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Assessing Stream Grazer Response to Stress: A Post-Exposure Feeding Bioassay Using the Freshwater Snail *Lymnaea peregra* (Müller)

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Snails are important prey items for fish, crayfish and leeches (Lodge 1986) and are an integral component of freshwater communities. Their grazing activities strongly influence the dynamics of the macrophyte epiphyte system by reducing shading and competition for nutrients (Bronmark 1989), through a reduction in biomass (Dillon and Davis 1991) and through significant shifts in algal species composition (McCormick and Stevenson 1989). The removal or reduction of snail populations from freshwater could therefore have significant consequences for community structure and function.

Reduced feeding rate is one mechanism that can potentially explain reductions in survival, growth and reproduction occurring in toxicant-exposed organisms (Jensen et al. 2001) and in the case of grazers, to indirect effects on algal community structure (McCormick and Stevenson 1989). Generally bioassays employ mortality as an endpoint, however, sublethal endpoints are commonly recognised as more sensitive, as often the first reaction of an organism to stress is a physiological one (Gerhardt 1996). Salice and Roesijadi (2002) noted reduced food consumption when *Biomphalaria glabrata* were exposed to cadmium. A significant reduction in feeding rate has also been demonstrated by Allen et al. (1995) following the exposure of *Daphnia magna* to a range of metals and organic chemicals under laboratory conditions. Feeding rate depression caused by toxic stress has been reported by McWilliam and Baird (2002) in post-exposure studies using *Daphnia magna* and by Maltby et al. (1990) in their in situ bioassay with *Gammarus pulex.*

Feeding responses to toxic exposure generally persist in the period immediately after exposure (Taylor et al. 1998), due to the difficulty of determining processing rates during exposure in the field and in the laboratory, a post-exposure technique was employed in this study. In addition measuring feeding rate with *L. peregra* is difficult, as the amount eaten is hard to quantify, consequently we used egestion rate as a surrogate for ingestion rate. In other words, we take advantage of the fact that impairment of feeding rate may lead to a subsequent reduction in rate of egestion. Cadmium was chosen as a model toxicant, as its speciation in the test medium employed is well understood (Barata et al. 2002).

The purpose of this study was to investigate the feeding response of *Lymnaea peregra* in the laboratory to the heavy metal cadmium, and to develop protocols for

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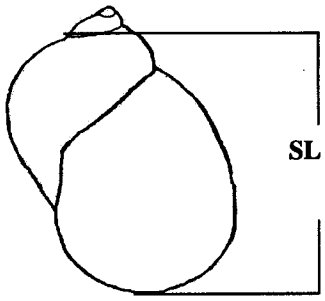

Figure 1. Shell dimension used as measurement of length (SL). This was the longest axis through the shell excluding the final two whorls or 'spire'.

a bioassay to use in toxicity testing procedures under laboratory and field conditions.

MATERIALS AND METHODS

L. peregra (shell length 5.9 to 13.55 mm, n = 50) were collected from Howietoun Fishery (56° N; 3°57 W), Stirling, Scotland and maintained under laboratory conditions for 48 hours prior to employment in the bioassays. Snails were held in 6 L plastic aquaria containing 5L of aerated artificial culture medium (11.1 g/L $KHCO_3$, 15.0 g/L KNO_3, 96.1 g/L $MgSO_4$, 79.9 g/L $NaHCO_3$ and 294.1 g/L $CaCl_2$) at 20°± 1°C and a light/dark cycle of 16h:8 h. Each tank containing 10 snails which were fed with 0.5 g of Tetramin fish food (TetraWerk, Germany). The measure of size used in this bioassay was the length of the longest shell axis excluding the final two whorls or spire (Figure 1). The longest shell axis has a siginificant relationship to both wet and dry biomass as outlined in Table 1.

Table 1 Regression of shell length vs. tissue weight. a and b are the regression coefficients presented with standard error in parentheses, r^2 is the coefficient of determination.

Shell length against	*N*	a	b	r^2	p
Wet weight (mg)	60	0.47 (0.15)	2.29 (0.13)	0.87	<0.001
Dry weight (mg)	60	0.09 (0.06)	2.24 (0.26)	0.56	<0.001

Food was supplied to individual snails in the form of a 'spinach mat'. This method of feeding attempts to simulate a diatom mat. Spinach mats require approximately one day to produce where as diatom mats can take up to 17 days. Spinach mats were prepared by boiling spinach in culture medium (1 g W/W spinach to 1ml culture medium). The spinach was then macerated using a hand-blender (Moulinex, Turbomix) for 2 minutes and diluted 10 fold with culture medium. Fifteen ml of

this dilution was filtered onto a pre-washed, dried and weighed filter paper (47 mm Fisher GFGC) and dried at 60° overnight. Spinach mats were then weighed (± 0.1 mg) to calculate the weight of food supplied.

Individual *L. peregra* were placed randomly into jars, each containing a spinach mat as a food source, and were covered with mesh (<1 mm) secured by an elastic band. Ten jars containing snails and 3 blank controls (without snail) were placed into 6 L tank containing 5 L of aerated culture medium. Snails were then allowed to feed for 24-hours.

Following the 24-hour feeding period, snails were removed from each jar and length was measured using calipers (Moore & Wright plastic dial caliper, 150 mm x 0.1 mm). Length provides a fast, repeatable measure, which may be taken several times during an experiment while causing minimal stress to the animal. Faecal pellets were collected from the surface of each spinach mat using a 3 mL plastic pipette. Faeces from each individual were filtered onto a pre-soaked and pre-weighed filter paper and dried at 60 °C for 24-hours after which time the dry weight was measured to the nearest 0.1 mg. The spinach mat was removed, dried at 60 °C for 48 hours and weighed. Ingestion rate (I_r mg dry weight spinach/mm shell length/day) and egestion rate (E_r mg dry weight faeces/mm shell length/day) of each surviving *L. peregra* was calculated using equation 1:

$$E_r \text{ or } I_r : \frac{f_1 - (f_o \times C_f)}{SL} \qquad \text{(Equation 1)}$$

where f_1 is the dry weight of the filter paper containing faeces or spinach (mg), f_0 is the initial dry weight of the filter paper (mg), SL is the shell length along the longest axis of *L. peregra* (mm) and C_f is the correction factor given by the average of the weight difference between pre and post-filtrate of control filter papers.

Stock solution containing 1 mg Cd/L culture medium was prepared from cadmium standard solution (Fisher Scientific, New Jersey, U.S.A.: Atomic Absorption Cadmium Standard Solution) within 24-hours of experimentation. Again, 60 snails were collected and maintained in the laboratory for 48 hours before use. Eight 6 L plastic tanks were filled with 5 L of culture medium containing cadmium concentrations 0, 10, 100, 250 or 500 µg/L. Snails were randomly assigned to 5 groups of 12 jars, each containing a pre-made spinach mat as a food source. Each jar was covered with <1 mm mesh secured by an elastic band and placed into one of the 5 tanks containing cadmium contaminated culture medium. Egestion rates were measured after 24-hours, at which time the exposure solutions were changed. Egestion rate was measured again after 48 hours and snails were measured for length. Finally, organisms` were transferred to cadmium-free culture medium for an additional 24 hour period to determine post-exposure egestion rates.

Water samples for cadmium analysis were taken at 0 hours, 24-hours and 48 hours from each tank and fixed with HNO_3 (1% final concentration). Water samples were stored at 4°C for up to 1 month before being analyzed directly on a Unicam GF90 graphite-furnace atomic absorption spectrophotometer (detection limit 0.5 µg Cd/L).

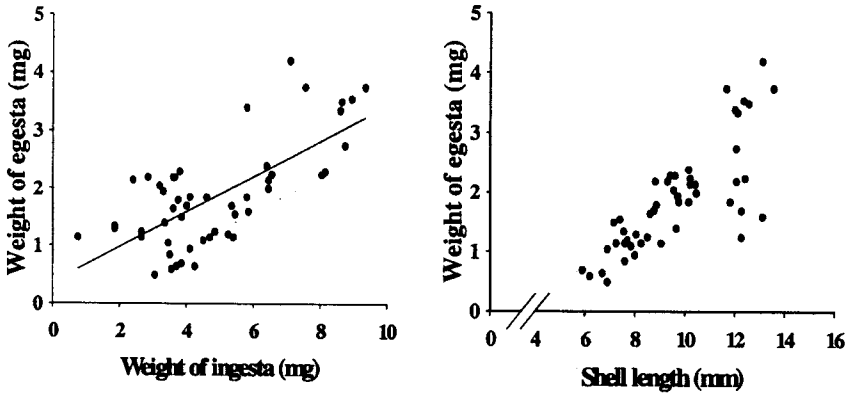

Figure 2. Weight of egesta (mg) taken over a 24-hour period from 50 snails, against (a) weight of ingesta over a 24 hour period. Coefficients of the linear regression mode fitted a = 0.37 (SE = 0.23); b = 0.045; $r^2 = 0.49$; $p<0.001$; and (b) shell length.

Data are given as mean ± standard deviation. All statistics were performed using Minitab version 12.1. Regression was performed on length and both wet and dry weight as well as with egesta and ingesta, with the later as the independent variable. One way analysis of variance was performed to determine if amount egested under contaminated conditions were significantly different from the control.

RESULTS AND DISSCUSSION

All 50 snails used in this investigation survived. Average ingestion and egestion rates for snails in this experiment were 0.51 ± 0.17 mg/mm/d and 0.19 ± 0.062 mg/mm/d respectively. The variability in egestion rate does appear to be in part a product of the range of snail size used in this investigation (Figure 2a). When snails with a shell length greater than 8mm are used the average ingestion rate becomes 0.48 ± 0.17 mg/mm/d, whereas the egestion rate becomes 0.21 ± 0.05 mg/mm/d. In order to reduce variability future investigations will use snails with a shell length of 8 mm or larger, this length also corresponds to the approximate length at which *L. peregra* reach maturity (Lam and Calow 1989).

A significant relationship ($r^2 = 0.49\%$) exists between amount ingested and egested for *L. peregra*, (Figure 2b) over the range of shell length. Egestion rate can therefore be used as a surrogate measure for ingestion.

Cadmium concentrations measured at the start of exposure periods differed by less than 15% from nominal concentrations, therefore concentrations in this study were reported as nominal. Past studies have shown that the cadmium loss in this system was negligible over 24 hours. The effects of cadmium on egestion rate of *L. peregra* over the two, 24-hour exposure periods to cadmium and a subsequent 24-hour post-exposure period are shown in Figure 3. During the first and second 24-hour periods of exposure there were no significant differences between egestion rate at any of the cadmium concentrations. The mean control egestion rate for the normal egestion rates usually recorded for this species and therefore considered an

Table 2 Actual versus nominal concentrations (μg/L) of cadmium at t = 0 of first 24 hour exposure and t = 0 of the second 24 hour exposure.

Nominal	N	Actual	
		t = 0 (1^{st}) Mean (SD)	t = 24 (2^{nd}) Mean (SD)
0	3	0.1 (0.0)	0.1 (0.0)
10	3	11.5 (0.3)	10.2 (0.02)
100	3	106.2 (5.1)	90.7 (7.5)
250	3	262.2 (9.5)	241.1 (8.3)
500	3	489.4 (19.4)	469.5 (22.4)

anomaly. As the same animals were used throughout the test period, egestion rate should have remained stable in the control treatment (Figure 3b). This depression in control egestion rates may be due to a satiation effect on feeding. In the post-exposure recovery period, the control egestion rate increased to a level comparable to that in the initial 24-hour period. During this recovery period, there was a significant difference between egestion rate for snails exposed to cadmium and the control (one way Anova F=3.36, p=0.016). A significant depression in post-exposure egestion rate signaled an inhibition of feeding in *L. peregra*, following cadmium exposure over 48 hours. Measuring the post- exposure effect of a contaminant permits delayed effects and organism recovery to be assessed. Organisms exposed to cadmium may exhibit delayed effects as recorded by Brent and Herricks (1998). Following exposure to a range of cadmium concentrations, *Ceriodaphina dubia*, *Hyalella azteca* and *Pimephales promelas* exhibited delayed effects, resulting in increasing immobility for up to 172 hours after exposure (Brent and Herricks 1998). In the same study, organisms regained mobility following exposure to phenol indicating recovery following exposure which may have been missed with out the post-exposure period.

Gomot (1998) reported inhibition of egg laying in *Lymnaea stagnalis* exposed to 400 μg/L of cadmium and hatching inhibition at 200 μg/L cadmium following a 49-day test period. In this study, effects on *L. peregra* were realized at 50 μg /L with an estimated EC_{50} for egestion rate of 5.6 (SE = 12.5) μg/L (unpublished data) for cadmium indicating that feeding inhibition is an ecologically relevant non-lethal endpoint that can be used in a short-term bioassay. Feeding inhibition is a relevant ecological endpoint, as prolonged energy deficit can lead to reduced fecundity (Baird et al. 1991). Van der Steen et al. (1973) also recorded a reduction in egg laying following a 1 to 7 day starvation period in *L. stagnalis*. Population level effects have already been linked to depressed feeding rates measured at individuals level in other species (Jensen et al. 2001), and it is thought that similar mechanisms could jeopardize the replacement of the generations in *L. peregra*. Further work is being carried out on energy budget modeling to predict long term consequences of contaminant exposure for *L. peregra*.

Collecting and weighing feces *of L. peregra* has proved a reliable measure of feeding activity. This laboratory bioassay was able to detect reduced egestion rates at environmentally relevant levels of cadmium contamination. In short-term exposure studies the use of such a bioassay as a quick screening tool for assessing

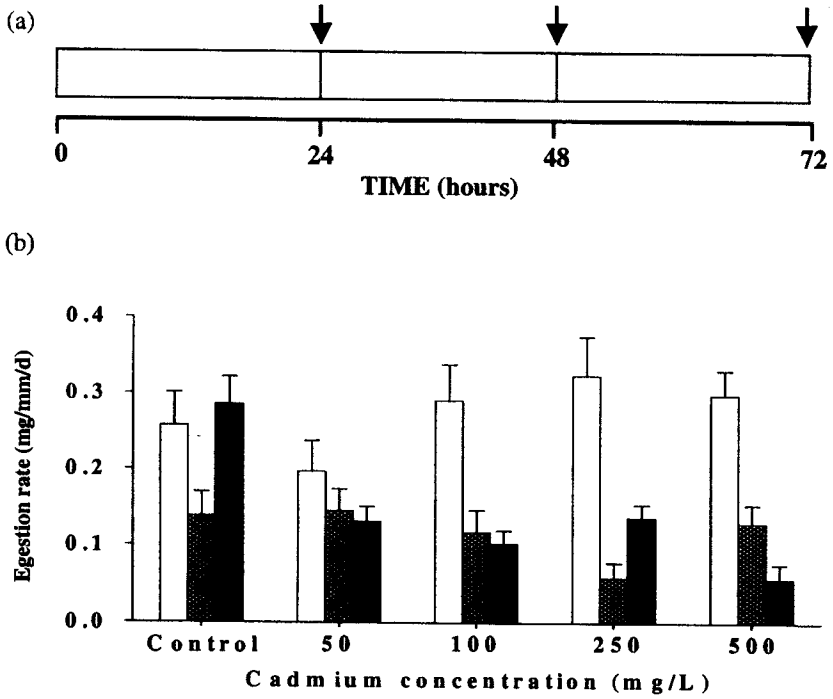

Figure 3. (a) Experimental design of the feeding study. Exposure period is indicated in grey; arrows show the times of egesta collection and renewal of the test medium. (b) Mean egestion rate (± s.e.) against cadmium concentration. White bars represent the first 24-hour period, hatched bars represent the second 24-hour period and black bars represent the post-exposure period.

effect of contaminants is valuable. It is envisaged that egestion rate in *L. peregra* can be adapted for use as a sensitive functional endpoint in an in situ bioassay to assess water quality in rivers and lakes.

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