Antarctic Science 18 (1), 15–22 (2006) © Antarctic Science Ltd Printed in the UK DOI: 10.1017/S0954102006000022

Cholinesterase activities in the adductor muscle of the Antarctic scallop *Adamussium colbecki*

STEFANO BONACCI*, ILARIA CORSI and SILVANO FOCARDI

Department of Environmental Sciences "G. Sarfatti", University of Siena, Via P.A. Mattioli 4, I-53100 Siena, Italy
**bonacci@unisi.it*

Abstract: Antarctica is regarded as one of the most pristine parts of the Earth but even this remote ecosystem is affected by contamination and high levels of certain heavy metals, such as cadmium, which may occur naturally in Antarctic waters. The bivalve scallop *Adamussium colbecki* is considered a key species of Antarctic benthic ecosystems and a sensitive target for bioaccumulation of xenobiotics and metals. Since cholinesterases (ChEs) in the adductor muscle of *A. colbecki* presumably play a prominent physiological role through regulation of swimming movements, the main aims of this study was to characterize ChE activities in adductor muscle of *A. colbecki* and to investigate their sensitivity to organophosphate pesticides and heavy metals. The results suggest that an acetylcholinesterase-like enzyme in the adductor muscle of the scallop has low sensitivity to organophosphates but was significantly inhibited by exposure to cadmium.

Received 1 December 2004, accepted 27 June 2005

Key words: aquatic pollutants, benthos, bivalve, Cd, ChE, DFP

Introduction

The remote continent of Antarctica is regarded as one of the most pristine areas of the Earth. However, several studies have reported organic xenobiotics and heavy metals in biotic and abiotic matrices of the Antarctic marine environment and their bioaccumulation in the marine food chain (Fuoco *et al.* 1991, 1996, Bargagli 2000). It is therefore now widely recognized that Antarctica is affected by man-made contaminants and naturally occurring heavy metals. Most trace elements have both anthropogenic and natural origins (Bargagli 2000). Sanchez-Hernandez (2000) reported that metal contents in nearshore environments may be predominantly controlled by phenomena not directly linked to anthropogenic contamination and that the high levels in biota do not necessarily indicate anthropogenic impact. In particular, the Southern Ocean exhibits very high concentrations of cadmium**,** regarded as a major biotoxic element and pollutant (Bordin *et al*. 1987, Nolting *et al*. 1991, Bargagli *et al*. 1996). In most Antarctic offshore environments the input of metals due to geochemical and anthropogenic sources and from long-range transport is negligible, but concentrations of Cd in seawater and biota may be higher than in water and organisms from polluted coastal areas (Berkman & Nigro 1992, Bargagli *et al*. 1996, Bargagli 2000, Sanchez-Hernandez 2000). High concentrations of Cd were measured by Rainbow (1989) in Antarctic crustaceans. It has been inferred that Cd enrichment in seawater and biota may be due to upwelling of deep nutrient-rich water, as the Southern Ocean is isolated and there is no evidence of wind-borne or anthropogenic input of this metal (Bargagli *et al*. 1996).

Adamussium colbecki (Smith, 1902) is an endemic Antarctic scallop that is considered a key species of the Antarctic benthic marine ecosystem (Mauri *et al.* 1990, Berkman & Nigro 1992, Chiantore *et al.* 1999, Cerrano *et al.* 2001). Swimming movements are performed by shell-clapping which seems to be significant for escape from predators and locally unfavourable conditions (Ansell *et al.* 1998), enabling at least some of the population to avoid mortality, as in other scallop species (Peterson *et al.* 1982, Vacchi *et al.* 2000). Swimming is achieved by contraction of the adductor muscle, which forces water out of the shell cavity through a pair of small jets located dorsally on either side of the hinge. This method of locomotion seems very effective in spite of the low temperatures of Antarctic waters (Stocchino 1992, Ansell *et al*. 1998).

Research has been done into bioaccumulation of xenobiotics and metals by *A. colbecki*. Although bioaccumulation is mainly influenced by the level a species occupy in the food chain, Focardi *et al*. (1993) found higher levels of organochlorine contaminants in whole body of *A. colbecki* than in any other invertebrate or fish species hierarchically located at higher levels. Strong accumulation of Cd (a metal usually considered an environmental contaminant) has been reported (Mauri *et al*. 1990, Bargagli *et al.* 1996). Scallops exposed *in vivo* to Cd and Cu for seven days showed high levels of both metals in gills which for Cu decreased sharply during 14 days of decontamination, whereas Cd levels did not vary (Berkman & Nigro 1992). The higher cadmium levels in tissues of *A. colbecki* than in those of other bivalve species, including Mediterranean ones (Brooks & Rumsby 1965, Viarengo *et al*. 1993), may be related to the intense feeding rate of the Antarctic scallop (Palmer & Rand 1977, Chiantore *et al.*

1998, 1999). These results suggest that *A. colbecki* may be a suitable species for studying the effects of Cd contamination in Antarctic marine biota. In light of this, and under the consideration of its circum-Antarctic distribution (Dell 1972, Berkman & Nigro 1992) the scallop was recently proposed for monitoring anthropogenic impact near Antarctic scientific bases (SCAR 1994, 1996). In addition, Viarengo *et al*. (1997) suggested the possible utilization of metallothioneins as a biomarker of Antarctic marine pollution utilizing the Antarctic scallop *A. colbecki* as a bioindicator. Moreover, it has been inferred that populations of *A. colbecki* could collapse under the effect of limited anthropogenic pressure (Berkman 1990). Since the scallop is considered a key species for pelagic-benthic coupling in the littoral system (Chiantore *et al*. 1998) and is an important secondary producer in the Antarctic benthic system (Ansell *et al*. 1998), insult or chronic stress disrupting the population structure of *A. colbecki* may also significantly affect higher ecological levels of the Antarctic nearshore marine ecosystem (Fossi 2000).

Cholinesterases (ChEs) are a class of serine hydrolases ubiquitous in the animal kingdom and are presumed to have evolved through mutation of an ancestral esterase form, achieving higher specificity for hydrolyzing choline esters. They play a prominent role in nerve impulse transmission (Silver 1974, Massoulié *et al*. 1993). Vertebrate ChEs are now classified as acetylcholinesterase (AChE, EC 3.1.1.7) and less specific butyrylcholinesterase, also known as pseudocholinesterase or non-specific esterase (BChE, EC 3.1.1.8) (Silver 1974, Massoulié & Toutant 1988). AChE hydrolyses the neurotransmitter acetylcholine (ACh) at cholinergic synapses in all living organisms and occurs in many molecular forms (Talesa *et al*. 2002). AChE is mainly, but not exclusively, present in the synaptic cleft (Massoulié *et al*. 1993) and is assumed to be a target enzyme of organophosphates (OPs) and carbamates (CBs) in most species (Boone & Chambers 1996). Recent studies provide evidence that ChE activities may also be affected by a wide range of contaminants, including heavy metals (Bocquené *et al*. 1990, Najimi *et al*. 1997, Guilhermino *et al*. 1998). Inhibition of AChE results in accumulation of the neurotransmitter ACh in neuromuscular junctions, causing hyperpolarization of the post-synaptic membrane and overstimulation of cholinergic receptors, both signs of cholinergic toxicity (Carlock *et al*. 1999, Pope 1999). While ChEs have been extensively studied in vertebrates, less research has been done on other species, particularly molluscs, where the classification is much more ambiguous. Recent attempts have been made to classify ChE types in marine invertebrata by substrate preference and sensitivity to specific inhibitors, as is done for vertebrate cholinesterases (Sturm *et al*. 1999, Chuiko 2000). The same methodology has been applied in studies involving marine bivalve species (Basack *et al*. 1998, Talesa *et al*. 2001,

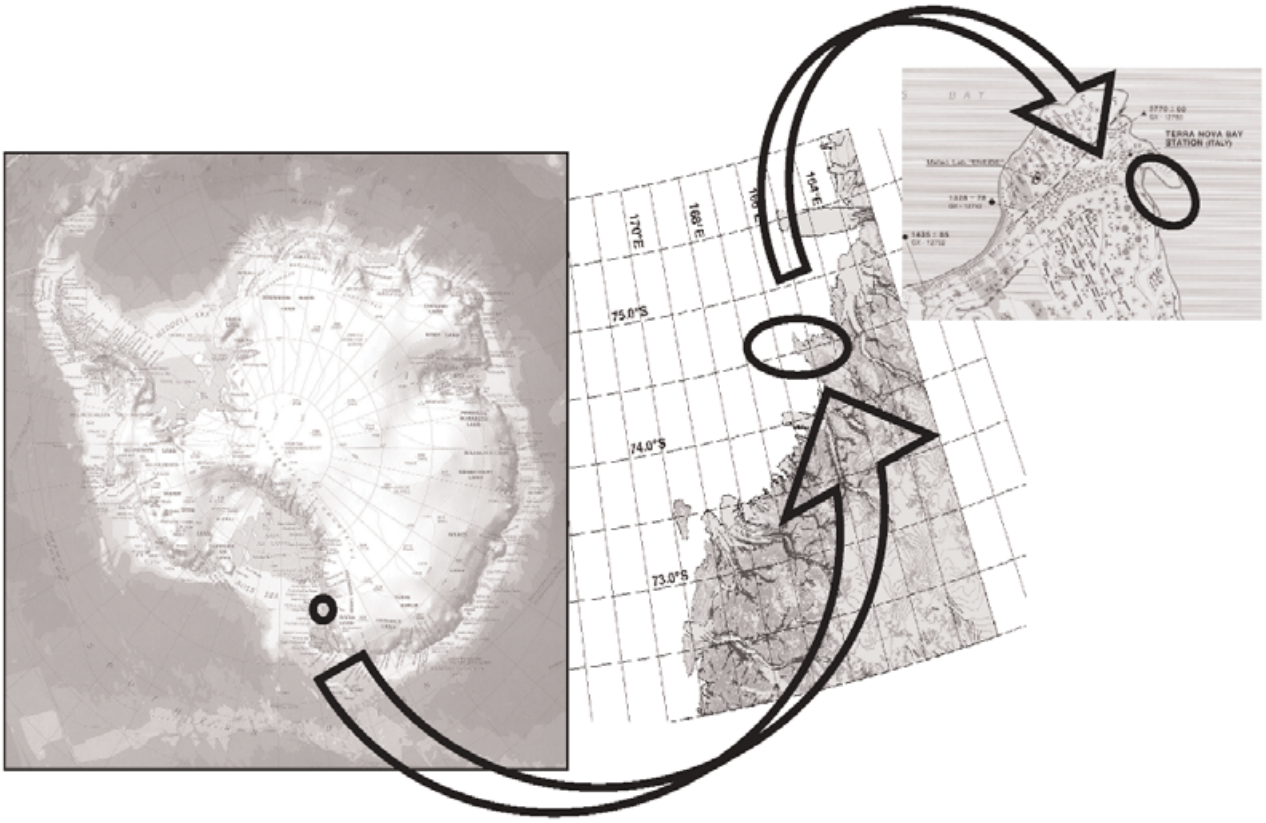

Fig. 1. Map of the Terra Nova Bay Italian scientific station in Antarctica, Ross Sea, showing location of the sampling site for *A. colbecki*.

2002, Galloway *et al*. 2002).

In view of the prominent ecological role played by ChE activities in the adductor muscle of *A. colbecki* (transmission of nerve impulses and regulation of swimming movements) the main aims of the present research were:

1) to characterize ChE activities in adductor muscle of *A. colbecki*, and
2) to investigate their sensitivity to exposure to pollutants, such as the OP insecticide diisopropyl fluorophosphate (DFP) and heavy metal Cd.

Materials and methods

Sample collection

Specimens of *A. colbecki* (n = 14) were collected in 2001, 2002 and 2003 from a coastal site at the outlet of Road Bay, near the Italian Antarctic Station BTN (now called Mario Zucchelli Station, MZS) (74°41'S, 164°04'E) (Terra Nova Bay, Ross Sea) (Fig. 1). After collection by SCUBA divers, the scallops were immediately shipped to the base, where adductor muscles were excised, frozen in liquid nitrogen to prevent enzyme deterioration and stored at -80°C until biochemical analysis.

Crude extract preparation

Adductor muscle tissue aliquots from each one of the various scallops were pooled and subsequently homogenized with buffer (0.1M Tris-HCl, pH 7.2, 0.25M sucrose, 0.1% Triton X-100) in a 1:5 ratio (w/v) and centrifuged at 2100 rpm for 10 minutes. The resulting pellet containing cell debris was discarded and the supernatant fraction used, preferably fresh, for assay of enzyme activities, or stored at -80°C. All procedures were carried out with particular concern for keeping tissues at 4°C.

Biochemical determinations

ASCh, BSCh, PrSCh, 5,5'-dithiobis-2-dinitrobenzoic acid (DTNB), Tris-HCl, Triton X-100, *tetra* (monoisopropyl) pyrophosphor-tetramide (Iso-OMPA), eserine, 1,5-bis (4-allyldimethylammoniumphenyl) pentan-3-one dibromide (BW284C51), DFP and $CaCl_2$ were obtained from Sigma Aldrich Chemie Gmbh. BIORAD Protein was purchased from Bio-Rad Laboratories Gmbh. ChE activities were measured by the method of Ellman *et al*. (1961). In general, thiocholine esters were hydrolyzed by serine-dependent esterases (ChEs) to yield thiocholine. Subsequent combination with DTNB forms a yellow anion, 5-thio-2-nitrobenzoic acid, which absorbs strongly at λ = 405 nm. Reaction rates were quantified at 30°C using a BIO-RAD microplate reader (Model 550) measuring the rate of change of absorbance at 405 nm for 5 min after addition of substrate. Three thiocholine esters (ASCh, BSCh and PrSCh) were used as substrates. Initial assay conditions in the reaction mixture (final volume 300 µl) were as follows: 25 mM Tris-HCl buffer (pH 7.6, 1 mM $CaCl_2$), 40µl DTNB (0.333 mM, final concentration) and 40 µl of sample. The reaction was started by adding 40 µl of substrate (2 mM, final concentration). Spontaneous hydrolysis was determined in the absence of supernatant. ChE activities were expressed as enzyme units x mg total proteins^{-1}. One enzyme unit (IU) was defined as the amount of enzyme which catalyses hydrolysis of 1 µmol of substrate per minute. Total proteins were measured as described by Bradford (1976), using bovine serum albumin as standard, values being expressed as mg total protein ml^{-1} supernatant.

Sensitivity to inhibitors was determined by incubating the reaction mixture at 10^{-9}–10^{-2} M range (10^{-9}–10^{-3} M range for BW284C51) for 15 min, after which residual activities were determined using ASCh. Selective inhibitors currently used to classify vertebrate ChEs were eserine, BW284C51 and iso-OMPA, considered selective for ChEs, AChE and BChE, respectively (Silver 1974, Mikalsen *et al*. 1986, Massoulié & Toutant 1988, Sturm *et al*. 1999). The OP insecticide used was DFP, a member of a class of well-known ChEs inhibitors (Ozretic & Krajnovic-Ozretic 1992, Bocquené *et al*. 1997, Talesa *et al*. 2002). For heavy metals, cadmium was tested as potentially affecting ChE activities (Galgani *et al*. 1990, Najimi *et al*. 1997). Stock solutions were prepared at 75 or 7.5 mM in suitable carrier (reaction buffer for Cd, methanol for all the others) and subsequently diluted to the desired concentrations.

Statistic analyses

All determinations were performed at least in quadruplicate and results were expressed as mean ± standard deviation. Statistical significance between means was determined using one-way analysis of variance (ANOVA). Differences with $P < 0.05$ were considered significant. Statistical analysis was carried out using Statgraphics 5.1 software (StatSoft, USA).

Results and discussion

All substrates were hydrolysed by ChEs of adductor muscle of the Antarctic scallop, in the following order: ASCh > PrSCh > BSCh. The highest ChE activities (2.29 ± 0.13 IU mg prot^{-1}) were noted when ASCh was used as substrate. ChE activities were one third this level (0.60 ± 0.05) for BSCh and one half (1.57 ± 0.14) for PrSCh.

Results are in line with previous observations reporting the occurrence of ChE activities able to cleave the above substrates in tissues of the Antarctic scallop (Bonacci *et al*. 2004). Our data seems also in line with results for many bivalve species from temperate areas, where a rank of

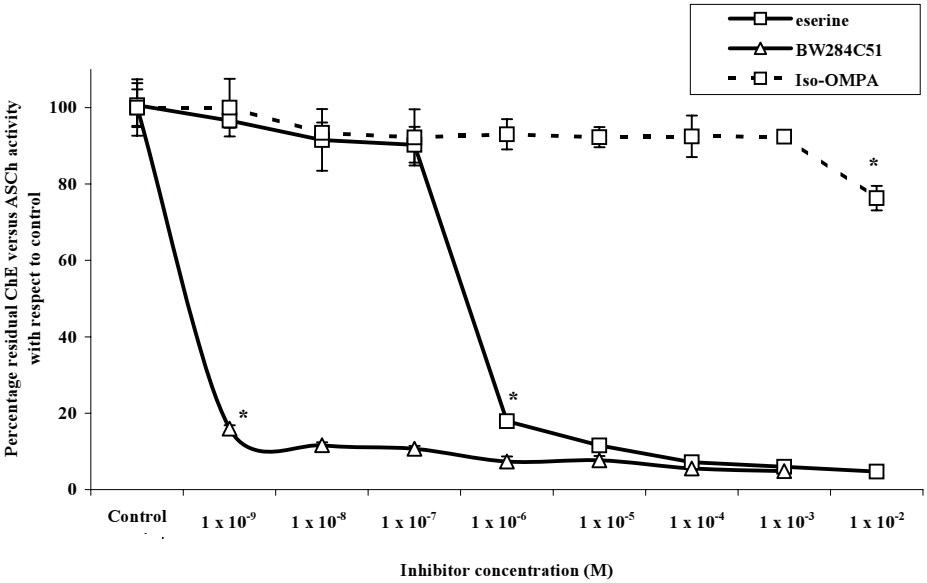

Fig. 2. Effect of 15 min *in vitro* exposure to eserine, BW284C51 and Iso-OMPA on ChE versus ASCh activity in *A. colbecki* adductor muscle crude extract. All values are mean ± standard deviation of at least four determinations expressed as percentage residual activity with respect to samples exposed to carrier only. * = exposure dose at which inhibition of activity starts to be significant with respect to controls.

substrate preference was also observed. Mora *et al*. (1999) reported higher ChE activities in S9 extract from whole body of *Mytilus galloprovincialis* (Lamarck, 1819) and *Corbicula fluminea* (Müller, 1774) using ASCh and PrSCh, and lower ChE versus BSCh activity in both organisms. Galloway *et al*. (2002) observed a similar order of substrate sensitivity in haemolymph of *Mytilus edulis* (Linnaeus, 1758): ASCh >> PrSCh > BSCh, although BSCh was the preferred substrate when homogenates of whole body were used. These discrepancies may reflect the great variability of ChE enzymes and their polymorphism in different tissues (Massoulié *et al*. 1993) which has already been reported not only in vertebrates and in invertebrates, including bivalves (Galgani *et al*. 1990, Le Bris *et al*. 1995, Bocquené *et al*. 1997, Escartin & Porte 1997, Najimi *et al*. 1997, Basack *et al*. 1998, Mora *et al*. 1999, Owen *et al*. 2002, Talesa *et al*. 2002, Brown *et al*. 2004).

Specific inhibitors

Eserine, BW284C51 and Iso-OMPA are known to selectively inhibit ChEs, AChEs and BChEs (Massoulié & Toutant 1988). *In vitro* exposure to the above inhibitors was tested on ChE activities in adductor muscle from *A. colbecki* to further distinguish enzymes occurring in the Antarctic scallop. ASCh was used as substrate (Massoulié *et al*. 1993). The results are shown in Fig. 2.

In vitro exposure to eserine (ChEs inhibitor) (10^{-9}–10^{-2} M) resulted in an IC_{50} of 1.58 x 10^{-6} M, since ChE activity was drastically inhibited at 10^{-6} M and over. Some residual activity (4.7% with respect to controls) was still present at the highest dose (10^{-2} M eserine).

The effect of BW284C51 (10^{-9}–10^{-4} M), an AChE inhibitor, on ChE versus ASCh activity resulted in an IC_{50} of 1.05 x 10^{-9} M. Marked inhibition occurred even at the lowest dose (15.9 % of residual ASCh-cleaving ChE activity at 10^{-10} M BW284C51). Residual activity was 4.8% at the highest dose (10^{-4} M).

Exposure to Iso-OMPA (BChE inhibitor) only significantly reduced ChE versus ASCh activity (76.3% of residual activity) at the highest concentration (10^{-2} M). Lower doses did not significantly affect ChE.

Inhibition were ranked BW284C51 > eserine > Iso-OMPA for capacity to inhibit ChE versus ASCh activity in tissues of *A. colbecki*.

Our results are in line with those obtained in a previous study in which ChE activity in adductor muscle of the Antarctic scallop exposed to Iso-OMPA (3 mM) was slightly inhibited (21%) when ASCh was used as substrate. Moreover, exposure to the same concentration of BW284C51 resulted in almost complete inhibition (> 90%) of ChE vs ASCh activity in gills (Corsi *et al*. 2004a, 2004b).

These results are in line with studies carried out in several bivalve species from temperate areas. Escartin & Porte (1997) reported only a negligible inhibitory effect (14–16%) of Iso-OMPA (10^{-4} M) on ASCh-cleaving ChE activity in gills and digestive gland of *M. galloprovincialis*. In the same study, exposure to eserine in the concentration range 10^{-6}–10^{-4} M resulted in a dose-dependent inhibitory effect on ChE versus ASCh activity in both tissues. Much lower levels of inhibition were recorded in tissues of the Mediterranean mussel than in the Antarctic scallop; however ChE versus ASCh activities in tissues of *M. galloprovincialis* were far more sensitive to eserine than Iso-OMPA, as we observed in *A. colbecki*. Galloway *et al*. (2002) reported IC_{50} < 10^{-5} M for eserine in *M. edulis* haemolymph which is higher than recorded by us in adductor muscle. A higher sensitivity to BW284C51 than to eserine was also reported by Talesa *et al*. (2001) for ChE isoforms purified from haemolymph and whole body of *M. galloprovincialis*.

Since the inhibition by eserine is a characteristic feature

of ChEs (Massoulié & Toutant 1988) enzyme activity versus ASCh is presumably totally or largely due to ChEs, ruling out activity due to any other classes of esterases, such as A-esterases, carboxylesterases (CbEs) and C-esterases, that may occur in tissues of the Antarctic scallop. Moreover, the above enzymes are known to have low specificity towards thiocholine esters (Maxwell 1992). A similar hypothesis was also formulated by Valbonesi *et al*. (2003) with regard to ASCh-cleaving activities in gills of *M. galloprovincialis* and *Ostrea edulis* (Linnaeus, 1758).

High sensitivity to inhibition by BW284C51 is a feature of vertebrate AChE (Silver *et al*. 1974); indeed, Mikaelsen *et al*. (1986) reported total inhibition of true-AChE activity in rat plasma and cerebral cortex after incubation for 30 min with 10^{-6} M BW284C51. We therefore hypothesize that enzymes which contribute to ASCh-cleaving activity in adductor muscle of *A. colbecki* share at least the main characteristics of true AChE, in line with our previous hypothesis on the presence of AChE in the Antarctic scallop (Corsi *et al*. 2004a).

In addition, low sensitivity to Iso-OMPA is a feature of vertebrate AChE. This inhibitor is often used to distinguish AChE and BChE activities (Massoulié & Toutant 1988). For instance, in avian species, total inhibition of BChE activity occurred after *in vitro* exposure of mixed plasma enzymes to 10^{-5} M iso-OMPA for 5 min. (reviewed by Fairbrother *et al*. 1991). This seems to confirm that the ASCh-cleaving activity in *A. colbecki* adductor muscle may be due to ChEs with at least the main of true AChE. A similar point of view is sustained by Escartin & Porte (1997), who considered residual ASCh-cleaving activity in gills and digestive gland of *M. galloprovincialis* following *in vitro* exposure to Iso-OMPA (10^{-4} M) to be due to AChE. They concluded that the ASCh-dependent ChE activities in this bivalve respond to inhibitors in a similar way to true-AChE.

On the basis of its substrate preference for ASCh, high sensitivity to eserine and BW284C51 and low sensitivity to Iso-OMPA, we infer that the ChE versus ASCh activity in adductor muscle of *A. colbecki* can be classified as an AChE-type enzyme. The presence of more than one ChE form, contributing to the observed hydrolysis of ASCh, is suggested by detectable ChE activity towards substrates other than ASCh.

Aquatic pollutants

Figure 3 shows the effects of *in vitro* exposure to DFP and $CdCl_2$ (in the concentration ranges of 10^{-9}–10^{-3} M and 10^{-9}–10^{-2} M, respectively). DFP elicited a significant reduction in ChE versus ASCh activity (74.7% residual activity) at 10^{-3} M, while lower doses did not significantly affect ChEs.

The present results seem to confirm our previous report on the effects of *in vitro* exposure to OP insecticides on *A. colbecki* ChEs (Corsi *et al*. 2004b). Significant concentration-dependent inhibition of ChE versus ASCh activity was observed in gills of the Antarctic scallop after pre-incubation with 0.1–60 μM chlorpyrifos. Almost total inhibition was observed at the highest dose (IC_{50} = 10^{-9} M) which accounts for the susceptibility of *A. colbecki* ChEs to inhibition by OP insecticides.

The observed low sensitivity of ChE versus ASCh activity in adductor muscle seems to suggest that sensitivity to OPs is variable, depending on the pesticide to which the organism is exposed, as shown by several studies with mussels (Galgani *et al*. 1990, Bocquené *et al*. 1997, Basack *et al*. 1998, Valbonesi *et al*. 2003). It may also be hypothesised that ChE activities in adductor muscle are resistant to OPs, unlike the enzymes found in gills (Corsi *et al*. 2004b). Similar conclusions were made in several bivalves following exposure to pesticides which showed differences in sensitivity of ChEs in different tissues (Escartin & Porte 1997, Mora *et al*. 1999). Similar results

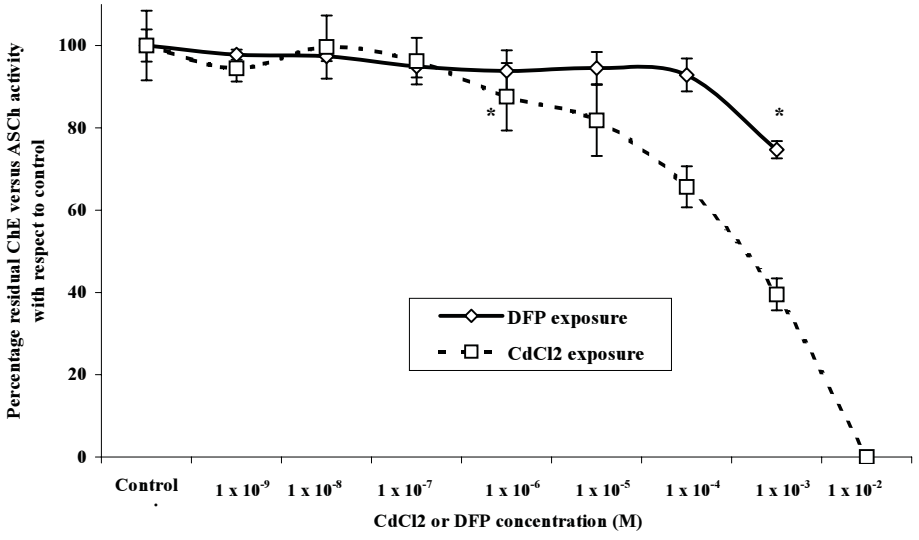

Fig. 3. Effect of 15 min *in vitro* exposure to DFP and $CdCl_2$ on ChE versus ASCh activity in *A. colbecki* adductor muscle crude extract. All values are mean ± standard deviation of at least four determinations expressed as percentage residual activity with respect to samples exposed to carrier only. * = exposure dose at which inhibition of activity starts to be significant with respect to controls.

were obtained in other bivalve species, in which DFP (10^{-9}–10^{-2} M) was found ineffective in inhibiting ChEs purified from hemolymph and whole body of *M. galloprovincialis* (Talesa *et al*. 2001). Talesa *et al*. (2002) also reported IC_{50} values ranging from not detectable to 10^{-6} to 10^{-4} in the bivalve clam *Scapharca inaequivalvis* Bruguière (1789), inferring that this relatively low sensitivity to DFP may be an adaptive response of specimens to a polluted environment. On the other hand, Galloway *et al*. (2002) found an IC_{50} of 8.2 x 10^{-4} M for ChE versus ASCh activity in hemolymph of *M. edulis* after 15 min exposure *in vitro* to DFP, while 90% inhibition was elicited by 1 mM DFP. Our results suggest that ChE activities in adductor muscle of *A. colbecki* have lower sensitivity to DFP exposure than those of other bivalve species. ChE forms resistant to OPs and mechanisms such as detoxification by carboxylesterases (CbEs) and A-esterases could be involved in this tolerance (Gupta *et al*. 1985, Boone & Chambers 1996).

ChE versus ASCh activity was inhibited by $CdCl_2$ in a dose-dependent manner with an IC_{50} of 4.57 x 10^{-4} M. The activity was significantly inhibited (87.6% residual activity) for concentrations of 10^{-6} M and over. The highest concentrations of $CdCl_2$ resulted in total depletion of activity.

Although the mechanism by which ChEs are inhibited by heavy metals is unclear, our results sustain the hypothesis that metal cations inactivate AChE and BChE (Masson *et al*. 1996, Guilhermino *et al*. 1998). Cd^{2+} is known to inhibit BChE from human serum (Sarkarati *et al*. 1999) and sheep brain (Çokuğras & Tezcan 1993) and it has been suggested that human serum BChE has more than one binding motif for divalent cations (Bhanumathy & Balasubramanian 1996, 1998).

In our previous study, *in vivo* exposure to Zn^{2+} (400 ppm) did not seem to affect ChE activities in the Antarctic scallop, as only slight, non significant differences in ASCh- or BSCh-cleaving ChE activities were observed between exposed and control organisms (Corsi *et al*. 2004b).

Various studies have demonstrated the potential of Cd^{2+} exposure to affect ChE activities in bivalve species: significant inhibition of ChE versus ASCh activity with respect to controls was found in *M. galloprovincialis* and *Perna perna* (Linnaeus, 1758) exposed to $CdCl_2$ at 10^{-2} M, whereas a slight non-significant decrease was observed for Cd^{2+} at a dose of 10^{-7} M (78% and 83% residual activity in *M. galloprovincialis* and *P. perna*, respectively) (Najimi *et al*. 1997). Bocquené *et al*. (1990) incubated crude extract from *M. edulis* with $CdCl_2$ 10^{-3} M for 40 min and observed a 17% reduction in ChE versus ASCh activity with respect to unexposed samples.

To sum up, the results of the present study suggest that ChE activities in adductor muscle of *A. colbecki* are sensitive to Cd^{2+} and susceptibility seems to be higher than in other bivalve species studied to date. Direct comparison with previous studies is difficult, however, due to differences in exposure protocols, biochemical determination techniques and the tissues investigated. Moreover, it is not entirely legitimate to draw conclusions about ChE sensitivity to metals, as different enzyme behaviours have been shown to depend on whether exposure is *in vivo* or *in vitro* (Thaker & Haritios 1989, Najimi *et al*. 1997). Nevertheless, the present results suggest some sensitivity of ChE activities in adductor muscle of the Antarctic scallop to Cd^{2+} exposure, also highlighting the potential of the use of such enzyme system in *A. colbecki* as a biomarker for monitoring Antarctic marine anthropogenic impacts.

What could be the effect on ChE activities in wild scallops? High Cd^{2+} levels have been reported in Antarctic marine waters (Bargagli *et al.* 1996, Bargagli 2000) and the significant ability of *A. colbecki* to bioaccumulate cadmium (up to 2.8 and 26.51 μg Cd g^{-1} wet weight in gills and digestive gland, respectively, Viarengo *et al*. 1993, about 1.0 and 100.0 μg Cd g^{-1} dry weight, in muscle and digestive gland, respectively, Bargagli *et al*. 1996) suggests that it might exert a significant toxicological effect on sensitive enzymes. Nevertheless, Viarengo *et al.* (1993) observed a statistically significant increase of the metallothioneins content in the gills of *A. colbecki* specimens *in vivo* exposed to 625 nM copper for three days. Consequently, it was suggested that metallothioneins may play a fundamental role in the studied species in the buffering of intracellular metal, ensuring a correct detoxification of the excess of heavy metal cations. The above might be a way for *A. colbecki* to avoid Cd (or other heavy metals) excess to cause severe physiological dysfunctions, such as affection of ChE activities.

Nevertheless, since the prominent physiological role played by ChE enzymes in adductor muscle enables swimming movements, our results show that *in vivo* studies and more detailed knowledge on the sensitivity of these enzymes to heavy metals are needed.

Conclusions

The present results confirm the occurrence of ChE activities in adductor muscle of the Antarctic scallop *A. colbecki*. Patterns of response to exposure to specific inhibitors suggest that these enzymes share certain features with true AChE. The low susceptibility to the OP insecticide, DFP, of enzymes from adductor muscle is in line with results from other bivalves from temperate areas. On the other hand, ChE activity was found to be sensitive to Cd exposure and this has toxicological implications due to the high levels to which this metal concentrates in the Antarctic scallop. Further experiments in which *A. colbecki* is exposed to other pesticides and heavy metals *in vivo* and *in vitro* are needed before any other conclusion regarding the sensitivity of these enzyme activities can be drawn.

Acknowledgements

The present study was performed in the framework of the Italian National Programme for Antarctic Research (PNRA) and is part of S. Bonacci's PhD. thesis. We thank Dr Helen Ampt for revising the English and Dr Mauro Alberti for supplying Antarctic maps. We thank the referees, especially Dr Mariachiara Cantore for his considerable help improving this paper.

References

Ansell, A.D., Cattaneo-Vietti, R. & Chiantore, M. 1998. Swimming in the Antarctic scallop *Adamussium colbecki*: analysis of *in situ* video recordings. *Antarctic Science*, **10**, 369–375.

Bargagli, R., Nelli, L., Ancora, S. & Focardi, S. 1996. Elevated cadmium accumulation in marine organisms from Terra Nova Bay (Antarctica). *Polar Biology*, **16**, 513–520.

Bargagli, R. 2000. Trace metals in Antarctica relate to climate change and increasing human impact. *Review in Environmental Contamination and Toxicology*, **166**, 129–173.

Basack, S.B., Oneto, M.L., Fuchs, J.S., Wood, J.S. & Kesten, E.M. 1998. Esterases of *Corbicula fluminea* as biomarker of exposure to organophosphorous pesticides. *Bulletin of Environmental Contamination and Toxicology*, **61**, 569–576.

Berkman, P.A. 1990. The population biology of the Antarctic scallop *Adamussium colbecki* (Smith, 1902) at New Harbor, Ross Sea. *In* Kerry, K.R. & Hempel, G., *eds*. *Antarctic ecosystems: ecological change and conservation.* Berlin: Springer, 281–288.

Berkman, P. A. & Nigro, M. 1992. Trace metals concentrations in scallops around Antarctica: extending the Mussel Watch Programme to the Southern Ocean. *Marine Pollution Bulletin*, **24**, 322–323.

Bhanumathy, C.D. & Balasubramanian, A.S. 1996. Evidence for a Zn^{2+} binding site in human serum butyrylcholinesterase. *Biochemical Journal*, **315**, 127–131.

Bhanumathy, C.D. & Balasubramanian, A.S. 1998. Selective inactivation of butyrylcholinesterase with metal chelators suggests there is more than one metal binding site. *The International Journal of Biochemistry & Cell Biology,* **30**, 695–705.

Bocquené, G., Galgani, F. & Truquet, P. 1990. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. *Marine Environmental Research*, **30**, 75–89.

Bocquené, G., Roig, A. & Fournier, D. 1997. Evidence for the presence of a soluble acetylcholinesterase insensitive to organophosphate and carbamate indicators. *FEBS Letters*, **407**, 261–266.

Bonacci, S., Browne, M.A., Dissanayake, A., Hagger, J.A., Corsi, I., Focardi, S., Galloway, T.S. 2004. Esterase activities in the bivalve mollusc *Adamussium colbecki* as a biomarker for pollution monitoring in the Antarctic marine environment. *Marine Pollution Bulletin*, **49**, 445–455.

Boone, J.S. & Chambers, J.E. 1996. Time course of inhibition of cholinesterase and aliesterase activities, and non protein sulfhydryl levels following exposure to organophosphorus insecticides in mosquitofish *(Gambusia affinis*). *Fundamentals and Applied Toxicoogy*, **29**, 202–207.

Bordin, G., Appiou, U. & Treguer, P. 1987. Repartitions horizontale et verticale du cuivre, du manganese et du cadmium dans le secteur indien de l'Ocean Antarctique. *Oceanological Acta*, **10**, 411–420.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248–254.

Brooks, R.R. & Rumsby, M.G. 1965. The biogeochemistry of trace metals element uptake by some New Zealand bivalves. *Limnology and Oceanography,* **10**, 521–527.

Brown, M., Davies, I.M., Moffat, C.F., Redshaw, J. & Craft, J.A. 2004. Characterization of coline esterases and their tissue and subcellular distribution in mussel (*Mytilus edulis*). *Marine Environmental Research*, **57**, 155–169.

Carlock, L.L., Chen, W.L., Gordon, E.B., Killeen, J.C., Manley, A., Meyer, L.S., Mullin, L.S., Pendino, K.J., Percy, A., Saragent, D.E. & Seaman, L.R. 1999. Regulating and assessing the risk of cholinesterase-inhibiting pesticides: divergent approaches and interpretations. *Journal of Toxicology and Environmental Health*, **2**, 105–160.

Cerrano, C., Puce, S., Chiantore, M., Bavestrello, G. & Cattaneo-Vietti, R. 2001. The influence of the epizoic hydroid *Hydractinia angusta* on the recruitment of the Antarctic scallop *Adamussium colbecki*. *Polar Biology*, **24**, 577–581.

Chiantore, M., Cattaneo-Vietti, R, Albertelli, G., Misic, C. & Fabiano, M. 1998. Role of filtering and biodeposition by *A. colbecki* in circulation of organic matter in Terra Nova Bay (Ross Sea, Antarctica). *Journal of Marine Systems*, **17**, 411–424.

Chiantore, M., Cattaneo-Vietti, R., Povero, P. & Albertelli, G. 1999. The population structure and ecology of the Antarctic scallop *Adamussium colbecki* in Terra Nova Bay. *In* Faranda, F., Guglielmo, L. & Ianora, A. *eds*. *Ross Sea ecology.* Milan: Springer, 563–573.

Chuiko, G.M. 2000. Comparative study of acetylcholinesterase and butyrylcholinesterase in brain and serum of several fish: specific activities and in vitro inhibition by DDVP, an organophosphorous pesticide. *Comparative Biochemistry and Physiology*, **127**, 233–242.

Çokugras, A.N. & Tezcan, E.F. 1993. Inhibition kinetics of brain butyrylcholinesterase by Cd^{2+} and Zn^{2+}, Ca^{2+} or Mg^{2+} reactivates the inhibited enzyme. *International Journal of Biochemistry*, **25**, 1115–20.

Corsi, I., Bonacci, S., Santovito, G., Chiantore, Castagnolo, L. & Focardi, S. 2004a. Preliminary investigation on cholinesterases activity and gonadal histology in *Adamussium colbecki* from Terra Nova Bay. *Chemistry and Ecology*, **20**, S79–S87.

Corsi, I., Bonacci, S., Santovito, G., Chiantore, M., Castagnolo, L. & Focardi, S. 2004b. Cholinesterase activities in the Antarctic scallop *Adamussium colbecki*: tissue expression and effect of $ZnCl_2$ exposure. *Marine Environmental Research*, **58**, 401–406.

Dell, R.K. 1972. Antarctic benthos. *Advances in Marine Biology*, **10**, 1–216.

Ellman, G.L., Courtney, K.D., Andres Jr., V. & Featherstone, R.M. 1961. A new rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **7**, 88–95.

Escartin, E. & Porte, C. 1997. The use of cholinesterase and carboxylesterase activities from *Mytilus galloprovincialis* in pollution monitoring. *Environmental Toxicology and Chemistry*, **16**, 2090–2095.

Fairbrother, A., Marden, B.T., Bennet, J.K., Hooper, M.J. 1991. Methods used in determination of cholinesterase activity. *In* Mineau, P., *ed*. *Chemicals in agriculture.* Vol. 2*: Cholinesterase–inhibiting insecticides*. New York: Elsevier Science, 37–71.

Focardi, S., Bargagli, R. & Corsolini, S. 1993. Organochlorines in antarctic marine food chain at Terra Nova Bay (Ross Sea). *Korean Journal of Polar Research*, **4**, 73–77.

Fossi, M.C. 2000. *Biomarkers: strumenti di diagnosi e prognosi ambientale*. Firenze: Rosini Editrice S.r.l., 135 pp.

Fuoco, R., Colombini, M.P. & Abete, C. 1991. Evaluation of pack melting effect on polychlorobiphenyl content in sea water samples from Terra Nova Bay, Ross Sea (Antarctica). *Annali di Chimica*, **81**, 383–394.

Fuoco, R., Colombini, M.P., Ceccarini & A., Abete, C. 1996 Polychlorobiphenyls in Antarctica. *Microchemical Journal*, **54**, 384–390.

Galgani, G., Galgani, F. & Truquet, P. 1990. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. *Marine Environmental Research*, **30**, 75–89.

Galloway, T.S., Millward, N., Browne, M.A., Depledge, M.H. 2002. Rapid assessment of organophosphorous / carbamate exposure in the bivalve mollusc *Mytilus edulis* using combined esterase activities as biomarkers. *Aquatic Toxicology*, **61**, 169–180.

Guilhermino, L., Barros, P., Silva, M.C., Soares, A.M.V.M. 1998. Should the use of inhibition of cholinesterases as a specific biomarker for organophosphate and carbamate pesticides be questioned? *Biomarkers*, **3**, 157–163.

Gupta, R.C., Patterson, G.T. & Dettbarn, W.-D. 1985. Mechanism involved in the development of tolerance to DFP toxicity. *Fundamental of Applied Toxicology,* **4**, S96–S105.

Le Bris, H., Maffart, P., Bocquené, G., Buchet, V., Galgani, F. & Blanc, G. 1995. Laboratory study on the effect of dichlorvos on two commercial bivalves. *Aquaculture*, **138**, 139–144.

Masson, P., Froment, M.T., Bartels, C.F. & Lockridge, O. 1996. Asp 70 in the peripheral anionic site of human butyrylcholinesterase. *European Journal of Biochemistry*, **235**, 36–48.

Massoulié, J. & Toutant, J.-P. 1988. Vertebrate cholinesterases: structures and types of interaction. *Handbook of Experimental Pharmacology*, **86**, 167–224.

Massoulié, J., Pezzementi, L., Bon, S., Krejci, E. & Vallette, F.-M. 1993. Molecular and cellular biology of cholinesterases. *Progress in Neurobiology*, **41**, 31–91.

Mauri, M., Orlando, E., Nigro, M. & Regoli, F. 1990. Heavy metals in the Antarctic scallop *Adamussium colbecki*. *Marine Ecology Progress Series*, **67**, 27–33.

Maxwell, D.M. 1992. Detoxication of organophosphorous compounds by carboxylesterases. *In* Chambers, J.E. & Levi, P.E., *eds. Organophosphate, chemistry, fate and effects*. San Diego, CA: Academic Press, 183–199.

Mikalsen, A., Andersen, R.A. & Alexander, J. 1986. Use of ethropropazine and BW284C51 as selective inhibitors for cholinesterases from various species. *Comparative Biochemistry and Physiology C Comparative Pharmacology*, **83**, 447–449.

Mora, P., Michel, X. & Narbonne, J.-F. 1999. Cholinesterase activity as a potential biomarker in two bivalves. *Environmental Toxicology and Pharmacology*, **7**, 253–260.

Najimi, S., Bouhaimi, A., Daubèze, M., Zekhnini, A., Pellerin, J., Narbonne, J.F. & Moukrim, A. 1997. Use of acetylcholinesterase in *Perna perna* and *Mytilus galloprovincialis* as a biomarker of pollution in Agadir Marine Bay (south of Morocco). *Bulletin of Environmental Contamination and Toxicology,* **58**, 901–908.

Nolting, R.F., De Baar, H.J.W., van Bennekom, A.J. & Masson, A. 1991. Cadmium, copper and iron in the Scotia Sea, Weddell Sea and Weddell/Scotia confluence (Antarctica). *Marine Chemistry,* **35**, 219–243.

Owen, R., Buxton, L., Sarkis, S., Toaspern, M., Knap, A. & Depledge, M. 2002. An evaluation of hemolymph cholinesterase activities in the tropical scallop, *Euvola* (*Pecten*) *ziczac*, for the rapid assessment of pesticide exposure. *Marine Pollution Bulletin*, **44**, 1010–1017.

Ozretic, B. & Krajnovic-Ozretic, M. 1992. Esterase heterogeneity in mussel *Mytilus galloprovincialis*: effects of organophosphate and carbamate pesticides *in vitro*. *Comparative Biochemistry and Physiology C Comparative Pharmacology*, **103**, 221–225.

Palmer, J.B. & Rand, G.M. 1977. Trace metal concentration in two shellfish species of commercial importance. *Bulletin of Environmental Contamination and Toxicology*, **18**, 512–519.

Peterson, C.H., Ambrose, W.G. & Hunt, J.H. 1982. A field test of the swimming response of the bay scallop (*Argopecten irradians*) to changing biological factors. *Bulletin of Marine Science*, **32**, 939–944.

Pope, C.N. 1999. Organophosphorous pesticides: Do they all have the same mechanism of toxicity? *Journal of Toxicology and Environmental Health-Part B Critical Reviews*, **2**, 161–181.

Rainbow, P.S. 1989. Copper, cadmium and zinc concentrations in oceanic amphipod and euphasiid crustaceans, as a source of heavy metals to pelagic seabirds. *Marine Biology*, **103**, 513–518.

Sanchez-Hernandez, J.C. 2000. Trace element contamination in Antarctic ecosystems. *Review of Environmental Contamination and Toxicology*, **166**, 83–127.

Sarkarati, B., Çokugras, A.N. & Tezcan, E.F. 1999. Inhibition kinetics of human serum butyrylcholinesterase by Cd, Zn and Al: comparison of the effects of metal ions on cholinesterases. *Comparative Biochemistry and Physiology - Part C Comparative Pharmacology*, **122**, 181–190.

SCAR 1994. Coastal and shelf ecology of the Antarctic Sea Ice Zone. Science plan and implementation plan. *SCAR Report*, No. 10, 20 pp.

SCAR 1996. Monitoring of environmental impacts from science and operations in Antarctica. *Scientific Committee on Antarctic Research (SCAR), Council of Managers of National Antarctic Programs (COMNAP)*, July 1996, 110 pp.

Silver, A. 1974. *The biology of cholinesterases*. Amsterdam: North Holland Publishing, 596 pp.

Stocchino, C. 1992. Attività svolte dall'Italia nel campo dell'oceanografia fisica in Antartide nel periodo 1986–1990. *In* Gallardo, V.A., Ferretti, O & Moyano, H.G.I., *eds*. *Oceanografia in Antartide*. Conception, Chile: ENEA, 13–20.

Sturm, A., da Silva de Assis, H.C. & Hansen, P.-D. 1999. Cholinesterases of marine teleost fish: enzymological characterization and potential use in the monitoring of neurotoxic contamination. *Marine Environmental Research*, **47**, 389–398.

Talesa, V., Romani, R., Antognelli, C., Giovannini, E. & Rosi, G. 2001. Soluble and membrane-bound acetylcholinesterase in *Mytilus galloprovincialis* (Pelecypoda: Filibranchia) from the northern Adriatic Sea. *Chemico-Biological Interactions*, **134**, 151–166.

Talesa, V., Romani, R., Antognelli, C., Giovannini, E. & Rosi, G. 2002. Different expressions of organophosphate-resistant acetyl-cholinesterases in the bivalve mollusk *Scapharca inaequivalvis* living in three different habitats. *Environmental Toxicology and Chemistry*, **21**, 102–108.

Thaker, A.A. & Haritos, A.A. 1989. Mercury bioaccumulation and effects onm soluble peptides, proteins and enzymes in the hepatopancreas of the shrimp *Callianassa thyrrene*. *Comparative Biochemistry and Physiology - Part C Comparative Pharmacology*, **94**, 199–205.

Vacchi, M., Cattaneo-Vietti, R., Chiantore, M. & Dalu, M. 2000. Predator-prey relationship beyween the nototheniid fish *Trematomus bernacchii* and the Antarctic scallop *Adamussium colbecki* at Terra Nova Bay (Ross Sea). *Antarctic Science*, **12**, 64–68.

Valbonesi, P., Sartor, G. & Fabbri, E. 2003. Characterization of cholinesterase activity in three bivalves inhabiting the North Adriatic sea and their possible use as a sentinel organisms for biosurveillance programmes. *Science of the Total Environment,* **312**, 79–88.

Viarengo, A., Canesi, L., Mazzucotelli, A. & Ponzano, E. 1993. Cu, Zn and Cd content in different tissues of the Antarctic scallop *Adamussium colbecki*: role of metallothionein in heavy metal homeostasis and detoxication. *Marine Ecology Progress Series,* **95**, 163–168.

Viarengo, A., Ponzano, E., Pondero, F., Fabbri, R. 1997. A Simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. *Marine Environmental Research*, **1**, 69–84.