BEHAVIORAL RESPONSES TO SUBLETHAL CADMIUM EXPOSURE WITHIN AN EXPERIMENTAL AQUATIC FOOD WEB

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Abstract—The foraging and predator-avoidance behaviors of mayfly (Baetis tricaudatus) nymphs and predator–prey interactions were investigated under differing combinations of cadmium concentration and predation risk. Predators consisted of stonefly (Kogotus nonus) nymphs, juvenile brook trout (Salvelinus fontinalis), and longnose dace (Rhinichthys cataractae), either alone or in combination. Organisms were exposed to 0, 0.5, or 5.0 μg/L Cd for 7 d (invertebrates) or 30 d (vertebrates) prior to observation in artificial stream channels. Behavioral observations (20-min duration) were made both day and night over a 24-h period. Mayfly feeding rate was assessed by image analysis of grazed diatom mats cultured on ceramic tiles. Both predators and cadmium produced significant effects on the behavior of B. tricaudatus and K. nonus nymphs, with mayflies showing a hierarchical response in the presence of multiple predators. A significant increase in activity was observed in S. fontinalis exposed to 5.0 μg/L Cd, with significant decreases in capture efficiency of prey items apparent at 0.5 μg/L Cd. Conversely, the foraging behavior of R. cataractae was unaffected by cadmium, possibly as a result of species-specific cadmium tolerance. Such sublethal effects of a contaminant on the energy budgets of individuals and the interactions between species have implications for community structure in aquatic food webs.

Keywords—Behavior Predator–prey interactions Benthic invertebrate Brook trout Longnose dace

INTRODUCTION

The initial response of an individual to contaminant exposure is a possible change in its behavior, due to visual or chemoreception (olfaction, taste) damage [1], or irritation [2]. Failure to avoid exposure, either through relocating to a more favorable environment or avoiding the consumption of contaminated food items, may result in reduced fitness and survival, eventually leading to detrimental effects at the population level [3]. Such a behaviorally based approach to toxicity studies provides a promising means of detecting ecologically relevant effects of contaminants (see reviews by [4±7]). By linking toxic effects at the level of the individual organism to those observed at the population and community levels, behavioral endpoints help address some of the limitations of traditional toxicity-testing procedures.

In a behavioral toxicity test, behavioral disruption is substituted for the physiological endpoints typical of conventional tests [4]. The current study focused on components of locomotion, foraging, and predator avoidance as behavioral indicators of toxic effects. Each of these endpoints is relevant ecologically because they relate to components of individual fitness and can alter the structure of communities through direct effects (e.g., emigration) and indirect effects (e.g., loss of food resource). Toxicants have been found to affect locomotion in several ways including preference/avoidance responses [7], free locomotor activity (hypo/hyperactivity [2,8]) and locomotor components (turning frequency or angular orientation [6]). Foraging behavior also has been a sensitive indicator of toxicity (e.g., [5]). Contaminants may mask natural odors, reduce motivation to respond to a natural attractant, alter the function of the taste mechanism, or disrupt other sensory systems for detecting prey [1,5]. Finally, contaminant exposure may modify the responses of an organism to predation risk through disruption of evolved predator defenses, such as shelter-seeking activities [9], schooling [10], sensory functions [11], and prey movement (hypo/hyperactivity [12]). Alterations to these behavioral mechanisms may serve to increase vulnerability to predators and thus increase the extinction probability of an affected organism.

Using cadmium as a model contaminant, the current study attempts to determine whether sublethal levels of aqueous cadmium exposure can produce ecologically significant effects within an aquatic food web. Experimental food webs were constructed using the diatom Nitzschia sp. as the primary producer, nymphs of the mayfly Baetis tricaudatus and the stonefly Kogotus nonus as invertebrate grazers and predators, respectively, and juvenile brook trout (Salvelinus fontinalis) and longnose dace (Rhinichthys cataractae) as vertebrate predators. The species co-occur in nature (e.g., [13]), and thus represent a subset of a natural riverine food web. In addition, mayflies and salmonids have been shown to be sensitive to many aquatic toxicants, making them important indicators of pollution. Ecologically significant effects of cadmium exposure were measured in terms of altered foraging behaviors and predator avoidance trade-offs (energy acquisition) and activity (energy expenditure) by the study organisms. In the process, the study also aimed to establish the utility of certain behavioral endpoints, such as invertebrate drift and grazing rates and the prey capture efficiency, reaction distance, and activity of predators, as sensitive indicators of sublethal cadmium contamination. Ultimately, we hoped that the study would provide an alternative, potentially nondestructive, method of effects...
testing based on the hypothesis that behavioral endpoints can resolve more subtle responses to environmental contamination than traditional toxicological approaches.

METHODS

Collection of benthic invertebrates

_Baetis tricaudatus_ and _K. nonus_ nymphs (0.5 ± 0.1 cm and 2.0 ± 0.2 cm in length, respectively, excluding cerci) were collected from Big Hill Springs, a pristine, spring-fed, first-order, foothills stream located 30 km west of Calgary, Alberta, Canada. _Baetis tricaudatus_ and _K. nonus_ are the numerically dominant mayfly and stonefly species, respectively, and cadmium levels at the site are negligible (<0.1 μg/L). Aspects of the ecology of both species have been studied previously at this site (e.g., _B. tricaudatus_ [14]; _K. nonus_ [15]). Big Hill Springs also has been employed as a collection site for _B. tricaudatus_ toxicity testing [16].

Organisms were sampled using a U-shaped net sampler (0.25-mm mesh size; see Scrimgeour et al. [17]). Uninjured nymphs of the required size were transferred quickly via pette (mayflies) or fine tweezers (stoneflies) to aerated stream water in separate 10-L coolers. No mortalities occurred during transportation to the laboratory, and nymphs were acclimated over a 3- to 4-d period to designated laboratory temperature (12 ± 1°C), photoperiod (16:8-h light:dark), and water (dechlorinated City of Saskatoon, SK, Canada, tap water: hardness = 156 mg/L CaCO₃, < 0.1 μg/L Cd) conditions. Nymphs were maintained under these conditions for a minimum of 48 h prior to exposure to cadmium solutions.

Collection of fish species

Juvenile _R. cataractae_ (6.0 ± 0.5 cm fork length) were obtained by electrofishing from Jumpingpound Creek, a fourth-order foothills stream located 30 km northwest of Calgary, Alberta, Canada. Aspects of _R. cataractae_ ecology had been studied previously at this site (e.g., [13,18]). Juvenile _S. fontinalis_ (6.5 ± 0.5 cm fork length) were obtained from Fort Qu’Appelle Hatchery (Fort Qu’Appelle, SK, Canada). Cadmium levels at both locations were negligible (<0.1 μg/L).

Collected individuals were split between a number of 20-L coolers containing aerated stream or hatchery water. No mortalities occurred during transportation, and individuals were acclimated over a 3- to 4-d period to laboratory conditions identical to those described previously for benthic invertebrates. Fish were maintained under these conditions for a minimum of 48 h prior to exposure to cadmium solutions.

Food sources

_Baetis tricaudatus_ nymphs were maintained on laboratory-cultured _Nitrosia_ sp. diatom mats (strain F110; Department of Fisheries and Oceans, Winnipeg, MB, Canada) at a rate of 1 mat/8 mayflies/d. Diatom batch cultures were maintained in exponential growth phase and subcultured onto ceramic tiles (6.25 cm²) to form uniform, single-species diatom mats within 6 to 8 d. All cultures were grown in S-diatom medium [19] at 18 ± 1°C, under a 16:8-h light:dark photoperiod. _Kogotus nonus_ nymphs were fed at a rate of five to six mayfly nymphs/stonefly/d.

Fish species were fed at a rate of 2% body weight/aquarium/d of commercial starter trout pellets, with live prey organisms (field-collected _B. tricaudatus_ nymphs and/or laboratory-cultured _Chironomus tentans_ larvae) being substituted as live food items every 3 to 4 d.

Cadmium solutions and exposure regime

Cadmium exposure solutions were prepared by the dilution of a 10-mg/L stock solution (CdCl₂·2H₂O; Sigma Chemical, St. Louis, MO, USA) with synthetic test water [20]. Stock solutions were renewed every 3 d and stored at 4°C.

Following acclimation to laboratory conditions, benthic invertebrates were distributed between two groups of tanks, with one group per species. A group of tanks consisted of 3 × 21-L glass aquaria each containing 5 L of dechlorinated tap water and a nominal cadmium concentration of either 0 (control), 0.5, or 5.0 μg/L. Each aquarium was aerated vigorously. The density of organisms per tank typically was 100 to 150 mayflies or six to eight stoneflies. Organisms remained in the exposure media for 7 d prior to an experiment. During this time, the media were not replaced, but any dead individuals and exuviae were removed. The feeding regime was maintained on a daily basis, until 24 h prior to an experiment when the organisms were deprived of food to standardize hunger level.

In a similar fashion, acclimated fish were distributed between two groups of tanks, with one group per species. A group of tanks consisted of 3 × 76-L glass aquaria each containing 60 L of dechlorinated city water and a nominal cadmium concentration of either 0 (control), 0.5, or 5.0 μg/L. Each aquarium was aerated vigorously. Typically the density of organisms was six fish per tank. A static-renewal procedure was adopted where 20 L of aquaria water were replaced with fresh dechlorinated tap water on a daily basis. Each 20-L volume of replacement water was made up to the cadmium concentration specific to each aquarium and was delivered from separate, labeled carboys. Fish waste and uneaten food were removed using a hand-pump siphon prior to each water exchange. Water temperature, dissolved oxygen, and pH were monitored following each water exchange to ensure and maintain consistent experimental conditions. Organisms were held under these conditions for a period of 30 d, after which time they were employed in experiments. The feeding regime was maintained on a daily basis, until 48 h prior to an experiment, when the organisms were deprived of food to standardize hunger level.

Water samples were obtained from each aquarium every 3 to 5 d for cadmium analysis.

Artificial stream channels

Behavioral tests were conducted in artificial stream channels [21]; Fig. 1. Each experimental unit consisted of a long, rectangular Plexiglas® (Röhm, Darmstadt, Germany) flume (170 × 27 × 17 cm) with semicircular ends. A white, plastic central divider served to split the stream in half as well as provide a background against which predator and prey behaviors could be observed clearly. A current speed of 4 cm/s was maintained using a diaphragm-pump system. A series of submerged airlines fitted at opposing ends of the stream forced the water to flow around the flume in a loop, in addition to providing aeration. Screen partitions fronted with coarse Nitex® (Sefar, Heiden, Switzerland) netting were placed between the central divider and the outer wall of the flume. These helped to compartmentalize the flume while still allowing relatively unimpeded water flow.

Each stream received 20 L of dechlorinated city water and a nominal cadmium concentration of either 0 (control), 0.5, or 5.0 μg/L. Figure 1 illustrates the typical arrangement of food web and stream elements at the beginning of a behavioral test.
Experimental design

In order to determine the effects of predators on B. tricaudatus behavior and the responses of K. nonus and fish to each other, a series of food webs was devised. The structure of each web was such that B. tricaudatus nymphs were exposed to no predators, one predator type (invertebrate or vertebrate), or both predator types (invertebrate and vertebrate). The following combinations were tested: Nitzschia sp. + B. tricaudatus, Nitzschia sp. + B. tricaudatus + K. nonus, Nitzschia sp. + B. tricaudatus + S. fontinalis, Nitzschia sp. + B. tricaudatus + R. cataractae, Nitzschia sp. + B. tricaudatus + K. nonus + S. fontinalis, and Nitzschia sp. + B. tricaudatus + K. nonus + R. cataractae.

Each organism combination was investigated under the three cadmium treatments (0, 0.5, and 5.0 μg/L), with one artificial stream channel per treatment (see below). All test organisms in a given treatment channel originated from holding tanks of the corresponding cadmium concentration. Diatom mats were exposed to the required cadmium concentration for 48 h prior to an experiment.

The experimental procedure was as follows. Predators (if required by the test: One fish and/or two stoneflies) were placed in the left-hand section of the stream. Fish were transferred using a fine-mesh net, with fine tweezers being used for stonefly nymphs. Using wide-tipped tongs, eight cultured diatom mats were then placed randomly throughout the testing arena along with 40 glass beads (4-mm diameter; Fisher Scientific, Pittsburgh, PA, USA). The beads served as prey refuges from predators while still allowing the prey species to be observed. Using a wide-bore pipette, 12 mayfly nymphs were then introduced into the prey presentation section. An additional number of nymphs were placed into the prey holding section. After a 15-min acclimation period, the screen partition separating predator and prey was removed and the organisms allowed to interact for 20 min, during which time the testing arena was videotaped (Sony®, Tokyo, Japan; DCR-TRV900 low-light digital camcorder). Any mayflies that were consumed during this observation period were replaced with individuals from the prey holding section to maintain constant numbers.

The experiment was terminated at the end of the observation period and the test species either removed to separate holding tanks (see below) or sampled for cadmium analysis.

Due to limitations in both space and stream channel numbers, channels were replicated (three per treatment) in time rather than space. This required the removal of food web elements (diatom patches, mayflies, and predators, if present) to separate, labeled holding tanks at the end of an observation period. A fresh group of food web elements was then substituted in the replicate test that followed.

Experiments were repeated at four times throughout a 24-h period: Two-day observation sessions (beginning at 09:15 and 15:15), when the streams were illuminated, and two night sessions (beginning at 21:15 and 03:15), when the streams were in darkness. Night sessions were videotaped using low-intensity red-light lamps [12, 13] that did not noticeably affect the behavior of predators or prey. Each replicate group of food web elements was observed at the four time periods, which permitted the resulting data to be analyzed statistically using repeated-measures analyses of variance.

Behavioral observations

During review of the videotapes, a series of behavioral observations was made for all predator and prey species. Table 1 illustrates the types of behavior observed for mayfly nymphs, stonefly nymphs, and juvenile fish, respectively. In addition to measuring B. tricaudatus drift rate, tile positioning by the species also was quantified (Table 1). Positioning refers to the number of mayflies using the top surfaces of ceramic tiles (i.e., diatom patches) at the end of an experiment and is an attempt at measuring risk-taking by foraging individuals in the presence of predators [22]. In addition, B. tricaudatus grazing rate was measured by image analysis of diatom patch surface area before and after an experiment (SigmaScan Ver 5.0.0; SPSS, Chicago, IL, USA).

Predator foraging behavior often is described by the sequence of events involved in predation, namely: Search, encounter, attack, and capture of the prey item [23]. Observed behaviors were categorized as either attempts at search or han-
Table 1. Descriptions of behavioral observations made for: (A) *Baetis tricaudatus* nymphs; (B) *Kogotus nonus* nymphs; (C) juvenile *Salvelinus fontinalis* and *Rhinichthys cataractae*. [R] = resting; [A] = active foraging

<table>
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<tr>
<th>A</th>
<th>Behavior</th>
<th>Description</th>
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<tbody>
<tr>
<td>Drift rate</td>
<td>Number of mayflies leaving substrate and drifting during observation period</td>
<td></td>
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<tr>
<td>Tile positioning</td>
<td>Number of mayflies present on upper tile surfaces at end of observation period</td>
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<tr>
<th>B</th>
<th>Behavior</th>
<th>Description</th>
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<tr>
<td>Search behavior:</td>
<td></td>
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<tr>
<td>Sit and wait [R]</td>
<td>Body stationary on substrate</td>
<td></td>
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<tr>
<td>Swimming [A]</td>
<td>Directional movement in the water column</td>
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<td>Handling behavior:</td>
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<tr>
<td>Attack [A]</td>
<td>Quick burst of movement directed towards prey item</td>
<td></td>
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<tr>
<td>Capture [A]</td>
<td>Prey grasped, followed by at least partial consumption</td>
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<td>Swallow [A]</td>
<td>Complete consumption of prey</td>
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<th>C</th>
<th>Behavior</th>
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<tr>
<td>Search behavior:</td>
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<tr>
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<td>Body stationary on substrate</td>
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<tr>
<td>Swimming [A]</td>
<td>Movement in the water column or near the substrate</td>
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<td>Rooting [A]</td>
<td>Probing of the substrate with snout</td>
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<tr>
<td>Handling behavior:</td>
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<tr>
<td>Attack [A]</td>
<td>Quick burst of movement directed towards prey item</td>
<td></td>
</tr>
<tr>
<td>Capture [A]</td>
<td>Intake of prey item into mouth</td>
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<tr>
<td>Mastication [A]</td>
<td>Upward movement of head in a vertical arc, with distinct opercular motion indicating chewing</td>
<td></td>
</tr>
<tr>
<td>Swallow [A]</td>
<td>Distinct head and opercular movement ceasing, with no re-emergence of prey item</td>
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On this basis, reaction distance and three behavioral indices (capture efficiency, active time proportion, handling time proportion) were calculated for both stonefly nymphs; (B) *Kogotus nonus* nymphs; (C) juvenile *Salvelinus fontinalis* and *Rhinichthys cataractae*. [R] = resting; [A] = active foraging.

Capture efficiency (CE) was defined as

$$CE = \frac{C}{A}$$  \hspace{1cm} (1)

where $C$ = total successful attacks (i.e., captures without re-emergence of prey item); $A_t$ = total attacks.

Handling time began with initiation of an attack and ended when prey escaped or were swallowed [25]. Proportion of the foraging bout spent handling prey items ($HTP$) was defined as

$$HTP = \frac{T_{H'}}{T_T}$$  \hspace{1cm} (2)

where $T_{H'}$ = total handling time and $T_T$ = total foraging time.

**RESULTS**

Due to the high variability of the datasets in this section, error bars are absent from many of the figures for the purpose of clarity.

*Baetis tricaudatus* behavior

Observations of *B. tricaudatus* (mayfly) behavior revealed significant effects of cadmium, predators, and time of day on the study. Statistical analyses were performed using the StatView statistics package (Ver 5.0.1, SAS Institute, Cary, NC, USA). Data sets were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene’s test) prior to statistical analysis.

Data were analyzed using repeated-measures analyses of variance. Factors (and levels) tested were Cd concentration (0, 0.5, 5.0 μg/L), predator (none, stoneflies only, trout only, dace only, trout + stoneflies, dace + stoneflies), and time (09:15, 15:15, 21:15, 03:15). When significant effects were detected in the model, the Student-Newman-Keuls test was used to examine the differences between all possible pairs of means. This post hoc test was applied to the means of between-subjects factors only, as repeated observations on a given subject are correlated, and therefore the means based on these groupings (i.e., within subjects factors) are correlated. The significance level for all tests was $\alpha = 0.05$.

Cadmium results for water and diatom/tissue samples were prepared for cadmium analysis (Perkin-Elmer, Norwalk, CT, USA, 5000 atomic adsorption spectrophotometer; see [26] for analytical procedures). Cadmium results for water and diatom/tissue samples were reported as μg/L and μg/g dry weight, respectively.

Invertebrate species were sampled by first transferring individuals into carbonated water to anaesthetize them (C. Bartata, University of Stirling, Stirling, UK, personal communication) and then removing them to dry, labeled (date, species, cadmium exposure concentration) centrifuge tubes to be placed in a −70°C freezer for 4 h. Tube contents were then freeze-dried (LABCONCO® [Kansas City, MO, USA] Freeze Dry System) and analyzed for cadmium.

Individual fish first were transferred to a clove oil anesthetic bath (see [27]) for 10 min. Concentrations of clove oil used were 40 mg/L for *S. fontinalis* and 30 mg/L for *R. cataractae*. When successfully anaesthetized (cessation of swimming; slow, shallow opercular movement), individuals were killed by spinal severance. In contrast to invertebrates, fish have a large mass of tissue in which the concentrations of most trace elements are subject to feedback regulation (see [28]). Thus, partitioning of metals within the body of the organism is important. To this end, the gills, liver, gut, and muscle of individuals were dissected using a stainless steel scalpel and plastic forceps. After removal of any gut contents, tissue samples were placed in separate, dry, labeled (date, species, tissue type, cadmium exposure concentration) centrifuge tubes and freeze-dried prior to analysis for cadmium.

Statistical analyses

All analyses were performed using the StatView statistics package (Ver 5.0.1, SAS Institute, Cary, NC, USA). Data sets were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene’s test) prior to statistical analysis.

Data were analyzed using repeated-measures analyses of variance. Factors (and levels) tested were Cd concentration (0, 0.5, 5.0 μg/L), predator (none, stoneflies only, trout only, dace only, trout + stoneflies, dace + stoneflies), and time (09:15, 15:15, 21:15, 03:15). When significant effects were detected in the model, the Student-Newman-Keuls test was used to examine the differences between all possible pairs of means. This post hoc test was applied to the means of between-subjects factors only, as repeated observations on a given subject are correlated, and therefore the means based on these groupings (i.e., within subjects factors) are correlated. The significance level for all tests was $\alpha = 0.05$.

$$ATP = \frac{T_{R'} - T_{R}}{T_T}$$  \hspace{1cm} (3)

where $T_{R}$ = total resting time.

Sampling for cadmium analysis

Following completion of the final observation period, all food web elements and water samples were prepared for cadmium analysis (Perkin-Elmer, Norwalk, CT, USA, 5000 atomic adsorption spectrophotometer; see [26] for analytical procedures). Cadmium results for water and diatom/tissue samples were reported as μg/L and μg/g dry weight, respectively.
Food web responses to sublethal cadmium exposure

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Fig. 2. Effects of cadmium and predator type on *Baetis tricaudatus* (A) drift rate, (B) tile positioning, and (C) grazing rate (measured as % change in diatom mat cover).

**A**

![Graph showing drift rate vs. nominal aqueous cadmium concentration](image)

**B**

![Graph showing mean number of mayflies vs. nominal aqueous cadmium concentration](image)

**C**

![Graph showing change in diatom mat cover vs. nominal aqueous cadmium concentration](image)

The drift rate ($p < 0.01$). The effects of cadmium were most pronounced at the higher concentration (Fig. 2A), with 5.0 $\mu$g/L producing a significant decrease in drift rate irrespective of the presence of predators.

Predators appeared to have differing effects on *B. tricaudatus*. Nymphs tended to decrease their drift rate in the presence of *R. cataractae* (longnose dace), with the presence of *S. fontinalis* (brook trout) having little effect, except at 5.0 $\mu$g/L Cd, probably because trout were more active (see below). When a fish species was augmented with *K. nonus* (stonefly) nymphs, the presence of stoneflies served to modify the drift response, producing an increase in both cases. The presence of *K. nonus* nymphs alone resulted in a significant increase in *B. tricaudatus* drift rate (Fig. 2A).

Although significant, the drift response of *B. tricaudatus* to time-of-day was complex. Examination of the data and the interactions between factors revealed no clear patterns. This also was true for tile-positioning behavior by the species.

As with drift rate, cadmium, predators, and time of day also had significant effects on *B. tricaudatus* tile positioning ($p < 0.01$). Cadmium produced decreases at both concentrations in the absence of predators, with the greatest reduction occurring at 5.0 $\mu$g/L (Fig. 2B).

Again, the response of *B. tricaudatus* to predators varied with predator type, with fish species producing decreases in the number of nymphs occupying diatom mats, irrespective of the presence of stoneflies. Only the presence of *K. nonus* nymphs in the absence of additional predator species resulted in an increase in tile-positioning behavior by *B. tricaudatus* nymphs.

Both cadmium and predators affected the grazing rate of *B. tricaudatus* ($p < 0.01$). In the absence of predators, cadmium produced a slight, but significant, increase at 5.0 $\mu$g/L (Fig. 2C).

The effect of predators was more complex. In the absence of cadmium, the presence of predators resulted in an apparent increase in *B. tricaudatus* grazing rate. This effect was modified with increasing cadmium concentration, resulting in a decrease in grazing rate at 5.0 $\mu$g/L when predators were present (Fig. 2C).

As grazing rate is a measure of the depletion in diatom mat area, and because *B. tricaudatus* nymphs have a general tendency to be both less active and less likely to be present on mat surfaces in the presence of predators, these declines in mat area at lower cadmium concentrations may be due to the increased activity of benthic predators at these concentrations, rather than actual mayfly feeding behavior (see below).

*Kogotus nonus* behavior

The most striking effect of cadmium and vertebrate predators on *K. nonus* behavior was a significant reduction in *K. nonus* activity ($p < 0.05$). At night, when nymphs tended to be most active (Fig. 3), the presence of vertebrate predators and cadmium (5.0 $\mu$g/L) produced significant decreases in activity. A Student-Newman-Keuls test showed the reduction in active time proportion by *K. nonus* to be similar in the presence of either fish species. The higher level of activity in some treatments at 09:15 (Fig. 3) may be an indication that the 15-min acclimation period prior to the initiation of an experiment was not long enough for these individuals.

The analysis of other elements of *K. nonus* foraging behavior provided little information due to the low frequency of predation attempts. A total of nine attacks on prey items were made throughout all the experiments, only two of which were successful (both at 0.5 $\mu$g/L of cadmium: One in the presence of trout, one in the absence of additional predator species).

These results show that the activity of *K. nonus* is greater in the absence of predators and cadmium, declining significantly when fish species are introduced and/or cadmium concentration increased. This variation in activity by nymphs is proposed as the mechanism responsible for the observed declines in mayfly grazing rate.

In the absence of fish species or cadmium, much of the activity of *K. nonus* involves walking on the substrate as a search tactic (D.J. Riddell, personal observation), a behavior...
that, if individuals were to walk across tile surfaces frequently, would result in loss of diatom mat area due to sloughing. Indeed, review of the videotapes revealed this to be the case, as stonefly nymphs attempted to search for or attack mayflies present on diatom patches. Therefore, as this behavior decreased with increasing cadmium or the introduction of predators, so too did the loss of diatom mat area.

Walking behavior by stoneflies, by increasing the encounter rate with mayfly nymphs, also is responsible for the increased drift rate and tile positioning observed in *B. tricaudatus* when *K. nonus* is present as the sole predator species. Mayfly nymphs frequently entered the drift to escape the predator or crawled to the upper surface of tiles if *K. nonus* individuals were walking on the floor of the stream channel between the tiles (D.J. Riddell, personal observation).

Salvelinus fontinalis behavior

The capture efficiency of juvenile *S. fontinalis* was affected significantly by increasing cadmium concentration and the presence of stoneflies (*p* < 0.01), but not by time of day (*p* > 0.05). Cadmium produced a reduction in capture efficiency at both 0.5 and 5.0 μg/L, with no significant difference observed in the responses between the two concentrations. Trout failed to capture any mayflies when *K. nonus* were present; this resulted in the obvious differences in capture efficiency illustrated in Figure 4A.

Neither cadmium nor stonefly nymphs had any effect on *S. fontinalis* reaction distance, although the behavioral measure responded significantly to time of day (*p* < 0.05). This result is influenced by the absence of attacks by control fish during the hours of darkness (Fig. 4B).

Cadmium concentration and time of day were found to have significant effects on the active time proportion of juvenile *S. fontinalis* (*p* < 0.001), with no response observed to the presence of stoneflies. Only the higher cadmium concentration produced a significant increase in activity (Fig. 4C) and the active time proportion of individuals tended to increase throughout the day, with fish being most active at night (Fig. 4C).

The proportion of time spent by trout in handling prey items remained unaffected by either cadmium, the presence of stoneflies, or time of day (*p* > 0.05).

Rhinichthys cataractae behavior

The capture efficiency of dace was not affected by cadmium or by the presence of stoneflies, but an interaction between these two factors and time of day was observed (*p* < 0.05). Although this interaction was significant, examination of the data revealed no clear patterns.

Cadmium and time of day had no effect on *R. cataractae* reaction distance, although the behavioral measure responded significantly to the presence of stoneflies (*p* < 0.05). Reaction distance was reduced in the presence of stoneflies, this result being influenced by the absence of attacks by fish at 5.0 μg/L of cadmium when stoneflies were present (Fig. 5A).

Only time of day was found to have a significant effect on the active time proportion of juvenile *R. cataractae* (*p* < 0.001), with no response observed to either cadmium or the presence of stoneflies (*p* > 0.05). In general, fish tended to be more active at night (Fig. 5B). However, unlike stoneflies, the variation in activity by dace did not contribute to increased loss of diatom mat area, as review of the videotapes revealed
that dace tended to avoid the tiles when in contact with the substrate (D.J. Riddell, personal observation). As with brook trout, the proportion of time spent by dace handling prey items remained unaffected by either cadmium, the presence of stoneflies, or time of day ($p > 0.05$).

**Cadmium analyses**

For all test species, whole body (invertebrates) or tissue (fish) cadmium concentrations were approximately linear functions of exposure concentration (Fig. 6). Cadmium concentration in the commercial trout food presented to fish as a maintenance diet was determined to be $0.36 \pm 0.04 \mu g/g$ dry weight. The relationship between nominal versus measured aqueous cadmium concentrations in aquaria is described by:

$$Y = 0.017 + 0.744X$$

The regression models explained 97 to 98% of the variability in uptake by invertebrates, with $B. tricaudatus$ tending to accumulate more cadmium per gram of dry weight than $K. nonus$ nymphs (Fig. 6A).

For $S. fontinalis$ and $R. cataractae$ (Fig. 6B and C, respectively), gills accumulated more cadmium than any other tissue, with levels in dace tending to be greater than those in trout. The liver, gut, and muscle tissues of brook trout and the liver of longnose dace all accumulated cadmium to a similar degree. The muscle and gut tissues of dace contained similar levels of cadmium, but at half the concentration present in the same tissues of trout.

**DISCUSSION**

Standard acute and chronic toxicity tests, though providing important information, tend to be limited in their ecological applications due to their typical emphasis on single species and physiological effects. By investigating effects on individuals, such tests ignore the indirect effects of pollutants and fail to address the complex dynamics possible within natural communities. The use of multispecies tests in the current study provides an important link, by way of ecologically relevant behavioral interactions, between toxicological effects at the individual and community levels. The results of species interactions obtained in the absence of cadmium confirm observations reported by other researchers for the same, or similar, species. In addition, sublethal levels of cadmium were shown to produce significant effects, with aqueous concentrations as low as $0.5 \mu g/L$ resulting in impaired capture efficiency in juvenile brook trout. Current Canadian water quality guidelines [29] set limits for cadmium at $0.2 \mu g/L$ for the protection of aquatic life.
Species behavior in the absence of cadmium

The presence of predators in ecological systems can have strong impacts on a variety of prey behaviors (see reviews [30,31]). By forcing behavioral trade-offs and altering prey activity rates, predators can influence feeding and growth rates, competitive ability, and, in turn, local prey density and community structure [30].

Our study has shown *B. tricaudatus* behavior to be affected significantly the presence of predators. However, the particular response of the species was dependent on predator type.

By using tactile and hydrodynamic cues to detect prey, attacks by stonefly nymphs often are initiated at short ranges and generally occur at or near the substrate [11]. Thus, an effective escape response for prey in the presence of these predators often is to release from the substrate and enter the drift (e.g., [32]). In contrast, many vertebrate predators, such as fish, use visual cues to attack invertebrate prey that are either on the substrate or drifting [33]. Therefore, entering the drift or being exposed on substrate surfaces are unsafe behaviors in the presence of these predators and prey should either stay hidden in refugia or drift only at times when this activity is safe (e.g., at night [33]).

The results of the current study confirm these assertions. A significant increase in *B. tricaudatus* drift rate was observed in the presence of *K. nonus* alone. However, stoneflies were found to have less effect on *B. tricaudatus* behavior when fish species were present. As reported by Peckarsky and McIntosh [34], this reduced impact of stoneflies on mayfly behavior likely is due to reduced stonefly activity in the presence of fish, even though individual stoneflies were not at risk of predation in the current study because fish were gape-limited. Similar species responses also have been reported by McIntosh and Peckarsky [12]. *Baetis tricaudatus* nymphs tended to reduce their presence on diatom mats when either *S. fontinalis* or *R. cataractae* were present, although only the presence of *R. cataractae* produced a decrease in mayfly drift rate. The differing drift response to the two vertebrate predators may be due to perceived differences in predation risk posed by the predators as a result of their contrasting foraging behaviors. As visual predators, salmonids show either crepuscular or diurnal patterns of feeding activity, depending on food availability and predation risk [35], whereas dace appear to avoid predation or competition with sympatric species by foraging nocturnally [18]. Baetid mayfly larvae typically are nocturnal in the presence of trout [22]. Therefore, *B. tricaudatus* nymphs may have reduced their rate of drift during the day but increased drift at night when the foraging ability of trout is reduced. As a consequence, this may have resulted in little change in drift rate in the presence of trout when compared with controls over a 24-h period. The results of statistical analyses indicated a significant interaction between drift rate and time of day. This may suggest a periodicity of behavior by *B. tricaudatus* nymphs, but the direction of the effect could not be determined due to the complex nature of the interaction.

An interaction between cadmium and trout activity also may have been responsible for the observed *B. tricaudatus* drift rate (see Effects of cadmium on species behavior section).

Such behavioral responses by *B. tricaudatus* to the two types of predators studied here would appear to conflict; increasing drift in response to *K. nonus* would make prey more vulnerable to fish, and decreasing activity and increasing use of refugia in response to fish may make prey more vulnerable to *K. nonus*. However, the results of the current study suggest that *B. tricaudatus* nymphs show a hierarchical response (sensu [12]) to the presence of multiple predators as drift and the number on tile surfaces were reduced whenever fish were present, irrespective of the presence of stoneflies. Because capture efficiencies of trout and dace were superior to those of stoneflies (see also [36]), fish, therefore, may be perceived as presenting a greater predation risk to mayfly nymphs than stoneflies.
flies. A reduction in stonefly activity in the presence of fish serves to reduce the predatory ability of *K. nonus* even further.

Due to the reduction in stonefly activity in the presence of fish, one would expect to witness an increase in attacks on mayflies by fish as the retreat of stoneflies to refuges resulted in the displacement of mayflies and their corresponding flight into the drift. However, although an increase in mayfly drift was apparent over that observed in the presence of fish alone, no increase in attacks on mayflies by fish was observed. It is possible that the presence and occasional movement of two large stoneflies may have been a distraction to fish, particularly at night, when stoneflies were most active. This may have been particularly true for hatchery-reared trout, as they had little experience of other aquatic species, and could account for the observed increase in nighttime activity by trout.

**Effects of cadmium on species behavior**

Sublethal levels of aquatic pollutants can limit the feeding, distribution, orientation, and reproductive behavior of aquatic organisms (e.g., [37]). A general decrease in invertebrate activity was observed in the current study, with exposure to cadmium producing decreases in both mayfly drift rate and tile positioning and a reduction in the activity of stoneflies. Previous studies have produced contrary results, demonstrating increases in drift and activity by aquatic macroinvertebrates in response to chemical contamination (e.g., [38]).

It is possible that, in our study, invertebrate activity became reduced with increasing cadmium concentration due to the closed nature of the stream system. The use of such a system for behavioral observations could have modified behavioral responses by preventing emigration at higher cadmium concentrations. When organisms do not relocate to a new location and remain in a contaminated environment, they may experience a reduction in locomotory activity or other behaviors [39]. Such a reduction in activity could be a consequence of increased energy demand due to contaminant acclimation or detoxification [40]. An increase in energy demand may have been the mechanism responsible for increased grazing by *B. tricaudatus* with increasing cadmium concentration in the absence of predators.

The observed increase in *B. tricaudatus* drift rate at 5.0 μg/L Cd in the presence of trout may have resulted from hydrodynamic disturbance due to an increase in trout swimming activity at this concentration (see below).

Cadmium contamination was found to have little or no effect on the behavior of *R. cataractae*, while the foraging behavior of *S. fontinalis* was affected significantly. Increasing cadmium concentration resulted in a significant increase in the activity of *S. fontinalis*, with significant declines in capture efficiency also being observed. Possible reasons for the different susceptibilities of species to cadmium are discussed in the next section.

The increase in activity observed in *S. fontinalis* exposed to cadmium has a number of possible explanations. Studies dating from the 1920s and 30s suggest that fish exposed to aqueous heavy metal concentrations suffocated due to damage to the gills and/or clogging of the lamellae with mucus (see [41] for review of this earlier work), a condition referred to as coagulation film anoxia [42]. Fish with high oxygen demands, such as salmonids [43], generally have a larger number of secondary lamellae that are spaced closer together than in species with lower requirements. Thus, the increase in activity observed in fish exposed to sublethal levels of cadmium may be due to behavior designed to dislodge mucus from gills and/or increase water flow (and, therefore, oxygen diffusion) across the gills.

The ability of metals to cause irritation of the skin or mucus membranes in bluegill (*Lepomis macrochirus*) was investigated by Ellgaard et al. [8]. The authors reported a dose-dependent increase in swimming behavior for fish exposed to zinc (at 0.1 and 5.0 mg/L), cadmium (at 0.1 and 0.25 mg/L), and chromium (at 0.05, 2.4, and 24.0 mg/L). The level of hyperactivity plateaued after 3 d, remaining constant for the remainder of the two-week studies. However, whether the observed behavioral response to metal contamination was due to external irritation (as suspected for copper [2]) or direct effects on the nervous systems of individuals (as suggested by Smith [44]) remains unknown.

Increases in the routine metabolism of fish due to increases in activity have the obvious potential to reduce the energy available for reproduction and growth of an individual. Such impacts on energy budgets are further exacerbated by a reduction in energy intake (i.e., capture efficiency) in juvenile *S. fontinalis* exposed to sublethal cadmium levels. Further investigations of the effects of cadmium on this particular component of fish foraging behavior will be reported in future papers.

**Cadmium uptake by species**

Cadmium analyses of whole-organism *B. tricaudatus* and *K. nonus* samples suggested that, over a 7-d period, *B. tricaudatus* accumulate more cadmium per g dry weight from a contaminated environment than *K. nonus*. The observed differences in uptake may be related to the differences in size of the two species and their surface area to volume ratios, specifically.

A considerable amount of metal uptake from the aqueous phase can occur in invertebrates due to adsorption onto the carapace [45]. Metals adsorbed in this way contribute greatly to the whole organism’s metal body burden and do not necessarily penetrate into the internal tissues and organs [46] because transport across the exoskeleton is extremely slow. Shedding of the carapace during ecdysis can lead to a significant reduction in metal burden [45], suggesting that, at least in some circumstances, adsorption of waterborne metals can contribute more to total body burden than dietary uptake. Surface-metal uptake is known to be a function of body size, with larger organisms being capable of greater metal uptake, but generally having lower concentrations weight for weight due to their smaller surface area to volume ratios [46,47]. Metal binding by metallothionein [48] or intracellular granular structures [49] may aid in the excretion of metals by invertebrates [49,50]. Given the above, the surface-bound metal fraction can contribute greatly to the total body burden of individual invertebrates and could account for the differences between species observed here.

In fish, the gill is a key site for metals uptake, but organs such as the liver and kidney can become more susceptible at chronic exposure levels [28] as the contaminant is detoxified and eliminated. For example, following 38-week exposures to 3.4 μg/L Cd, the kidney, liver, and gill tissues of first- and second-generation brook trout accumulated cadmium in a time- and dose-dependent fashion [51]. The kidney, liver, and gill accumulated the greatest amount of cadmium, approximately 50, 10, and 7 mg/g dry weight, respectively. The kidney continued to accumulate cadmium after exposure ceased, probably
as a result of the redistribution of cadmium from storage in the liver [28].

In the current study, gills were found to accumulate more cadmium than any other tissue, although at much lower concentrations than in the study by Benoit et al. [51], likely due to our much shorter exposure period (four weeks). The levels of cadmium present in the liver tissue of *S. fontinalis* and *R. cataractae* at the higher cadmium concentration (1.75–2.0 µg/g at 5.0 µg/L aqueous Cd) also are considerably lower than those obtained by Benoit et al. [51], suggesting that cadmium had yet to reach levels requiring detoxification by this organ.

Differences in cadmium accumulation also were observed between fish species, with levels in gills tending to be greater in dace than in trout. Levels in the liver were similar between species, but levels of cadmium in the muscle and gut tissues of dace were half the concentrations present in the same tissues of trout. Studies by Brown et al. [52] and Olsson and Kille [53] investigated differential accumulation of cadmium across fish species and found that much of the difference can be attributed to the presence and nature of the intracellular proteins to which cadmium is bound, as well as to differences in species’ metabolic rates.

Work by Thomas et al. [54] suggests that much of the cadmium taken up by rainbow trout (*Oncorhyncus mykiss*) is bound to a nonmetallothionein protein that is inferior to metallothionein at sequestering cadmium. A subsequent study by Olsson and Kille [53] revealed that both the structure and regulatory potential of metallothionein differs between rainbow trout and other fish species and is a contributing factor to the cadmium sensitivity observed in trout.

**CONCLUSION**

In summary, exposure to sublethal levels of cadmium resulted in significant effects on the behavior of all species, except longnose dace, possibly as a result of species-specific cadmium tolerance. Both capture efficiency and active time proportion proved to be sensitive endpoints to the effects of cadmium on species interactions, with aqueous concentrations as low as 0.5 µg/L, resulting in impaired capture efficiency in brook trout. Impaired foraging by affected individuals has the potential to alter the structure of aquatic communities by modifying energy flow and trophic interactions. When in combination with increased activity levels, as observed in exposed brook trout, the energy budget of such organisms becomes further compromised, with potentially negative effects on growth, survival, and reproduction. Such effects on species’ vital rates will be reported in future papers.

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Food web responses to sublethal cadmium exposure

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