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Biomarker responses of mussels exposed to earthquake disturbances

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ABSTRACT

The green-lipped mussel, *Perna canaliculus* is recognised as a bioindicator of coastal contamination in New Zealand (NZ). Mussels (shell length 60–80 mm) were collected from three intertidal areas of Canterbury in the South Island of NZ prior to extreme earthquake disturbances on 22nd February 2011, and 9 months later in October 2011. 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. Metal levels in tissues were site specific, and mostly unaffected by earthquake disturbances. Physiological biomarkers were negatively affected by earthquake disturbances and mussels from the Port of Lyttelton had higher negative scope for growth post-earthquake. Metallothionein-like protein in the digestive gland correlated with metal content of tissues, as did catalase activity in the gill and lipid peroxidation values for the digestive gland. This research demonstrates that physiological and other biomarkers are effective at detecting the effects of multiple stressors following seismic disturbances.

1. Introduction

Mussels are used internationally to assess the health of coastal ecosystems and risks associated with food safety (Whyte et al., 2009). They tolerate fluctuations in salinity, temperature and oxygen levels, and these attributes, along with their sessile nature, general abundance and annual availability favour their use as a bioindicator (Goldberg, 1986). Mussels accumulate contaminants in proportion to their availability in the environment (e.g. Boening, 1999), an attribute used in the Mussel Watch programme (Goldberg, 1986; Nakata et al., 2012; Marigomez et al., 2013; Melwani et al., 2014; Regoli et al., 2014). Exposure to stressors induces sub-lethal effects in mussels, which can be monitored as biomarkers of exposure and effect. Biomarkers are defined as any measurable change in behavioural, physiological, cellular, biochemical, or molecular response, including disruption of homeostasis, and damage at the cellular and molecular levels (e.g. Depledge, 1994; Tsangaris et al., 2010; Chandurvelan et al., 2012, 2013a; 2013b).

Mussels are particularly effective as biological indicators of stressors in coastal areas (Luoma and Rainbow, 2010; Elliott and Quintino, 2007), especially estuaries, where the main anthropogenic stressors include contaminants such as trace metals, from industrial and domestic waste (de los Rios et al., 2015). Trace metals can be harmful to a wide range of aquatic species, are persistent, non-biodegradable, and can be lethal above a certain threshold (Rainbow, 2007). In molluscs, trace metals accumulation is more efficient in the tissues than in shells (Brown and Depledge, 1998; Perez-Lopez et al., 2003), but shell metal concentrations can be useful for detecting long term changes in environmental metal levels (Cravo et al., 2002). Trace metal concentrations in bivalves can fluctuate widely amongst tissues with exceptionally high concentrations found in the digestive gland, making this tissue particularly sensitive to contaminants and suitable for biomonitoring (Chandurvelan et al., 2013a). Bivalve tissue metals are expected to correlate with both anthropogenic inputs and habitat disturbances (Cain and Luoma, 1986). Coastal areas are subjected to natural stressors including extreme weather events, earthquake activity, tsunamis and terrestrial sediment inputs and although such disturbances are common, few studies have evaluated their

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effects on marine bivalves.

Among the natural stressors, those likely to cause the greatest disturbance to coastal settings and the biota therein, are earthquakes. Strong seismic activity is characterised by shaking and ground deformation, resulting in changes in elevation, sediment and contaminant inputs (Marsden et al., 2015). During and following earthquake activity, sessile organisms such as marine bivalves are vulnerable to shearing, mortality displacement and shifts in shore level. Marine bivalves are thought to be able to recover quickly from the effects of earthquake disturbances (Castilla et al., 2010), but there has been no previous research on the sub-lethal effects of earthquake disturbances on marine mussels.

In the current study we investigated biomarker responses of the green-lipped mussel (*Perna canaliculus*) following exposure to earthquake disturbances. In September 2010, a M_w 7.2 earthquake hit the Canterbury region. The epicentre for this earthquake was 40 km away from the city, but as a shallow event it caused significant shaking and minor damage. In February 2011, a smaller (M_w 6.2) earthquake struck, but very close to the city of Christchurch causing severe shaking (Measures et al., 2011; Reid et al., 2012). This earthquake resulted in damaged sewerage infrastructure in the Estuary and regular input of untreated human waste into Christchurch waterways. Further away from the epicentre of the second earthquake, minor effects were observed in intertidal habitats at the Port of Lyttelton and minimal disturbances were recorded in coastal areas of Banks Peninsula. The potential environmental stressors resulting from earthquake disturbances in the Estuary and the Port included surface disruption of sediment containing known trace metals and other contaminants, the input of large quantities of subsurface (liquifaction sediment) and increased nutrients due to the failure of sewerage systems.

We measured tissue metal concentrations in specific tissues and biomarker responses in mussels between two seismic events (i.e. in January 2011), and again following the more substantial February seismic activity (in October 2011), in three sites from around the Canterbury region. One site was in the Avon-Heathcote Estuary/Ihutai, where, prior to 2010, treated domestic sewage and industrial effluent were discharged directly into the Estuary. The second site was near the commercial port of Lyttelton, and the third, a relatively uncontaminated, reference site at Pigeon Bay within Banks Peninsula. We measured physiological biomarkers (e.g. clearance rate, respiration rate, excretion rate, scope for growth) at both sampling events, and in addition measured 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, 2015) in the latter sampling.

This study therefore evaluates the effectiveness of *P. canaliculus* as an indicator of natural and anthropogenic coastal disturbances. Removal of treated sewage from the Avon- Heathcote Estuary/Ihutai in March 2010 had been expected to improve mussel health, while exposure to contaminants in 2011, post quake, was expected to result in increased physiological stress. We predicted that physiological changes of the mussels would be detected using a suite of biomarkers combined with measurement of the metal content in the gill, mantle, digestive gland and foot. This is the first study to evaluate the effects of multiple stressors including earthquake disturbances on mussel biomarkers.

2. Methods

2.1. Study area, site descriptions and mussel collections

The sites for this study were in the Canterbury region of the South Island of New Zealand, the Avon-Heathcote Estuary/Ihutai, Pigeon Bay, and the Port of Lyttelton (Table 1). Pigeon Bay on Banks Peninsula was selected as the reference site (Chandurvelan et al., 2015), being some distance from built up areas and known contaminant inputs.

Collections of up to 30 green-lipped mussels (shell length 60–80 mm) were made from low tide intertidal sites in January (pre-earthquake) and October 2011 (post-earthquake). 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 laboratory was completed within 6 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. They were stored at 4 °C until processed.

2.2. 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.

In the present study, medium sand (particle size < 500 μm) was collected from areas close to the mussels to compare the metal content of the various sites and to determine if environmental conditions had changed as a result of the earthquakes. All sediments were dominated by fine sand (<250 μm) with an average particle size about 90 μm. 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) at 90 °C for 60 min. Once cooled, the samples were made up to 20 ml and left overnight before the sediment samples were diluted using 2% Ultrapure HNO_3 for analysis (Marsden et al., 2015). The trace metal concentration in the sediment is expressed as μg g^{-1} dry weight sediment. We used a USEPA method (USEPA 200.8) to determine total recoverable metals in the sediment. This method is widely used by regulatory agencies across New Zealand to determine metal sediment concentrations and as such enables comparison with previous studies as well as the ANZECC sediment quality guidelines which are based on total metal concentrations.

Trace metal analyses of arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in mussel tissues and sediment samples from the Avon-Heathcote Estuary/Ihutai were carried out using the methodology described in Chandurvelan et al. (2015) 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 sediment reference SRM 2976 (National Institute of Standards and Technology, US). The recoveries for the certified reference materials were acceptable.

2.3. Biomarker measurements

2.3.1. 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