Science of the Total Environment 511 (2015) 663–675

Contents lists available at ScienceDirect

Science of the Total Environment

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

# Assessment of a mussel as a metal bioindicator of coastal contamination: Relationships between metal bioaccumulation and multiple biomarker responses

Rathishri Chandurvelan [a], Islay D. Marsden [a,*], Chris N. Glover [a], Sally Gaw [b]

[a] School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
[b] Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

## HIGHLIGHTS

- Multiple biomarker responses were measured in mussels from 6 sites.
- Metal content of mussel tissues correlated with specific biomarker responses.
- Clearance rate, biochemical and cytogenotoxic biomarkers reflected contaminant levels.
- Mussels are confirmed as effective bioindicators for coastal metal contaminants.

## ARTICLE INFO

*Article history:*
Received 20 August 2014
Received in revised form 27 November 2014
Accepted 21 December 2014
Available online xxxx

Editor: Eddy Y. Zeng

*Keywords:*
*Perna canaliculus*
Trace metals
Sediment
Metal bioaccumulation
Biomarkers
Biomonitoring

## ABSTRACT

This is the first study to use a multiple biomarker approach on the green-lipped mussel, *Perna canaliculus* to test its feasibility as a bioindicator of coastal metal contamination in New Zealand (NZ). Mussels were collected from six low intertidal sites varying in terms of anthropogenic impacts, within two regions (West Coast and Nelson) of the South Island of NZ. Trace elements, including arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn), were measured in the gills, digestive gland, foot and mantle, and in the surface sediments from where mussels were collected. Metal levels in the sediment were relatively low and there was only one site (Mapua, Nelson) where a metal (Ni) exceeded the Australian and New Zealand Interim Sediment Quality Guideline values. Metal levels in the digestive gland were generally higher than those from the other tissues. A variety of biomarkers were assessed to ascertain mussel health. Clearance rate, a physiological endpoint, correlated with metal level in the tissues, and along with scope for growth, was reduced in the most contaminated site. Metallothionein-like protein content and catalase activity in the digestive gland, and catalase activity and lipid peroxidation in the gill, were also correlated to metal accumulation. Although there were few regional differences, the sampling sites were clearly distinguishable based on the metal contamination profiles and biomarker responses. *P. canaliculus* appears to be a useful bioindicator species for coastal habitats subject to metal contamination. In this study tissue and whole organism responses provided insight into the biological stress responses of mussels to metal contaminants, indicating that such measurements could be a useful addition to biomonitoring programmes in NZ.



## 1. Introduction

There is international interest in the fate of contaminants in the marine environment where increasingly they are being detected in locations that had previously been regarded as pristine (Macdonald et al., 2000). In New Zealand (NZ), where coastal areas have been traditionally regarded as relatively clean, monitoring studies have detected hotspots of contaminants including trace metals (e.g., Craw et al., 2005). Trace metals are particularly important because they can be harmful to a wide range of aquatic species, are persistent, non-biodegradable, and can be lethal above a certain threshold (Rainbow, 2007). Exposure to metals can also induce sublethal effects in organisms, including disruption of homeostasis, and damage at the cellular and molecular levels (e.g., Tsangaris et al., 2010). Additionally, these effects may significantly reduce the survival capacity of the organism by increasing susceptibility to diseases and damage (de Montaudouin et al., 2010).

Sessile filter-feeding organisms in contaminated environments will often reflect environmental degradation, both in terms of their ability

* Corresponding author at: School of Biological Sciences; University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand.
*E-mail addresses:* rch118@uclive.ac.nz (R. Chandurvelan), islay.marsden@canterbury.ac.nz (I.D. Marsden), chris.glover@canterbury.ac.nz (C.N. Glover), sally.gaw@canterbury.ac.nz (S. Gaw).

http://dx.doi.org/10.1016/j.scitotenv.2014.12.064
0048-9697/

to accumulate toxicants in their tissues, and through the impacts of such exposure on key biological processes (Rainbow and Phillips, 1993). These bioindicator species therefore represent a mechanism for monitoring environmental health, and indeed taxa such as bivalves have been used in this manner (Rainbow, 2002). Bivalve species such as mussels are able to tolerate fluctuations in salinity, temperature and oxygen levels, and these attributes, along with their sessile nature, general abundance and annual availability, make them favourable as a bioindicator (Goldberg, 1986). Furthermore, numerous laboratory and experimental studies have shown that mussels accumulate trace metals in proportion to the availability of metals in the environment (e.g., Boening, 1999). This ability has been used in the Mussel Watch programme (Goldberg, 1986), a monitoring initiative that has successfully determined spatial and temporal changes in contaminants in several countries (e.g., Nakata et al., 2012; Marigomez et al., 2013; Melwani et al., 2014; Regoli et al., 2014). Mussels have also been used to assess the biological impacts of contaminants, but until recently (Chandurvelan et al., 2012, 2013a,b), there have not been any such studies on any NZ bivalve. These "biomarker" responses (any measurable change in behavioural, physiological, cellular, biochemical, or molecular response; Depledge, 1994) provide a robust approach for determining the impacts of organisms to environmental stressors.

*Perna canaliculus* is an ecologically- and culturally-significant mussel species found low in the intertidal zone on open coasts of rocky shores and estuaries in New Zealand (NZ) (Powell, 1979). It is also a major aquaculture species (Pickering, 2009). We recently investigated multiple biomarker responses following laboratory exposure of *P. canaliculus* to acute and subchronic waterborne Cd. In both exposure scenarios, a number of biomarker responses were found that correlated significantly with Cd levels in the tissues. These included physiological changes such as clearance rate (Chandurvelan et al., 2012), levels of metallothionein-like protein (MTLP) in the gill and digestive gland (Chandurvelan et al., 2013a), and nuclear aberrations in branchial cells (Chandurvelan et al., 2013b).

In the present study we evaluated the effectiveness of *P. canaliculus* as an indicator for coastal contaminants. We hypothesised that exposure to contaminants would result in a stress response which could be detected by a suite of biomarkers. Three sites in each of two regions of the South Island of NZ were selected, with each region consisting of two sites with a history of contaminant inputs and a reference site that was regarded as clean or distant from known contaminant inputs. We considered it necessary to sample contrasting regions because the two coastal regions have very different exposure levels, currents and contaminant inputs. The metal content in the gill, mantle, digestive gland and foot were measured and a sediment sample from the mussel collection locations was also taken to assess environmental metal values. This is the first study in NZ to measure multiple biomarker responses and relate them to the metal content in the tissues of an intertidal bivalve. We tested *P. canaliculus* as an effective bioindicator species for environmental metals, and determine which, if any, biomarkers might be of particular use for detecting metal contaminants in NZ coastal habitats.

## 2. Material and methods

### 2.1. Study area and site description

The study area consisted of three sampling sites within two regions located in the South Island of NZ (Fig. 1, Latitude −41.76–37.52, Longitude 171.56–175.32), chosen according to their different sources and degrees of metal contamination. Carters Beach was the designated as the cleanest of the sites and used as reference site for the West Coast region. Mussels are scarce along these very exposed coasts and collecting was unsafe in other areas that had been considered. Adele Island was chosen as the reference site for the Nelson region (Fig. 1; Table 1) because of a lack of known contaminant inputs. A single reading of temperature, salinity and pH at each sampling site was recorded using a hand held YSI recorder.

### 2.2. Sample collection

Adult green-lipped mussels (n = 30 from each sampling site; 60–80 mm shell length) were collected at low tide from the six sampling sites during October 2011, by cutting the byssus threads attaching them to the substratum. They were transferred to the laboratory in polyethylene bags half-filled with seawater from the collection site and aerated using an air-bubbler. Transportation to the University of Canterbury was completed within 24–48 h and mussels were maintained in a 12 h light/dark cycle at 15 °C in fresh seawater that had been collected from each site. Sediment samples (~0 to 2–5 cm depth; n = 3) were collected from each site using a corer and placed in 50 ml acid-washed polypropylene tubes in a cooler. On reaching the laboratory, they were stored at 4 °C until processed.

### 2.3. Trace metal analysis

Mussels (n = 5–7) from each study site were dissected and gill, digestive gland, mantle and foot tissue stored at −80 °C until analysis. After thawing, 0.2 g wet weight of each of the tissues was dried at 60 °C, transferred into acid-washed tubes, and digested at 90 °C for 1 h using 5 ml of 50% $HNO_3$. Each sample was diluted appropriately using 2% Ultrapure $HNO_3$, before being analysed for metal content as described below. Trace metal concentrations in mussel tissues were expressed as μg $g^{-1}$ dry weight tissue.

Sediments were dried at 60 °C for 48 h, and after transfer to clean polyethylene bags, were crushed to obtain uniform grain sediment size prior to acid digestion. Approximately 1 g of bulk sediment (not sieved and not washed) was weighed and acid digested in 5 ml 50% $HNO_3$ (Analar grade) and 10 ml of 20% HCl (Analar grade) made up to 20 ml at 90 °C for 60 min. Once cooled overnight, the sediment samples were diluted using 2% Ultrapure $HNO_3$ for analysis (Marsden et al., 2014). The trace metal concentration in the sediment is expressed as μg $g^{-1}$ dry weight sediment.

Trace metal analyses of arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in mussel tissue and sediment samples were carried out using the methodology described in Chandurvelan et al. (2012) using inductively coupled plasma mass spectrophotometry (ICP-MS; Agilent 7500cx, Agilent Technologies, USA). QA/QC was achieved by using certified reference mussel tissues SRM 2702 and SRM 2976 (National Institute of Standards and Technology, US). The percentage recoveries for the different trace metals in the sediment standard reference material are reported in Supplementary Table 1.

### 2.4. Biomarker measurements

#### 2.4.1. Physiological biomarkers

The clearance rate, absorption efficiency, respiration rate, excretion rate and oxygen to nitrogen ratio (O:N) were measured in individual mussels (n = 5 or 6) for each sampling site. Detailed protocols for all of these physiological biomarker measurements are given in Chandurvelan et al. (2012). Scope for growth (SFG) was calculated using the equation of Widdows et al. (1995) based on ingested energy (based on a *Tetraselmis chuii* algal culture with 1.33 mg particulate organic matter per litre), estimated energy loss (whereby the excretion of 1 μg $NH_4$–N $h^{-1}$ is equivalent to energy loss of 0.0249 J $h^{-1}$) and energy respired (the respiration rate multiplied by 0.456 (J μmol$^{-1}$ $O_2$), the heat equivalent of oxygen uptake; Gnaiger, 1983).

Condition index (CI) is an integrated measure of overall organism health (Davenport and Chen, 1987). To determine CI, mussels (n = 6) from each sampling site were dissected and the soft tissues and the shell were dried separately at 60 °C for 48 h (Davenport and Chen, 1987). The CI was calculated using the equation, CI = W × 100/S,