Science of the Total Environment 472 (2014) 36–48

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Lysosomal and tissue-level biomarkers in mussels cross-transplanted among four estuaries with different pollution levels

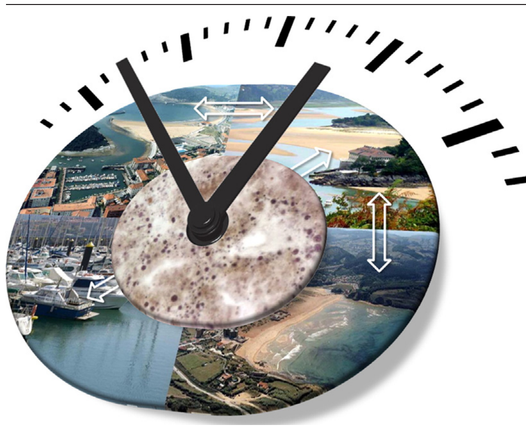
Xabier Lekube, Urtzi Izagirre, Manu Soto, Ionan Marigómez *

CBET Research Group, Research Centre for Experimental Marine Biology and Biotechnology (PIE-UPV/EHU), University of the Basque Country UPV/EHU, Areatza, 48620 Plentzia-Bizkaia, Basque Country, Spain

HIGHLIGHTS

- Biomarker sensitivity, rapidity, durability and reversibility were investigated in the field.
- Lysosomal and tissue-level biomarkers reflected environmental pollution/stress levels.
- These biomarkers were sensitive and rapid (2 d) and, to a large extent, reversible.
- Tissue-level biomarker responsiveness was durable (3–4 wk cross-transplantation).
- Lysosomal responses durability was not established due to confounding factors.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form 22 October 2013
Accepted 22 October 2013
Available online xxxx

Keywords:
Lysosomal structural changes
Lysosomal membrane stability
Changes in cell-type composition
Mussels
Biomarkers
Transplantation

ABSTRACT

A 3–4 wk cross-transplantation experiment was carried out in order to investigate the sensitivity, rapidity, durability and reversibility of lysosomal and tissue-level biomarkers in the digestive gland of mussels. Four localities in the Basque coast with different levels of chemical pollution and environmental stress were selected. Lysosomal membrane stability (LP) and lysosomal structural changes (Vv_L; S/V_L; Nv_L) and changes in cell-type composition in digestive gland epithelium (Vv_{BAS}) were investigated to determine short (2 d) and mid-term (3–4 wk) responses after cross-transplantation. Mussels from Txatxarramendi presented $Vv_{BAS} < 0.1$ $\mu m^3/\mu m^3$ (unstressed) whilst $Vv_{BAS} > 0.12$ $\mu m^3/\mu m^3$ was recorded in mussels from Plentzia (moderate stress) and $Vv_{BAS} > 0.2$ $\mu m^3/\mu m^3$ in Arriluze and Muskiz (high stress). Accordingly, LP < 10 min (high stress) was recorded in mussels from Muskiz and Arriluze and LP ~ 15 min (low-to-moderate stress) in those from Plentzia and Txatxarramendi. According to the Vv_L, S/V_L and Nv_L data, a certain lysosomal enlargement was envisaged in mussels from Arriluze in comparison with those from Txatxarramendi and Plentzia. Mussels from Muskiz exhibited a peculiar endo-lysosomal system made of abundant tiny lysosomes (low Vv_L and high S/V_L and Nv_L values). Lysosomal and tissue-level biomarkers were responsive after 2 d cross-transplantation between the reference and the polluted localities, which indicated that these biomarkers were quickly induced and, to a large extent, reversible. Moreover, the tissue-level biomarker values were maintained during the entire period (3–4 wk) of cross-transplantation, which evidenced the durability of the responsiveness. In contrast, comparisons in the mid-term were unfeasible for lysosomal biomarkers as these exhibited a seasonal winter attenuation resulting from low food availability and low temperatures. In conclusion, lysosomal enlargement and membrane stability and

* Corresponding author at: Research Centre for Experimental Marine Biology and Biotechnology (PIE-UPV/EHU), Areatza, 48620 Plentzia-Bizkaia, Basque Country, Spain. Tel.: +34 94 6012690; fax: +34 94 4648500.
E-mail address: ionan.marigomez@ehu.es (I. Marigómez).

0048-9697/$ – see front matter
http://dx.doi.org/10.1016/j.scitotenv.2013.10.075

changes in cell-type composition were sensitive, rapid and reversible responses to changes in environmental stress whilst durability of the response could not be demonstrated for lysosomal responses by interferences with the seasonal variability.

1. Introduction

Mussels have been worldwide used since the 1970s as sentinel organisms in biomonitoring programmes in order to assess chemical pollution in coastal, estuarine and continental waters (Goldberg, 1975). Native mussels collected from the field are used since the start of the mussel watch pollution monitoring programme (Goldberg, 1975); however, most recently there has been an increasing use of transplanted mussels that are collected from a reference site or a farm and deployed in cages in the monitoring site for several weeks (Andral et al., 2011; Brooks et al., 2012; Da Ros and Nesto, 2005; Da Ros et al., 2000, 2002; Marigómez et al., 2013; Nasci et al., 1999; Nigro et al., 2006; Regoli, 1992; Regoli et al., 2004; Wepener et al., 2008; Zorita et al., 2006). Caging-based biomonitoring has the advantage of ensuring comparable biological samples and therefore reducing their natural variability (De Kock, 1983). Likewise, this approach is also believed to facilitate the investigation of areas lacking native specimens (Andral et al., 2011). Caging experiments can be also useful to determine the durability and reversibility of the biological responses to pollution; e.g., by transplanting mussels from polluted to less polluted or reference sites (Da Ros et al., 2000; Lopes et al., 2012; Serafim et al., 2011).

In mussels, the digestive gland epithelium is comprised of digestive and basophilic cells. Under normal physiological condition digestive cells outnumber basophilic cells but in response to environmental stress, including exposure to pollutants, the relative proportion of basophilic cells increases (Cajaraville et al., 1990; Garmendia et al., 2011; Marigómez et al., 1990; Zaldibar et al., 2007b). This change in cell-type composition is a reversible tissue-level biomarker that results from digestive cell loss and concomitant basophilic cell hypertrophy rather than to basophilic cell proliferation, as believed in the earliest reports (Zaldibar et al., 2007a). Since basophilic cells appear practically devoid of β-glucuronidase and N-acetyl-β-hexosaminidase lysosomal enzyme activities (Izagirre et al., 2009) the occurrence of changes in cell-type composition may have consequences for the measuring and interpretation of lysosomal biomarkers (Marigómez et al., 2002). This is why in the present study both changes in cell-type composition and lysosomal responses were investigated in parallel.

Lysosomal responses to pollutants in mollusc digestive gland are considered effect biomarkers indicative of general stress (Marigómez et al., 2005a, 2005b; Moore et al., 2007). The most common response includes membrane destabilisation and lysosomal enlargement (Cajaraville et al., 1995; Etxeberria et al., 1994; Lowe et al., 1981; Marigómez et al., 1996; Moore et al., 1978), which recently have been demonstrated not to depend strictly on each other (Izagirre and Marigómez, 2009). Lysosomal membrane destabilisation appears to constitute an early onset of biological disturbance whilst enlargement would represent an integrated sign of cellular damage (Marigómez et al., 2005a). Lysosomal responses against organic and metallic pollutants have been reported in field (Domouhtsidou and Dimitriadis, 2001; Etxeberria et al., 1995; Krishnakumar et al., 1994; Marigómez et al., 2005a, 2006; Regoli, 1992; Zorita et al., 2006) and laboratory studies (Harrison and Berger, 1982; Lowe et al., 1981; Marigómez and Baybay-Villacorta, 2003; Marigómez et al., 2005b; Moore, 1988), as well as a consequence of environmental factors such as temperature (Moore, 1976), salinity changes (Bayne et al., 1981; Marigómez et al., 1991), hypoxia (Moore et al., 1979), food availability (Moore et al., 1987), tidal cycle (Izagirre et al., 2008; Tremblay and Pellerin-Massicote, 1997) and reproductive stress (Bayne et al., 1978). Lysosomal responses to pollutants may also consist of enlargement with concomitant increase in numbers (Etxeberria et al., 1994; Marigómez et al., 1996; Moore et al., 1978) or lysosomal shrinkage (Cajaraville et al., 1995; Marigómez et al., 1996; Marigómez and Baybay-Villacorta, 2003). Provided that the marked seasonal variability is taken into account, the changes in lysosomal size and numbers are clearly indicative of health impairment in sentinel mussels, which in turn reveals impairment in ecosystem health (Etxeberria et al., 1995; Garmendia et al., 2010, 2011; Marigómez et al., 1996; Regoli, 1992).

Presently, a cross-transplantation experiment between four estuaries with different levels of pollution was carried out in the Basque coast. Previous reports on pollution levels and biomarkers in the study area revealed marked differences in the levels and biological effects of pollution in the estuaries of Bilbao, Plentzia and Urdaibai (Etxeberria et al., 1995; Marigómez et al., 1996, 2013; Orbea et al., 1999; Soto et al., 1995, 1996). In addition, the Barbadun estuary was also included in the study because a large oil refinery is situated in its vicinity. A battery of biomarkers, including antioxidant enzymes, peroxisomal proliferation, lysosomal responses and tissue-level biomarkers was applied, together with chemical analysis of pollutant tissue burdens. The results on PAH and PCB tissue burdens, peroxisome proliferation and antioxidant enzymes were published (Orbea and Cajaraville, 2006).

The present work deals with lysosomal responses and changes in cell-type composition in the digestive gland epithelium (tissue-level biomarker) in an attempt to determine their sensitivity, rapidity, durability and reversibility under field conditions.

2. Material and methods

2.1. Transplantation experiment design

Cross-transplantation of mussels (*Mytilus galloprovincialis*) was carried out between four different estuaries in the Basque coast in early November 1998. Caged mussels were deployed in Muskiz in the Barbadun estuary (43° 20′N, 3° 06′W), Arriluze in the Bilbao estuary (43° 20′N, 3° 01′W), Plentzia in the Plentzia estuary (43° 25′N, 2° 57′W) and Txatxarramendi in the Urdaibai estuary (43° 19′N, 2° 40′W) (Fig. 1). Muskiz is located at the left bank of the Barbadun estuary in the vicinity of a big oil refinery and some metallurgical industries. Arriluze is a marina located at the right bank of the Bilbao estuary; which has been subjected to severe urban and industrial impact for decades and was still considered heavily polluted in the 1990s (Azkona et al., 1984; Orbea et al., 1999, 2002; Soto et al., 1995, 1996). However, as a result of the increased captures and treatment of wastewater discharges, pollutant load to the Bilbao estuary was significantly reduced from 1993 to 2003 (García-Barcina et al., 2006). Plentzia was located at the front of a small fishing harbour in the Plentzia estuary; where a certain industrial and agricultural activities take place 15–20 km upstream at the high course of the river. Txatxarramendi, considered as the reference locality, is a small island in the left bank of the Urdaibai estuary mouth. This estuary was declared Biosphere Reserve by UNESCO in 1984 and considered, in the 1990s, a well-preserved estuary with relatively low levels of organic pollutants and heavy metals (Belzunce et al., 2004; Orbea et al., 2002).

Mussels (4.5–6 cm shell length) were collected from the lowest intertidal zone at the lowest month-tide in Txatxarramendi (reference site) and transplanted to Arriluze, Muskiz and Plentzia where they were caged (150 mussels per cage) in 20 L fishing nets and immersed at 1–2 m depth (bottom-moored with a floating device and a buoy or attached to existing structures). At the same time, mussels of the same size-class and tide level were collected from these localities and caged in Txatxarramendi. In addition, native mussels from each locality were