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Cadmium toxicity and snail–digenean interactions in a population of *Lymnaea* spp.

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Abstract

The toxicity of cadmium to a population of *Lymnaea peregra* and *L. stagnalis* naturally infected with a range of digeneans and collected from a number of sites in the lower Thames Valley, UK was investigated. Lymnaeid snails were exposed to $100\,\mu g\,l^{-1}$ cadmium and the effects on host survival and emergence of cercariae recorded. Overwintered *L. peregra*, but not *L. stagnalis*, showed significantly reduced survival compared to seasonally infected snails, i.e. snails which have acquired an infection during the spring or summer. A significant increase in survival with increasing snail size was demonstrated for *L. stagnalis* and for seasonally infected *L. peregra* only. Only *L. stagnalis* infected with *Diplostomum spathaceum* and *L. peregra* infected individually with *D. spathaceum*, *Sanguinicola inermis*, *Echinoparyphium recurvatum* and *Notocotylus attenuatus* demonstrated a significantly reduced survival compared to laboratory-bred controls. The exposure of *L. stagnalis* to cadmium resulted in a significant reduction in the emergence of *D. spathaceum* over a 5-day period but cadmium-exposed *L. peregra* showed no difference in the emergence of *E. recurvatum* cercariae over a 3-day exposure period. The mechanisms and importance of metal toxicity to snail–digenean interactions are discussed.

Introduction

Molluscs have been extensively used to study the effects of environmental pollution (Phillips & Rainbow, 1993). Pulmonates, in particular, are one of the most important taxonomic groups investigated because acute effects may be observed within a very short time (Abd Allah *et al.*, 1999). The effects of heavy metal toxicity on host–parasite interactions in freshwater snails have been the subject of several investigations. Metal-induced changes in host survival (Guth *et al.*, 1977; Stadnichenko *et al.*, 1998), cercarial emergence (Abd Allah *et al.*, 1997) and the physiochemical properties of host haemolymph (e.g. Stadnichenko *et al.*, 1993) have been studied in both naturally and experimentally infected snails. However, experimental studies are often undertaken using snails of uniform size simultaneously infected with the same number of parasites, and results derived from such experiments may not accurately reflect what can occur in natural systems. Indeed Dreyfuss *et al.* (2000) found that snails collected from a site polluted with household refuse and herbicides demonstrated an increased susceptibility to infection with miracidia of *Fasciola hepatica* with increasing snail body size compared with snails from unpolluted sites. Nevertheless, little is known about how the seasonality of naturally infected snails may affect their vulnerability to pollution.

Freshwater lymnaeid snails within the UK have been shown to host a variety of larval digeneans (Nasir, 1984). Pike (1968) demonstrated seasonal changes in the occurrence of digeneans in freshwater snails, with peaks of infections occurring in June and August. The factors mainly responsible for seasonal occurrence were considered to be changes in the life-cycle of the snail host and environmental temperature, which may affect parasite development. Many infected snails survive over winter

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because of lower temperatures, but then die during the early summer months (Pike, 1968). The aim of the present paper is to investigate the effect of cadmium toxicity on a population of naturally infected *Lymnaea* sp. snails from the lower Thames valley. The rich diversity of the cercarial fauna of this area was established by Adam & Lewis (1993), who recorded eight species of cercariae from *L. peregra*, with a 10.8% prevalence of infection in the snail population. Cadmium toxicity to snail survival will be compared in both seasonally infected, i.e. snails which have acquired an infection during the spring or summer, and overwintered *L. peregra* and *L. stagnalis* for snails of different size and with infections of different digenean species. In addition, the effect of cadmium toxicity on cercarial emergence of two of the most prominent species (*Diplostomum spathaceum* and *Echinoparyphium recurvatum*) from this snail population will be studied. Cadmium is a common constituent of industrial and mining effluents and in the UK some of the highest recorded concentrations in polluted freshwater have been recorded from the River Tawe, Wales where concentrations have been as high as 160 $\mu g\,l^{-1}$ (Vivian & Massie, 1977).

Materials and methods

A stock solution of 100 $mg\,l^{-1}$ cadmium was prepared by dissolving cadmium chloride ($CdCl_2.5/2H_2O$) in distilled water to give the correct concentration of metal ions. Test solutions of 100 $\mu g\,l^{-1}$ were obtained by diluting the stock solution in distilled water. Synthetic soft water (25 $mg\,l^{-1}$ $CaCO_3$, pH 7.85) was prepared using procedures described by HMSO (1969).

Samples of test solutions were analysed for metal loss after 24 h from soft water incubated at 20°C. Solutions were analysed on a Perkin Elmer Optima 3300 Inductively Coupled Plasma-Atomic Emission Spectrometer calibrated with a 1% nitric acid blank and a standard which consisted of 1000 $\mu g\,l^{-1}$ of cadmium in 1% nitric acid. The accuracy of data was assessed by analysing a certified reference material (NIST SRM 1643d) along with the samples, and a relative error was calculated to be 0.00037%.

Lymnaea peregra and *L. stagnalis* naturally infected with a range of digenean cercariae were collected from a number of sites in the lower Thames valley (National Grid Reference SU985697, SU750710, SU779724, SU775525, TQ160694). Overwintered snails, with an active emergence of cercariae, were collected during April–May, while seasonal infections, i.e. snails which have acquired an infection during the spring or summer, were collected during September–October. No overwintered snails were observed to survive this far into the season. Cercariae were identified according to Nasir (1984). Snails were allowed to acclimatize to laboratory conditions for 72 h in aerated aquaria containing soft water (HMSO, 1969).

Survival studies were undertaken using both overwintered and seasonally infected snails, and laboratory bred controls, bred from uninfected snails collected from the same field sites, exposed to 100 $\mu g\,l^{-1}$ cadmium in 500 ml of soft water at 20°C. Snails were fed on a small amount of lettuce as required and the water was changed daily. The time to death and shell size were noted for each snail. Results were analysed with an SPSS computer package using the Mann-Whitney test and Spearman Rank correlation coefficient.

Studies on cercarial emergence patterns were undertaken using seasonally infected snails. *Lymnaea stagnalis* infected with *Diplostomum spathaceum* ($n = 6$ per treatment) were individually placed in 500 ml of soft water at 20°C, in a 12 h light/dark regime, and the daily cercarial emergence rate was recorded for 5 days. Snails were then exposed to 100 $\mu g\,l^{-1}$ cadmium for a further 5 days, and the cercarial emergence was recorded daily. Snails were fed on small amounts of lettuce as required. Additional emergence studies were undertaken using *L. peregra* infected with *Echinoparyphium recurvatum* ($n = 6$ per treatment) individually placed in 200 ml of soft water at 20°C, in a 12 h light/dark regime (0800–2000), and the hourly cercarial emergence rate from 0800–2000 and the total overnight emergence rate (2000–0800) were recorded for a period of three days. Snails were then exposed to 100 $\mu g\,l^{-1}$ cadmium for a further 3 days, and the hourly cercarial emergence and overnight emergence rates recorded. Snails were fed on small amounts of lettuce as required. Results from this experiment were analysed with an SPSS computer package using a repeated measures ANOVA or one-way ANOVA.

Results

The water analysis demonstrated that there was only a small loss of dissolved metals (10%) from the test solution over 24 h.

Exposure of snails to cadmium induced several effects on their survival. Survival of overwintered snails compared with seasonally infected ones demonstrated that with *L. stagnalis*, there was no difference in survival (Mann-Whitney test, $P = 0.866$). However, survival of *L. peregra* seasonally infected snails was significantly (Mann-Whitney test, $P = 0.002$) longer in the cadmium solution than for overwintered snails. An examination of combined overwintered and seasonal data showed that, for *L. stagnalis*, there was a significant positive correlation ($r_s = 0.3653$, $P = 0.0236$) between survival and snail size, with an increase in survival occurring with increasing snail size (fig. 1a). However, no such correlation was apparent for the combined *L. peregra* data ($r_s = -0.2579$, $P = 0.1174$). Nevertheless, an examination of the two data sets separately showed that seasonally infected *L. peregra* did demonstrate a positive correlation ($r_s = 0.6789$, $P = 0.0468$) but overwintered snails did not ($r_s = 0.3067$, $P = 0.1239$) (fig. 1b).

The combined overwintered and seasonal survival data for *L. stagnalis* infected with different species of digeneans against the survival of laboratory-bred controls showed that there was no significant difference in the survival of snails infected with *E. revolutum* or *Cercariae chislehurstensis*. However, *L. stagnalis* infected with *D. spathaceum* demonstrated a significantly reduced survival compared with controls (Mann-Whitney test, $P = 0.011$) (fig. 2). There was no difference in survival between snails infected with the three different digenean species.

The survival of infected *L. peregra* was significantly

reduced compared with controls for all snails and their particular species of parasite infection (Mann-Whitney test- *Sanguinicola inermis* $P = 0.002$, *D. spathaceum* $P = 0.006$, *E. recurvatum* $P < 0.001$, *Notocotylus attenuatus* $P = 0.011$). Differences in survival between snails infected with particular parasite species was only significant when comparing snails infected with furcocercariae and echinostome cercariae (Mann-Whitney test- *S. inermis/E. recurvatum* $P = 0.027$, *D. spathaceum/E. recurvatum* $P = 0.010$) (fig. 3). The survival of *L. stagnalis* and *L. peregra* infected by *D. spathaceum* showed no significant difference when exposed to the cadmium solution (Mann-Whitney test, $P = 0.874$).

The exposure of infected snails to cadmium produced a

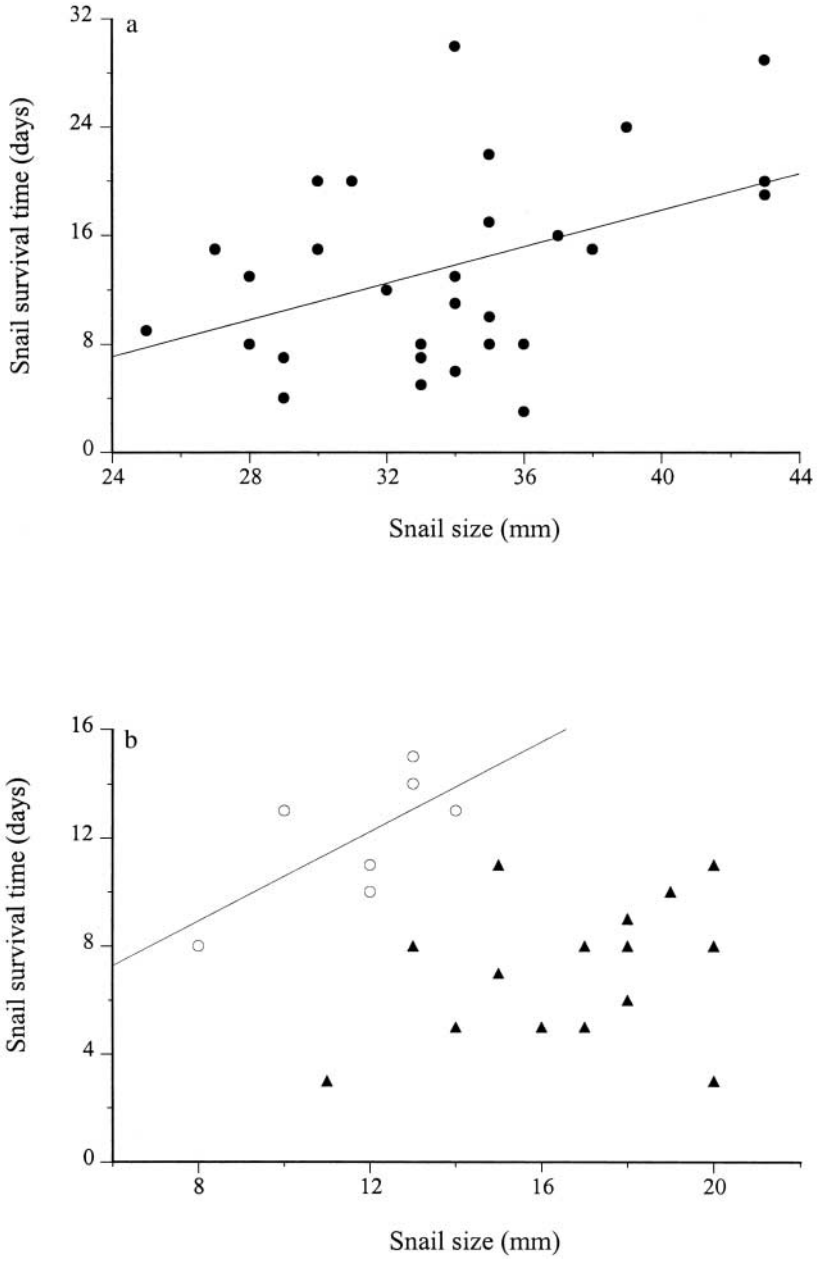

Fig. 1. The relationship between snail size and survival time of parasite-infected snails exposed to 100 $\mu g l^{-1}$ cadmium. a, *Lymnaea stagnalis*. b, *L. peregra* (▲, overwintered snails, sampled in April and May; ○, seasonal snails, sampled in September and October). There is no line of best fit for *L. peregra* overwintered snails because there was no correlation; *L. stagnalis* is grouped as one population because there was no difference between overwintered and seasonal snails.

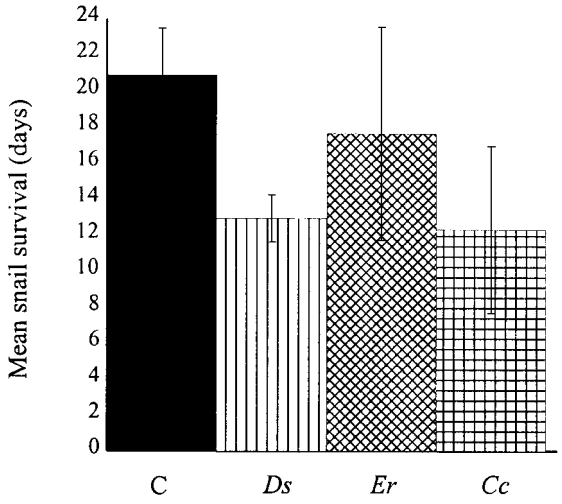

Fig. 2. A comparison of the survival of uninfected (control) *Lymnaea stagnalis* (C) with snails infected with *Diplostomum spathaceum* (*Ds*), *Echinostoma revolutum* (*Er*), *Cercariae chislehurstensis* (*Cc*) exposed to $100\,\mu g\,l^{-1}$ cadmium. Error bars are standard errors.

number of effects on cercarial emergence patterns. During the pre-exposure period (days 1–5) there was no difference in the emergence of *D. spathaceum* cercariae between control and exposed *L. stagnalis* (repeated measure ANOVA, $F = 3.043$, $P = 0.109$). However, exposure to cadmium induced an immediate reduction in the daily cercarial emergence rate (fig. 4). Daily emergence of cercariae continued to decrease over the 5-day exposure period. However, an overall comparison of cercarial emergence patterns of cadmium exposed snails from days 1–5 with days 6–10 revealed no significant difference (repeated measures ANOVA, $F = 3.558$, $P = 0.087$). A comparison of cercarial emergence on day 5 with day 10 showed that there was a significant decrease in cercarial emergence (one-way ANOVA, $F = 5.921$, $P = 0.032$). Over the entire 10-day period cercarial emergence was found to be significantly different in snails exposed to $100\,\mu g\,l^{-1}$ cadmium and controls (repeated measures ANOVA, $F = 5.933$, $P = 0.033$) and also for days 6–10 (repeated measures ANOVA, $F = 7.094$ $P = 0.022$). However, the reduced cercarial emergence appeared to be the result of metal-induced mortality of several snail hosts in the test population. The reduced cercarial emergence was only apparent in dying *L. stagnalis* and two of the six snails died during the exposure period.

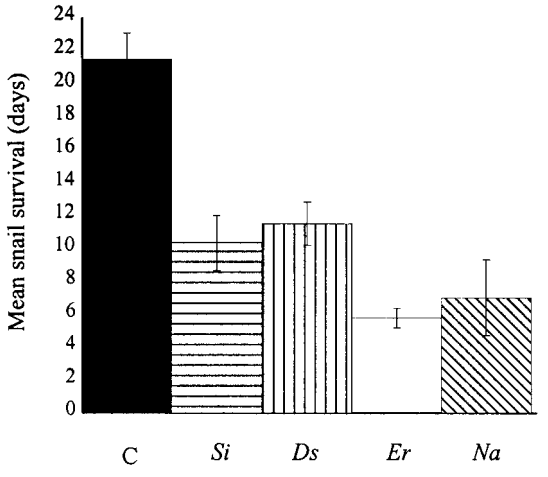

Fig. 3. A comparison of the survival of uninfected (control) *Lymnaea peregra* (C) with snails infected with *Sanguinicola inermis* (*Si*), *Diplostomum spathaceum* (*Ds*), *Echinoparyphium recurvatum* (*Er*), and *Notocotylus attenuatus* (*Na*) exposed to $100\,\mu g\,l^{-1}$ cadmium. Error bars are standard errors.

The exposure of *L. peregra* infected with *E. recurvatum* to $100\,\mu g\,l^{-1}$ cadmium is shown in fig. 5. No snails died during the course of the experiment and a 3-day exposure to cadmium had no significant effect on the pattern of cercarial emergence between days 1–3 and days 4–6 (repeated measure ANOVA, $F = 1.086$, $P = 0.322$). There was also no difference between days 3 and 6 (one-way ANOVA, $F = 0.986$, $P = 0.344$). A comparison of the hourly cercarial emergence rates between days 1–3 and days 4–6 showed no significant difference at any time period, including overnight emergence, between exposed and unexposed periods. There was also no significant change in the daily emergence peak between exposed and unexposed periods. No significant difference in cercarial emergence occurred between control and $100\,\mu g\,l^{-1}$ cadmium exposure for any parameter investigated (repeated measures and one-way ANOVA, $F \leq 1.203$, $P \geq 0.298$).

Discussion

The survival of infected snails was severely reduced by exposure to cadmium. The toxicity of cadmium to parasitized snails has not previously been investigated at this metal concentration ($100\,\mu g\,l^{-1}$). Guth *et al.* (1977), using much higher zinc concentrations of $24–75\,mg\,l^{-1}$ found a rapid mortality for snails infected with *Schistosomatium douthitti* and *Trichobilharzia* sp., with a 100% mortality occurring within 12 h. In contrast, Abd Allah *et al.* (1997), using much lower concentrations of 0.075–100 μM of cadmium, lead, and mercury against *Biomphalaria glabrata* experimentally infected with *Schistosoma mansoni*, found that metal-induced mortality took weeks before there was a significant effect on the snail population.

In the present study, the toxicity of cadmium to a population of *Lymnaea* sp. naturally infected with several species of digeneans, although derived from a relatively small experimental population, has demonstrated several interesting points. The different susceptibility of over-wintered *L. peregra* and *L. stagnalis* to cadmium may be species-specific and may reflect either the 'health' of the host species or the environmental conditions in which hibernation took place. The increased survival of *L. stagnalis* with increased snail size may be due to the larger specimens being more efficient at binding cadmium to metallothioneins and other low-molecular weight

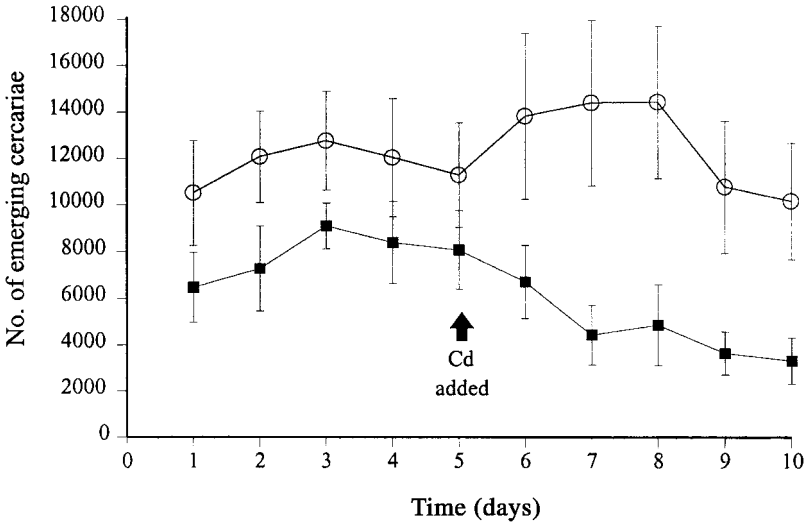

Fig. 4. The emergence of *Diplostomum spathaceum* cercariae from *Lymnaea stagnalis* exposed to 100 $\mu g l^{-1}$ cadmium (■, cadmium, ○, control). Error bars are standard error.

proteins, or to a longer time required for cadmium uptake, or to an increased resistance to unfavourable environmental conditions due to a superior ability of larger snails to aestivate (Godan, 1983). During aestivation, the metabolic activity of snails declines and they stop feeding. Under natural uncontaminated conditions, aestivation allows a snail to survive during unfavourable periods (Laskowski & Hopkin, 1996). However, when exposed to long term contamination, snails are endangered by a prolonged decrease in consumption rate which may eventually lead to death by starvation rather than direct toxicity. However this effect is difficult to assess in short ecotoxicological experiments as snails are able to aestivate for several weeks (Laskowski & Hopkin, 1996). In the present study, the additional nutritional burden and physiological stress imposed by parasitic infections could easily induce the more rapid mortality demonstrated. This may be more acute in smaller snails and in smaller snail species for which nutritional 'reserves' are more limited. Indeed, Badger & Oyerinde (1996) noted that the mortality of *Biomphalaria pfeifferi*, harbouring a mature infection of *S. mansoni*, increased under aestivation compared with those snails which had only been recently infected prior to undergoing aestivation, which the authors attributed to an increased nutritional burden caused by the larger parasites. In addition, the differing survival of snails infected with specific parasite species may reflect the pathology that can be induced on the snails by different intramolluscan stages. In particular, rediae actively feed on the digestive gland which is the main organ of metal accumulation and its destruction may impair host survival by interfering with the metal storage capacity of infected snails. Indeed, Evans *et al.* (2001) found significantly lower accumulated levels of metals in parasitized marine snails than uninfected ones.

The increased mortality of infected snails exposed to heavy metals may have a number of additional consequences for host–parasite interactions. In particular, the present study has demonstrated that the emergence of cercariae from exposed snails can be altered if snails suffer from the extra stress induced by a toxicant. Evans (1982) postulated two reasons for a depressed rate of emergence in snail hosts exposed to low metal concentrations: (i) a reduced snail activity or (ii) a build-up of heavy metal concentrations within the snail tissues which adversely affected parasite development. Host activity is linked with the parasite emergence rate. For example, Anderson *et al.* (1976) found that the maximum emergence of *Trichobilharzia ocellata* coincided with periods of peak activity of the host *L. stagnalis*, and snail activity is reduced in the presence of heavy metals (e.g. Evans, 1982; Laskowski & Hopkin, 1996). However Yescott & Hansen (1976) and Abd Allah *et al.* (1997) believe that adversely affected parasite development was responsible for reduced cercarial emergence from metal-exposed hosts. In contrast, Evans *et al.* (2001) found that parasitized snails collected from a metal-polluted site accumulated significantly lower levels of metals than those from an unpolluted site which may be a product of parasite-induced pathology of the metal storage capacity of the digestive gland. In the present study, reduced cercarial emergence is probably a consequence of metal-induced snail mortality rather than any physiological effects on the parasite. This is in agreement with the findings of Evans (1982) for high concentrations of copper or zinc exposure on *L. peregra* infected with *N. attenuatus*. Indeed, Morley (unpublished observations) found that the emergence of *D. spathaceum* from *L. stagnalis* exposed to zinc demonstrates signs of recovery at 100 $\mu g l^{-1}$, possibly indicating that the initial depression in the rate of cercarial emergence may be associated with an initial increased stress response by the host, leading to an alteration in activity before acclimation occurs.

It is possible that longer exposure periods to lower

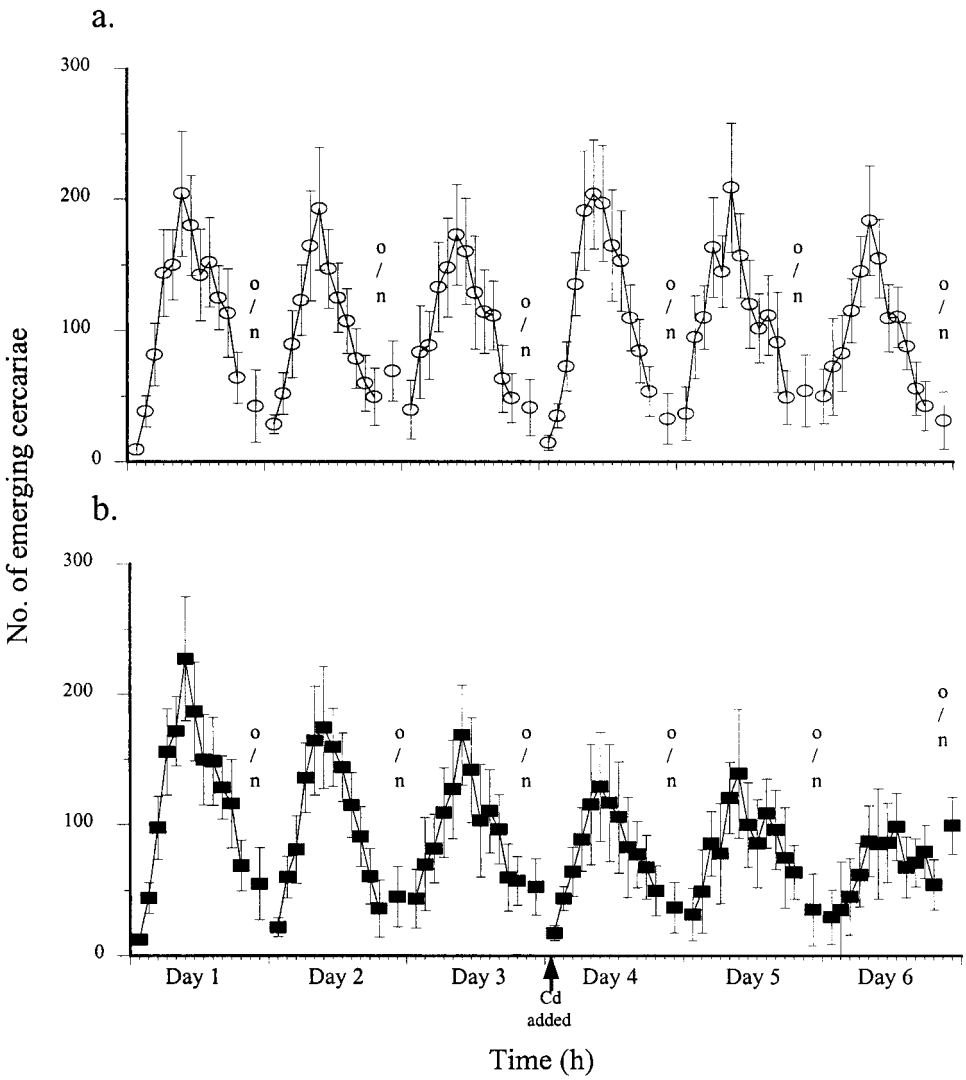

Fig. 5. The toxicity of 100 $\mu g l^{-1}$ cadmium to the emergence of *Echinoparyphium recurvatum* cercariae from *Lymnaea peregra*. (x axis indicates hourly emergence from 0800–2000; o/n overnight emergence) (a. control ○, b. cadmium ■). Error bars are standard errors.

metal concentrations than those used in the present study may have a more significant impact on parasite emergence. Abd Allah *et al.* (1997) found that a 6-week exposure to metal concentrations no higher than 100 μM caused a significant reduction in *S. mansoni* cercarial emergence from *B. glabrata*, which the authors attributed either to the termination of sporocyst development or to the death of the sporocyst. Toxicity to the intramolluscan stages may, therefore, in the short term influence the maturation or emergence of cercariae into the snail tissue. Interestingly, Cross *et al.* (2001) found that cercariae which emerged from snails collected from a metal-polluted site had a significantly reduced survival compared to control cercariae. Establishing whether cercarial survival is affected by exposure of the snail host to short-term acute metal concentrations would be a suitable subject for further study.

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