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Influence of Food-Capture Nets on Cadmium Uptake by Net-Spinning Caddisfly (Trichoptera: *Hydropsychidae*) Larvae

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The use of stream-dwelling invertebrates as biomonitors of environmental pollution *in situ* (eg. Clements *et al*. 1988a; Vuori and Kukkonen 1996) and also as test organisms in laboratory bioassay (eg. Clements *et al*. 1988b; Hatakeyama 1989; Postma *et al*. 1996) and/or field biomanipulation experiments (eg. Clements *et al.* 1988b; Stuijfzand *et al*. 1999) is well established. Caddisfly (Order:Trichoptera) larvae, in particular, have become important as test organisms (eg. Carlson 1966; Camargo 1991; Cain *et al.* 1992; Clements *et al*. 1988a; Greve *et al*. 1998; van der Geest *et al*. 1999; Stuijfzand *et al.* 1999) because they are widely distributed in nature, are important decomposers of organic matter and are a food source for fish and birds (van der Geest *et al*. 1999). Furthermore, they are hardy, their collection for study is simple and inexpensive and they are relatively tolerant of many trace metals (Cain *et al.* 1992; Balch and Evans 1999; Balch *et al*. 2000). In recent years, Hydropsychid caddisfly larvae have been used, increasingly, to study sub-lethal effects of metals and other contaminants especially in laboratory experiments (Greve *et al*. 1998; van der Geest *et al*. 1999; Balch *et al*. 2000).

One issue with respect to the use of caddisflies that is often overlooked in these types of studies, is how their feeding behavior may affect their performance as test organisms. Species within the genus, *Hydropsyche,* feed by collecting seston from the flowing river or stream water in protein-rich silken nets that they spin (Fuller *et al*. 1983). The surfaces of these newly spun nets are an ideal site for growth of microorganisms and their associated extracellular polysaccharides or biofilms (Meyer-Reil 1994). In organic-rich waters, the nets often take on a brown stain from the water (personal observation). Thus, even in a bioassay system with no particles or particulate-bound metals, the presence of the nets in the test system may increase the apparent uptake of the metal by the organism. This is because free metal ions in solution may sorb to the net structure or attached biofilm; subsequent grazing on the net and associated material then may add to the body burden of the caddisfly (Schlekat *et al*. 1999).

A further complication is that not all of the metal present in solution will be in the form of free ions. Metals bound to other ligands, such as dissolved

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organic carbon (DOC) may not be available for uptake by the organism. Thus, the presence of DOC can reduce both the uptake (Hart 1982; Wang 1987; Stackhouse and Benson; Penttinen *et al.* 1998) and toxicity (Wang 1987; Penttinen *et al.* 1998) of metals to aquatic invertebrates. While this problem is general to all bioassay tests, it has particular implications for studies conducted using net-spinning organisms. On one hand, sorption of metal to DOC may decrease the bioavailability and body burden of the metal in the larvae. On the other hand, sorption of DOC-metal complexes and free metal ions to the nets and associated biofilms, could result in an increase in metal body burdens. Net grazing behavior could therefore increase the uptake of metals from solution over that from gill membrane transport alone.

The objective of this study was to determine the potential for nets to affect uptake of cadmium (Cd) by *Hydropsyche* larvae. Specifically, we wanted to determine if an exposure route existed from net grazing behavior. If this route existed, we also wanted to determine the relative magnitude of it compared with a respiratory route via the gill membranes. Lastly we wanted to determine whether or not DOC, which typically suppresses the transfer of metals across gill membranes, might actually augment dietary uptake of Cd by facilitating metal sorption to net surfaces.

MATERIALS AND METHODS

Caddisfly larvae (4^{th} instar) of the genus *Hydropsyche* (mostly *H. betteni*) were gathered from a riffle zone in Thomson Creek, Peterborough, Ontario ($44^{0}17'N;78^{0}19'W$), which originates as an overflow from the Otonabee River. Individual larvae were removed with forceps from overturned rocks and placed into tubes ('Trent Tubes') for transportation to the laboratory. There was no mortality of larvae due to sampling and transport. The Trent Tube consists of an S-shaped, transparent, 5-cm i.d. PVC tube containing 50 individual chambers. These chambers are covered with Nitex netting, which prevents the escape of the larvae while being large enough to allow good circulation of water and added food. Only one organism is placed into each chamber thus providing it with a unique refuge (see Balch and Evans 1999 for a complete description).

The experiment consisted of three treatments to determine if the presence of the capture nets influences the uptake of Cd by *Hydropsyche* larvae and also if Cd uptake is affected by the presence of DOC: A) exposure to elevated Cd concentrations, B) exposure to both elevated Cd and DOC concentrations and C) exposure to river water only (control). Three mixing tanks, representing the three treatments (A, B and C), were established. Each mixing (treatment) tank supplied water to 4 exposure tanks, two of which contained larvae that were left undisturbed and allowed to construct capture nets ('with-net' group) and two tanks from which the capture nets

were removed on a daily basis ('without-net' group). Thus, there were 6 groups (i.e. Treatment A-'with net', Treatment A-'without net', Treatment B-'with net', Treatment B-'without net', Treatment C-'with net', Treatment C-'without net') with 1 replicate/group, resulting in 12 exposure tanks in total.

All experiments were conducted in a flow-through system using sand-filtered water from the Otonabee River. Prior to the commencement of the experiment, ~25 *Hydropsyche* larvae were placed into each of 12 Trent Tubes (1 per exposure tank) and allowed to acclimatize to the river water for two days. The larvae were fed a mixture of Tetramin™ flake fish food, freeze dried *Tubifex* and live *Artemia* during this 2-day period. Cadmium was added to two of the mixing tanks (Treatment A and B) in the form of ^{114}Cd isotope (99.3% as 114), to bring the total Cd concentration in the tanks (mixing and exposure), to ~400 ng/L. This concentration (~4X ambient levels) was selected to elevate the Cd concentration above natural levels but not to an unrealistically high concentration, or one that might result in toxicity to the organism.

After exposure to the experimentally-added isotope, the concentration of ^{114}Cd in the organism will have been derived from 2 sources: 1) the experimentally-added isotope and 2) ^{114}Cd present from all other sources including that present in the organism at the start of the experiment and any additional 'natural' Cd obtained during the experiment. Correction for ^{114}Cd from the latter was determined by measuring another of the naturally-occurring Cd isotopes in each organism at the conclusion of the test. Thus final reported concentrations of the uptake isotope, ^{114}Cd, represent only experimentally-added or 'excess' ^{114}Cd.

Dissolved organic carbon, in the form of commercially-available fulvic acid (Quimica Foliar s.a. de c.v, Mexico) was added to one mixing tank (Treatment B) to bring the final concentration of DOC to ~12 mg/L (~2X ambient levels). The DOC and ^{114}Cd were allowed to equilibrate for 24 hours in the mixing tank prior to the start of the experiment. T= 0, was defined when the water from the mixing tanks was allowed to circulate through the exposure tanks.

On Days 0, 6, 12 and 18, water samples were collected from each of the 3 mixing tanks and analyzed for Cd as well as for water 'quality' parameters (dissolved O_2, DOC, Cl, F, SO_4, pH and temperature). On the same days, two *Hydropsyche* larvae were retrieved from each of the 12 exposure tanks (24 larvae in all) and analyzed for ^{114}Cd. In the 'without-net' exposure tanks, the nets were removed from the larvae daily by gently nudging the larvae out of their housing unit. The larvae were then placed back into a clean chamber (within the same Trent Tube), and the nets were dried and combined for later Cd analysis.

On Days 6 and 12, after 2 larvae had been sampled from each tank, the *Hydropsyche* remaining in the tanks were again allowed to feed on a mixture of Tetramin™ flake fish food, freeze dried *Tubifex* and live *Artemia.* Cadmium concentrations were measured in the dried foodstuffs to ascertain whether or not they contained significant amounts of ^{114}Cd (obtained from sorption of experimentally-added ^{114}Cd) that might contribute to uptake in the larvae.

Hydropsyche larvae samples were air dried for 3 to 5 days followed by oven drying at 60°C overnight. Individual larvae, combined net samples and food samples were digested separately for 3 hr in 250 µL of trace grade nitric acid along with a 50 µL spike of ^{112}Cd which was used as an internal standard for isotope dilution analysis (Longerich 1989). Acid digests were later diluted to 10 mL with distilled-deionized water. The samples were analyzed on an Elan 6000 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS), using flow injection sample introduction. Excess ^{114}Cd concentrations (i.e. corrected for the ^{114}Cd contribution from naturally-occurring Cd) in the water and animal digestates were calculated using isotope dilution (ibid). Certified reference material, NIST1566a oyster tissue was used for quality control of the trace metal analysis.

RESULTS AND DISCUSSION

Cadmium-114 concentrations in the mixing tanks averaged (±1 s.d.) 366±9 ng/L (Treatment A) and 378±16 ng/L (Treatment B). There was no apparent change in [^{114}Cd] throughout the duration of the experiment. Water quality parameters measured over the course of the experiment averaged 9.6±0.80 mg dissolved O_2/L (n=12), 5.07±0.15 mg DOC/L in Treatments A and C, and 11.9 mg/L in Treatment B, 9.10±0.33 mg Cl/L, 0.40±0.04 mg F/L, 7.06 ± 0.17 mg SO_4/L (n=9 for all 3 parameters). Water temperature varied between 12 and 16°C and pH averaged 7.48±0.23. There was no excess ^{114}Cd detectable in any of the foodstuffs (Tetramin™ fish flakes, freeze dried *Tubifex* and live *Artemia*) analyzed.

The *Hydropsyche* larvae remained active in all treatments for the duration of the experiment and no gross differences in behavior were observed in any of the groups. The larvae built net structures in all three treatments within the first 24 hours of placement in the housing system. The survival rates were 100% in all the treatments where the *Hydropysche* were allowed to maintain their nets; however, for the 6 tanks where the nets were removed from the larvae, survival rates ranged between 81 and 100%. It is probable that the increased mortality in the group without nets resulted from mechanical injury associated with frequent removal from the housing. Dry weights of the larvae did not vary significantly among the 3 treatments for each sampling day (1-way ANOVA; $p>0.05$ for each of the 4

days), nor did they vary from day-to-day for each treatment (1-way ANOVA; $p>0.05$ for each of the 3 treatments).

On Days 6, 12 and 18, ^{114}Cd concentrations in the larvae (ug/g dw) were compared for the various groups (i.e. Treatment A – 'with net' vs 'without net', Treatment B – 'with net' vs 'without net'; 'With net' – Treatment A vs Treatment B and 'Without net' – Treatment A vs Treatment B), using a 1-way ANOVA for each test (Table 1). Excess ^{114}Cd concentrations in the *Hydropsyche* contained in the control tank (Treatment C) generally averaged <0.05 µg/g dw, and are therefore not discussed further. Significant differences ($p<0.05$) in metal concentrations between the two treatments (i.e. Cd vs Cd+DOC) or between the 'with net' versus the 'without net' group of animals were found in 4 of the 12 tests. The lack of significance in the other 8 tests is due, partly, to the large variability in the data, which will be discussed in further detail later.

Table 1: ANOVA results testing for differences in *Hydropsyche* [^{114}Cd]'s in Treatment A vs B and in the 'With net' vs 'Without net' groups of animals.[1]

	'With net' group *Treatment A vs*			'Without net' group *Treatment A vs*		
	Day 6	*Day 12*	*Day 18*	*Day 6*	*Day 12*	*Day 18*
Treatment B	ns	0.002*	0.029*	ns	0.002*	Ns
	Treatment A *'With net' group vs*			Treatment B *'With net' group vs*		
	Day 6	*Day 12*	*Day 18*	*Day 6*	*Day 12*	*Day 18*
Without net	ns	0.001*	ns	ns	ns	Ns

[1] Numbers reflect 'p' values for each of the 3 sampling days. ns = not sig., * = sig. at 5%

The effect of DOC on Cd uptake is shown in Figure 1. The presence of DOC in Treatment B ('squares') appears to mitigate (lower) the uptake of Cd both by *Hydropsyche* allowed to maintain their nets (Figure 1a) and also those that had their nets removed (Figure 1b). Significant differences in ^{114}Cd concentration (Table 1) were found between Treatment A and Treatment B on Day 12 (for both 'with-' and 'without net' groups) and on Day 18 ('without' nets only). Thus the presence of the DOC may be affecting sorption of Cd to the nets themselves. Measured ^{114}Cd levels in the nets obtained from Treatment A, containing only Cd (78 µg/g), were slightly higher than those measured in the nets from Treatment B, containing both Cd and DOC (62 µg/g), which would support the theory that the presence DOC is reducing sorption of Cd to the nets. However, the lack of replication makes it difficult to say this conclusively.

The data in Figure 1 also show that uptake of Cd during the 'with net'

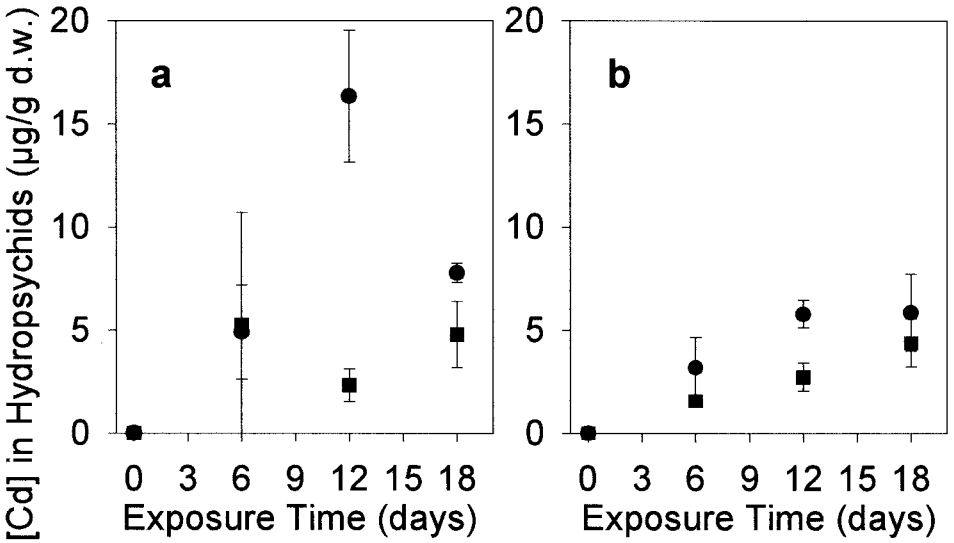

Figure 1. Comparison of ^{114}Cd uptake by *Hydropsyche* in the presence (squares) and absence (circles) of DOC; a - with nets, b - without nets. Points represent average ± 1 s.d. of 4 larvae (i.e. 2 tanks X 2 larvae/tank).

exposures was more variable (coefficients of variation, CVs, ranging between 6 and 103%) than that observed during the 'without net' exposures (CVs ranging between 10 and 47%).

The variability likely arises from the fact that biofilm growth on nets will be variable and the behavior of individual larvae with respect to their nets will differ over time. For both treatments, the uptake rate in the absence of the nets ('squares') is relatively linear over the course of the experiment, whereas when the nets are available to the larvae, uptake is more erratic.

Despite the variability in the data, larvae that were allowed to maintain their nets appear to have higher body burdens of Cd than larvae that had their nets removed. The highest levels of ^{114}Cd measured during the experiment (average±1 s.d. = 16.3±3.2 ug/g dw) were found in the 'with net' group of larvae. This, together with the significant ^{114}Cd levels in the nets (78 and 62 ug/g in Treatments A and B, respectively), would imply that the nets provide an increased uptake of Cd to the animals. The variability within the 'with net' groups for both treatments suggests that the nets are having an impact on the availability of the free Cd metal. The free Cd is binding to the nets and associated biofilms and is becoming available to the *Hydropsyche* larvae through net grazing behavior. Although trends were consistent for all the treatment groups, the lack of more statistically-significant differences (Table 1) may be attributed to small sample size and sample variability within the net group treatments;

however, additional uptake experiments at similar Cd concentrations, conducted using *Hydropysche* larvae (D. Evans, unpublished data), indicated that an increase in sample size to 12 individuals per sample period (i.e. 3 larvae/tank X 4 tanks) still resulted in high variability in measured Cd burdens in the presence of nets (CVs as high as 80%). In any event, it is evident that Cd is binding to the net structure readily, with or without the presence of added DOC.

In summary, the net building strategy of *Hydropsyche* larvae potentially increases the risk of metal uptake. Toxicity tests based on free metal ions do not take this strategy into account when determining water quality objectives. Therefore, these objectives may not provide adequate protection for all aquatic organisms. The results of this study demonstrate the intricate interplay between biology and geochemistry in determining the fate of metals in organisms. They also indicate the difficulty of undertaking artifact-free bioassay experiments. Future studies of toxicity and fate of contaminants should recognize this interplay in both study design and interpretation of observations.

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