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# Correlation of transcriptomic responses and metal bioaccumulation in *Mytilus edulis* L. reveals early indicators of stress

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ABSTRACT

Marine biomonitoring programs in the U.S. and Europe have historically relied on monitoring tissue concentrations of bivalves to monitor contaminant levels and ecosystem health. By integrating 'omic methods with these tissue residue approaches we can uncover mechanistic insight to link tissue concentrations to potential toxic effects. In an effort to identify novel biomarkers and better understand the molecular toxicology of metal bioaccumulation in bivalves, we exposed the blue mussel, *Mytilus edulis* L., to sub-lethal concentrations (0.54 μM) of cadmium, lead, and a Cd + Pb mixture. Metal concentrations were measured in gill tissues at 1, 2, and 4 weeks, and increased linearly over the 4 week duration. In addition, there was evidence that Pb interfered with Cd uptake in the mixture treatment. Using a 3025 sequence microarray for *M. edulis*, we performed transcriptomic analysis, identifying 57 differentially expressed sequences. Hierarchical clustering of these sequences successfully distinguished the different treatment groups demonstrating that the expression profiles were reproducible among the treatments. Enrichment analysis of gene ontology terms identified several biological processes that were perturbed by the treatments, including nucleoside phosphate biosynthetic processes, mRNA metabolic processes, and response to stress. To identify transcripts whose expression level correlated with metal bioaccumulation, we performed Pearson correlation analysis. Several transcripts correlated with gill metal concentrations including *mt10*, *mt20*, and contig 48, an unknown transcript containing a wsc domain. In addition, three transcripts directly involved in the unfolded protein response (UPR) were induced in the metal treatments at 2 weeks and were further up-regulated at 4 weeks. Overall, correlation of tissue concentrations and gene expression responses indicates that as mussels accumulate higher concentrations of metals, initial stress responses are mobilized to protect tissues. However, given the role of UPR in apoptosis, it serves as an early indicator of stress, which once overwhelmed will result in adverse physiological effects.



## 1. Introduction

Historically, national and international "mussel watch" programs including the NOAA National Status and Trends (NS&T) have monitored ecosystem health through the chemical analyses of contaminant levels in marine bivalve tissues (Goldberg, 1986; Cantillo, 1998). As sessile, ecologically-important coastal species, marine bivalves, in particular the blue mussel *Mytilus edulis* L., bioaccumulate a broad spectrum of organic and inorganic contaminants (Kimbrough et al., 2008). They are good surrogates for monitoring water quality because their soft tissues integrate exposure over time, even when water concentrations are close to detectable levels or when contaminant levels are temporally variable (Viarengo and Canesi, 1991; Blackmore and Wang, 2003).

Several attempts have been made over the last 30 years to link contaminant body burdens with toxicity. While also referred to as the Critical Body Residue approach, recent work has adopted the term tissue residue approach (TRA), a term that encompasses both organic and inorganic contaminants, individual tissues and whole bodies (Meador et al., 2008). The theoretical basis for this approach assumes that the concentration of a contaminant at the site of toxic

*Abbreviations:* AOP, adverse outcome pathway; BDL, below detection limits; DET, differentially expressed transcript; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; EST, expressed sequence tag; FDR, false discovery rate; GEO, gene expression omnibase; GO, gene ontology; HSP, heat shock protein; ICP-MS, inductively coupled plasma mass spectrometer; *mt*, metallothionein (gene); MT, metallothionein (protein); NS&T, National Status and Trends; POP, persistent organic pollutant; RDX, 1,3,5-trinitro-1,3,5-triazacyclohexane; ROS, reactive oxygen species; RT-qPCR, quantitative reverse transcription PCR; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ, TCDD toxic equivalents; TNT, 2,4,6-trinitrotoluene; TRA, tissue residue approach; UPR, unfolded protein response.

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action (i.e. the effective dose) is proportional to the concentration of that contaminant in the whole body and individual tissues. The closer the measurement of "dose" is in proximity to the target site, the more consistent responses between individuals or species will be, because major sources of variability such as bioavailability, uptake, toxicokinetics, and sequestration are bypassed (Meador et al., 2008). Therefore, by measuring either whole body or specific tissue contaminant concentrations, the TRA should provide a more accurate dose than measuring water or sediment contaminant concentrations, and should be more closely correlated with adverse effects (McCarty and Mackay, 1993; Meador et al., 2008). The TRA has proven to work very well for some classes of toxicants including chlorophenols and nonpolar organic compounds that elicit narcosis. In these cases, the assumption that whole body residues are proportional to the biologically active concentration at the target site holds up well (Meador et al., 2008). However, due to the variety and efficiency of mechanisms that have evolved in various species for metal uptake, detoxification and internal sequestration, tissue residues of metals may not correspond well to target site concentrations (Chapman, 1997; Rainbow, 2002; Luoma and Rainbow, 2005). Therefore, understanding the relationship between tissue concentrations and the biologically active concentration of metal (BAM) is not straightforward (Vijver et al., 2004; Adams et al., 2011).

In addition to chemical analysis, many monitoring programs have recently incorporated biological measures of environmental health (i.e. biomarkers). A variety of biochemical, physiological and behavioral biomarkers has been developed for mussels and other bivalves in order to identify contaminant exposure, toxicological effects, and susceptibility to additional stressors (Decaprio, 1997; Shaw et al., 2011; Schettino et al., 2012). For example, the United Kingdom's "Ecoman" monitoring program incorporates multiple biomarkers to prioritize sites for hierarchical risk assessment (Galloway et al., 2006).

Over the past 10 years, 'omic approaches have begun to provide additional opportunities for identifying novel biomarkers and unraveling modes of action in non-model organisms (Snape et al., 2004; Poynton et al., 2007; Poynton and Vulpe, 2009; Veldhoen et al., 2012). Transcriptomic, proteomic, and metabolomic biomarkers are likely to be among the first responses to contaminant stress, preceding that of physiological and behavioral biomarkers. Because of their enormous potential value to marine monitoring programs, there has been much interest in developing 'omic resources and conducting transcriptomic studies on indicator organisms such as blue mussels. Venier et al. (2006) described a cDNA microarray for *Mytilus galloprovincialis* containing 1700 unique cDNAs, which provided transcriptome profiles capable of discriminating between different pollutants and between reference and impacted sites. Since then a number of additional studies have investigated transcriptomic responses in *Mytilus* spp. when exposed to okadaic acid (Manfrin et al., 2010), different mixtures of pollutants (Dondero et al., 2010, 2011; Canesi et al., 2011), heat or salinity stress (Lockwood et al., 2010; Lockwood and Somero, 2011; Mohamed et al., 2014), immunological stress (Venier et al., 2011; Philipp et al., 2012) and ocean acidification (Hüning et al., 2013). These studies have contributed mechanistic insight into the stress response of mussels and provided valuable biomarkers for use in monitoring programs. Some studies have even developed strategies for incorporating the complex data inherent in transcriptomic studies into monitoring databases. For example, Shaw et al. (2011) used gene expression profiling along with a suite of biomarkers within the Mussel Expert System (MES) to rate the level of stress experienced in mussels collected within the Tamar River and Estuary.

Used in concert with TRA, 'omics methods should provide mechanistic information to link tissue concentrations to toxic effects. For example, because gene expression responds to some portion of the biologically active internal dose of a chemical, when used in combination with TRA, transcriptomics may be able to relate tissue concentrations to the biologically active concentration. This may be particularly helpful for the study of metal accumulation where presently, a lack of mechanistic detail related to the sequestration and detoxification of metals has prevented researchers from precisely estimating biologically active metals (BAMs) from total metal concentrations (Rainbow, 2002; Adams et al., 2011). Through expression profiles of cellular toxicity, 'omics' methods may identify biochemical pathways that are adversely impacted by BAMs and also identify pathways that trigger metal sequestration and detoxification pathways (e.g. metallothionein and antioxidant production) that mitigates toxicity and/or builds up the non-bioavailable metal pool. In addition, gene expression is more likely to be correlated with tissue metal concentrations than with water or sediment exposure concentrations. Despite the potential advantages of combining transcriptomics and TRA, only a few studies to date have made correlations between tissue concentrations and gene expression responses (Nakayama et al., 2006; Gong et al., 2012).

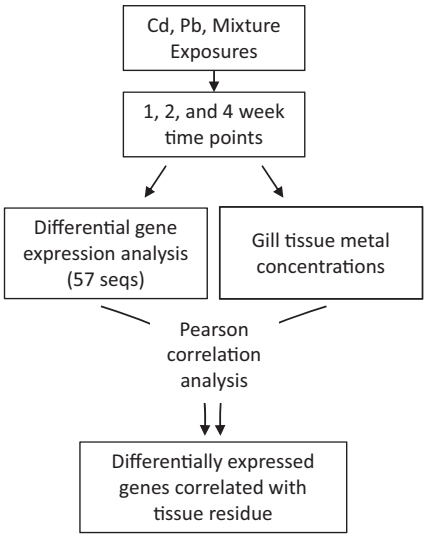


**Fig. 1.** Overview of research approach.

The objective of the present study was to identify differentially expressed genes, through a transcriptomic approach, that correlated with metal accumulation in *M. edulis* gill tissue. Our approach, outlined in Fig. 1, involved 1-, 2- and 4-week exposures to cadmium, lead, and a Cd + Pb mixture, and subsequent measurement of gill gene expression responses and the corresponding gill metal concentrations in individual mussels. We first identified genes that were differentially expressed in each of the treatments and timepoints. From this reduced gene set, we then performed Pearson correlation analysis to identify genes whose expression level correlated with metal concentrations in the gills of the same mussels. Our goal was to provide monitoring programs with gene expression biomarkers that respond in a dose-dependent manner to specific metal bioaccumulation. In addition, we wanted to uncover insights into the mode of action and cellular toxicity of these metals through transcriptomic analysis, to help improve upon the TRA for metals in bivalves.

## 2. Materials and methods

### 2.1. Experimental animals

One hundred and twenty *M. edulis* between 50 and 60 mm in length were collected in January 2011, from Bourne, MA (41.7402EN, 70.6157EW). Historical data from this NS&T site