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Research Paper

Selenium speciation influences bioaccumulation in *Limnodynastes peronii* tadpoles

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ABSTRACT

Despite being essential for animal health and fitness, Se has a relatively narrow range between deficiency and toxicity, and excess Se can cause a variety of adverse effects in aquatic organisms. Amphibians are particularly vulnerable to contaminants during larval aquatic life stage, because they can accumulate toxic ions through various routes including skin, gills, lungs and digestive tract. Few attempts have been made to understand the tissue-specific accumulation of trace elements, including the impacts of chemical speciation in developing amphibian larvae. We used radiolabelled ^{75}Se to explore the biokinetics and tissue distributions of the two dominant forms occurring in surface waters, selenite (SeIV) and selenate (SeVI). Tadpoles of the native Australian frog *Limnodynastes peronii* were exposed to Se in both forms, and live-animal gamma spectroscopy was used to track accumulation and retention over time. Tissue biodistributions were also quantified at the end of the uptake and depuration phases. Results showed the bioconcentration of SeIV to be 3 times greater compared to SeVI, but rates of elimination were similar for both forms. This suggests a change of Se speciation within the organism prior to excretion. Depuration kinetics were best described by a one-phase exponential decay model, and tadpoles retained approximately 19% of the accumulated Se after 12 days of depuration in clean water. Selenium bioaccumulation was greatest in digestive and excretory organs, as well as the eye, which may directly relate to previously reported Se-induced impairments. Results demonstrate how the use of radiotracing techniques can significantly improve our understanding of trace element toxicokinetics and tissue distributions in developing amphibians. From an environmental monitoring perspective, the findings highlight the importance of considering chemical speciation as this could influence the accuracy of risk assessment.

1. Introduction

Selenium (Se) naturally and ubiquitously occurs in the environment in both organic and inorganic forms. Despite being essential to most living organisms, anthropogenic activities, such as mining and agriculture, can introduce toxic levels of Se into aquatic environments (Lemly, 2004). In the worst cases, Se pollution stemming from such activities has been associated with local extinctions of several fish species (Lemly, 2002). In other instances, elevated Se has been reported to adversely impact growth, development, behaviour and reproduction, and to cause oxidative stress and deformities in aquatic biota (Hamilton, 2004; Janz et al., 2010). Selenium may pose serious long-term threats to aquatic ecosystems because it has the capacity to bioaccumulate and biomagnify in the food chain, making it difficult for ecosystems to recover from Se contamination (Lemly, 1985).

Importantly, the bioavailability and toxicity of Se depends not only on its concentration in the environment, but also its speciation (Franz et al., 2011; Kleinow and Brooks, 1986a; Maier and Knight, 1993). Se can exist in five oxidation states (VI, IV, 0, −I, −II), with Se oxyanions selenite (SeIV) and selenate (SeVI) representing the dominant forms of dissolved Se in surface waters. In aquatic systems, the relative abundance of SeIV and SeVI often vary substantially, and is generally dependent on the regional geology and the different anthropogenic sources of Se that are present. Selenite is more prevalent in systems receiving contaminated discharges from coal fly ash or oil refineries, whereas agricultural activities and run-off from crop irrigation typically result in greater mobilization of selenate (Maher et al., 2010). The different forms of Se are known

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to behave differently with respect to their sorption, bioavailability and mobility in aquatic ecosystems. In general, Se solubility has been observed to increase with increasing redox potential, and while both forms are considered readily soluble in water, SeVI has greater water solubility compared to SeIV (Masscheleyn and Patrick, 1993). As such, the bioaccumulative potential and toxicity of inorganic Se species has been shown to differ in some organisms, with SeIV generally exhibiting greater bioavailability and toxicity compared to SeVI (Franz et al., 2011; Kleinow and Brooks, 1986a; Maier et al., 1993). However, while the accumulation kinetics and toxicity of aquatic Se has been well documented for various fish and invertebrates, much less is known regarding Se toxicokinetics in amphibians (Hopkins et al., 2006; Janz et al., 2010).

Amphibians are highly susceptible to the bioaccumulation of metals and metalloids, in part because they may take up toxic ions through multiple exposure pathways (*i.e.*, skin, gills and diet), during their various life stages. They are extremely vulnerable to pollutants in general, and are currently listed amongst the most threatened organisms on the planet with many species apparently suffering local and regional declines (Monastersky, 2014). Considering their sensitivity to pollutants and globally threatened status, it is critical that we strive to understand the various factors that may be driving the toxicity of widespread environmental pollutants to amphibians. This holds particularly true for amphibians during sensitive larval developmental stages, when Se can be readily accumulated from contaminated aquatic environments (Lanctôt et al., 2016; Unrine et al., 2007).

The present study investigated accumulation and depuration kinetics as well as the resultant tissue distributions of Se administered in the dissolved forms of selenite and selenate, in striped marsh frog (*Limnodynastes peronii*) tadpoles. Radiotracing techniques were applied to monitor the bioaccumulation kinetics of Se in live tadpoles. This technique has the advantage of being non-lethal, and therefore provides robust longitudinal data on individual organisms that is ideal for kinetic assessments.

2. Materials and methods

2.1. Animals

A single fertilized *L. peronii* egg mass was collected from an ephemeral pool in Elanora, Queensland, Australia and hatched in the laboratory in natural pond water (QLD Government Permit No. WISP16587715). After hatching, water levels were slowly increased and replaced with reconstituted moderately hard water made according to standard test guidelines (US EPA, 2002). This water was used for control and diluent water. Tadpoles were held in a 52 L clear plastic container filled with aerated water 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 during acclimation and experimentation. 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). Tadpoles exhibited no obvious signs of stress during acclimation or experimentation. All aspects of experimentation were conducted at the Australian Nuclear Science and Technology Organisation (ANSTO), approved by the ANSTO Animal Care and Ethics Committee (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. SeIV and SeVI exposure: uptake and depuration

Sodium selenite (as Na_2SeO_3) and sodium selenate (as Na_2SeO_4) salts (BioReagent ≥ 98%, Sigma-Aldrich, Germany) were neutron activated to produce the radioisotopes ^{75}SeIV and ^{75}SeVI, respectively, at the OPAL reactor, ANSTO, Sydney, Australia. Activated salts were dissolved in Milli-Q water to produce stock solutions for both Se species. Selenium speciation of each stock solution was confirmed using ion chromatography (Dionex LC20). The neutron activation/irradiation process did not affect the species of either Se radioactive stock solution (data not shown). Stock solutions were diluted to 1.2 μg/L SeIV (37 kBq/L) or SeVI (35 kBq/L) and equilibrated in moderately hard water for at least 12 h prior to experimentation. Exposure concentrations were verified by inductively coupled plasma mass spectrometry (ICP-MS; Varian 820MS Quadropole; all samples run with internal standard correction for matrix and drift correction) and the species of Se within each exposure solution was confirmed using ion chromatography. Exposure concentration was selected based on the revised US EPA guideline for Se in lentic system (US EPA, 2016). Radiation doses were maintained significantly lower than previously established LD_{10} for amphibian tadpoles (Landreth et al., 1974; Panter, 1986).

Tadpoles (snout-vent length (SVL): 11 ± 1 mm; total length: 29 ± 3 mm) of Gosner stage 30–33 (Gs; Gosner, 1960) were individually transferred to 12 square 1.125 L polypropylene containers (Decor, Tellfresh; hereafter referred to as exposure chambers) containing 750 mL of 1.2 μg/L SeIV or SeVI. An additional 4 tadpoles were transferred to 4 containers containing 750 mL of Se-free control water. Exposure chambers were randomly positioned in the laboratory and tadpoles were transferred to treatments at 10 min intervals to compensate for the counting time required for radioanalysis (see Section 2.3.2). Tadpoles were exposed for 7 days, after which they were transferred to clean exposure chambers containing 750 mL of control water for a 12-day depuration period. The exposure water was renewed daily to ensure consistent exposure levels. Subsamples of exposure solutions collected both before and after water renewals were radioanalysed, and confirmed via analysis using ICP-MS, to verify exposure activity and Se concentrations, respectively. Tadpoles were fed equal amounts of Sera Micron® twice daily throughout the experiment (10% of body weight/day) in experimental solutions. Our previous study showed that feeding tadpoles in water containing dissolved ^{75}Se had no effect of Se bioaccumulation compared to tadpoles fed in clean water (Lanctôt et al. a, under review). Each chamber contained an internal polypropylene basket, which allowed the tadpoles to be removed from the chamber and rinsed with ease prior to radioanalysis. Constant aeration was provided in all tests via a compressed air line fed through a hole drilled in the lid of each chamber. Holding and exposure water physicochemical parameters were as follows: pH: 7.4 ± 0.2, temperature: 26 °C ± 0.9, DO: 86 ± 10%, conductivity: 318 ± 8 μS/cm.

2.3. Gamma-spectrometry

2.3.1. Daily radioanalysis of water samples and live tadpoles

The emission of gamma rays from water samples and exposed tadpoles was quantified using a 1.5 × 1.5 inch Lanthanum Bromide (LaBr) detector. The LaBr detector was held within a lead chamber attached to a multichannel spectrometer (Canberra InSpector 1000 with IPROL-1 LaBr probe) and connected to a PC equipped with spectra analysis software (Genie 2000). Varying count times (from 5 to 15 min) were used to ensure propagated counting error rate was less than 5% for all measurements. Gamma counts were corrected for background and for radioactive decay. Efficiencies for the detector were estimated using geometric matched standards and appropriate corrections applied to the count rates. Geomet-

ric standards for whole tadpoles were made by adding 0.6 mL of standard ^{75}Se solution (3800 kBq/L) to the same 6 mL tubes used for live animal radioanalysis (see Section 2.3.2). This volume was approximately the same volume as the tadpoles. ^{75}Se emissions were counted between 234 and 293 keV (peak at 264 keV). Daily checks for detector response were made using an in-house ^{75}Se standard.

Live tadpoles were radioanalysed every 24 h during the accumulation and depuration phases of the experiment. Tadpoles were removed from their respective exposure chambers by removing polypropylene internal baskets, and rinsed following a pre-established procedure to remove any loosely adsorbed radionuclides (Cresswell et al., 2015). Briefly, tadpoles were consecutively transferred between 4 different rinse solutions (250 mL) for 10 s each: 1 × non-active control water, 2 × 0.1 mM ethylenediaminetetraacetic acid (EDTA, pH 7.6) and 1 × non-active control water. Separate rinse containers were used for each treatment. Following rinsing, each tadpole was transferred to a 6 mL plastic tube (H 50 × D 15 mm) containing 4 mL of non-active control water. Tadpoles were constrained to the bottom 4 mm of the tubes using plastic cylindrical inserts (H 40 × D 11 mm) closed at one end with 1 mm mesh to inhibit the tadpoles from swimming up the tube. Tubes were placed at the center of the LaBr detector and all tadpoles were oriented in the same direction for counting. Each animal was counted for 5–15 min each to determine ^{75}Se activity. After counting, tadpoles were removed from the tubes and weighed by in a pre-weighed petri dish. They were then developmentally staged based on the Gosner staging table (Gosner, 1960) and photographed on a 10 mm × 10 mm grid for measurement of SVL and total length using ImageJ image analysis software (National Institutes of Health, USA). Tadpoles were then returned to their respective test chambers. Morphometric measurements were used to calculate condition factor (K = [weight/SVL^3] × 100) for each tadpole.

To assess the possible influence of minor tadpole movements during live animal radioanalysis, three tadpoles from each treatment were radioanalysed multiple times (5 min each). After each measurement, the tube was fully removed from the lead chamber, moved slightly and then replaced and re-counted. This was performed three times for each animal, and provided a relative standard deviation for the radioanalysis procedure of 2 ± 1%.

2.3.2. Tissue dissection, digestion and radioanalysis

Following the live-animal radioanalysis, three tadpoles from each treatment and two from control were euthanized by immersion in 3-aminobenzoic acid ethyl ester (MS222; Sigma–Aldrich) dissolved in water (i.e., on day 7 of exposure and again on day 12 of the depuration). Tadpoles were not fed for 24 h prior to euthanasia in order to allow clearance of the digestive tract (Altig and McDearman, 1975; Pryor and Bjorndal, 2005). Euthanized tadpoles were rinsed with reverse osmosis (RO) water, blotted dry on a Kimwipe™, staged and measurements of SVL (mm), tail length (mm) and whole-body wet weight (mg) were taken. Whole tail, gut (intestine and stomach), liver, mesonephros, gills and eyes were dissected from treated tadpoles. Tools were washed carefully using RO water, EDTA and ethanol between each tissue and animal. Dissected tissues were rinsed with RO water, blotted dry on a lint-free tissue and weighed to obtain the wet weight (mg) of each tissue. Tissues were dried at 60 °C in 10 mL plastic tubes. Whole bodies of control tadpoles also dried. Dry weights were obtained and samples were stored at room temperature until further processing and analysis.

Dried tissues were digested with a mixture of nitric acid and hydrochloric acid (Merck, GR Grade, Germany; 9 parts HNO_3: 1 part HCl) following a modified protocol from the US EPA Method 200.3 (1996). Briefly, 0.9 mL HNO_3 (69%) and 0.1 mL HCl (37%) were added to each 10 mL tube containing the dried samples, and mixed well. Caps were loosened and samples were equilibrated with the acid mixture for 1 h at room temperature. Samples were then digested for 1 h at 95 ± 5 °C in water bath and cooled at room temperature for 1 h. Each sample (1 mL acidic mixture) was then transferred to 6 mL borosilicate glass vials. Tubes were rinsed twice with 1 mL deionized (DI) water and this was also collected to ensure that the entire sample was saved (3 mL final volume). All tissues were digested in this manner in order to ensure a standard counting geometry for radioanalysis. Radioactivity associated with the different body compartments was then measured with an automated gamma counter (Wizard^2, Perkin-Elmer, Australia) and standardised using geometrically-matched, mixed-isotope in-house standards, verified by HPGe gamma spectrometry.

2.4. Statistical analysis

The influx rates (I_u, μg/g/d) were calculated using the slope of the linear regression line of Se accumulation during the first 6 d of exposure with the intercepts set at zero (Creighton and Twining 2010). In order to consider slight differences in exposure concentrations, accumulation rate constants (k_u, mL/g/d) were also calculated from the linear regression between BCF and time (first 6 days) with the intercepts set at zero. It is important to note that there would likely have been efflux processes occurring during the first 6 days and therefore I_u and k_u calculated are inclusive of such efflux. As the main goal was to compare between SeIV and SeVI kinetics, it is likely that the efflux processes during the exposure period would have been very similar for the two forms (see below for depuration rate analysis). We believe that the determination of I_u and k_u from 0 to 6 days is sufficient for this comparison. Bioconcentration factors (BCFs, mL/g) were calculated for each Se form by dividing the whole body or organ Se concentrations on day 7 of uptake by the average dissolved Se concentration in the test solution. The retention efficiencies (RE, %) were calculated by fitting a first order decay model: $RE_t = RE_0 \times e^{-ke \times t}$, where RE is the percentage of Se remaining in the tadpoles at time t (d), and k_e (d^{-1}) is the depuration rate constant for Se loss, and used the corresponding y-intercept to estimate Se retention efficiency (Hervé-Fernández et al., 2010; Lee et al., 2006). Depuration half-lives ($t_{1/2}$, d) are calculated on the exponential part of the depuration curve using $t_{1/2} = \ln(2)/k_e$.

Differences in size (i.e., SVL and weight), K, development, Se concentration and biodistribution were analyzed using full factorial two-way ANOVAs with treatments (SeIV and SeVI) and time as factors. Differences in BCF, I_u, k_u, k_e, $t_{1/2}$ between treatments were analyzed using unpaired t test. Full factorial two-way ANOVAs were also used to test differences in Se concentration, BCF and retention between the different tissues and treatments. Tukey HSD post hoc comparisons were used to determine specific differences between the different tissues. In all cases, data were first analyzed for normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's test) to ensure parametric analysis was appropriate. Percentage data were arcsine square root transformed prior to analysis. The significance level was set at $\alpha \leq 0.05$ for all tests. Analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Longitudinal verification of water chemistry

Measured concentration throughout the 7-d exposure to SeIV was 1.56 ± 0.08 μg/L (52 Bq/mL), 0.4 μg/L over the nominal concentration of 1.2 μg/L, whereas SeVI was 1.12 ± 0.04 μg/L (37 Bq/mL), 0.1 μg/L below our target concentration. Concentrations fluctuated by less than 10% between water changes, with the average of fresh

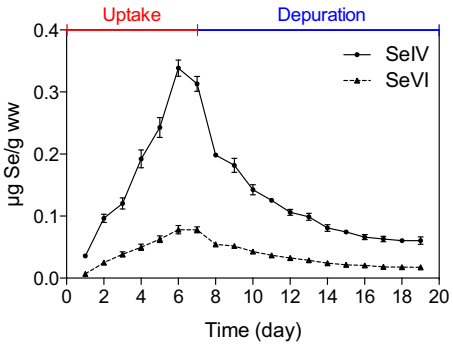

Fig. 1. Concentration (µg Se/g wet weight) of selenite (SeIV) and selenate (SeVI) measured in *L. peronii* tadpoles throughout a 7-d exposure (uptake) followed by 12 days in clean water (depuration). Values are means ± SE; n = 3-6.

Table 1
Influx rate constant (I_u, µg/g/d), accumulation rate constant (k_u, mL/g/d), bioconcentration factor (BCF, mL/g wet weight), retention efficiency (RE, %), elimination rate (k_e, d^{-1}) and depuration curve half time ($t_{1/2}$, d) of Se in whole tadpoles exposed to radiolabelled selenite (SeIV) or selenate (SeVI) for 7 days followed by 12 days of depuration in clean water. Values are mean ± SD. Asterisks indicate significant differences between treatments based on unpaired *t*-tests with α = 0.05.

	SeIV	SeVI
I_u	0.051 ± 0.006	0.013 ± 0.003*
k_u	32.4 ± 3.8	11.3 ± 2.3*
BCF	201 ± 19	69 ± 10*
RE	19.0 ± 3.2	19.1 ± 1.9
k_e	0.32 ± 0.03	0.31 ± 0.05
$t_{1/2}$	2.15 ± 0.22	2.30 ± 0.38

solutions being 1.59 µg/L for SeIV and 1.14 µg/L for SeVI compared to 1.53 and 1.11 µg/L, respectively, after 24 h. ^{75}Se was not detected in controls and depuration water after 15-min counts. No mortalities were observed throughout the study duration. Tadpole size, condition (*K*) and development did not vary between treatments (Supplementary Fig. 1; SVL: $p = 0.75$; weight: $p = 0.06$; *K*: $p = 0.25$; Gs: $p = 0.24$).

3.2. Accumulation and retention kinetics of SeIV and SeVI in whole tadpoles

Two-way ANOVA revealed a significant time * treatment interaction ($F_{(18,118)} = 37.8$, $p < 0.001$) as well as a significant main effect of time ($F_{(18,118)} = 97.3$, $p < 0.001$) and treatment ($F_{(1,118)} = 1167$, $p < 0.001$). Results show that tadpoles accumulated and retained significantly more SeIV than SeVI throughout the exposure (Fig. 1). Accumulation of SeIV and SeVI was linear for the first 6 days of exposure and appeared to reach a steady state on day 7. The influx rate (I_u, µg/g/d), calculated from the linear regression of Se body burdens during the first 6 d of uptake, was 4 times greater for SeIV ($R^2 = 0.90$) compared to SeVI ($R^2 = 0.83$) ($t = 14.26$, df = 10, $p < 0.0001$; Table 1). The bioconcentration factor (mL/g wet weight) of tadpoles after 7 days of exposure was 3-times higher for SeIV than SeVI ($t = 14.93$, df = 10, $p < 0.0001$; Table 1). Similarly, the accumulation rate constant calculated using the BCF (k_u, mL/g/d) was also significantly greater for SeIV ($R^2 = 0.90$) compared to SeVI ($R^2 = 0.83$) ($t = 11.60$, df = 10, $p < 0.0001$; Table 1).

Once transferred to clean water, Se elimination in tadpoles followed an exponential decrease (*i.e.*, first-order elimination kinetics, $R^2 > 0.95$; Fig. 2). The efflux rate was not dependent on the Se form, as the k_e values did not differ significantly between treatments (t = 0.51, df = 4, $p = 0.636$; Table 1). Retention efficiencies did not differ significantly between Se forms ($t = 0.039$, df = 4, $p = 0.971$; Table 1), and tadpoles from both treatments retained 17–25% of the initial Se at the end of the depuration phase. This represents whole-body concentrations (average ± SD) of 0.06 ± 0.011 µg SeIV/g ww and 0.02 ± 0.002 µg SeVI/g ww at the end of the depuration phase.

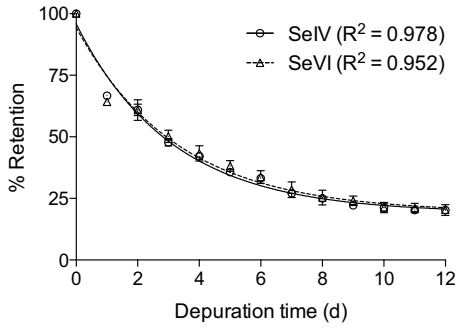

Fig. 2. Selenium retention (mean ± SE) throughout the 12-d depuration phase in *L. peronii* tadpoles exposed to selenite (SeIV) and selenate (SeVI). Regression equations are $y = 76.8 \times e^{-0.32t} + 19.1$ for SeIV and $y = 75.1 \times e^{-0.31t} + 19.4$ for SeVI.

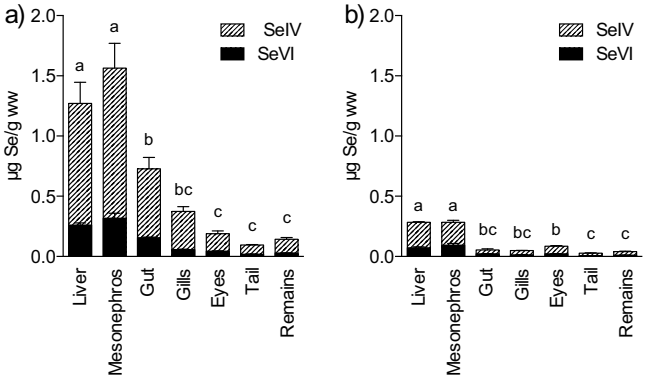

Fig. 3. Selenium concentration (µg/g wet weight) in dissected organs of *L. peronii* tadpoles exposed to selenite (SeIV) or selenate (SeVI) on (a) day 0 and (b) day 12 of depuration. Values are means ± SE; n = 3. Letters indicate significant differences between the various tissues, based Tukey HSD post hoc test with α = 0.05.

3.3. Biodistribution of SeIV and SeVI in tadpoles

A two-way ANOVA was conducted to examine differences between treatments and tissues Se concentration at the start and end of the depuration phase, and revealed a significant interaction between the effects of treatments and tissues on Se accumulation on day 0 ($F_{(6,41)} = 17.7$, $p < 0.0001$) and 12 of depuration ($F_{(6,41)} = 63.5$, $p < 0.0001$). A significant treatment effect revealed that higher concentrations of SeIV were taken up in all organs on day 0 ($F_{(1,41)} = 139.9$, $p < 0.0001$) and 12 of depuration ($F_{(1,41)} = 447.0$, $p < 0.0001$; Fig. 3). Main effect analysis also revealed significant differences amongst tissues on day 0 ($F_{(6,41)} = 40.7$, $p < 0.0001$) and 12 of depuration ($F_{(6,41)} = 205.9$, $p < 0.0001$). Post hoc analysis showed that livers and mesonephros accumulated more Se regardless of exposure species compared to other tissues (gut, gills, eyes, tail and remains) (Tukey HSD, $p < 0.05$; Fig. 3).

Likewise, two-way ANOVA analysis revealed significant interaction between treatments and tissues for BCF ($F_{(6,41)} = 14.0$, $p < 0.0001$). Main effect analysis revealed that BCF was greater for SeIV than SeVI in all dissected tissues ($F_{(1,41)} = 111.2$, $p < 0.0001$) and BCF differed amongst tissues ($F_{(6,41)} = 45.3$, $p < 0.0001$). Post hoc analysis showed that bioconcentration of SeIV and SeVI was greatest in liver and mesonephros compared to other tissues (Tukey HSD, $p < 0.05$; Table 2). On the other hand, no significantly treatment * tissue interaction were observed for Se retention ($F_{(6,41)} = 0.49$, $p = 0.814$). Main effect analysis revealed that tadpoles retained more SeIV than SeVI in all dissected tissues

Table 2
Bioconcentration factor (BCF) and retention (%) of selenite (SeIV) and selenate (SeVI) in dissected organs of *L. peronii* tadpoles after 12 days of depuration following a 7-day exposure. Values are means ± SEM; n = 3. Letters indicate significant differences between the various tissues, based Tukey HSD post hoc test with $\alpha = 0.05$.

	BCF		% Retention	
	SeIV	SeVI	SeIV	SeVI
Liver	814.4 ± 195.3^{a}	232.5 ± 26.6^{a}	22.4 ± 0.8^{ace}	27.5 ± 5.3^{ab}
Mesonephros	1002.3 ± 227.5^{a}	282.7 ± 63.7^{a}	18.2 ± 1.8^{ac}	29.5 ± 8.5^{ab}
Gut	465.9 ± 105.6^{b}	138.5 ± 7.2^{b}	7.6 ± 2.1^{b}	14.9 ± 1.1^{a}
Gills	239.7 ± 44.0^{bc}	51.5 ± 5.3^{c}	13.1 ± 1.2^{ab}	23.5 ± 7.0^{ab}
Eyes	121.5 ± 26.1^{c}	38.7 ± 6.2^{c}	45.5 ± 4.8^{d}	51.5 ± 6.9^{c}
Tail	60.0 ± 4.5^{c}	17.6 ± 2.7^{c}	27.7 ± 5.1^{e}	38.2 ± 2.7^{bc}
Remains	91.6 ± 16.5^{c}	25.8 ± 3.5^{c}	27.0 ± 4.4^{ce}	38.7 ± 4.8^{bc}

($F_{(1,41)} = 38.2$, $p < 0.0001$) and that retention differed amongst tissues ($F_{(6,41)} = 39.7$, $p < 0.0001$). Post hoc analysis showed that the eyes retained the highest proportion of SeIV and SeVI throughout the depuration and that guts retained the least (Tukey HSD, $p < 0.05$; Table 2).

Contrary to Se tissue concentrations and BCF, the relative proportion of Se accumulated in tadpole tissues was not influenced by Se form and no significantly treatment * time interactions were observed for any tissues (Fig. 4, two-way ANOVA $p > 0.05$). Main effect analysis revealed significant decreases in Se biodistribution between day 0 and 12 of depuration in gut ($F_{(1,8)} = 85.1$, $p < 0.0001$), gills ($F_{(1,8)} = 36.8$, $p < 0.0001$) and mesonephros ($F_{(1,8)} = 9.0$, $p = 0.017$) regardless of treatment (Fig. 4a, d and e, respectively). On the other hand, Se biodistribution in tail ($F_{(1,8)} = 89.3$, $p < 0.0001$) and eyes ($F_{(1,8)} = 722.1$, $p < 0.0001$) exhibited significant increases through depuration (Fig. 4b and f, respectively). No significant differences in Se biodistribution were observed in liver tissue between time points ($F_{(1,8)} = 3.1$, $p = 0.12$; Fig. 4c).

4. Discussion

4.1. Tadpoles accumulated significantly more SeIV than SeVI

Results demonstrate that *L. peronii* tadpoles readily accumulate both selenite and selenate from water when exposed to environmentally relevant concentrations. However, tadpoles accumulated selenite at a much faster rate than selenate. Greater selenite bioaccumulation has also been reported in other species (Franz et al., 2011; Kleinow and Brooks, 1986a). Speciation differences in accumulation likely relate to differences in metabolism between Se oxyanions. Once absorbed, selenate must first be reduced to selenite prior to further metabolism (Burk and Hill, 2015; Kleinow and Brooks, 1986a). This rate-limiting step results in selenite being more rapidly metabolized and incorporated into proteins and a greater proportion of selenate being eliminated prior to biotransformation (Burk and Hill, 2015). Importantly, increased selenite accumulation is consistent with increased toxicity that has generally been identified for this form of Se compared to that reported for selenate (Franz et al., 2011; Kleinow and Brooks, 1986a; Maier et al., 1993).

The greater bioavailability of selenite is an important consideration for hazard assessment, since larval amphibians (and other aquatic species) are likely at greater risk of experiencing Se toxicity in waterways where selenite is the predominant form. For example, monitoring data suggests that systems receiving discharge from coal fly ash or oil refineries commonly have high selenite concentrations (Maher et al., 2010). From a monitoring perspective, our results highlight the relevance of considering Se speciation when establishing site-specific water quality guidelines. Importantly, major monitoring programs do not currently make such distinctions. For example, the U.S. EPA chronic water quality criterion does not discriminate between different selenium forms (US EPA, 2016). Understanding the comparative bioavailability of different chemical species and how they are incorporated or eliminated by sensitive biota may therefore help to improve environmental risk assessment.

4.2. Tadpoles exposed to SeIV and SeVI had similar depuration kinetics

Interestingly, while Se uptake differed between species, elimination kinetics and retention efficiencies were similar for the two forms. Depuration for both Se oxyanions followed a simple exponential kinetic model and tadpoles retained approximately 19% of accumulated Se regardless the exposure form. Retention efficiencies for both species were in the range previously reported in other embryo and tadpole studies (Browne and Dumont, 1979; Lanctôt et al. b, under review), as well as fish studies (Xu and Wang, 2002; Creighton and Twining, 2010). Similarities in depuration kinetics between species suggest a change of Se speciation within the organism prior to excretion (Kleinow and Brooks, 1986a), which is in accordance with the biotransformations required for protein incorporation. Once inorganic Se species have been taken up, Se in the form of selenite (selenate is reduced to selenite) is reduced to selenide, which is then transported to the liver where it is further metabolized (Burk and Hill, 2015). In most organisms, Se is predominantly accumulated as selenomethionine (SeMet) and selenocysteine (SeCys) regardless of exposure form (Misra et al., 2012; Phibbs et al., 2011a, 2011b), although rates of biotransformation are known to differ between forms. Speciation analysis in fish showed a concentration dependent shift in the dominant species with increasing Se burdens, with SeCys-like forms being predominant at lower Se concentrations and SeMet-like species at higher concentrations (>5 ug/g dw) (Janz et al., 2014; Phibbs et al., 2011a). In amphibians, the proportion of Se incorporated into proteins is thought to be retained throughout tadpole metamorphosis (Rowe et al., 2011; Snodgrass et al., 2004, 2003), and will not likely be eliminated unless proteins are catabolized (Franz et al., 2011). Examining efflux rates is therefore important since this may help explain differences between organisms' abilities to cope with contaminant burdens.

Retention of Se throughout metamorphosis may pose additional risks beyond the individual animals, due to potential transgenerational effects through maternal transfer or transference to terrestrial food webs through predator-prey interactions. For example, water snakes (*Nerodia fasciata*) were found to contain elevated Se concentrations as a result of feeding on amphibians and fish from coal-impacted sites (Hopkins et al., 2002, 1999). Thus, Se accumulation in larval amphibians may not only impact juveniles and adults after metamorphosis, but can also have impacts on organisms that rely on them as a food source (Snodgrass et al., 2004). In other studies, maternally transferred Se burdens were linked to reduced hatching success and increased malformation rates in offspring (Hopkins et al., 2006; Massé et al., 2015). These examples further highlight the importance of understanding biokinetics of Se (and other trace pollutants) through metamorphosis, including differences amongst oxyanions.

4.3. Selenium biodistribution

Liver and mesonephros accumulated the highest concentrations of both SeIV and SeVI, followed by gut > gills > eyes > tails. Partitioning of Se in hepatic and renal tissue is consistent with previous studies in fish (Alquezar et al., 2008; Kleinow and Brooks, 1986a; Mackay et al., 1975) and frogs (Lanctôt et al. b, under review). Hepatic accumulation most likely relates to the important role of the

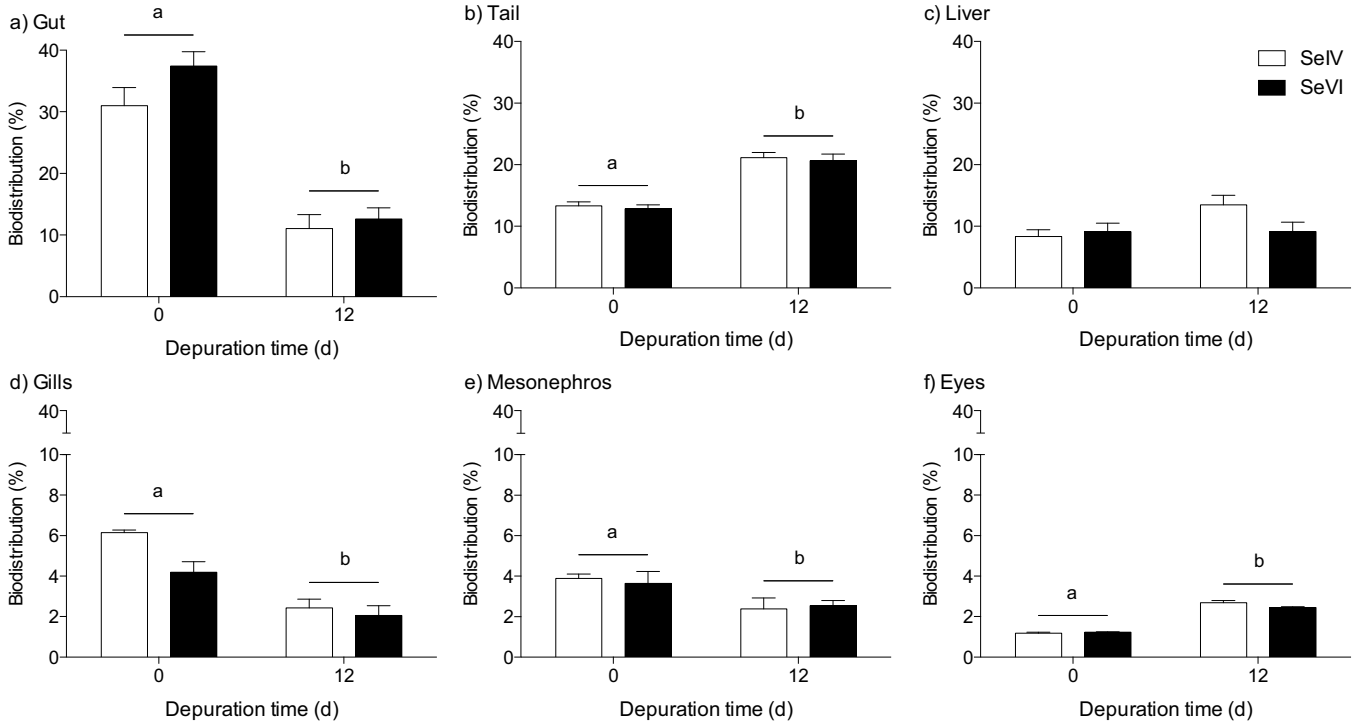

Fig. 4. Proportion (%) of Se in tissues (μg/g wet weight) relative to total body concentrations (i.e., sum of all tissues) in *L. peronii* tadpoles exposed to selenite (SeIV) or selenate (SeVI) at the beginning and end of the depuration phase (day 0 and 12, respectively) following a 7-d exposure. Values are means ± SE; n = 3. Letters indicant significant differences between time points (α = 0.05).

liver is Se metabolism, elimination and assimilation, whereas accumulation in renal tissue corresponds to the role of the mesonephros in excretion (Burk and Hill, 2015). Kidneys have been identified as a primary site of Se accumulation in various species, and urine a primary route of excretion (Alquezar et al., 2008; Kleinow and Brooks, 1986b). Accumulation of Se in liver and mesonephros can therefore have damaging consequences on these tissues, including histopathological damage and oxidative stress (Miller et al., 2007; Sorensen et al., 1984; Sorensen, 1988).

Despite relatively high Se burdens in the gut at the end of the uptake phase, retention in the gut was low compared to other organs, especially for selenite. As previously suggested, Se losses in the gut during prometamorphic growth (Gs 31 to Gs 37) likely relate to a combination of elimination and absorption via this organ (Lanctôt et al. b, under review). This is again consistent with fish and mammalian studies in which dissolved and dietary Se oxyanions were found to be readily absorbed from the gastrointestinal tract and partially eliminated through the feces (Kleinow and Brooks, 1986b; Pedrosa et al., 2012). In tadpoles, the process of metamorphosis introduces other considerations that may contribute to observed losses. Specifically, the gut undergoes drastic remodeling during development, and Se has been hypothesized to be further eliminated through the shedding of apoptotic cells during metamorphic tissue degeneration and remodeling. The influence of complex physiological and morphological changes that occur to the tadpole gut during metamorphosis may represent unique mechanisms for uptake and elimination that warrant further investigation.

Larval specific tissues, such as gills and tails, do not represent a site of long-term storage since these tissues regress through metamorphosis. However, excess Se has been linked to cytoxicity in these tissues and this could have an impact on tadpole respiration or mobility during larval stages (E. Sorensen et al., 1984). In accordance with our previous study, Se accumulation and retention in the tail was relatively high compared to other tissues during growth and development, and biodistribution increased through depuration (Lanctôt et al. b, under review). Nevertheless, Se biodistribution in the tail and gills of tadpoles in that study was found to decrease during metamorphic climax as tissues degenerate. As suggested for the gut, Se associated with apoptotic cells may be either eliminated and/or redistributed during metamorphic tissue remodeling, and transference of Se burdens from degenerated cells to surrounding cells may lead to concentrated tissue burdens.

Finally, our results indicate that tadpole eyes retain the greatest proportion of Se regardless of the exposure form and showed that the relative proportion of Se in the eyes increased through depuration. This likely relates to role of selenoproteins in ocular development and function (Flohé, 2005), and the incorporation of Se into sulfur proteins (Choudhury et al., 2015; Shearer et al., 1984), although speciation assessments is necessary to confirm the fate of Se accumulated in the eyes of tadpole. It has recently been demonstrated that Se (exposed as dissolved SeIV and dietary SeMet) was accumulated predominantly within the lens-core and lens-epithelium of tadpoles (Lanctôt et al. b, under review) and fish (Choudhury et al., 2015). These previous studies demonstrate that Se partitioning within the eye lens is not influenced by speciation or exposure route. Importantly, ocular accumulation beyond the necessary levels may result in Se-induced impairments, including cataracts and distorted shape (Flohé, 2005; Lemly, 1997, 1993; Massé et al., 2015; Shearer et al., 1987; Woock et al., 1987). Ocular impairments have been hypothesized to result from increased oxidation due to selenomethionine incorporation into crystalline proteins (Choudhury et al., 2015). We suspect that similar mechanisms apply across species, but more research is needed to elucidate the mechanisms of ocular deformities in amphibians.

5. Conclusion

Selenium has been garnering increasing interest as a contaminant of concern due to industrial sources of this element in the

environment, but there is a recognised paucity of information regarding Se accumulation and toxicity in amphibians. This study used radiotracing techniques to explore speciation differences in Se toxicokinetics and tissue distributions during larval amphibian development. Findings demonstrate differential uptake and retention of the two major Se oxyanions, SeIV and SeVI, in striped marsh frog exposed to environmental concentrations. The findings highlight the importance of considering chemical speciation for improving risk assessment, a practice that is uncommon in major monitoring frameworks. Additional work is however needed to assess the impact of environment conditions on Se accumulation, and specific consideration is needed to compare bioaccumulation of inorganic species to organic forms from diet and water. Further investigations into changes in Se biodistribution and speciation in amphibians will increase our understanding of Se biotransformation and toxicokinetics of Se species in these sensitive organisms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2017.03.009.

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