Marine Ecology. ISSN 0173-9565

ORIGINAL ARTICLE

Adaptation of the antioxidant defence system in hydrothermal-vent mussels (*Bathymodiolus azoricus*) transplanted between two Mid-Atlantic Ridge sites

Rui Company[1], Angela Serafim[1], Richard Cosson[2], Aline Fiala-Médioni[3], David R. Dixon[4] & Maria João Bebianno[1]

1 CIMA, Faculty of Marine and Environmental Sciences, University of Algarve, Faro, Portugal
2 Laboratory of Marine Biology, University of Nantes, ISOMER, Nantes, France
3 University P.M. Curie, Observatoire Océanologique, Banyuls sur Mer, France
4 National Oceanography Centre, Waterfront Campus, European Way, Southampton, UK

Keywords
Antioxidant enzymes; *Bathymodiolus azoricus*; hydrothermal vents; lipid peroxidation; transplant experiment.

Correspondence
M.J. Bebianno, CIMA, Faculty of Marine and Environmental Sciences, University of Algarve, Faro, Portugal.
E-mail: mbebian@ualg.pt

Accepted: 19 October 2006

doi:10.1111/j.1439-0485.2006.00125.x

Abstract

The vent mussel *Bathymodiolus azoricus* is the dominant member of the Northern Mid-Atlantic Ridge (MAR) hydrothermal megafauna, and lives in an environment characterized by temporal and spatial variations in the levels of heavy metals, methane and hydrogen sulphide, substances which are known to increase reactive oxygen species levels in the tissues of exposed organisms. To evaluate the effects of two contrasting hydrothermal environments on the antioxidant defence system of this vent mussel species, a 2-week transplant experiment was carried out involving mussels collected from the relatively deep (2300 m), and chemical rich, Rainbow vent field. These were transplanted to the shallower (1700 m), and relatively less toxic, Lucky Strike vent field. To achieve this objective, levels of superoxide dismutase, catalase (CAT), total glutathione peroxidase (GPx), selenium-dependent glutathione peroxidase and lipid peroxidation (LPO) were measured in the gills and mantle tissues of resident and transplant mussels before and after the transplant experiment. With the exception of CAT, the gills of the transplanted mussels had significantly higher antioxidant enzyme activity compared with the basal levels in the donor (Rainbow) and recipient (Lucky Strike) populations; whereas the antioxidant enzyme levels in the mantle tissues of the transplants reflected the baseline levels of activity in the native Lucky Strike mussels after 2 weeks. In contrast, LPO levels were significantly higher in both tissue types in the transplants than in either the source or the recipient populations, which suggested a response to hydrostatic pressure change (note, the transplant animals were brought to the surface for transportation between the two vent fields). The fact that the Rainbow mussels survived the transplant experience indicates that *B. azoricus* has a very robust constitution, which enables it to cope behaviourally, physiologically and genetically with the extreme conditions found in its naturally contaminated deep-sea environment.

Problem

Although hydrothermal vent environments are characterized by extremely high temperatures, low oxygen and are rich in potentially harmful chemical species, mainly hydrogen sulphide (H_2S), methane (CH_4) and heavy metals (Mn, Fe, Cu, Zn, Ag and Cd), they are paradoxically home for some luxuriant animal communities

(Lonsdale 1977; Corliss *et al.* 1979). The mussel *Bathymodiolus azoricus* is one of the most common species at MAR vents sites and its capacity to survive in a metal-rich and highly variable environment has been extensively studied over the past decade (*e.g.* Cosson 1997; Géret *et al.* 1998; Pruski & Dixon 2003).

During recent years, measurement of chemical compounds (metals and others) in pure end-member hydrothermal vent fluids was able to better characterize the extreme environmental conditions of the vent sites (Table 1). A large range of temperature (185–324 °C) and pH (3.4–5.0) is observed in Lucky Strike fluids compared with other Mid-Atlantic Ridge vent sites (Douville *et al.* 2002) (Table 1). Lucky Strike is relatively depleted in CH_4 (0.5–0.97 mM), while high concentrations of H_2S (0.6–3.4 mM) are present in the fluids compared with Rainbow (Douville *et al.* 2002). Lower metal content is found in Lucky Strike fluids than those found in Rainbow, particularly for Ag (4.7–25 nM), Cd (18–79 nM), Cu (2–30 μM), Fe (70–920 μM), Mn (77–540 μM) and Zn (2–40 μM) (Douville *et al.* 2002) (Table 1). On the other hand, Rainbow hydrothermal fluids are uniform in composition and influenced by phase separation (Douville *et al.* 1997, 1999). Fluid temperature in Rainbow is higher (365 °C) compared with Lucky Strike, just beneath the boiling point at that depth, extremely acidic (pH = 2.8) and relatively low in H_2S content (1.0 mM) (Table 1) (Douville *et al.* 2002). The metal concentrations observed in Rainbow fluids are the highest observed in the MAR hydrothermal area, particularly for Ag (47 nM), Cd (130 nM), Cu (140 μM), Fe (24000 μM), Mn (2250 μM) and Zn (160 μM), which are several fold higher than those found in Menez-Gwen and Lucky Strike fluids (Douville *et al.* 2002) (Table 1). However, *B. azoricus* does not live near the emission of pure vent fluids, but rather in diffuse venting areas, and therefore experiences much less harsh conditions. In this context, the characterization of the fluids that surround the mussel beds is better described in Desbruyères *et al.* (2001) and reflects how microhabitat fluids can differ from pure end-member fluids. The concentrations of metals in the mussel beds are considerably lower than those reported for the end-member fluids (Desbruyères *et al.* 2001).

Table 1. Temperature, pH and concentration of chemical species in the end-member fluids of two different MAR vent fields (Lucky Strike and Rainbow) compared to average seawater (adapted from Douville *et al.* 2002).

Site	Lucky Strike 37°17′ N, 32°16′ W	Rainbow 36°13′ N, 33°54′ W	Seawater
T (°C)	185–324	365	–
pH	3.4–5.0	2.8	7.8
H_2S (mm)	0.6–3.4	1.0	~0
CO_2(mm)	8.9–28	<16	–
CH_4(mm)	0.5–0.97	2.2–2.5	~0
Ag (nm)	4.7–25	47	0.023
Cd (nm)	18 -79	130	0.7
Cl (mm)	413–554	750	546
Co (μm)	<2	13	<2
Cu (μm)	<2–30	140	0.0033
Fe (μm)	70–920	24000	0.0045
Mn (μm)	77–450	2250	0.0013
Ni (μm)	<2	3	<2
Si (mm)	8.2–16	6.9	<0.2
Zn (μm)	<2–40	160	0.028

Oxidative stress is generally defined as a disruption of the balance between the levels of oxidants (reactive oxygen species – ROS) and reductants (antioxidants) in the organisms (Granot & Kohen 2004). ROS include a variety of both radical and non-radical molecules that can be produced under natural conditions but are frequently enhanced by the presence of toxic compounds (Matés 2000). To avoid ROS-induced injury to tissues, a complex antioxidant system, consisting of both enzymatic and non-enzymatic defences, has evolved. Traditionally, antioxidants have been defined as substances that prevent the formation of ROS or other oxidants, scavenge them, or repair the damage they cause (Sies 1991; Halliwell 1995).

Metals, as well as other toxic compounds like hydrogen sulphide and methane present in hydrothermal vents, are known to increase the production of ROS. These include the superoxide anion radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($OH^{\bullet}$) (Darley-Usmar *et al.* 1995), which are subsequently detoxified by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases. SOD converts $O_2^{-\bullet}$ to H_2O_2, CAT converts H_2O_2 to water present in peroxisomes and glutathione peroxidase (GPx) detoxifies H_2O_2 and organic hydroperoxides produced by lipid peroxidation (LPO) present in mitochondria and in the cytosol.

This pro-oxidant/antioxidant balance and detoxification of potentially damaging ROS is crucial for cellular homeostasis (Winston & Di Giulio 1991; Livingstone 2001). The antioxidant defences in *B. azoricus* from five hydrothermal vent sites near Azores Triple Junction were recently found to be tissue-specific and site-dependent, reflecting the importance of the environmental metal concentrations in the oxidative status of these mussels (Bebianno *et al.* 2005). Also, the exposure of *B. azoricus* to ROS-inducing metals (Cd, Cu and Hg) in controlled laboratory experiments proved to interfere with antioxidant protection in these bivalves (Company *et al.* 2004, 2006). However, the capacity of *B. azoricus* to respond to environmental changes *in situ* was never assessed. Therefore, the aim of the present study was to evaluate the

effects of two contrasting vent fields in the adaptation of antioxidant parameters of the deep-sea mussel *B. azoricus* using a transplant experiment between two contrasting vent fields, Rainbow and Lucky Strike. During this investigation the following questions were postulated:

1 What is the adaptation potential of the antioxidant defence system in *B. azoricus* from different Mid-Atlantic Ridge hydrothermal vents?

2 Can the environment that mussels occupy influence some of their biochemical responses?

3 Can transplanted mussels change and adapt their antioxidant enzymatic systems to become protected in new environments?

4 Can the organisms prevent oxidative damage (lipid peroxidation) after being manipulated during transplant experiments?

Material and Methods

Bathymodiolus azoricus were collected from two hydrothermal vent sites located in the Azores Triple Junction (ATJ) in the MAR: Rainbow (36°13′ N; 33°54.1′ W, 2300 m) (6.76 ± 0.57 cm, n = 10) and Lucky Strike (37°17′ N; 32°16′ W, 1700 m) (7.67 ± 0.66 cm, n = 10). At the same time, another group of mussels (6.26 ± 0.92 cm, n = 10) was transplanted from Rainbow to Lucky Strike vent site using the remote operated vehicle Victor6000 during the EU-funded ATOS cruise (Sarradin *et al.* 2001) for a 2-week period (Fig. 1). The mussels used in the transplant experiment were transplanted to the sea surface in a closed, but not pressure-sealed container, at approximately 5–6 °C, and were kept on board ship in a static aquarium tank at 7 °C. It took approximately 36 h between the time of mussel recovery at Rainbow and their eventual deployment at Lucky Strike. The transplanted mussels, approximately 100 in total, were housed in a small mesh fish trap to prevent them from escaping at Lucky Strike.

Both indigenous organisms (from Rainbow and Lucky Strike) and transplanted mussels were dissected on board, with gills and mantle isolated and immediately frozen in liquid nitrogen until further analysis.

Antioxidant enzymes

Antioxidant enzymatic activities were determined in the gills (tissue + symbionts) and mantle of *B. azoricus* after homogenization in 20 mM Tris buffer, pH 7.6, containing 1 mM of EDTA, 0.5 M of saccharose, 0.15 M of KCl and 1 mM of DTT. The homogenates were centrifuged at 500 *g* for 15 min at 4 °C to precipitate large particles and centrifuged again at 12,000 *g* for 45 min at 4 °C to precipitate the mitochondrial fraction. Supernatants were purified on a Sephadex G-25 gel column to remove low molecular weight proteins.

Superoxide dismutase activity (EC 1.15.1.1) was determined by measuring the reduction of cytochrome *c* by the xanthine oxidase/hypoxanthine system at 550 nm (McCord & Fridovich 1969). One unit of SOD is defined as the amount of enzyme that inhibits the reduction of cytochrome *c* by 50%. SOD activity is expressed in U SOD mg^{-1} total protein concentrations.

Catalase activity (EC 1.11.1.6) was determined according to Greenwald (1985) by the decrease in absorbance at 240 nm because of H_2O_2consumption. The CAT activity is expressed as $mmoles \cdot min^{-1} \cdot mg^{-1}$ of total protein concentrations.

Glutathione peroxidase activities were measured following NADPH oxidation at 340 nm in the presence of excess glutathione reductase, reduced glutathione and corresponding peroxide (Lawrence & Burk 1976). The selenium-dependent glutathione peroxidase (Se-GPx) (EC 1.11.1.9) and Total GPx activities were measured by using H_2O_2 and cumene hydroperoxide respectively as substrates. GPx activities are expressed as $\mu moles \cdot min^{-1} \cdot mg^{-1}$ of total protein concentrations.

Total protein concentrations

The tissues were homogenized in 20 mM Tris buffer, pH 8.6, containing 150 mM of NaCl. The homogenates were centrifuged for 30 min at 30,000 *g* at 4 °C. Total protein concentrations were measured on supernatants by the Lowry method (Lowry *et al.* 1951) using BSA as reference

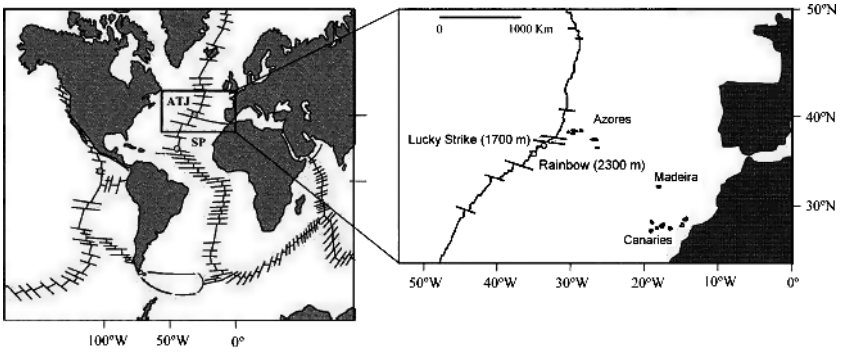

Fig. 1. Location of vent fields with detail of the Azores Triple Junction area (Lucky Strike, and Rainbow hydrothermal vent fields) (adapted from Bebianno *et al.* 2005).

standard material. Protein concentrations are expressed as $mg \cdot g^{-1}$ wet weight tissue.

Lipid peroxidation

Lipid peroxidation was determined in the supernatant used for total proteins quantification. The method described by Erdelmeier *et al.* (1998) measures the amount of malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) produced during decomposition of polyunsaturated fatty acid peroxides of membrane lipids. This procedure is based on the reaction of chromogenic reagent, N-methyl-2-phenylindole (R1), where two moles of R1 react with one mole of either MDA or 4-HNE at 45 °C for 60 min to yield a stable chromophore with maximal absorbance at 586 nm. The levels of MDA + 4-HNE were estimated at 586 nm using malonaldehyde bis (tetrametoxypropan, SIGMA) as standard. The concentrations of LPO compounds in the gills and mantle of *B. azoricus* were expressed as nmoles of MDA + 4-HNE g^{-1} total protein concentrations.

Statistical analysis was performed using STATISTICA for Windows v.5.1. The data were previously tested for normality and homogeneity and analysed by analysis of variance (ANOVA) to determine significant statistical differences between mussels from Rainbow, Lucky Strike and transplanted mussels regarding antioxidant enzymatic activity (SOD, CAT and GPx) and LPO concentrations. A Duncan test was used to determine significant differences between groups for each variable. The level of significance was set at $P < 0.05$.

Results

The activities of SOD, CAT, total GPx, Se-GPx and LPO levels in the gills and mantle of native and transplanted mussels are presented in Fig. 2. Antioxidant enzymatic activities are tissue-specific, *i.e.* SOD and CAT are mainly present in the gills (ANOVA, $F_{1,54} = 31.128$, $P < 0.001$ for SOD; ANOVA, $F_{1,54} = 78.582$, $P < 0.001$ for CAT), while total and Se-GPx was mainly in the mantle tissue (ANOVA, $F_{1,54} = 30.877$, $P < 0.001$ for T-GPx; ANOVA, $F_{1,54} = 31.128$, $P < 0.001$), which was consistent with previous findings for the same sites (Bebianno *et al.* 2005). SOD activities (cytosolic and mitochondrial) were significantly higher in Lucky Strike mussels than at Rainbow, for both tissues types (ANOVA, $F_{2,54} = 101.337$, $P < 0.001$) (Fig. 2A and B).

Mussels from Lucky Strike also showed higher GPx activities compared with those from Rainbow, but only in

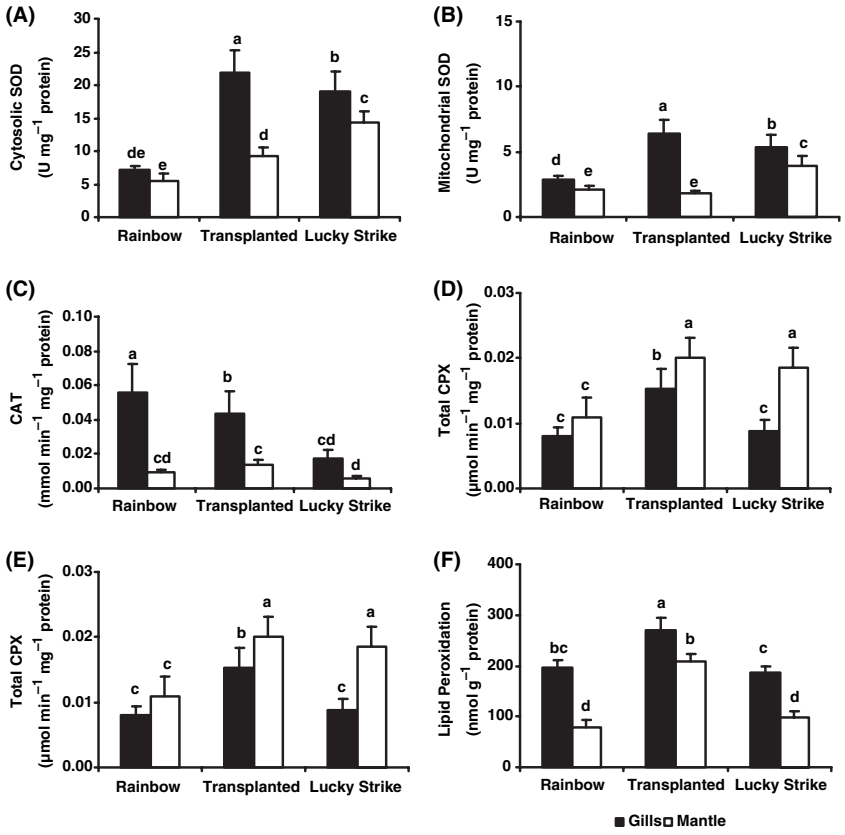

Fig. 2. The activity of cytosolic superoxide dismutase (SOD) (A), mitochondrial SOD (B), catalase (C), total glutathione peroxidase (D), selenium-dependent glutathione peroxidase (Se-GPx) (E) and lipid peroxidation (F) in the gills and mantle of native (Rainbow), host (Lucky Strike) and transplanted from Rainbow to Lucky Strike *Bathymodiolus azoricus*. Values followed by the same letter are not statistically different ($P > 0.05$) ($n = 10$).

the mantle (ANOVA, $F_{2,54} = 50.735$, $P < 0.001$), and showed no significant difference in the gills ($P > 0.05$) (Fig. 2D and E). In contrast, CAT activity was significantly higher in the gills of mussels from Rainbow (ANOVA, $F_{2,54} = 183.128$, $P < 0.001$), while no significant difference was observed in the activity of this enzyme in the mantle between the two sites ($P > 0.05$) (Fig. 2C). In the transplanted mussels (after 2 weeks), the activity of most antioxidant enzymes in the gills, excluding CAT, was significantly enhanced (induced) compared with baseline levels for either Rainbow or Lucky Strike ($P < 0.05$). CAT activity, on the other hand, showed a decrease in the gills of the transplanted mussels, compared with their original source population (Rainbow), and was higher than that in Lucky Strike mussels ($P < 0.05$) (Fig. 2B). In the mantle, cytosolic SOD, total GPx and Se-GPx followed a similar pattern, with a significant induction in transplanted mussels compared with source population, to levels close to those observed at Lucky Strike (Fig. 2A-E). Mitochondrial SOD and CAT in this tissue, however, remained unchanged in the transplanted organisms compared with their original site ($P > 0.05$) (Fig. 2B and C). LPO in all mussels was tissue-dependent (ANOVA, $F_{1,54} = 157.903$, $P < 0.001$), as it occurs for antioxidant enzymes, with higher levels in the gills ($P < 0.05$), but no significant differences were detectable between native mussels from Rainbow and Lucky Strike ($P > 0.05$). In transplanted *B. azoricus*, however, LPO concentrations in both tissues were significantly raised compared with these baseline levels ($P < 0.05$).

Discussion

The capacity of an organism to react to changes in its environment depends on the balance between the degree of short-term physiological plasticity and the limitations set by its genetic constitution. At hydrothermal vents, the spatially variable physiological characteristics of animals are often assumed to reflect short-term physiological adjustments to an irregular and dynamic chemical environment (Bergquist *et al.* 2004). Hydrothermal vents have been the focus of a new ecotoxicological perspective because of the presence of compounds considered toxic in other environments, such as hydrogen sulphide, methane, carbon dioxide and especially metals. Therefore, hydrothermal environments are considered 'Natural Pollution Laboratories' where the effects of toxic compounds can be studied in the indigenous organisms and can be used to predict the biochemical responses of coastal organisms living in highly polluted areas.

This study used a 2-week transplant experiment to assess the *in situ* adaptation potential of the antioxidant defence system in *Bathymodiolus azoricus* between two Mid-Atlantic Ridge hydrothermal vents. The transplanted mussels, originally from Rainbow, showed that most antioxidant enzymes, acquired or nearly acquired the specific activities of their host population (Lucky Strike), which demonstrated the primary role of the environment in determining the physiological characteristics of resident mussels, and also the capacity of *B. azoricus* to adapt to any externally forced changes in the abiotic character of its environment. It has already been established that metal accumulation in vent mussels is site-dependent and therefore, directly related to the composition and speciation of the metal cocktail in the vent fluid which surrounds the animals (Pruski & Dixon 2003). Also, it seems that some metal concentrations in *Bathymodiolus* tissues are related to vent field depth. A recent study showed that levels of Fe and Mn in *B. azoricus* tissues are directly related to bathymetric depth in both tissues while the opposite is the case for Cu in the mantle (Bebianno *et al.* 2005). Fe and Mn are the dominant metals in mussel tissues from Rainbow, while Zn, Cd and Ag were higher at Lucky Strike irrespective of tissue (Bebianno *et al.* 2005). Similar results were previously detected for the same species and tissues collected from Menez-Gwen and Lucky Strike (Géret *et al.* 1998; Rousse *et al.* 1998). Although mussels from Lucky Strike seem to be exposed to lower metal levels from hydrothermal fluids than those from Rainbow, they exhibit much higher SOD activity in both tissues, seemingly to counteract the production of superoxide anion radical ($O_2^{-\bullet}$), which suggests that metals alone may not explain the formation of ROS in *B. azoricus*, as already suggested by previous studies (Bebianno *et al.* 2005).

Similarly, the activity of GPx (both total and Se-dependent) was higher in mussels from Lucky Strike than Rainbow in the mantle tissue. Moreover, mussels from two environmentally different hydrothermal vent sites (Rainbow and Lucky Strike) have similar LPO levels, which implies a similar degree of membrane damage in their cells, supporting the idea that these mussels are exceptionally adapted to potentially toxic chemical species and spatial and/or temporal fluctuations in these conditions. After 2 weeks of transplantation, LPO levels in both the gills and mantle of *B. azoricus* were in fact higher than in the two native populations, suggesting that a period of adaptation is necessary when a drastic change in the environmental conditions occurs (as occurred during recovery and subsequent redeployment). In a recent study, where total oxyradical scavenging capacity was evaluated in transplanted coastal mussels (*Mytilus galloprovincialis*) in the Venice lagoon, the authors underline that the translocation of animals can be a source of additional stress and that transplantation methods require good practice to minimize stress that can affect the balance between prooxidant and antioxidant forces in the

mussels (Camus *et al.* 2004). In a similar transplant study, the LPO products (MDA) were also elevated in *M. galloprovincialis* moved from a clean site to several polluted areas in the Venice Lagoon (Pampanin *et al.* 2005).

The increase in LPO levels in transplanted *B. azoricus* seems to be followed by an induction of most of the antioxidant system (mainly SOD and GPx), which may serve to balance an increase of reactive oxidative damage inflicted as a result of hydrostatic pressure stress. It is only natural to assume that when hydrothermal organisms are transplanted between vent sites, especially those typified by markedly different depths, and in addition have to endure the added stress of surface recovery (1 bar pressure) during transit between sites, their physiology has to cope with a far greater stress than a simple chemistry change alone. However, only 2 weeks after being relocated, the antioxidant enzymes in these mussels exhibited an exceptional resemblance to those seen in the recipient population. This result shows that as a group *B. azoricus* and its relatives are extremely resistant organisms, with a great capacity to adapt to changed environmental circumstances. This has been observed previously in the field under conditions when vent activity has ceased; only mussels remain alive many months after other vent species have become extinct.

Summary

Transplant experiments using hydrothermal vent organisms are a relatively new area of research and provide a way to explore the adaptations which vent organisms have developed against a potentially hostile and fluctuating deep-sea habitat. Transplanted *Bathymodiolus azoricus*, which of necessity had to involve surface recovery at 1 bar pressure, showed a capacity to recover from this transient, albeit extreme change in conditions, which was at least partly counteracted by the induction of antioxidant defences during physiological adaptation to the new environment. These findings emphasize the importance of the environment in determining the physiological characteristics of vent mussels. In addition to confirming the findings of previous studies, these results highlight the adaptational capacity of *B. azoricus* to fluctuations in the abiotic part of its extremely hostile deep-sea environment.

Acknowledgements

The authors would like to thank Pierre M. Sarradin, chief scientist of ATOS cruise and D. Dixon, VENTOX project coordinator (EVK3 CT1999-00003) and the crew of N/O L'Atalante and Victor 6000 (IFREMER). R. Company was supported by an FCT PhD grant of the Ministry of Science and Technology of Portugal (reference SFRH/BD/904/2000). Part of this work was funded by the BICEP CSP of the National Oceanography Centre, Southampton.

References

Bebianno M.J., Company R.M., Serafim A.M., Camus L., Cosson R. & Fiala-Medioni A. (2005) Antioxidant systems and lipid peroxidation in *Bathymodiolus azoricus* from Mid-Atlantic Ridge hydrothermal vent fields. *Aquatic Toxicology*, **75**(4), 354–373.

Bergquist D.C., Fleckenstein C., Szalai E.B., Knisel J. & Fisher C.R. (2004) Environment drives physiological variability in the cold seep mussel *Bathymodiolus childressi*. *Limnology and Oceanography*, **49**(3), 706–715.

Camus, L., Pampanin, D.M., Volpato, E., Delaney, E., Sanni, S. & Nasci, C. (2004) Total oxyradical scavenging capacity responses in *Mytilus galloprovincialis* transplanted into the Venice lagoon (Italy) to measure the biological impact of anthropogenic activities. *Marine Pollution Bulletin*, **49**(9–10), 801–808.

Company R., Serafim A., Bebianno M.J., Cosson R., Shillito B. & Fiala-Médioni A. (2004) Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. *Marine Environmental Research*, **58**, 377–381.

Company R., Serafim A., Cosson R., Camus L., Shillito B., Fiala-Médioni A. & Bebianno M.J. (2006) The effect of cadmium on antioxidant responses and the susceptibility to oxidative stress in the hydrothermal vent mussel *Bathymodiolus azoricus*. *Marine Biology*, **148**, 817–825.

Corliss J.B., Dymond J., Gordon L.I., Edmond J.M., von Herzen R.P., Ballard R.D., Green K., Williams D., Bainbridge A., Crane K. & Andel T.H. (1979) Submarine thermal springs on the Galapagos Rift. *Science*, **203**, 1073–1083.

Cosson R.P. (1997) Adaptation des organisms hydrothermaux à la contrainte métallique. *Bulletin de la Société Zoologique de France*, **122**(2), 109–123.

Darley-Usmar V., Wiseman H. & Halliwell B. (1995) Nitric oxide and oxygen radicals: a question of balance. *FEBS Letters*, **369**, 131–135.

Desbruyères, D., Biscoito, M., Caprais, J.C., Colaço, A., Comtet, T., Crassous, P., Fouquet, Y., Khripounoff, A., Le Bris, N., Olu, K., Riso, R., Sarradin, P.M., Segonzac, M. & Vangriesheim, A. (2001) Variations in deep-sea hydrothermal vent communities in the Mid-Atlantic Ridge near the Azores plateau. *Deep-sea Research. Part I, Oceanographic Research Papers*, **48**, 1325–1346.

Douville E., Charlou J.L., Donval J.P., Knoery J., Fouquet Y., Bienvenu P. & Appriou P. (1997) Trace elements in fluids from the new Rainbow hydrothermal field (36°14' N, MAR): a comparison with other Mid-Atlantic Ridge fluids. *EOS Transactions American Geophysical Union*, **78**(46), 832.

Douville E., Bienvenu P., Charlou J.L., Donval J.P., Fouquet Y., Appriou P. & Gamo T. (1999) Yttrium and rare earth

elements in fluids from various deep-sea hydrothermal systems. *Geochimica et Cosmochimica Acta*, **63**, 627–643.

Douville E., Charlou J.L., Oelkers E.H., Bienvenu P., Jove Colon C.F., Donval J.P., Fouquet Y., Prieur D. & Appriou P. (2002) The rainbow vent fluids (36°14' N, MAR): the influence of ultramafic rocks and phase separation on trace metal content in Mid-Atlantic Ridge hydrothermal fluids. *Chemical Geology*, **184**, 37–48.

Erdelmeier I., Gerard-Monnier D., Yadan J.C. & Chaudiere J. (1998) Reactions of N-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. *Chemical Research Toxicology*, **11**, 1184–1194.

Géret F., Rousse N., Riso R., Sarradin P.M. & Cosson R. (1998) Metal compartmentalization and metallothionein isoforms in mussels from Mid-Atlantic Ridge; preliminary approach to the fluid-organism relationship. *Cahiers de Biologie Marine*, **39**, 291–293.

Granot, E. & Kohen, R. (2004) Oxidative stress in childhood health and disease states. *Clinical Nutrition*, **23**(1), 3–11.

Greenwald R.A. (1985) *Handbook of Methods for Oxygen Radical Research*. CRC Press, Boca Raton, FL.

Halliwell, B. (1995) Antioxidant characterization. Methodology and mechanism. *Biochemical Pharmacology*, **49**, 1341–1348.

Lawrence, R.A. & Burk, R.F. (1976) Glutathione peroxidase activity in selenium-deficient rat liver. *Biochemical and Biophysical Research Communications*, **71**, 952–958.

Livingstone D.R. (2001) Contaminated-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin*, **42**, 656–666.

Lonsdale P.F. (1977) Deep-tow observations at the Mounds Abyssal Hydrothermal Field, Galapagos Rift. *Earth and Planetary Science Letters*, **36**, 92–110.

Lowry O.H., Rosenbrough N.J., Farr A.L. & Randall R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.

Matés J.M. (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology,* **153:83–104.**

McCord J.M. & Fridovich I. (1969) Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry*, **244**(22), 6049–6055.

Pampanin, D.M., Camus, L., Gomiero, A., Marangon, I., Volpato, E. & Nasci, C. (2005) Susceptibility to oxidative stress of mussels (*Mytilus galloprovincialis*) in the Venice Lagoon (Italy). *Marine Pollution Bulletin*, **50**(12), 1548–1557.

Pruski A.M. & Dixon D.R. (2003) Toxic vents and DNA damage: first evidence from a naturally contaminated deep-sea environment. *Aquatic Toxicology*, **64**, 1–13.

Rousse N., Boulegue J., Cosson R.P. & Fiala-Médioni A. (1998) Bioaccumulation des métaux chez le mytilidae hydrothermal *Bathymodiolus* sp. de la ride médio-atlantique. *Oceanologica Acta*, **21**(4), 597–607.

Sarradin P.M., Desbruyères D., Dixon D., Almeida A., Caprais J.C., Colaço A., Company R., Cosson R., Cueff V., Dando P.R., Etoubleau J., Fiala-Medioni A., Gaill F., Godfroy A., Gwynn J.P., Hourdez S., Jollivet D., Khripounoff A., Lallier F., Laulier M., Le Bris N., Martins I., Mestre N., Pruski A.M., Rodier P., Santos R.S., Shillito B., Zal F., Zbinden M. (2001) ATOS cruise R/V l'Atalante, ROV Victor, June 22nd–July 21st 2001. *InterRidge News*, **10**(2), 18–20.

Sies, H. (1991) Oxidative stress: from basic research to clinical application. *The American Journal of Medicine*, **91**, 31S–38S.

Winston G.W., Di Giulio R.T. (1991) Pooxidant and antioxidant mechanisms in aquatic organisms. *Aquatic Toxicology*, **19**, 137–161.