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Examining waterborne and dietborne routes of exposure and their contribution to biological response patterns in fathead minnow (*Pimephales promelas*)

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ABSTRACT

The objectives of the current study were: (i) to gain a better understanding of the relative importance of water and diet as routes of exposure causing toxicity in fathead minnow (FHM) exposed to metal mining effluents (MME) using a full factorial water/food experimental design (Experiment 1), and (ii) to assess differences in the effects of food quality on toxicity by comparing FHM fed both a live and frozen diet of *Chironomus dilutus* (Experiment 2). The results showed significant increases in general water quality parameters (e.g., hardness, conductivity) and various metals in the effluent treatment waters compared to control waters, with maximum increase seen in the multi-trophic streams. Metals accumulation (Rb, Al, Se, Sr, Tl, Ce, Co, Cu, Pb) effects of both waterborne and multi-trophic exposures were significant in one or more fathead minnow tissue type (muscle, gonads, liver, larvae) relative to those in the control systems. Condition factor and liver somatic index (LSI) of FHM were also significantly affected in both exposures by one or both routes of exposure (water and/or diet). In addition, cumulative total egg production and cumulative spawning events were significantly affected by both waterborne and dietborne exposures, with maximum effect found in the multi-trophic streams. These results suggest that under environmentally relevant exposure conditions, trophic transfer of metals may lead to greater reproductive effects and increased metal toxicity in fish. It also indicates that metals are assimilated in tissues differently depending on the quality of the food (live vs. frozen). Overall, it appears that the multi-trophic bioassay provides an important link between the laboratory and field, which may allow for a more realistic assessment of the true impact of MME's in the environment.

1. Introduction

Drainage from historical mining activities, mine waste, process mill tailings, mine effluent and refining activities can enter surrounding watersheds and become sources of metals (e.g., Cu, Ni, Zn, Cd, Co), metalloids (e.g., Se, Si, As, Sb) and non-metals (e.g., P, S, Cl) in the sediments, water and biota (fish, benthic invertebrates, biofilm). However, due to the complexities of aquatic ecosystems, our understanding of how elements and elemental mixtures affect the food chain is limited. Previous studies conducted by our research group have shown waterborne effects in both fish (decreased egg size, increased tissue metal burdens) and invertebrates (reduced hatching success, reduced emergence) when exposed to the treated metal mining effluents (MME) (Hruska and Dubé, 2004; Dubé et al., 2005). Similar effects of metal mining effluent exposure on fish have also been demonstrated in both lab-based as well as field-based studies (Bradley and Morris, 1986; Eastwood and Couture, 2002; Couture and Rajotte, 2003; Pyle et al., 2005).

There is no dispute that waterborne exposure is an important route of metal uptake in aquatic organisms and has been the basis for setting environmental water quality criteria and standards for metals in Canada and the USA (US EPA, 1983; CCME, 1999). However, metals are assimilated from the water and the diet very differently in the tissues of invertebrates and fish (Meyer et al., 2005). Dietborne exposure can result in whole body burdens, (often several orders of magnitude higher), or specific tissue burdens far greater than caused by waterborne exposure alone (Meyer et al.,

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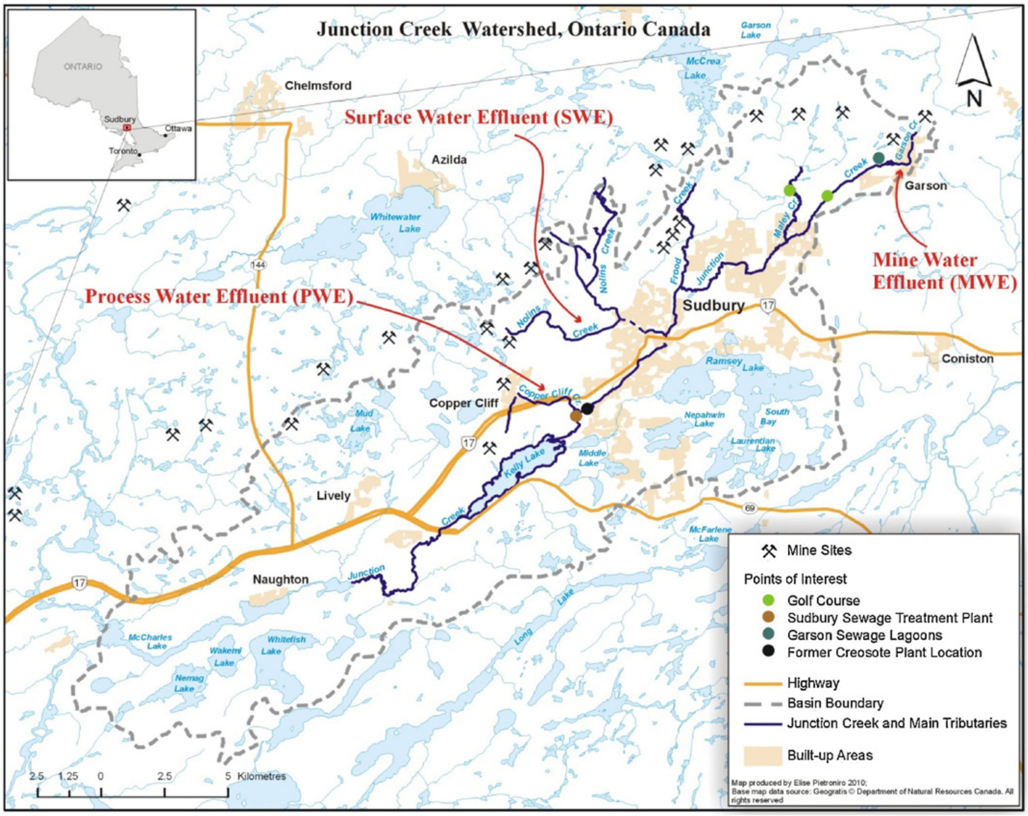

Fig. 1. Location of Junction Creek watershed near Sudbury, Ontario, Canada, and its main tributaries (map produced by Elise Pietroniro, 2010).

2005; Boyle et al., 2008). Even low levels of exposure in the water could lead to acutely toxic levels of dietary exposure in higher level predators due to biomagnification and trophic transfer up the food chain (Fisher and Hook, 2002; Campbell et al., 2005). Dietborne metals such as Cu, Ni, Zn, Se can also reduce the survival, growth and reproduction of aquatic invertebrates and fish (DeSchamphelaere and Janssen, 2004). More recent studies have demonstrated that diet can be an important pathway of metal exposure in fish leading to reproductive impairment and toxicity (Rickwood et al., 2006, 2008; Rasmussen et al., 2008; Ng and Wood, 2008; Boyle et al., 2008; Muscatello et al., 2008).

It is often assumed that the waterborne exposure pathway is primarily mediated through the gills and the dietary pathway is mediated through other internal target organs, such as the gut and liver. However, metals accumulated from diet can be transported to the gill, and can subsequently affect the gill structure and function, suggesting that the dietary pathway is more important than previously identified (Hoar and Randall, 1984; DeSchamphelaere and Janssen, 2004). Nevertheless, it is well documented that the gill is usually the primary organ of metal accumulation if the exposure is predominantly waterborne, whereas gill accumulation of metals is much less relative to that in other vital organs (e.g., liver and gut) during dietary exposure.

The objectives of our current study are two-fold: (i) to examine the effects of exposure through diet, through water or through both routes of exposure using a fully factorial exposure design in the laboratory (Experiment 1), and (ii) to examine the role of food type by assessing differences in fathead minnow (FHM) responses when fish were fed a live diet of *Chironomus dilutus* (multi-trophic) vs. when fish were fed a frozen, laboratory-prepared diet of *C. dilutus* (Experiment 2). This information is critical to bettering our understanding of the significance of diet for chronic toxicity testing of MMEs to reproducing FHM and their offspring and to consider how this information may affect our interpretation and application of different bioassays.

2. Methods and materials

2.1. Source area of mining effluents

The study site was located in Junction Creek, Sudbury, ON, Canada ∼400 km north of Toronto, Ontario, Canada from January to May, 2009. Junction Creek spans a length of about 25 km and can vary in width between 3 and 16 m. The main branch of the creek flows in a south westerly direction from the community of Garson, ON, through the city of Greater Sudbury, ON, terminating at Kelly Lake (Fig. 1). It contains five main tributaries and receives several point and non-point source inputs, which may affect the system in a cumulative manner, including urban runoff, historical and current atmospheric deposition from mining activities, municipal wastewater treatment and landfill seepage (Jaagumagi and Bedard, 2001). It is also the final receiving environment of three treated MME discharges. The MME's are all treated by conventional hydroxide precipitation and subsequent pH adjustment prior to discharging into Junction Creek (Rickwood et al., 2008). It should be noted that all effluent water was collected directly from the final discharge area following treatment. However, the nature of the MME varies among the three mine discharges. This study was conducted using only one of the MME discharges, a process water effluent (PWE) diluted to its natural environmental concentration in Junction Creek of 45% PWE comprised of a mixture of several mining inputs (e.g., mining, milling, smelting and refining from the Sudbury area. The 45% PWE was chosen for this study since previous research undertaken by our lab has shown it to elicit the greatest