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Evidence of oxidative stress in wild freshwater mussels (*Lasmigona costata*) exposed to urban-derived contaminants

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ABSTRACT

The physiological effect of complex mixtures of anthropogenic contaminants on aquatic organisms is not well understood. This study employed a suite of sub-cellular biomarkers and general health measurements to assess the effect of urban-derived contaminants on wild freshwater mussels. Adult *Lasmigona costata* were collected from four sites in the Grand River (ON, Canada) that receive incremental amounts of municipal wastewater effluents and road runoff. Biomarkers of metal exposure, oxidative stress, and general health were examined in the gills of wild mussels. Concentrations of nine metals as well as the metal-binding protein, metallothionein (MT), were significantly higher ($p < 0.05$) in mussels living downstream of the urban area. For example the concentrations of Pb, Cr and Zn were five-fold, and Ag more than 20 fold higher in mussels collected downstream of 11 municipal wastewater treatment plants and four cities compared to levels in upstream mussels. Downstream mussels showed evidence of oxidative stress, such that lipid peroxidation (LPO) (as thiobarbiturate reactive substances) was significantly elevated and the antioxidant capacity against peroxyl radicals (ACAP) was significantly decreased ($p < 0.01$) in downstream mussels compared to upstream mussels. Regarding general health indicators, although gill lipid concentrations were similar across sites, protein concentration was significantly ($p < 0.001$) higher in mussels collected from the upstream reference site compared to all downstream sites. The trends observed indicate that there are physiological effects in mussels associated with chronic exposure to complex urban inputs and that some biomarkers respond to municipal wastewater effluent and road runoff exposure in a cumulative manner. The observed oxidative stress response (ACAP) along with the elevation in MT, suggest that even though the defense mechanisms in the chronically exposed mussels have been activated, there is still an excess of reactive oxygen species that result in oxidative damage. The physiological effects of exposure reported in this study correspond with previously reported whole-organism impacts and declines in freshwater mussel populations in the urban-impacted region of this watershed.

1. Introduction

Chronic exposure to complex environmental contaminants can lead to a variety of impairments in aquatic organisms. While organisms can tolerate some amount of contaminant-induced stress, long-term exposure can exhaust repair and coping mechanisms resulting in negative impacts at higher levels of biological organization. According to the cascade of biological events triggered by stress (Selye, 1976), sub-cellular changes can provide evidence of impairment before irreversible effects are evident at the organism or population level. Sub-cellular measurements can elucidate the compromised physiological state of exposed organisms and if ecological relevance of the biological measurement, or biomarker has been established, potentially the health of the organism and the population as well. While some biomarkers are believed to demonstrate exposure to a specific group of contaminants (e.g. metallothionein), others such as oxidative stress can be used to indicate the cumulative effects of exposure to complex mixtures of contaminants, such as those found in urban rivers.

The Grand River is the largest river in Ontario, running 280 km from the village of Grand Valley to Lake Erie (Fig. 1). While the watershed drains mostly agricultural and forested land, the Grand River also flows through a large urban area (population >900,000; GPS Group, 2010) and receives input from 30 municipal wastewater treatment plants (MWWTP). These cumulative inputs create a complex mixture of agriculturally and urban-derived contaminants that pose a risk to aquatic ecosystems. Long-term water quality

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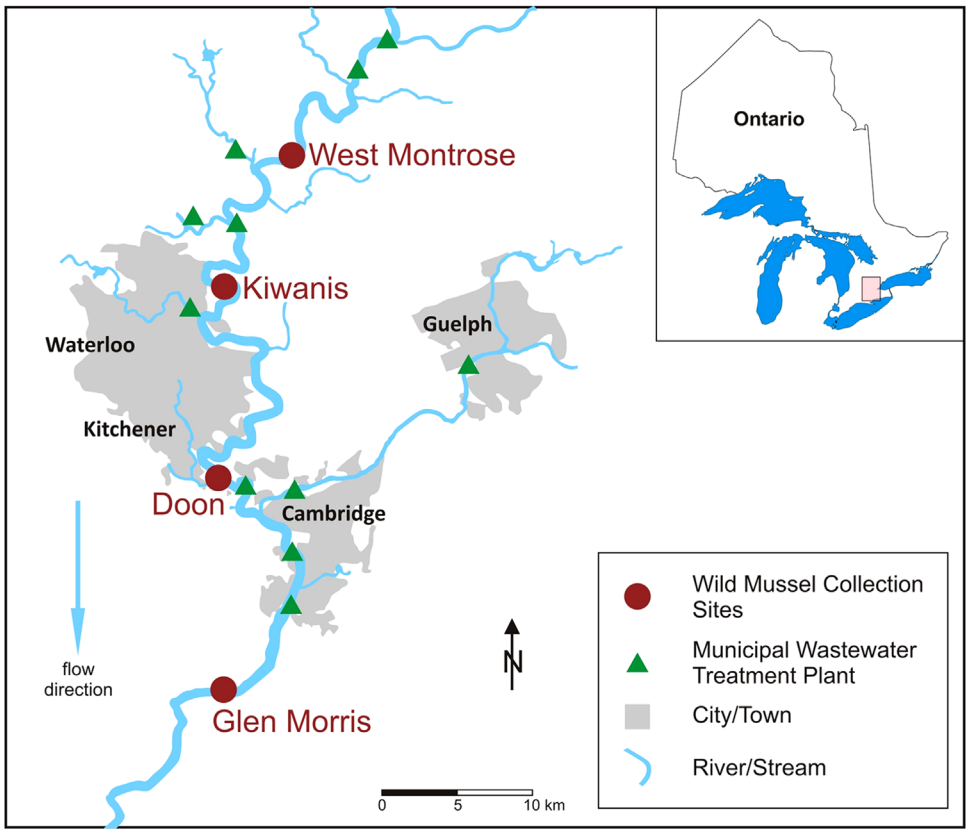

Fig. 1. Map of study area indicating the location of sites where wild *Lasmigona costata* were collected as well as the location of cities and municipal wastewater treatment plants releasing effluent into the study area in the Grand River (ON).

monitoring data indicate that total phosphorus, nitrates, ammonia and some metals (Al, Fe and Pb) periodically exceed the Canadian Water Quality Guidelines for the Protection of Aquatic Life in the urbanized region of the river (Cooke, 2006). Furthermore, a wide range of agricultural chemicals, pharmaceuticals and personal care products has been reported in the Grand River (Frank and Logan, 1988; Metcalfe et al., 2010). Recent investigations have demonstrated that the poor water quality of this watershed negatively impacts aquatic biota. For instance, there are reports of feminization of wild male fish (Tetreault et al., 2011) and altered fish communities downstream of MWWTP (Brown et al., 2011), as well as evidence that 25–50 percent of the freshwater mussel species once found in this watershed have been extirpated (Kidd, 1973; Metcalfe-Smith et al., 2000). In addition, wild freshwater mussels living downstream of multiple urban inputs (MWWTP and road runoff) have reduced condition factor and lifespan (mean age) compared to mussels living upstream of the urban area (Gillis, 2012). While both Kidd (1973) and Metcalfe-Smith et al. (2000) have suggested that 'pollution' contributed to the loss of mussel diversity in the Grand River, no direct physiological impact of the exposure has been demonstrated in wild mussels.

Therefore, in order to quantify contaminant-induced physiological stress and examine the coping mechanisms of chronically exposed mussels, a suite of stress biomarkers was employed. Wild mussels were collected from four sites along the Grand River (ON). These sites receive incrementally increasing levels of contaminant input from urban development such that the final site was downstream of 11 MWWTPs and four cities. Water quality deteriorates moving downstream through the study area, such that ammonia, chloride and phosphorus increase by 3 to 10 fold between the upstream reference site and the final downstream site (Gillis, 2012). Gill tissue metal levels and metallothionein concentrations were quantified in order to confirm the exposure gradient. Biochemical measurements were examined to assess the capacity of wild mussels to deal with oxidative stress (antioxidant capacity against peroxyl radicals (ACAP)) and quantify the damage caused by oxidative stress (lipid peroxidation). In addition, general health indicators were examined (lipid and protein levels) to determine if sub-cellular stress biomarkers correspond to tissue level effects.

2. Materials and methods

2.1. Study area

Four study sites spanning approximately 50 km of the Grand River in the Kitchener–Waterloo–Cambridge area (Ontario, Canada) were selected for wild mussel collections (Fig. 1). Beginning with the furthest upstream, the sites are referred to as West Mountrose, Kiwanis, Doon, and Glen Morris. West Mountrose is the local reference site and although it is downstream of two small tertiary wastewater treatment plants, it is located upstream of the large urban area. Kiwanis is located within the city of Waterloo but upstream of any significant MWWTPs. Doon is also located within the urban area but is downstream of the Waterloo wastewater treatment plant which in 2010, employed secondary treatment and served 126,000 residents (Region of Waterloo, 2011). The final site, Glen Morris is located downstream of four cities (total population >900,000; GPS Group, 2010) and eleven MWWTPs including the Kitchener wastewater treatment plant which in 2010 served 226,000 using secondary treatment (Region of Waterloo, 2011). The Glen Morris site is also located downstream of the influence of another river (Speed R.) which overall, drains a less developed area.

2.2. Mussel collection and tissue preparation

Lasmigona costata (fluted-shell mussel) (Rafinesque, 1820) was chosen for this study because it is found throughout the study area (Metcalfe-Smith et al., 2000) and is classified by Ontario's Natural Heritage Information Center, as 'secure at present' (NatureServe, 2010). In August 2010 fifteen adult mussels were collected from each site while wading and using water viewers (wooden boxes with glass