission. Moreover, Dailianis et al. (2003) noted that the existence of cholinergic transmission in the peripheral nervous system in molluscs has not so far been demonstrated.

Many studies have shown potential interest in the measurement of AChE activity in invertebrates as a specific biomarker in coastal waters and rivers (Moulton et al. 1996, Varela & Augspurger 1996). This AChE activity could be inhibited by the presence of organophosphorus compounds and carbamates (Zinckl et al. 1987, Day & Scott 1990, Devi & Fingerman 1995, Amiard-Triquet et al. 1998, Galgani & Bocquené 1998), as well as by heavy metals in mussels (Payne et al. 1996, Dellali et al. 2001), fish (Najimi et al. 1997) and clams (Hamza-Chaffai et al. 1998, Dellali et al. 2001).

Our results have shown that in July there was significant ($p < 0.05$) AChE inhibition in all tissues of OH mussels as compared to the other months. This variation in enzymatic activity could be related to seasonal physiological conditions and/or the degree of exposure of the mussels to pollutants. In this context, Taleb & Boutiba (2007) recorded high levels of Zn, Cd, Pb, Cu, Ni, Fe and Mg bioaccumulated by *M. galloprovincialis* from OH, as well as a significant monthly variation with a peak of contamination in July 2000, since these molluscs are continually exposed to untreated domestic and industrial sewage. Moreover, further studies demonstrated that a reduction in AChE activity may be an indicator of heavy metal pollution (Bocquené et al. 1997, Najimi et al. 1997, Amiard-Triquet et al. 1998, Hamza-Chaffai et al. 1998).

The reduction in the activities of this enzyme in the OH mussels during April and July and in those from Mrf in March may, according to Bocquené et al. (1997), be related to the inhibiting effect of the phycotoxins released into the water column during the phytoplankton bloom that generally occurs during the spring and summer. The Chl *a* concentrations found in our

study area (Table 1) may reflect the influence of these phytoplankton bloom periods on AChE activity.

Compared to the Mrf mussels in October and April (Fig. 5), an unexpected finding was that the OH mussels, which are continuously exposed to environmental stress, exhibited significantly ($p < 0.05$) low AChE activity in the haemolymph and the gills during the autumn and spring. According to Eichhorn et al. (1969), heavy metals can alter AChE activity not only by inhibiting it, but also by stimulating the catalytic function of the enzyme, indicating a state of stress resulting from disturbance to the mussels sampled at this station. A probable explanation for this could be the impact of the polluting activities of the zinc electrolytic plant at Ghazaouet City, situated on the coast approximately 30 km to the east of this aquaculture farm, and of the pesticides applied to the agricultural land in this coastal area.

In view of this contrasting geographical variation in AChE concentrations, it appears that the activity of this enzyme may be affected not only by chemical pollutants (heavy metals, pesticides, detergents, hydrocarbons) (Day & Scott 1990, Devi & Fingerman 1995, Payne et al. 1996, Najimi et al. 1997, Amiard-Triquet et al. 1998, Galgani & Bocquené 1998, Hamza-Chaffai et al. 1998), but also by generic stress factors, such as the water temperature (Bocquené et al. 1997, Dellali et al. 2001), salinity (Scaps & Borot 2000, Dellali et al. 2001) and phycotoxins (Bocquené et al. 1997). It should be noted for this purpose that the OH mussels are exposed to continual osmotic stress as a result of the domestic and industrial wastes discharged by the city of Oran, especially in the summer. This corresponds perfectly with the significantly higher AChE activity generally observed in July than at other times of the year. On the other hand, the difference in the water temperature in the various sampling periods and the lowest salinity during July (Table 1) may also be factors able to affect AChE levels.

4. Conclusions

The damage caused to the lysosomal membrane and ADN appears to be a universal marker for evaluating the effects of stress on marine organisms such as bivalves. Indeed, NRRT results, which reflect responses to environmental stress, showed that the variation in the destabilization of the lysosomal membrane of *M. galloprovincialis* haemocytes depends on pollution levels and reflects a difference in the health of the mussels sampled at the two sites. However, further research into the MN test is needed if it is to be an adequate reflection of the genotoxic effect of water quality in our coastal study area.

The levels of AChE inhibition in certain tissues of *M. galloprovincialis* illustrate well the interest in the use of this specific biomarker as an early warning tool of possible contamination in coastal ecosystems.

Within the framework of the active biomonitoring project using caged mussels that we have just undertaken in the same area, the chemical and biomarker analysis will provide a clear indication of the degree of stress syndrome induced by pollutants in these marine bivalves.

The results of this study to examine environmental stress by means of recommended biomarkers (UNEP 1997, Ringwood et al. 1999, UNEP/RAMOGÉ 1999, Bocquené & Galgani 2004) will help to fill gaps in the ecotoxicology data regarding the pollution of Algerian coastal waters.

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