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Uptake and tissue distributions of cadmium, selenium and zinc in striped marsh frog tadpoles exposed during early post-embryonic development

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ABSTRACT

Metals and metalloids released through anthropogenic activities can accumulate in aquatic organisms, resulting in adverse effects in sensitive species. We investigated the influence of feeding regime and exposure complexity (i.e., mixture) on bioaccumulation kinetics and body distribution of common metal(loid) pollutants in *Limnodynastes peronii* during early post-embryonic development. Tadpoles were exposed to radiolabelled ^{109}Cd, ^{75}Se and ^{65}Zn alone and in a mixture for 4 days, followed by 3 days depuration in clean water. One group was fed directly in exposure aquaria, whereas a second group was transferred to clean water for feeding, to investigate the potential influence of sorption to food on uptake. Bioconcentration factor and retention was observed to be greatest for Se. Results demonstrate that tadpoles accumulated and retained half the amount of Cd when exposed in mixture, suggesting that Se and/or Zn may have antagonistic effects against Cd uptake. Additionally, tadpoles fed directly in exposure water accumulated 2–3–times more Cd and Zn compared to tadpoles fed in clean water, indicating that the presence of food particles is an important factor that may influence uptake. Interestingly, this had a negligible impact on Se uptake. The study reveals how exposure conditions can influence the bioaccumulation of metal(loid)s, highlighting experimental factors as important considerations for both controlled toxicity experiments and for understanding exposure risks for amphibian populations.

1. Introduction

Metal pollution has long been recognized as an important environmental concern, since these elements can adversely impact aquatic animals and ecosystem health (Luoma, 1983; Rainbow, 2002; Wang, 1987). Many trace metals and metalloids (hereafter referred to as metals) occur naturally in the environment and are essential for the health and maintenance of physiological homeostasis in vertebrates. However, anthropogenic activities such as the combustion of fossil fuels, agriculture, mining and other processes can introduce unnatural levels of metals into aquatic environments. This introduces the risk of adverse effects in sensitive aquatic species, since metals are often highly persistent and bioaccumulative (Deb and Fukushima, 1999). The potential for toxicological impacts resulting from metal pollutants has been well studied for a number of aquatic species, but in general such research efforts have primarily focused on fish and invertebrates (Atchison et al., 1987; DeForest and Meyer, 2015; Rainbow, 2002). In these organisms, reported effects include (but are not limited to) tissue damage, decreased immunity, changes in behaviour, altered growth rates and nutritional status, effects on digestive enzyme activities, efficiency of food assimilation, carbohydrate metabolism, teratogenic, mutagenic and gonadotoxic effects, damage to lipid, protein, and peptide metabolism, as well as effects on productivity and life cycles. Importantly, metal toxicity has been found to be highly variable and is frequently dependent on the physicochemical characteristics of the environment (Luoma, 1983; Wren and Stephenson, 1991). As such, it is extremely important to understand the factors influencing the bioavailability and bioaccumulation of metals in understudied aquatic organisms in order to properly assess their risks under varying environmental conditions.

When considering aquatic species, some animals are inherently more susceptible than others to the potential for adverse toxicity associated with industrial contaminants. In particular, amphibians are expected to be quite vulnerable to metal bioaccumulation during larval aquatic life stages, due to their highly permeable skin and gills during this timeframe (Linder et al., 2010; Wake and Vredenburg, 2008). However, despite evidence that amphibians may be more sensitive to metals and other industrial pollutants during aquatic developmental stages (Ferrari et al., 1993; Herkovits et al., 1997; Melvin and Trudeau, 2012), metal bioaccumulation in larval amphibians has received relatively little attention. This represents an important shortcoming, since

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the limited studies that have explored bioaccumulation in amphibians have indeed linked metal uptake to severe physiological disruption (Davey et al., 2008; Lanctôt et al., 2016; Unrine et al., 2007; Zocche et al., 2013). However, previous studies with amphibians have typically focused on assessing metal burdens in field-captured individuals exposed to naturally occurring mixtures or single exposures in the laboratory. While such research is extremely important considering the threatened status of many amphibian populations globally (Monastersky, 2014), controlled mixture studies are necessary to fully explore and understand how common metal pollutants and mixtures are taken up by amphibians during sensitive larval-life stages.

To address some of the identified knowledge gaps, we investigated the bioaccumulation of three key elemental contaminants commonly occurring in industrial effluents, both alone and when exposed as a tertiary mixture, throughout early post-embryonic development in striped marsh frog (*Limnodynastes peronii*) tadpoles. Elements and forms were selected based on previous studies suggesting that they may pose high environmental risks, particularly due to their bioaccumulative nature and potential for additive and antagonistic interactions when present in mixtures (Dobrovoljc et al., 2012; Herkovits and Perez-Coll, 1990). Cadmium (Cd), a heavy metal with no known biological function in animals, has been associated with a number of reproductive and developmental effects in many species at relatively low concentrations (Hammons et al., 1978). Selenium (Se) is an essential micronutrient that plays an important role in reproduction, DNA synthesis, thyroid hormone metabolism and oxidative stress defense, although excess Se levels can also cause developmental abnormalities and reproductive failure in many species (Hamilton, 2004). Like Se, Zinc (Zn) is an essential trace element required for growth and reproduction, but is also the most ubiquitous heavy metal in the environment and can cause severe toxicological effects when present at concentrations above essential thresholds (Skidmore, 1964).

Radiotracing techniques have been shown to be highly valuable for studying trace element bioaccumulation, as they not only allow for environmental concentrations to be studied that may be undetectable with other analytical approaches, but also reduces the number of animals required by providing robust longitudinal data on individual organisms (Creighton and Twining, 2010; Cresswell et al., 2015, 2014; Metian et al., 2010). Gamma-emitting radioisotope tracers were used to trace low levels of the radiolabelled elements in individual live animals over time, and to assess their distribution within the organisms. While each of the studied metals has been deemed a risk to aquatic wildlife, little is know regarding the kinetics and interactions of these three elements, or other factors that may be important for influencing bioaccumulation and subsequent toxicity in developing amphibians. For this reason, we investigated the effect of trace metal mixture and feeding condition on the bioaccumulation kinetics and body distribution of Cd, Se and Zn in tadpoles exposed to environmentally relevant concentrations.

2. Materials and methods

2.1. Animals

L. peronii was chosen for its wide distribution in Australia, including areas in Central Queensland and New South Wales characterized by intensive mining and other industrial activities. Fertilized eggs were collected from an ephemeral pond in Elanora, QLD, and hatched in the laboratory in pond water from the collection site (QLD Government Permit No. WISP16587715). After hatching, water levels were slowly increased and replaced with reconstituted moderately hard water (MHW) made according to standard test guidelines (US EPA, 2002) (hereafter referred to as control water). Tadpoles were held in a 52 L tank filled with aerated control water renewed bi-weekly and fed Sera Micron® fry food (Sera, Heinsberg, Germany) *ad libitum* twice daily prior to experimentation. Laboratory conditions were maintained at 26 °C and 12:12 light:dark sequence. Tadpole survival was monitored twice daily and water physicochemical parameters (temperature, pH, dissolved oxygen and electrical conductivity) were monitored before and after each water renewal (i.e., bi-weekly in holding tank and daily in experimental tanks). All aspects of experimentation and sampling were approved by the Animal Care and Ethics Committee at the Australian Nuclear Science and Technology Organisation (ANSTO; ACEC Protocol No. P291), and performed in accordance with the guidelines of the Australian Code for the Care and Use of Animals for Scientific Purposes.

2.2. ^{109}Cd, ^{75}Se and ^{65}Zn exposure: uptake and depuration

^{109}Cd (as $CdCl_2$, $t_{1/2}$ = 462.6 days) was obtained from Eckert & Ziegler Isotope Products Inc., Valencia, CA. ^{75}Se (as Na_2SeO_3, $t_{1/2}$ = 119.8 days) and ^{65}Zn (as $ZnCl_2$, $t_{1/2}$ = 243.8 days) were produced at the ANSTO facility in Sydney, Australia. ^{109}Cd and ^{65}Zn stocks were dissolved in 0.1 M HCl and ^{75}Se stock was dissolved in Milli-Q water. Radioisotopes were diluted and equilibrated in control water for at least 12 h prior to use in experiments. There was no change in the measured pH after radionuclide addition (pH 7.6 ± 0.1).

Upon reaching Gosner stage 25 (Gs; Gosner, 1960), thirty tadpoles were individually transferred to square 1.125 L polypropylene containers (hereafter referred to as exposure chambers) containing 400 mL of control water or ^{109}Cd (520 kBq/L), ^{75}Se (25 kBq/L) and ^{65}Zn (230 kBq/L) alone and the full tertiary mixture for 4 days (uptake phase). Analysis of water samples by inductively coupled plasma mass spectrometry (ICP-MS; Varian 820MS Quadropole; all samples run with internal standard correction for matrix and drift correction) confirmed that water concentrations were equivalent to 0.5 µg Cd/L, 0.7 µg Se/L and 25 µg Zn/L (0.005, 0.009 and 0.38 µM, respectively). The exposure water was renewed daily to ensure consistent exposure concentrations. Concentrations were within the range commonly found in mine-impacted environments and were lower than Australian trigger values that are protective of ≥ 90% of freshwater species (ANZECC trigger values = 1.1 µg Cd/L, 18 µg Se/L and 38 µg Zn/L for the protection of 90% of species in freshwater system with moderately hard water) (ANZECC and ARMCANZ, 2000). Chambers were randomly positioned in the exposure room and tadpoles were transferred at 6 min intervals for measurements, to compensate for the counting time required for radioanalysis (as described in Lanctôt et al. (2017)).

During the exposure, tadpoles were fed equal amounts of non-spiked Sera Micron® (1 mg per animal) 1 h prior to radioanalysis and daily treatment renewals. Of the 6 tadpoles assigned to each treatment group, 3 were assigned to feeding group A and 3 to feeding group B. Tadpoles in feeding group A were removed from treatments, rinsed following the procedure described in Section 2.3 and transferred to a separate container containing 100 mL of clean water for feeding, thereby limiting their exposure to the dissolved isotopes (i.e., uptake via water only). After feeding and radioanalysis, tadpoles were returned to their exposure container. Group B were fed directly in their respective exposure chambers, and were therefore exposed to both dissolved isotopes and isotopes that potentially sorbed or complexed with food particulates. After the 4-day uptake phase, tadpoles were transferred to isotope-free water for 3 days of depuration. During the depuration phase, all tadpoles were fed in exposure chambers after their daily radioanalysis measurements. Water in experimental chambers was completely renewed every 24 h during both the exposure and depuration phases. Subsamples of exposure solutions collected before and after water renewals were radioanalyzed, and also analyzed via ICP-MS, to verify exposure activity and metal concentration, respectively. The concentration of radioisotope metals partitioned to the food in feeding group B was not quantified. Constant gentle aeration was provided in all tests via a compressed air line fed through a hole drilled into the lid of each chamber. Physicochemical parameters throughout the experiment were: pH: 7.6 ± 0.1, temperature: 25 °C ± 0.8, DO: 90 ± 9.6%,

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