

# Variation in Zn, C, and N isotope ratios in three stream insects

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## Abstract

Total Zn concentrations and Zn isotope ratios were measured, using multicollector inductively coupled plasma (ICP)-mass spectrometry (MS), in three species of aquatic insects collected from a stream in Peterborough, Ontario, Canada. Total Zn levels averaged  $193 \pm 88$   $\mu\text{g/g}$  dry weight (dw) in water striders (Heteroptera: Gerridae, *Aquarius remigis*) and were significantly higher than the concentrations measured in stonefly nymphs (Plecoptera: Perlidae, *Acroneuria abnormis*) and caddisfly larvae (Trichoptera: Limnephilidae, *Pycnopsyche guttifer*), i.e.,  $136 \pm 34$   $\mu\text{g/g}$  dw and  $125 \pm 26$   $\mu\text{g/g}$  dw, respectively. Average delta values for  $^{66}\text{Zn}/^{64}\text{Zn}$  in the water striders were approximately 0.7‰ lighter ( $-1.2\text{‰} \pm 1.0\text{‰}$ ) and were significantly different than those measured for stoneflies ( $-0.45\text{‰} \pm 0.62\text{‰}$ ) and caddisflies ( $-0.51\text{‰} \pm 0.54\text{‰}$ ). Nitrogen isotope ratios were significantly different ( $P < 0.05$ ) among the three species suggesting differences in trophic positioning. Similar to the Zn isotope ratios,  $\delta^{13}\text{C}$  values for the water striders ( $-28.61\text{‰} \pm 0.98\text{‰}$ ) were significantly different than those of the stoneflies and caddisflies, i.e.,  $-30.75\text{‰} \pm 1.33\text{‰}$  and  $-30.68\text{‰} \pm 1.01\text{‰}$ , respectively. The data suggest that the differences observed in Zn ratios relate to food source for these insects. Similar to their carbon sources, Zn in water striders appears to be primarily of terrestrial origin, and of aquatic origin for the other two species.

**Key words:** food source, stream insects, zinc, carbon, nitrogen isotope ratios



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## Introduction

With the tremendous advances in mass spectrometry (MS) techniques over the past few decades, the use of stable isotope ratios to determine the cycling and ultimate fate of elements in the aquatic environment has grown exponentially. Mass-dependent fractionation of lighter isotopes, such as C and N, has been well-studied. Variations in stable isotope ratios of  $^{13}\text{C}/^{12}\text{C}$  ( $\delta^{13}\text{C}$ ) and  $^{15}\text{N}/^{14}\text{N}$  ( $\delta^{15}\text{N}$ ) have been used extensively to examine food sources, trophic structure, and energy transfer and efficiency in lakes (Vander Zanden et al. 1999; Vander Zanden and Rasmussen 1999, 2001; Post 2002), reservoirs (Saito et al. 2001), and marine systems (Das et al. 2003). In addition, interest in the sources and trophic transfer of contaminants in aquatic systems has led to studies in which  $\delta^{15}\text{N}$  and (or)  $\delta^{13}\text{C}$  values were coupled with measurements of total metal concentrations in both freshwater and marine biota (e.g., Power et al. 2002; Das et al. 2003; Quinn et al. 2003; Larsson et al. 2007; Jardine et al. 2009, 2012; Senn et al. 2010; Jones et al. 2014).

Previous limitations in MS technology precluded rapid, precise, sensitive, and accurate measurements of stable isotope ratios of the transition elements and other heavier elements. However, developments

in multicollector (MC)-inductively coupled plasma (ICP)-MS technology have enabled investigations of stable isotope ratios for transition metals, such as Fe, Cu, and Zn, and also heavier elements, such as Hg, similar to those for lighter elements (see review by [Weiss et al. 2008](#)). Thus, the potential exists to use transition element stable isotope ratios to trace kinetic pathways of these elements in biological systems, similar to the approach used for the lighter elements, C and N. To date, most work has been directed towards isotopic fractionation of Hg (a known toxin) in various environmental compartments (e.g., sediments, zooplankton, and fish), which, in combination with C and N stable isotope values, has been used to trace sources of Hg ([Gantner et al. 2009](#); [Perrot et al. 2010, 2012](#)).

In humans, zinc is an essential nutrient that plays an important structural role in a number of proteins including those involved in metabolism, protein expression, and replication; however, acute and chronic zinc toxicities can occur at high Zn concentrations. Zinc also has several stable isotopes making it an ideal candidate for isotopic fractionation studies that can be extrapolated potentially to other transition metals. Zinc fractionation has been determined in a variety of materials (see review by [Cloquet et al. 2008](#)) including, for example, seawater ([Conway et al. 2013](#)), soil profiles ([Juilliot et al. 2011](#)), and plants ([Weiss et al. 2005](#); [Cavagnaro and Jackson 2007](#); [Viers et al. 2007](#)). Results from terrestrial plant studies suggest that the lighter isotope is taken up preferentially or is more available ([Deng et al. 2014](#)), although enrichment of heavy Zn isotopes ( $\delta^{66}\text{Zn} = 0.40\text{‰}$ – $0.72\text{‰}$ ) was observed from soil to root, followed by a depletion ( $\delta^{66}\text{Zn} = -0.10\text{‰}$  to  $-0.50\text{‰}$ ) in heavy Zn from root to shoot ([Tang et al. 2012](#)).

Despite evidence that Zn fractionation occurs in geological material, soils, plants, and water ([Cloquet et al. 2008](#)), there are few data on whether this is also the case in animals. In sheep, [Balter et al. \(2010\)](#) found that bone, muscle, serum, and urine were enriched in the heavy Zn isotopes relative to the diet, whereas the feces, red blood cells, kidney, and liver were enriched in light isotopes. [Jaouen et al. \(2013\)](#) reported that for animals and plants collected from Western Cape and Kruger National Park, South Africa, the bones of herbivores were significantly enriched in the heavier Zn isotopes relative to the plants, whereas the carnivores were slightly depleted in the heavier isotopes compared to the herbivores. Average zinc concentrations were similar in the animals ( $117 \pm 95 \mu\text{g/g}$ ) and plants ( $74 \pm 38 \mu\text{g/g}$ ), and no correlation was observed between total Zn concentration and isotopic composition. To our knowledge, there are no data on Zn fractionation in aquatic insects.

Differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values between trophic levels and from different food sources, respectively, have been used repeatedly to assess food sources, trophic structure, and energy transfer and efficiency in freshwaters ([Vander Zanden et al. 1999](#); [Vander Zanden and Rasmussen 1999, 2001](#); [Saito et al. 2001](#); [Post 2002](#)). Data collected from stream invertebrates indicated the potential for a significant difference in whole-body metal concentrations according to feeding strategy ([Smock 1983](#)). Thus, it is possible that Zn also may fractionate according to food sources or feeding strategy. Therefore, the purpose of this study was to measure Zn isotope ratios,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in three common stream insects, with different feeding strategies, collected from a stream in Ontario, Canada, to test whether Zn isotope fractionation occurs and if so, whether or not it is related to feeding strategy.

## Methods

### Collection of insects

Samples of three common stream insects, (1) northern caddisfly larvae (Trichoptera: Limnephilidae, *Pycnopsyche guttifer*), (2) common stonefly nymphs (Plecoptera: Perlidae, *Acroneuria abnormis*), and (3) adult water striders (Heteroptera: Gerridae, *Aquarius remigis*), were collected monthly

(28 May, 25 June, 26 July, and 25 August 2009) from a riffle section of Jackson Creek, located in Jackson Park, Peterborough, ON (44°19'W, 78°20'N). The stream is ~6–7 m wide and 20–50 cm deep (depending on the time of year), with a primarily pebble and cobble substrate (some boulders) at the sample location. Jackson Park has a walking trail that meanders through a mixed deciduous and coniferous woodland, estimated to have ~80% or greater canopy coverage.

Insects were collected by hand from the underside of rocks (stoneflies and caddisflies) or with hand-held nets (water striders) and then placed into pre-rinsed high density polyethylene (HDPE) 20 L buckets, containing ambient creek water, for transport back to Trent University. At the lab, the insects were allowed to depurate in 0.45 µm filtered river water (obtained from the Otonabee River, beside Trent University, via a pipeline that runs directly between the river and the lab) for a period of 24 h. Individual water striders were placed into 100 mL Erlenmeyer® flasks containing filtered river water, and the flask was covered with perforated tin foil. Individual caddisflies and stoneflies were positioned in a multi-compartment depuration chamber (the “Trent Tube”; see [Balch and Evans 1999](#) for details), which was covered at each end with 200 µm Nitex® netting. Up to eight animals were placed into each Trent Tube, which was then placed in a 30 × 45 × 15 cm Rubbermaid® tub containing filtered river water. A small pump continuously re-circulated the water in each tub, and air was pumped into the container to prevent hypoxia.

After 24 h depuration, the insects were placed into 1.5 mL capped polypropylene vials, freeze dried (Labconco Corp, Kansas City, Missouri, USA) for 24 h, and then weighed to obtain dry weight (dw); caddisflies were carefully removed from their stick houses after freeze drying. All samples were stored at room temperature until further analysis.

## Total Zn analysis

All samples were analyzed in the Water Quality Centre at Trent University. Sample preparation was carried out in a Class 10 000 metal free clean room using 18 MΩ water and double-distilled (dd) acids.

## Sample digestion

Insects were analyzed individually. Dried subsamples (5–10 mg) were placed into acid washed Teflon® tubes, to which 2 mL of concentrated dd HNO<sub>3</sub> was added. Samples were capped and digested on a hotplate for 6 h at 110 °C, then evaporated to dryness, and reconstituted to 2 mL with 7 N dd HCl (herein referred to as the “stock solution”). For total Zn analyses, a 50 µL subsample of the stock solution was diluted to 2 mL with 2% HNO<sub>3</sub> and then measured on an Elan DRC ICP-MS (PerkinElmer).

## Zn double spike

The use of a Zn double spike improved the dynamic range of the mass spectrometer for Zn isotope analyses, allowing for the accurate and precise determination of Zn isotope ratios using smaller sample quantities. A Zn double-spike was prepared consisting of an almost equimolar mixture of highly enriched isotopes <sup>67</sup>Zn and <sup>70</sup>Zn obtained from Isoflex (San Francisco, USA). The mixed double-spike was then added to both samples and the Zn isotope standard IRMM-3702. A double-spike inversion routine was developed (using MatLab) that provided values for instrumental fractionation, natural fractionation relative to IRMM-3702, and the spike-to-sample mixing proportions ([Galer 1999](#); [Rudge et al. 2009](#)). Because the spike was mixed with the sample prior to any sample processing, the value for instrumental mass discrimination expanded to include any artificial fractionation induced after the spike was added. The double-spike thus provides a very accurate monitor of sample integrity and is of utmost importance to identifying and rejecting potential outliers with highest confidence.

## Zn stable isotope measurement

### Matrix removal

The method for sample matrix removal and sample pre-concentration in preparation for Zn isotope ratio analysis was modified from [Maréchal et al. \(1999\)](#) and has been outlined in [White \(2007\)](#). First, 1.6 mL of AG MP1M resin (Bio-Rad Laboratories, Mississauga, Canada), mesh size 50–100, was washed and decanted ten times with 18 MΩ water and loaded into a 10-mL plastic chromatography column (Bio-Rad Laboratories). The settled resin was rinsed three times with 7 mL of 0.5 N dd HNO<sub>3</sub>, alternating with 2 mL of 18 MΩ water. The column was then conditioned with 6 mL of 2 N HCl. New resin was used for each sample.

Approximately 100 ng of Zn from the digested sample stock solution and 60 ng of the Zn double spike solution were brought up to 2 mL with 7 N HCl. The sample was then loaded onto the column, followed by a wash of 10 mL with 2 N HCl to elute the bulk sample matrix and Cu, which was discarded. The Zn was eluted in 20 mL of 0.5 N HNO<sub>3</sub>, which was evaporated and brought up again to 2 mL with 0.5 N HNO<sub>3</sub>. To determine Zn recovery from the column extraction, standards (IRMM-3702) which also contained the Zn double spike solution were treated in the same manner as the samples.

### MC-ICP-MS analysis

The purified Zn samples and the standards were analyzed on a Finnegan Neptune MC-ICP-MS. An Apex desolvating nebulizer (Elemental Scientific Inc., Nebraska, USA) was used for sample introduction, which improved sensitivity 3–10× compared to conventional spray chambers. Platinum skimmer and sampler cones were used to eliminate possible interferences from <sup>62</sup>Ni that can result from the use of conventional nickel cones. Standard-sample-standard bracketing was employed using the IRMM-3702 standard. Thus, all delta values are reported relative to IRMM-3702, which has a certified ratio for <sup>66</sup>Zn/<sup>64</sup>Zn of 0.56397.

### C/N stable isotope analysis

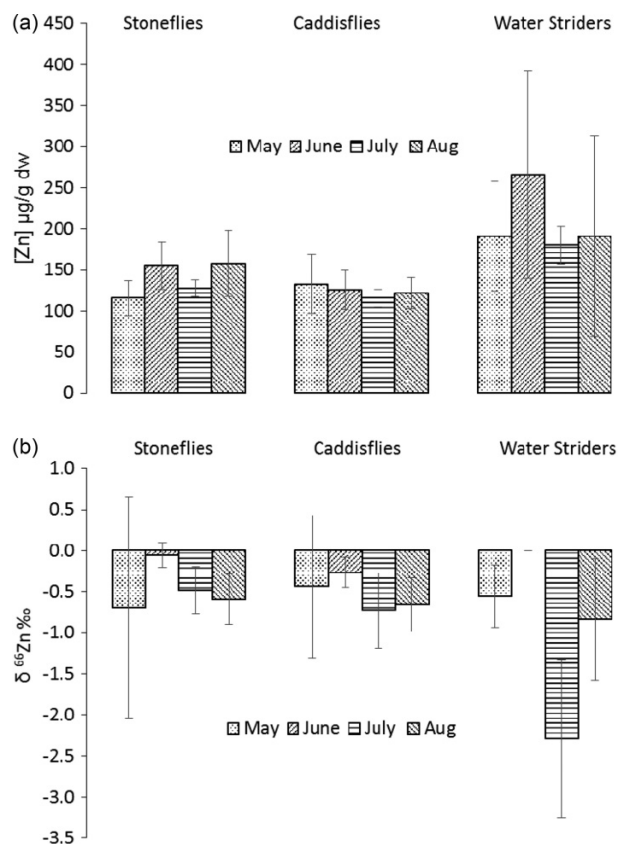
Dried samples were pulverized to a fine powder with a Wig-L-Bug® Mixer (Dentsply Corp., Philadelphia, USA). Approximately 0.2–0.4 mg of dried sample was weighed into tin capsules (Isomass Scientific, Alberta, Canada) and the samples analyzed on a Micromass (Isoprime) continuous-flow isotope ratio mass spectrometer (CF-IRMS) equipped with a Eurovector Elemental Analyser (EA) for sample introduction. Several blanks and certified reference materials (i.e., USGS 40 Glutamic Acid Standard (IAEA Certified Reference Material), dg(a1) Glutamic Acid Standard (working standard), and Casein (protein working standard)) were analyzed in conjunction with the samples, which were measured in duplicate or on occasion, in triplicate. Delta values are reported as <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N.

### Data analysis

All statistical analyses (*t*-tests, correlation, ANOVA, and regression) were performed using Microsoft Excel. *T*-tests were carried out between caddisflies and stoneflies, caddisflies and water striders, and stoneflies and water striders, to determine whether or not total Zn concentrations, and  $\delta^{66}\text{Zn}$  (<sup>66</sup>Zn/<sup>64</sup>Zn),  $\delta^{15}\text{N}$  (<sup>15</sup>N/<sup>14</sup>N) and  $\delta^{13}\text{C}$  (<sup>13</sup>C/<sup>12</sup>C) isotope fractionation (‰) was significantly different between each species ( $\alpha = 0.05$ ). Data are reported as mean  $\pm$  1 SD.

## Results

A total of 40 stonefly nymphs, 41 caddisfly larvae, and 42 water striders were collected on the 4 sampling days. Total Zn,  $\delta^{66}\text{Zn}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{13}\text{C}$  values were determined on individual insects,



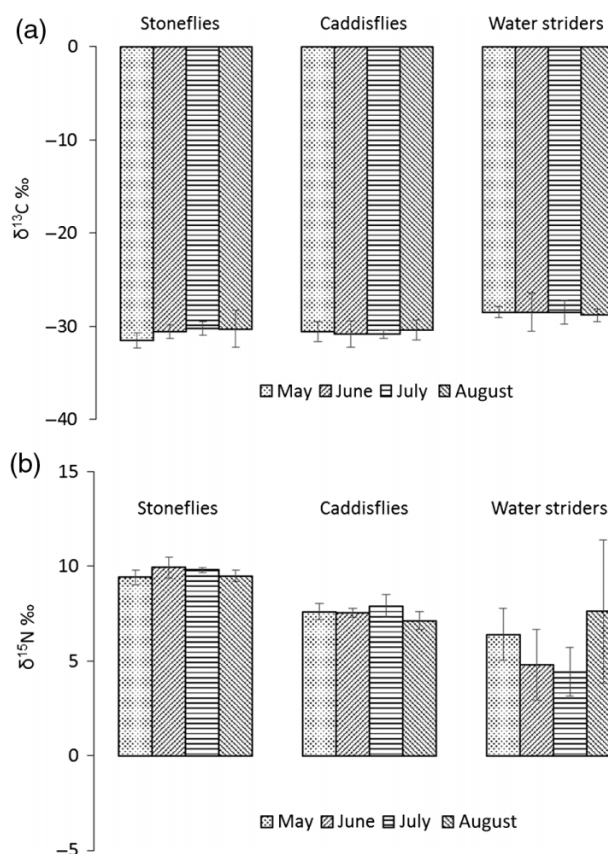
**Fig. 1.** (a) Monthly average total Zn concentrations ( $\mu\text{g/g dw}$ ) and (b)  $\delta^{66}\text{Zn}$  (‰) in stoneflies, caddisflies, and water striders collected from Jackson Creek, Peterborough, in May, June, July, and August 2009. Error bars represent 1 SD.

although each parameter was not always measured on each insect. As there was no difference in the average total Zn,  $\delta^{66}\text{Zn}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{13}\text{C}$  values in insects where all parameters had been measured compared to those where they had not, all data were pooled.

Average total Zn concentrations (Fig. 1a) were highest in the water striders ( $193 \pm 88.5 \mu\text{g Zn/g dw}$ ,  $n = 34$ ) and were significantly higher ( $P < 0.05$ ) than those measured in the stoneflies ( $136 \pm 34 \mu\text{g Zn/g dw}$ ,  $n = 38$ ) and caddisflies ( $125 \pm 26 \mu\text{g Zn/g dw}$ ,  $n = 40$ ), which were not significantly different from each other. Total Zn concentrations remained constant in all three insects, both as a function of time and also dw. Average dw of the stoneflies ( $0.045 \pm 0.026 \text{ g}$ ) and the caddisflies ( $0.040 \pm 0.010 \text{ g}$ ) were significantly higher than the average dw of the water striders ( $0.010 \pm 0.005 \text{ g}$ ).

Zn fractionation in the stoneflies and caddisflies did not vary significantly over the 4-month sampling period; however,  $\delta^{66}\text{Zn}$  in water striders was significantly lower ( $P < 0.05$ ) in July ( $-2.288\text{‰} \pm 0.662\text{‰}$ ) than in May and August (Fig. 1b; note no  $\delta^{66}\text{Zn}$  values were determined on water striders collected in June). When the data were combined, ANOVA revealed that  $\delta^{66}\text{Zn}$  values for water striders ( $-1.196\text{‰} \pm 1.030\text{‰}$ ) were significantly lighter (more negative) than those measured in the stoneflies ( $-0.453\text{‰} \pm 0.616\text{‰}$ ) and caddisflies ( $-0.512\text{‰} \pm 0.542\text{‰}$ ), which were similar to each other. There was no correlation between  $\delta^{66}\text{Zn}$  and total Zn concentrations for any of the insects (data not shown).

The results for C and N fractionation indicate a significant difference in  $\delta^{13}\text{C}$  values between caddisflies and water striders ( $-30.682\text{‰} \pm 1.012\text{‰}$  and  $-28.611\text{‰} \pm 0.981\text{‰}$ , respectively) and also between stoneflies and water striders ( $-30.754\text{‰} \pm 1.327\text{‰}$  and  $-28.611\text{‰} \pm 0.981\text{‰}$ , respectively)



**Fig. 2.** (a) Monthly average  $\delta^{13}\text{C}$  (‰) and (b)  $\delta^{15}\text{N}$  (‰) values in stoneflies, caddisflies, and water striders collected from Jackson Creek, Peterborough, in May, June, July, and August 2009. Error bars represent 1 SD.

but not between caddisflies and stoneflies (Fig. 2a). Water striders had the lowest and most variable  $\delta^{15}\text{N}$  signature ( $6.810\text{‰} \pm 2.726\text{‰}$ ; Fig. 2b), whereas stoneflies had the highest and least variable  $\delta^{15}\text{N}$  signature ( $9.604\text{‰} \pm 0.396\text{‰}$ ); caddisflies were intermediate ( $7.581\text{‰} \pm 0.513\text{‰}$ ). There was a significant difference ( $P < 0.05$ ) in  $\delta^{15}\text{N}$  values among all three species, that is, between caddisflies and stoneflies, between caddisflies and water striders, and between water striders and stoneflies.

There was no correlation between  $\delta^{66}\text{Zn}$  and  $\delta^{13}\text{C}$  or  $\delta^{66}\text{Zn}$  and  $\delta^{15}\text{N}$  in any of the insects (data not shown).

## Discussion

The average concentration of total Zn in the water striders is significantly higher than in the stoneflies and caddisflies. Even if one very high value for total Zn in one of the water striders (i.e.,  $544 \mu\text{g/g dw}$ ; Fig. 1a) is removed from the average, total Zn concentrations in the water striders ( $170 \pm 44 \mu\text{g/g dw}$ ) are still significantly higher than in the caddisflies ( $128 \pm 23 \mu\text{g/g dw}$ ) or stoneflies ( $141 \pm 34 \mu\text{g/g dw}$ ). This most likely represents bioaccumulation; water striders are predators and are ingesting and accumulating Zn from their (terrestrial) prey similar to what was observed by Quinn et al. (2003), who found that Zn concentrations increased with trophic level in a stream food web in Montana. In addition, lower Zn levels in the heavier caddisflies and stoneflies could reflect simple bio-dilution of the metal in these insects.

The Zn isotope ratios in the water striders indicate that the heavier isotopes are significantly depleted (more negative delta values) relative to those of the caddisflies and stoneflies, whereas there is no

significant difference between caddisflies and stoneflies (**Fig. 1b**). The large variability and more negative  $\delta^{66}\text{Zn}$  values for water striders in July could reflect an overlap of nymph and adult stages, given that they can coexist during the summer (Blanckenhorn and Fairbairn 1995). Evidence for lighter  $\delta^{66}\text{Zn}$  signatures in predators was reported by Jaouen et al. (2013) who observed that  $^{66}\text{Zn}/^{64}\text{Zn}$  was slightly depleted (by  $\sim 0.2\text{‰}$ ) in carnivore bones relative to herbivore bones. Depletion of  $^{66}\text{Zn}$  from the roots to shoots of higher plants (lettuce, tomato, rice, and Durham wheat) under varying exposure conditions (e.g., nutrients and Fe levels; Weiss et al. 2005; Jouvin et al. 2012) and moving from root to shoot to fruit and leaves in a variety of plants has also been reported (Viers et al. 2007; Cavagnaro and Jackson 2007). Assuming that terrestrial herbivores feed on terrestrial plants, then heavier isotope depletion would also be expected in their predators. This, therefore, suggests that diet may account for the observed differences in Zn fractionation among the stream insects.

If the observed fractionation of Zn is due to dietary sources, we can further examine why the heavy isotopes of Zn are depleted in the water striders compared to the other groups. The original selection of stream insects was based on different feeding styles and, therefore, potential differences in diet. *Pycnopsyche* spp. are shredders with a preference for microbially conditioned detrital material (Rong et al. 1995; Collins et al. 2016). *A. abnormis* is a primarily predatory stonefly with prey selection dependent on availability of prey items (Genito and Kerans 1999; Benke et al. 2001). In Jackson Creek primary prey items would be mayflies and caddisflies.

In contrast, water striders are entirely carnivorous and have a significant range in food choice, consisting primarily of terrestrial insects that land on the water surface (Smock 1983) and to a lesser extent, autochthonous aquatic larvae that float on the surface. The average  $\delta^{13}\text{C}$  value of  $-28.61\text{‰} \pm 0.98\text{‰}$  observed in the water striders is within the range of values reported for *A. remigis* in New Brunswick, Canada ( $\sim -26\text{‰}$  to  $-32.5\text{‰}$ ; Jardine et al. 2012) and is significantly heavier (i.e., less negative) than the  $\delta^{13}\text{C}$  values measured in our stoneflies and caddisflies (**Fig. 2a**). Jardine et al. (2012) found that *A. remigis* was only weakly linked to aquatic organic matter, indicating allochthonous (terrestrial) prey sources for these insects; in an earlier publication, Jardine et al. (2009) even advocated that adult water striders were potentially more appropriate as terrestrial, rather than aquatic sentinels of Hg sources. Alternately, the similarity in average  $\delta^{13}\text{C}$  values between the stoneflies ( $-30.754\text{‰} \pm 1.327\text{‰}$ ) and caddisflies ( $-30.682\text{‰} \pm 1.012\text{‰}$ ) indicates that they have comparable aquatic sources of dietary carbon. Jardine et al. (2012) found significant correlations between  $\delta^{13}\text{C}_{\text{insect}}$  and  $\delta^{13}\text{C}_{\text{periphyton}}$ , indicating a strong connection to autochthonous C sources, despite the fact that *Pycnopsyche* are known to shred terrestrial leaf litter. More recently, the importance of autochthonous bacterial and fungal growth on leaf litter as a dietary carbon source to *Pycnopsyche* and other stream invertebrates such as Ephemeroptera was documented (Chung and Suberkropp 2009; Halvorson et al. 2016; Collins et al. 2016). It appears that a primary source of C incorporated by stream insects, such as *Pycnopsyche*, comes from autochthonous bacteria and fungi that populate the terrestrial leaf litter. For this reason, their  $\delta^{13}\text{C}$  signature is more reflective of streamwater  $^{13}\text{C}$  than that of terrestrial vegetation. Given that caddisflies and mayflies are a primary food source for the stoneflies, it, therefore, makes sense that the  $\delta^{13}\text{C}$  signatures of the stoneflies and caddisflies are not significantly different. Thus, our C isotope ratio analyses corroborate the food source differences/similarities for these three organisms.

The lower  $\delta^{15}\text{N}$  values in the water striders compared to the stoneflies and caddisflies are perhaps contrary to what would be predicted, given that they are entirely carnivorous. The predatory stoneflies have a slightly higher signature than the caddisflies, which are shredders. Generally, a  $\sim 2.5\text{‰}$  to  $3.4\text{‰}$  enrichment in  $\delta^{15}\text{N}$  is assumed from diet to consumer (DeNiro and Epstein 1981; Post 2002). However, differences in diet-tissue  $^{15}\text{N}$  fractionation in stream invertebrates have been reported suggesting that  $^{15}\text{N}$  isotope signatures do not always follow this pattern in aquatic insects (Jardine et al. 2012). The method by which water striders feed can affect their  $\delta^{15}\text{N}$  values. McCutchan et al.

(2003) proposed that estimates of the trophic shift for nitrogen differ between “fluid-feeding” consumers (such as water striders, which suck out nourishment from their prey) and other consumers that eat the entire prey, so the lower  $\delta^{15}\text{N}$  values we measured in the water striders might reflect this feeding behavior. Also, species which undergo complete metamorphosis (caddisflies) might be expected to have a longer or more highly processed or more variable food chain length, which would also modify  $\delta^{15}\text{N}$  values. Thus, the differences in  $\delta^{15}\text{N}$  values among the stoneflies, caddisflies, and water striders may indicate slight dietary differences and (or) different feeding strategies resulting from the species or instar present during collection of the organisms.

Borrok et al. (2008) observed that in streams with low dissolved Zn concentrations, the heavier isotopes were more abundant, suggesting that the heavy isotopes are bound preferentially by dissolved organic carbon (DOC) and micro-organisms such as the bacteria and fungi populating detrital material in the water. The similarity in  $\delta^{13}\text{C}$  and  $\delta^{66}\text{Zn}$  between caddisfly larvae and stonefly nymphs suggests that the path of Zn incorporation and fractionation is similar to that of carbon. It is generally accepted that stream invertebrates take up the majority of their Zn from food (organic material) and not water (reviewed by Goodyear and McNeill 1999), further supporting the expectation of a heavier signature in these organisms relative to water striders. Water striders on the other hand, having a diet consisting primarily of terrestrial insects that would be feeding on plant parts depleted in the heavy isotopes, reflect their food source. Although we do not have isotope ratio data for terrestrial insects, given the correspondence between the C and Zn isotope ratios, it is reasonable to conclude that the differences observed between species relate to differences in Zn ratios in their diets.

In conclusion, our findings suggest that sources of Zn to these aquatic insects are similar to that of C; it may be possible to trace sources of Zn, and possibly other contaminants, in food webs using Zn isotope ratios in stream invertebrates with different feeding styles. Chen et al. (2008) used Zn isotope signatures to trace contaminants in river water; however, this required large volumes of water and extensive concentration of samples. Invertebrates already contain adequate concentrations of Zn for analysis, and, therefore, less sample preparation is required resulting in less chance of contamination. The use of water striders as a sentinel of Hg sources has already been proposed (Jardine et al. 2005). Furthermore, improvements in ICP-MS techniques have resulted in recent growth in studies where C, N, and Hg stable isotope ratios have been combined to address source partitioning and biogeochemical transformations of Hg in environmental compartments (Gantner et al. 2009; Perrot et al. 2010, 2012; Tsui et al. 2012).

Our findings contribute to the overall understanding of the biogeochemical cycling of Zn, specifically in freshwater communities. Future work will require analyzing a wider range of stream invertebrates for both Zn and C isotope fractionation, as well as analysis of specific food and water sources. By expanding the scope of the study, it should be possible to partition terrestrial and aquatic sources of Zn to biota, and from there, to identify all environmental sources (not simply food) of Zn and other transition metals.

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## Author contributions

Conceived and designed the study: RDE. Performed the experiments/collected the data: WW. Analyzed and interpreted the data: RDE, WW, HEE. Contributed resources: RBG. Drafted or revised the manuscript: RDE, HEE.

## Competing interests

The authors have declared that no competing interests exist.

## Data accessibility statement

All relevant data are within the paper.

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