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Evaluating the trophic transfer of selenium in aquatic ecosystems using caged fish, X-ray absorption spectroscopy and stable isotope analysis

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ABSTRACT

This research evaluated the dominant exposure pathways with regard to the bioaccumulation and trophic transfer of selenium (Se) in caged small-bodied fish inhabiting the receiving waters of a uranium-processing mill in northern Saskatchewan, Canada. A 21-day cage study was conducted using wild naïve lake chub (*Couesius plumbeus*) collected from a reference lake and caged in a reference and an exposure lake downstream of the mill discharge. Caged fish were fed commercially produced *Chironomus spp.* diets of 1.5 (basal – commercial food) and 5.5 (lab reared in Se-spiked water) μg Se/g (dry weight) at a feeding ration of 10 percent percent body weight/day. Lake chub fed the Se-spiked diet and caged in the reference lake showed increased whole-body Se concentrations compared to chub fed the basal diet after 21 days. Lake chub caged in the exposure lake from both the elevated Se and basal diet groups had significantly greater whole-body Se concentrations compared to the reference lake, and were not significantly different from each other. The use of stable carbon (C), nitrogen (N), and sulphur (S) isotope analyses indicated that alternate benthic food sources native to the exposure lake were likely consumed in conjunction with the controlled diets. Stable isotope analysis of both wild and caged lake chub indicated that the N and S isotopic signatures decreased with increasing Se exposure, which was reflective of the differences in isotopic signatures of the food sources. Dose-dependent substitution of Se for S in methionine as a consequence of dietary Se exposure was illustrated by a decreasing whole-body S isotope signature and an increasing proportion of selenomethionine-like compounds (as measured by synchrotron-based X-ray absorption spectroscopy) with increasing Se exposure. Speciation results from caged lake chub indicated that Se substituted for S in methionine was the dominant Se species found in caged lake chub exposed to dietary sources of Se.

1. Introduction

Previous characterisation of lakes downstream of the Key Lake uranium mill in northern Saskatchewan, Canada has indicated elevated Se concentrations in water, sediment, and biota (Muscatello et al., 2008; Golder Associates Ltd., 2008; Wiramanaden et al., 2010a). Using synchrotron-based X-ray absorption spectroscopy, selenomethionine-like compounds have been shown to be the dominant form (species) of Se accumulated in fish following both short-term and long-term Se exposure (Phibbs et al., 2011). The presence of predominantly selenomethionine-like compounds in fish exposed to elevated Se indicates it may be a suitable marker of elevated Se exposure (Phibbs et al., 2011).

Caging of small-bodied fish in contaminated aquatic systems has been shown to be an effective method of evaluating the bioavailability of trace elements in certain fish species (Pyle et al., 2001; Doebel et al., 2004; Palace et al., 2005; Allert et al., 2006; Oikari, 2006; Phibbs et al., 2011). Field-based cage studies are most suitable for evaluating impacts on fish species with high mobility or where the simulation of complex environmental conditions would be too difficult to conduct in laboratory studies (Palace et al., 2005). A standardized technique for caging small-bodied fish used by Palace et al. (2005) and adapted by Phibbs et al. (2011) has shown promising results in controlling fish interactions with the surrounding environment (water, sediment, and biota) and minimising stress caused by confinement. A species comparison between lake chub (*Couesius plumbeus*) and spottail shiner (*Notropis hudsonius*), two common small-bodied fish species inhabiting northern Canadian ecosystems, has shown that lake chub are well suited for measuring contaminant uptake in caging experiments (Phibbs et al., 2011).

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As an essential ultra-trace micronutrient, Se is required by fish in small amounts, but shows a narrow range between essentiality and toxicity (Janz et al., 2010). In aquatic environments Se is converted to organic Se species (organoselenides) by algae, macrophytes, and bacteria, which then bioaccumulate in higher food chain organisms (consumers) via dietary pathways (Feldmann, 1986; Gomez-Ariza et al., 1999; Orr et al., 2006; Stewart et al., 2010). Selenite can also be reduced by microorganisms in anoxic sediments to elemental selenium (Se^0), which is generally considered to be less bioavailable (Oremland et al., 1989; Wiramanaden et al., 2010b). Even at relatively low aqueous concentrations, bioavailable forms of Se bound to sediment are taken up by benthic invertebrates and bioaccumulate in aquatic food webs (Bowie et al., 1996; Lemly, 1997; Fan et al., 2002; Hamilton, 2004; Tsopelas et al., 2004; Simmons and Wallschlager, 2005). The mechanisms of Se biotransformation into organic forms by primary producers are not completely understood at this time; however, the metabolism of Se species into more toxic organic forms increases at higher trophic levels (Andrahennadi et al., 2007). The chemical species of Se may also be an important factor affecting uptake, mobility, and trophic transfer through the aquatic food web (Andrahennadi et al., 2007; Wiramanaden et al., 2010b). The trophic transfer of Se in aquatic food webs can lead to potential population impacts at higher trophic levels, such as fish and aquatic birds (Bowie et al., 1996; Lemly, 1997; Fan et al., 2002; Hamilton, 2004; Tsopelas et al., 2004; Simmons and Wallschlager, 2005; Janz et al., 2010).

Stable isotope analysis has been adapted to the field of aquatic toxicology, allowing researchers to track the movements of food and contaminants within an ecosystem (Cabana and Rasmussen, 1994). For example, stable isotope analysis of caged mussels has been successfully used to determine connections between marine pollution levels and high tissue concentrations (Deudero et al., 2009). By tracing the relative amounts of naturally produced carbon (C), nitrogen (N), and sulphur (S) isotopes from primary producers to the top of a food chain, it is possible to track food consumption and make inferences regarding biological cycling within an ecosystem, including the bioaccumulation of contaminants in higher trophic levels. Carbon (^{12}C and ^{13}C), nitrogen (^{14}N and ^{15}N), and sulphur (^{32}S and ^{34}S) isotopes are the most commonly used markers in aquatic ecotoxicology, because they have more than one isotope, are naturally abundant, and can be precisely measured (Lajtha and Michener, 1994; Connolly et al., 2004). The C isotope signature ($\delta^{13}C$) of an ecosystem is established during the assimilation of ^{13}C by primary producers and differs between terrestrial, freshwater, and marine environments based on the available carbon in each ecosystem (DeNiro and Epstein, 1978). The N isotope signature ($\delta^{15}N$) of an organism reflects its diet and the trophic position of the species analysed because it tends to increase during trophic transfer (DeNiro and Epstein, 1981; Cabana and Rasmussen, 1994). The S isotope signature ($\delta^{32}S$) of an ecosystem will be very narrow in a system that has not been involved in the sedimentary process and have a wider range of values in weathered or disturbed systems, which have undergone various degrees of biological sulphur cycling. A common example of this is through the reduction of sulphate to sulphide by bacteria in anaerobic environments resulting in increased ^{34}S of the remaining sulphate. Since $\delta^{32}S$ has the ability to vary over much smaller distances than can be expected with $\delta^{13}C$ and $\delta^{15}N$ it can be used as an effective tool in separating food sources with similar $\delta^{13}C$ and $\delta^{15}N$ signatures (Connolly et al., 2004; Croisetiere et al., 2009). In addition, $\delta^{32}S$ can aid in distinguishing between producers for different treatments (Connolly et al., 2004).

The objective of this study was to evaluate exposure pathways with respect to the bioaccumulation and trophic transfer of Se using caged lake chub. Lake chub collected from a reference lake were caged in both a reference lake and in a lake downstream of a uranium mill in Saskatchewan, Canada. Comparisons of dietary Se uptake were made by feeding caged lake chub a diet spiked with Se or a basal (normal Se) diet in both study lakes. Whole-body Se concentrations were compared between exposure and reference lakes, as well as between diets. Whole-body Se concentrations were evaluated in conjunction with Se speciation in fish and the stable isotope signatures of fish, experimental diets, and water. The overall goal of this research was to enhance our understanding of the dietary transfer of Se and the role of Se speciation in the aquatic ecotoxicology of this trace element.

2. Materials and methods

2.1. Study area

This research was conducted in the receiving waters downstream of the Key Lake uranium-processing mill, approximately 600 km north of Saskatoon in north-central Saskatchewan, Canada (57°11′N, 105°34′W). The Key Lake uranium milling operation processes high-grade uranium ore into yellowcake (U_3O_8) and releases approximately 5500 m^3/d of effluent characterised by elevated trace element concentrations (Muscatello et al., 2008). The research sites used in the present study included a reference lake (Yeoung Lake) and an exposure lake (Unknown Lake). The exposure lake is located in a small watershed approximately 4 km downstream of the uranium effluent release and the reference lake is located in a nearby unaffected watershed. For comparison, wild lake chub were collected and analysed from both the reference lake and the closest available exposure lake where lake chub were present (Delta Lake), located approximately 6 km downstream of Unknown Lake. The exposure lakes used in this study have been influenced by approximately 30 years of uranium milling effluent release and are characterised by elevated trace elements, hardness, conductivity, ammonia, and nitrate compared to nearby reference lakes (Muscatello et al., 2006, 2008; Golder Associates Ltd., 2008). Further details regarding these study lakes can be found in Muscatello et al. (2008), Wiramanaden et al. (2010a), and Phibbs et al. (2011).

2.2. Fish collection

Lake chub were selected for this feeding experiment because of their ubiquity across the Canadian boreal forest region and the availability of a reference population in the study area (Scott and Crossman, 1973; Muscatello et al., 2008). Lake chub were also chosen because of their demonstrated suitability for use in this type of caging experiment (Phibbs et al., 2011). Adult lake chub were captured from the reference lake using a combination of fyke nets and beach seine, and held in net pens for approximately 24 h. On day 1, lake chub ($n=120$) were transported to the caging sites in aerated tanks. Upon arrival at the study lakes, fish were lightly anesthetised using 0.1 g/L MS-222 (Tricaine, Sigma–Aldrich, Oakville, ON) before collecting initial size measurements (weight and fork length), and then deployed ($n=10$/cage) into the study lakes. At the conclusion of the 21-day feeding trials, the surviving fish were collected and immediately euthanized for future analysis (Se concentration or speciation) by administering an overdose (0.8 g/L) of MS-222. Body weight and fork length were recorded before whole-fish samples were frozen individually on dry ice in airtight containers. Samples were held on dry ice until they were transferred to a -80 °C freezer at the University of Saskatchewan. Condition factor (a general measure of fish health) was calculated using a length to weight relationship (weight/length^3 $\times$ 100). All sampling and experimental procedures involving animals in this study were conducted in accordance with the Canadian Council on Animal Care (University of Saskatchewan Animal Care and Use Protocol 20030088).

2.3. Feeding cage study

Feeding cages were deployed to determine short-term Se uptake rates and Se speciation in fish fed controlled diets and caged in either the reference or exposure lake. Six 0.5 m^3 feeding cages ($1.0 \times 1.0 \times 0.5$ m^3; $l \times w \times h$) covered with 1/4 in. (0.64 cm) nylon mesh were placed in the exposure lake (Unknown Lake) and in the reference lake (Yeoung Lake). Cage construction and design was adapted from Phibbs et al. (2011) and Palace et al. (2005). Each PVC cage was deployed approximately 1.5 m below the water surface. Previous work in these study lakes by Wiramanaden et al. (2010a) indicated that aqueous Se concentrations (total and dissolved) show little spatial variation within lakes, and the dissolved Se fraction was determined to be approximately equal to the total amount of Se in the water column. For all cages deployed the cage bottom and 30 cm of each side were covered with a plastic tarpaulin and the bottom of each cage was covered a 10 cm layer of clean silica sand (particle size 250–425 μm; Brock White Canada Inc., Saskatoon, SK) to attempt to restrict Se exposure in caged fish to only aqueous and controlled dietary sources. This barrier was implemented to eliminate the