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Contamination of a dredged-material disposal site (La Rochelle Bay, France). The use of the acetylcholinesterase activity of *Mytilus edulis* (L.) as a biomarker of pesticides: the need for a critical approach

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A single aspect of the toxic impact of a dredged material disposal site located near a mussel-farming zone was followed for eight months. Acetylcholinesterase activity (AChE) of *Mytilus edulis* was investigated as a biomarker for possible contamination by neurotoxic compounds (carbamates and/or organophosphorous pesticides). Our observations showed that the enzymatic activities (including AChE) of these harbour mussels were decreased in sites directly and indirectly influenced (according to hydrodynamic conditions) by the dumping of dredged sediments, suggesting possible contamination by pesticides. The strong correlations observed between AChE activity and growth parameters (length and weight) seems to show, however, that the enzyme activity is also indirectly controlled through growth restriction, which may imply limitation of the development of the nervous system in juveniles. The concentration of total proteins, as well as the spawning process also seem to disturb the assessment of AChE activity. These field observations clearly indicate that the use of this enzyme activity as a biomarker should proceed with caution. For example, the seasonal variability of such activity should be taken into account in a biomonitoring programme.

Keywords: Acetylcholinesterase activity, *Mytilus edulis*, dredged sediments, total protein content, growth, reproduction cycle.

Introduction

Dredging activities are operations involving perturbations of the marine environment, especially immediately around both the extraction and disposal sites. The oxidation of the dredged sediments during the dredging and dumping processes results in increased bioavailability of organic contaminants such as pesticides adsorbed on suspended particles (Melzian 1989).

For the past 20 years, the role of these sediments as sources of pesticides has been demonstrated in many field studies and bioassays (Holland *et al.* 1967, Coppage and Matthews 1974), and the neurotoxicity of compounds such as carbamates and organophosphates in aquatic organisms has become apparent. Initially, studies focussed on freshwater organisms because these compounds were widely used in agriculture. The contaminants persist in the aquatic environment (Streit and Kuhn 1994), and accumulate in the marine coastal sediments usually considered the final depot. Even at sublethal concentrations, these contaminants cause functional or structural damage within the most sensitive organisms,

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therefore their presence can be assessed by biochemical analyses (Heath *et al.* 1993). For example, the inhibition of the activity of acetylcholinesterase (AChE), an enzyme involved in the transmission of nerve impulses, by neurotoxic compounds is now widely accepted as a sensitive method to assess their toxic impact upon the marine environment (Bocquené *et al.* 1993, Beyers and Sikoski 1994). Shellfish display some similarities with insects in the structure of their nervous system (nerve fibres which connect different pairs of ganglia) and therefore represent one of the main targets of these pesticides (Bocquené and Galgani 1991).

A field experiment was conducted on different populations of *Mytilus edulis* (bivalve mollusc) immersed around a local dredged-material disposal site ('Le Lavardin', Bay of La Rochelle, France, figure 1) in order to follow their AChE activity. Mussels were used as indicator organisms because these filter-feeder invertebrates are known to accumulate and tolerate high levels of contaminants (organic or not) within their tissues, providing a time-integrated indication of the environmental quality (Regoli and Principato 1995). Furthermore, the dredged material disposal site is located near a highly productive farming zone of edible mussels (the Bay of Aiguillon, figure 1). The environmental quality in terms of pesticide contamination of this mussel-farming area is a major point of concern for the management of the dredged material, because this area previously experienced a lindane (organochlorine insecticide) contamination which resulted in high mortalities within the cultivated populations. At present, the lindane concentrations observed in Charente-Maritime remain the highest of all French coastal waters, even though they have regularly decreased for the last ten years (RNO 1995).

The primary aim of this field study was to evaluate the effects of the dumping of harbour-dredged sediments on the AChE activities of mussels transplanted around the local disposal site during their first year of development. Furthermore, some aspects of the influence of biological processes on this enzymatic activity were observed by following the growth parameters and the reproductive cycle of individuals originating from the same mussel population (Radenac *et al.* 1997).

Methods

Study area

The dredged-material disposal site of Le Lavardin is located on a large mudflat in the front part of the Bay of La Rochelle (north of the Bay of Biscay, figure 1A). The hydrodynamic conditions of the dump-site area are different from the local patterns, which are characterized by very high sedimentation rates. The presence of a trench (20 metres deep) along the disposal site allows the resuspension of the dumped material, and bathymetric studies showed that the depth of the disposal site sediments has increased by only 20 mm per year over the last 10 years (Vanderbach 1991). Dominant wind-driven currents, coming from a WSW direction (figure 1B), reach the Le Lavardin plateau (5 metres deep during low tides) via this trench. The difference in water depth allows these currents to acquire enough energy to resuspend settled material. At present, the harbour-dredged sediments are almost totally removed from the dump-site, and are spread out in a southerly direction by tidal currents (highest speeds reach 0·65 m s^{-1}), and by the wind-driven currents from WSW to ENE (Baron 1992). Tidal currents present a

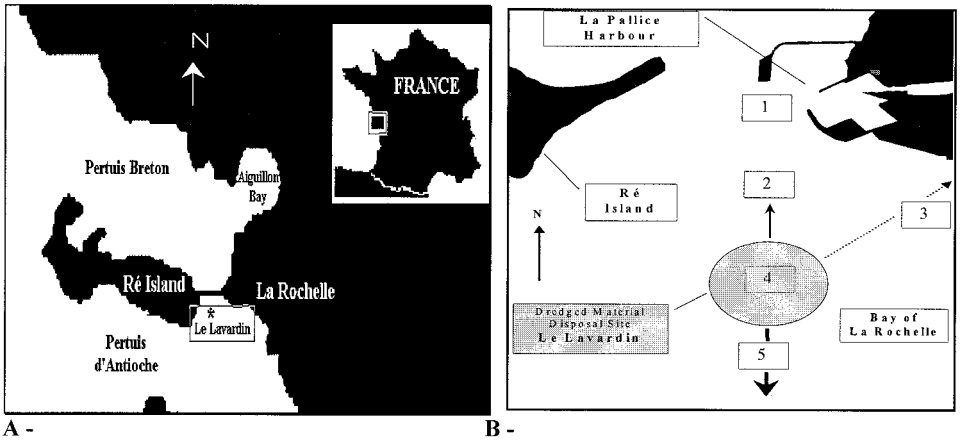

Figure 1. A – Localization of the dredged material disposal site of 'Le Lavardin'. B – Localization of the sampling stations.
: Tidal currents: Direction of *Southern component (time-integrated resultant)*; Direction of *Northern component*
: Direction of wind-driven currents

southern time-integrated 'resultant' if data of the direction of tidal currents are considered over one tidal cycle (figure 1B). Dredging operations continued over the study period and the volumes of harbour sediments regularly dumped in Le Lavardin ranged from 15 000 m^3 to 50 000 m^3 monthly (Radenac 1995).

Biomonitoring field study: AChE activities of mussel individuals

Juvenile mussels from the surrounding area, the Bay of Aiguillon (figure 1A), were transplanted onto five stations around the disposal site (figure 1B) in order to follow their AChE activity, growth, and reproductive cycle from July 1993 to the following May. The locations of the sampling stations were defined following the recommendations established by Baron (1992) in his study on hydrodynamic characteristics of the dump site (figure 1B).

Station 1: influenced by the harbour inputs.
Station 2: influenced by the northern component of the tidal current.
Station 3: influenced by the wind-driven current.
Station 4: dredged material disposal site.
Station 5: influenced by the southern component of the tidal current (time-integrated resultant).

The experimental installations immersed at each station consisted of vertical wooden poles on which mussel populations were fixed according to the method of *bouchot* (Radenac *et al.* 1997). For the study on AChE activities, ten mussels were randomly collected at each sampling date (July, September, November, March, and May) from each population and were kept deep-frozen (–80 °C) before analysis. Station 4 was temporarily inaccessible in March and mussels from this station were not sampled on that date.

Measurements of AChE activities were carried out on the ten individuals pooled together. In the laboratory, soft parts were excised f

suspended in 0·1 M Tris buffer, pH 7·8 (Bocquené *et al.* 1990). Then tissues were homogenized by Ultra-Turrax© and these extracts were centrifuged at 10 000 × g for 20 min at 4 °C. Supernatants were analysed for AChE activities. The colorimetric method of Ellman *et al.* (1961) was applied, as modified for microtitration plate reading (Galgani and Bocquené 1988). AChE activity was expressed as units min^{-1} mg^{-1} of total protein, one unit of AChE activity being the variation by 0·001 of the optical density. The total protein concentrations within the extracts (in μg μl^{-1}) were determined by the method of Bradford (Bocquené *et al.* 1993) using bovine serum albumin (BSA) as standard.

Concurrent studies on growth and reproductive processes were carried out over the same period. At each sampling date (except May), 60 mussels were collected at each station. The total shell length (TSL) and the ash-free-dry weight (AFDW) of each individual were then determined (Radenac 1996). The reproductive cycle was assessed by visual inspection of the different stages of gonad maturation (Radenac *et al.* 1997).

Statistical data analyses

The measures of the AChE activities were achieved in quadruplicate for each station, and mean activity (±standard deviation) of the quadruplicates was calculated. For the growth study, mean values and standard deviations of the 60 individuals were determined.

For each sample station and period, the normality of data was tested (Kolmogorov-Smirnov normality test, $p > 0{\cdot}05$) and the homogeneity of variance was verified (Bartlett's test, $p > 0{\cdot}05$). One-way analysis of variance (ANOVA) was then performed to assess differences of AChE activities first between stations at each sample date, and then between sample periods for each station. Critical F-ratios, which validated significant differences ($p < 0{\cdot}05$) were: $F_{crit} \geq 2{\cdot}37$ with d.f. =4 for the differences between stations and sample periods (except in March and for station 4 where $F_{crit} \geq 2{\cdot}60$ with d.f. =3). In all cases, the analyses of variance were combined with a Fisher's test to define homogeneous groups (table 1).

In order to relate growth parameters (AFDW and TSL) and the AChE activity data, a correlation matrix was performed with the mean values of each parameter calculated for each sample period and station. Then, the significance of the coefficients of correlation was validated by a Pearson's test (figure 3).

Results

The AChE activities recorded within mussels collected from the different stations are given in figure 2. From July to the following May, mean enzymatic activities ranged from 217·4 ±60·5 to 595·6 ±106·5 U min^{-1} mg^{-1} protein depending upon sampling station and time (figure. 2).

While the AChE activities appeared homogeneous in September, the statistical analyses established that there were significant differences between stations in November (table 1): the population directly immersed in the dump site (station 4) and the one influenced by the southern component of the tidal current (station 5) presented individuals with much lower AChE activities than were found at stations 1 and 3. A similar pattern appeared once again in May but at this time, the mussels of station 1, close to La Pallice harbour, also displayed slightly (but significantly)

Table 1. Results of one-way analyses of variance performed on AChE activity data of *Mytilus edulis* collected from July 1993 to May 1994 at five stations around the dump site of 'Le Lavardin'. Homogeneous groups are given (Fisher's test). Sample stations (1, 2, 3, 4, and 5) and months (T0: July, T1: September, T2: November, T3: March and T4: May) were considered as main sources of variation of the enzymatic activity. The asterisks (*) located on the same line define the homogeneous groups of stations (1, 2, 3, 4, and 5) or sampling dates (T1, T2, T3, T4, and T5). *n.d.*: not determined. *n.s.* not significant

Source of Variation:	AChE Activity	d.f.	F Factor	Sign Level	Homogeneous Groups (Fisher)				
Stations					1	2	3	4	5 (Station)
	July	4	0	1·00 n.s.	*	*	*	*	*
	September	4	0·65	0·64 n.s.	*	*	*	*	*
	November	4	22·55	0	*	*	*		
								*	*
	March	3	1·37	0·32 n.s.	*	*	*	*n.d.*	*
	May	4	40·75	0		*	*		
					*				
								*	*
Time					T0	T1	T2	T3	T4 (Time)
	Station 1	4	14·60	0			*		
						*		*	*
					*				
	Station 2	4	6·62	0·01			*	*	*
						*	*	*	
					*				
	Station 3	4	9·62	0			*	*	*
						*		*	
					*				
	Station 4	3	1·43	0·29 n.s.	*	*	*	*n.d.*	*
	Station 5	4	8·39	0				*	
						*	*		
					*		*		*

depressed activity. Conversely, no reliable location-based differences appeared in March.

One-way analyses of variance allow assessment of the temporal variation of AChE activity at each station (table 1). After a significant increase in the activity from July to November, the mussel populations of stations 2 and 3 showed steady enzymatic activities until May. Mussels from station 1 exhibited a significant decrease from November after the same initial increase. Populations from the dump site (station 4) and from the southern area (station 5) presented different temporal patterns. No significant differences of activity were established throughout the experimental period in station 4, AChE activities remaining lower than in other stations. For the population of station 5, the enzymatic activity was clearly depressed in November and in May (see below), altering the temporal pattern observed in stations 2 and 3.

Considering growth parameters (TSL and AFDW), we observed a rapid growth from July to the winter months. Then, the increase in length stopped until March and a decrease of AFDW was concurrently observed (Radenac *et al.* 1997). Observations of the gonads indicated that the decline of AFDW

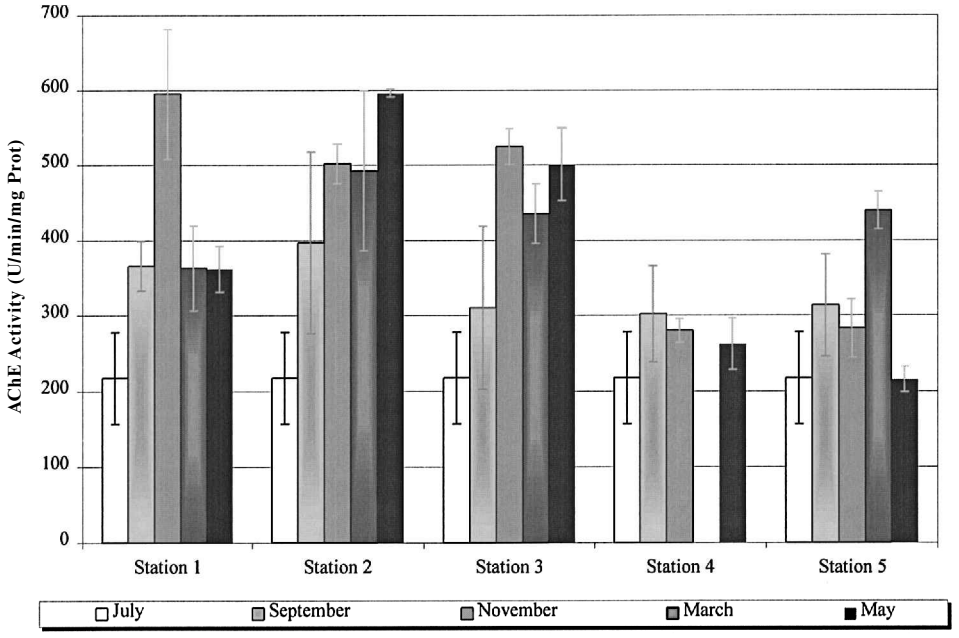

Figure 2. Evolution of the mean values (± standard deviation of the four replicates) of the AChE activities (U min^{-1} mg^{-1} protein) recorded within mussels from five different stations around the dredged material disposal site of the 'Le Lavardin' from July 1993 to May 1994.

spawning process (Radenac *et al.* 1997). Linear regressions between both growth parameters and the enzymatic activities are given in figure 3. Significant correlations were found in both cases ($r = 0{\cdot}70$, $p < 0{\cdot}05$ and $r = 0{\cdot}77$, $p < 0{\cdot}05$ for TSL *vs* AChE and AFDW *vs* AChE respectively).

Discussion

In this field study, the levels of AChE activity recorded within the soft parts of *Mytilus edulis* (from 217·4 to 595·6 U min^{-1} mg^{-1} protein) confirm the levels described in previous studies, where areas with relatively low concentrations of pesticides were investigated (Galgani and Bocquené 1990, Bocquené *et al.* 1990).

Laboratory treatments, however, could influence these levels of enzymatic activity. More precisely, the dilution of the crude sample throughout suspension and AChE measurement might affect the stability of the carbamylated (inhibited) enzyme. Actually, several studies carried out on the effects of carbamate insecticides showed that inhibition is still detectable several days after the contamination. For example, the inhibitory effects of a carbaryl treatment (0.1 µg l^{-1}) on the common prawn (*Palaemon serratus*) was clearly detected 29 days after contamination (Bocquené and Galgani 1991). This latter study confirmed the relative stability of the carbamylated AChE.

However, Galgani and Bocquené (1990) found that AChE from whole mussel was less sensitive to organophosphates than the enzyme extracted from *Palaemon serratus* and fish (*Pleuronectes platessa* and *Scomber scomber*). Despite this fact, *Mytilus edulis* remains a suitable target species to monitor the c[illegible]

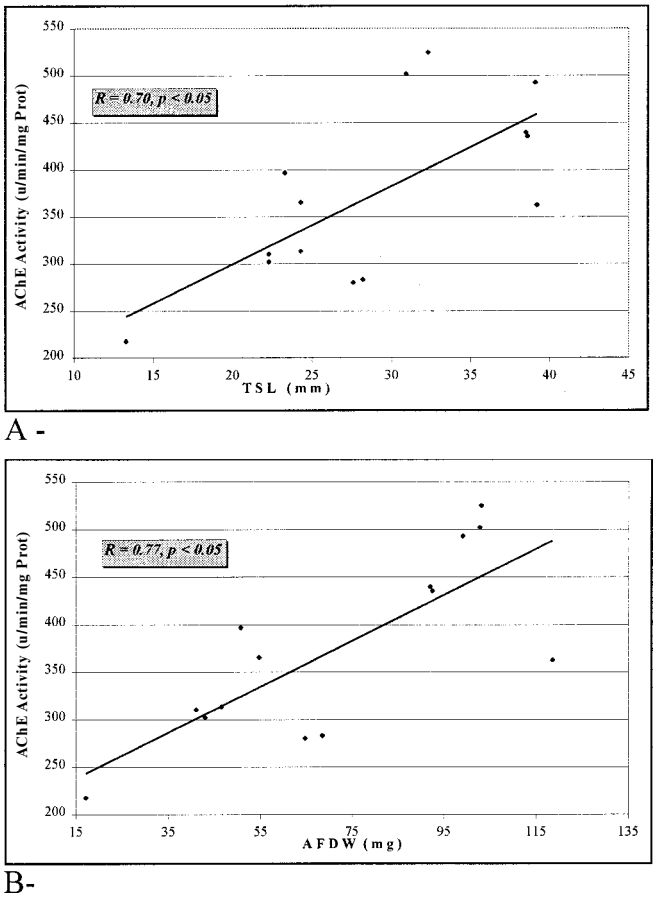

Figure 3. Linear regressions between AChE activity data (U min^{-1} mg^{-1} protein) and the growth parameters data recorded in *Mytilus edulis* collected from July 1993 and May 1994 at five stations around the dump site of 'Le Lavardin': A – Total Shell Length (TSL, in mm) *vs* AChE, B – Ash-Free Dry Weight (AFDW, in mg) *vs* AChE. The coefficients of correlation between the parameters and their significant are given (Pearson's test, n = 15).

environment, considering the simplicity of rearing and sampling, and its wide geographical distribution along the coasts which allows meaningful comparisons between field studies (Bocquené *et al.* 1990).

Finally, other molecules, especially organic forms of heavy metals, can cause reduced AChE activities (Bocquené *et al.* 1995). Moreover, some algal toxins have been reported to be strong AChE inhibitors. These anatoxins are mainly produced by algal blooms of cyanobacteria: *Microcystis aeruginosa*, *Anabaena flos-aquae*, and *Aphanizomenon flos-aquae*. These three species are fresh-water organisms, and although they tolerate brackish waters (up to 8 % salinity), they have never been found in marine waters (Carmichael 1994). The salinities recorded in our study area were close to 34 or 35 ‰ (Fichet unpublished data), which prevent the presence of these blue-green algal species.

Despite the interindividual variations of AChE activity which were not assessed by the method used, the depressed activities observed in the p

(station 4) or indirectly (station 5) influenced by the disposal site may indicate the presence of inhibitor compounds in these areas. Organophosphorous and/or carbamate could be introduced to Le Lavardin with the dredged harbour-sediments regularly dumped. After its dumping upon the disposal site, this dredged material is preferentially moved in a southerly direction according to the time-integrated resultant tidal current. The local hydrodynamic pattern can thus explain the reduction of the AChE activity observed in mussels of the station 5. This hypothesis should now be tested by chemical analyses on the concentrations of pesticides or other inhibitors in dredged sediments or/and dumped particles but such results are not presently available.

The direct harbour inputs seem to influence the enzymatic activity of mussels immersed in station 1 indicating possible pesticide contamination of the harbour sediments. But, this problem seems to be distinct from the possible contamination observed around the dredge disposal (station 5).

The use of an enzyme's activity as a biomarker of any toxic contamination requires a good knowledge of its temporal variability, related, for instance, to growth and reproductive behaviour (Bocquené *et al.* 1990). Few studies have investigated such variability within marine invertebrates in the context of field conditions. Biomonitoring programmes often focus on fish species (Holland *et al.* 1967, Olson and Christensen 1980, Bocquené *et al.* 1993), and only assess the enzymatic activities of indicator organisms at one sample date.

In the present study, following the AChE activities within the first year of the development of marine mussels allowed us to strongly correlate this enzymatic process with growth as measured by length and weight (figure 3). The values globally found throughout the experimental period confirmed this correlation. The AChE mean activity was $217{\cdot}4 \pm 60{\cdot}5$ U min^{-1} mg^{-1} protein when the juvenile mussels were distributed among the different stations (TSL $13{\cdot}3 \pm 1{\cdot}9$ mg and AFDW $17{\cdot}2 \pm 7{\cdot}6$ mg in average in July, Radenac *et al.* 1997). These activities reached values between 400 and 500 U min^{-1} mg^{-1} protein from November while mean values of TSL were around 39 mm, and AFDW were close to 100 mg. Some studies confirmed this trend for other species (*Aplysia* or fish), showing an increase of the AChE activity with growth due to the development of the nervous system of larval and juvenile individuals (Srivatsan *et al.* 1992, Heath *et al.* 1993). Conversely, other studies showed higher AChE activity in younger individuals of different fish species (Burgeot *et al.* 1996). Therefore, it seems that growth features and AChE activity are positively or negatively correlated according to the target species. In the case of *Mytilus edulis*, our observations seems to show that the AChE activity increases with the age of the individual, at least during the first year of development.

A physiological parameter such as the seasonal variation in the concentration of total proteins could also influence the levels of AChE activities recorded throughout the sampling period. For instance, the mid-winter apparent increase in AChE activity could be a result of protein loss in less active mussels exposed to the lower water temperatures. Although few active proteins (such as enzymes) are probably lost by this process (which mainly concerns structural proteins), the specific AChE activity (in U min^{-1} mg^{-1} total protein) seems to increase during autumn. In the same way, no apparent recovery of AChE activity was noticed in *Tigriopsus brevicornis* (copepods, crustacea) when individuals were removed from waters contaminated by pesticides. This seeming stability of the activity was due to

two concurrent processes which cancel each other: the increase of primary AChE activity, and the decrease of total protein content in fasting individuals, as feeding individuals used for controls proved (Bocquené unpublished data).

To corroborate such hypotheses, significant negative correlation appeared between the concentrations of total protein (expressed as mg total protein ml^{-1} extract) and AFDW at least until the spawning period ($r = 0{\cdot}80$, $p < 0{\cdot}05$ for [tot. prot.] *vs* AFDW). This correlation seems to prove that the protein content decreases during the first months of development of juvenile mussels, at least before the spawning period. Then, immediately after the spawning period (i.e. in March), the sexual activity seems to interfere with the activity of AChE which appeared spatially homogeneous. The spawning process observed in all populations between January and March, and proved by the macroscopic observations of the gonads and the sudden decline of the AFDW (Radenac *et al.* 1997), confirms the usual pattern of early sexual maturity in *Mytilus edulis* (Kautsky 1982, Sprung 1983). The depression of the AChE activity observed in stations 4 and 5 in November and May is thus concealed during the spawning period. Once again, the loss of proteins which occurs with spawning leads to an increase in the measured activity even if the concentration of the enzyme (AChE) remains low.

The low AChE activities recorded in November in populations 4 and 5 were also related to low growth parameters (figure 3). These mussels presented lower AChE activities (around 280 U min^{-1} mg^{-1} protein) and concurrently slower individual growth (statistically significant in terms of AFDW); about 35 % lower than the maximum weights observed in stations 2 and 3 (Radenac *et al.* 1997). Other studies have already shown the existence of an energetic cost of compensating toxic effects, even at low-level AChE inhibition (Beyers and Sikoski 1994). Energy expended to compensate adverse effects is then less available for other biological processes such as growth or reproduction. In contrast, the low AChE activities recorded in May near the harbour installations (station 1) were not related to low growth parameters, the mussels at this station displaying higher values in terms of AFDW and TSL (Radenac *et al.* 1997).

Conclusion

The measurements carried out on individual mussels focussed on the potential toxicity of particles suspended in water. According to our results, there were some indications of contamination by neurotoxic compounds within the Le Lavardin site (most clearly in November 1993 and in May 1994), and in the southern area, towards which harbour-dumped sediments were dispersed by the resultant of the tidal current. A reduction of the enzymatic activity was also detected in May in an area close to La Pallice harbour which could indicate, in this particular case, the influence of direct terrestrial inputs leading to pesticide contamination.

The observations performed during this field study give also some indications about the control of the AChE activity. It seems directly controlled by the presence of pesticide compounds (enzymatic inhibition), and indirectly by the limitation of growth. In this latter case, the presence of pesticides implies a shift of the energetic balance toward detoxification to the detriment of somatic growth. Consequently, the development of the nervous system of juvenile individuals may be reduced and AChE activity remains at a low level. One or both processes (direct inhibition and growth restriction) could depend on the level of pesticide contamination.

However, apart from the presence of pesticides in the environment, further parameters, such as an elevated density of individuals, could negatively affect the growth process, but this feature was not precisely assessed in this study. The physiological conditions, such as protein content, should also be taken into account to accurately assess the specific activity of individuals which present seasonal variations in protein concentrations. Similarly, the spawning process seems to disturb the assessment of AChE activity because the release of the gametes also leads to a loss of proteins, without an equivalent decrease in the concentration of AChE. In order to cancel all these phenomena which seriously influence the measurement of AChE activity, studies should be carried out on particular organs (e.g. gills) not influenced by these biological processes (Bocquené *et al.* 1997).

This field study is not precise enough to clearly and entirely understand the control mechanisms of the AChE activity briefly described earlier. It allowed us to put forward some hypotheses on the influence of biological processes (protein content, growth, and reproduction) upon the variability of such enzymatic activity within a bivalve mollusc species. Further studies should be carried out under controlled conditions to throw further light on our field observations. Chemical analyses should be performed to measure the pesticide concentration in water from the area where the mussels were initially collected. As noticed in some studies which focussed on a fish species (Baslow and Nigrelli 1964), the influence of other parameters as the external temperature, the salinity, or pH on the enzyme activity should be also assessed. The measurements of AChE activity should be followed over a long period of time to take into account the seasonal variability in the context of a biomonitoring programme. These results clearly showed that the use of an enzyme activity as a biomarker of toxic contamination should proceed cautiously.

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