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Toxic effects of cadmium and zinc on the transmission of *Echinoparyphium recurvatum* cercariae

N.J. Morley*, M. Crane and J.W. Lewis

School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, TW20 0EX, UK

Abstract

The toxicity of cadmium, zinc and Cd/Zn mixtures to the transmission of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae) cercariae into the snail second intermediate hosts was investigated at concentrations ranging from 100 $\mu g\,l^{-1}$ to 10 000 $\mu g\,l^{-1}$ in both soft and hard water. A differential response in the infectivity of metal-exposed cercariae into *Lymnaea peregra* and *Physa fontinalis* was demonstrated which was dependent on the snail species being infected. Exposure of *L. peregra*, *P. fontinalis*, and *L. stagnalis* to heavy metals caused a differing susceptibility to *E. recurvatum* cercariae depending on the snail species being exposed. The mechanism and effects of metal toxicity, together with the importance of the parasite/host strain on cercarial transmission are discussed.

Introduction

The successful transmission of digenean free-living stages into an appropriate host is one of the most important aspects of their biology which can be influenced by a wide variety of environmental factors, e.g. temperature (Pechneik & Fried, 1995). In many aquatic ecosystems pollutants are an additional environmental variable and any effect a toxic pollutant may have on digenean transmission would have wide ranging and long lasting effects on the parasite population. The toxicity of pollutants to parasite transmission has therefore formed the basis of a number of studies, especially on the toxicity of heavy metals to the transmission of cercariae of the medically important *Schistosoma mansoni* (Holliman & Esham, 1977; Abd Allah *et al.*, 1996).

However, toxicity studies on the transmission of other digenean species, especially where cercariae do not actively penetrate their target host are extremely limited. Evans (1982a,b) investigated copper and zinc toxicity to the transmission of *Echinoparyphium recurvatum* cercariae and *Notocotylus attenuatus* metacercariae respectively. Although the transmission of *N. attenuatus* into the definitive bird host was not significantly reduced by metal toxicity (Evans, 1982b), *E. recurvatum* over a range of metal concentrations (500–10 000 $\mu g\,l^{-1}$) and exposure periods (15–120 min), in both soft and hard water, experienced reduced transmission of cercariae into the snail second intermediate host, *Lymnaea peregra* (Evans, 1982a). Copper-induced effects were particularly severe and exposure of cercariae to 500 $\mu g\,l^{-1}$ for only 15 min caused significantly reduced infection rates (Evans, 1982a).

The present study was designed to extend the work of Evans (1982a) on metal toxicity to *E. recurvatum*. This digenean is a 45-collar-spined echinostome which is ubiquitous in the UK, adult worms being found in the intestine of a wide range of aquatic birds (McCarthy, 1999a). The first intermediate hosts are the aquatic molluscs *L. peregra* and *Valvata piscinalis* (McCarthy, 1990a), although *E. recurvatum* shows a low specificity for its second intermediate host having been recorded from a wide range of freshwater molluscs (Evans *et al.*, 1981). However, only *L. peregra*, *V. piscinalis*, and *Physa fontinalis* demonstrate a high degree of compatibility (Evans & Gordon, 1983a). Cercariae of *E. recurvatum* do not actively penetrate their target host as they lack penetration glands, instead entry is via the natural openings of the snail body (Adam & Lewis, 1992). Maximum transmission of cercariae is achieved approximately 2 h post-shedding from the first intermediate host with an initial dispersal phase of sub-maximal infectivity (Evans & Gordon,

*Fax: 01784 470756
E-mail: n.morley@rhul.ac.uk

1983b) and is temperature dependent (Adam, 1991; McCarthy, 1999b).

The aims of the present study are to investigate the toxicity of cadmium, zinc and a cadmium/zinc mixture to the transmission of *E. recurvatum* cercariae into the second intermediate snail host. Cadmium and zinc are related heavy metals, almost always occurring together in pollution incidents. They have been widely used in industry and are released into the environment as a by-product of ore smelting (Hellawell, 1986). In the UK, concentrations chronically polluting the aquatic environment have been recorded as high as 160 $\mu g\,l^{-1}$ for cadmium and 8800 $\mu g\,l^{-1}$ for zinc (Vivian & Massie, 1977). Previous studies (Morley *et al.*, 2001a,b) have demonstrated that cadmium and zinc have a wide ranging toxicity to the functional biology of free-living stages of digeneans. Unlike the previous study by Evans (1982a) the present study focuses on differences that may occur between different snail hosts with the following objectives:

1. To examine the effects of cadmium and zinc, both singly and in combination, on the transmission of metal exposed *E. recurvatum* cercariae into *L. peregra* and *P. fontinalis*.
2. To assess the influence of prior metal exposure of highly susceptible (*L. peregra* and *P. fontinalis*) and poorly susceptible (*L. stagnalis*) hosts on subsequent *E. recurvatum* infection.

Materials and methods

Stock solutions of 100 $mg\,l^{-1}$ cadmium and zinc were prepared by dissolving either cadmium chloride ($CdCl_2.5/2H_2O$) or zinc chloride ($ZnCl_2$) (Sigma Chemicals) in distilled water to provide the correct concentration of metal ions. Test solutions of 100 $\mu g\,l^{-1}$, 1000 $\mu g\,l^{-1}$, and 10,000 $\mu g\,l^{-1}$ metal ions were obtained by diluting stock solutions in distilled water and adding to synthetic soft (25 $mg\,l^{-1}$ $CaCO_3$, pH 7.85) or hard (250 $mg\,l^{-1}$ $CaCO_3$, pH 8.00) water which were prepared using procedures described by HMSO (1969).

Samples of test solutions were analysed for metal loss from soft water incubated at 20°C in the concentration range of 100–10 000 $\mu g\,l^{-1}$ at 0.5 h and 24 h. Solutions were analysed on a Perkin Elmer Optima 3300 Inductively Coupled Plasma-Atomic Emission Spectrometer which was calibrated with a 1% nitric acid blank and a standard which consisted of 1000 $\mu g\,l^{-1}$ of cadmium and zinc in 1% nitric acid. The accuracy of the data was assessed by analysing a certified reference material (NIST SRM 1643 d) along with the samples and was calculated to have a relative error of 0.00037% for cadmium and 0.00065% for zinc.

Lymnaea peregra, naturally infected with *Echinoparyphium recurvatum* were collected from Bushy Park, London (National Grid Reference TQ160694). Cercariae were identified according to Adam (1991) and Nasir (1984). Laboratory snails, of similar ages (4–6 weeks old, 3–6 mm in shell length), were bred from mature adults collected from the same habitat as *E. recurvatum* infected snails.

Studies on the transmission of metal exposed cercariae were undertaken with snails (*L. peregra* and *P. fontinalis*) ($n = 12$ per test solution) individually exposed to ten cercariae in 10 ml of either soft or hard water for 1 h at 20°C. All cercariae were between 75–90 min old and had been exposed for 30 min to either 100 $\mu g\,l^{-1}$, 1000 $\mu g\,l^{-1}$ or 10 000 $\mu g\,l^{-1}$ metal concentration (cadmium and zinc) or equal concentrations of 100 $\mu g\,l^{-1}$ Cd- 100 $\mu g\,l^{-1}$ Zn, 1000 $\mu g\,l^{-1}$ Cd- 1000 $\mu g\,l^{-1}$ Zn, or 10 000 $\mu g\,l^{-1}$ Cd-10 000 $\mu g\,l^{-1}$ Zn (Cd/Zn mixture), or distilled water as a control in 10 ml of either soft or hard water in 35 mm plastic Petri dishes. After exposure to the test solution, cercariae were briefly rinsed in distilled water before exposure to the test snail, so that the latter were not exposed to heavy metals.

Studies on the transmission of cercariae into metal exposed snails were undertaken using laboratory bred snails (*L. peregra*, *L. stagnalis*, and *P. fontinalis*) ($n = 12$ per test solution) exposed for 24 h in aerated glass beakers at 20°C to either 100 $\mu g\,l^{-1}$ Cd, 100 $\mu g\,l^{-1}$ Zn, or 100 $\mu g\,l^{-1}$ Cd + 100 $\mu g\,l^{-1}$ Zn mixture in 500 ml of soft water. Snails were then briefly rinsed in distilled water and individually exposed to ten cercariae (maximum age 75–90 min) for 1 h in soft water at 20°C.

In all studies snails were subsequently maintained in aerated aquaria for 24 h then shell length was recorded (to the nearest 0.5 mm) and the tissue examined for metacercarial cysts by squash preparations. Data were expressed as either a 'transmission success' (equation 1) or, for the figures where comparisons between snail species which had differing susceptibility to *E. recurvatum* was needed, as a transmission index (T.I.) (equation 2)

$$\text{Transmission success} = \frac{\text{Total no. cysts established}}{\text{Total no. of cercariae}} \times 100 \quad (1)$$

$$\text{T.I.} = \frac{\text{Total no. of cysts established in test solution}}{\text{Total no. of cysts established in control}} \quad (2)$$

If $\text{T.I.} > 1$ this implies increased transmission, and if $\text{T.I.} < 1$ this implies reduced transmission when compared with controls.

Data were transformed using a square root transformation and analysed using a factorial analysis of variance (ANOVA) implemented in the SPSS statistical software package.

Results

Chemical analysis of the test solutions revealed that a loss of dissolved metals over 0.5 h and 24 h occurred in most test solutions. The greatest loss occurred in the highest metal concentration (10 $mg\,l^{-1}$). Cadmium showed a greater concentration loss than zinc at the lower metal concentrations, whilst zinc had a greater loss at the higher metal concentrations. However losses were low over the experimental period, amounting to no more than 10% after 24 h.

The infectivity of metal-exposed *Echinoparyphium recurvatum* cercariae to *Lymnaea peregra* and *Physa fontinalis* is shown in tables 1 and 2 and fig. 1. Although for controls, the transmission success and the proportion of snail populations infected demonstrate a difference between the two species in both water mediums, these

Table 1. Transmission of metal-exposed *Echinoparyphium recurvatum* cercariae into the snail host *Lymnaea peregra*.

Test solution ($\mu g\,l^{-1}$)		Mean no. cysts per snail (± S.E.)	Mean no. cysts per infected snail (± S.E.)	% snails infected	Transmission success
a. Soft water					
Control		4.92 (0.76)	4.92 (0.76)	100	49.2
Cd	10 000	1.00 (0.39)	2.00 (0.52)	50.0	10.0
	1000	1.92 (0.66)	3.29 (0.78)	58.3	19.2
	100	5.58 (0.71)	5.58 (0.71)	100	55.8
Zn	10 000	3.92 (0.71)	4.70 (0.58)	83.3	39.2
	1000	4.33 (0.67)	4.73 (0.59)	91.7	43.3
	100	4.75 (0.78)	4.75 (0.78)	100	47.5
Cd/Zn mixture	10 000	0 (0)	–	0	0
	1000	2.83 (0.63)	3.78 (0.52)	75.0	28.3
	100	6.92 (0.57)	6.92 (0.57)	100	69.2
b. Hard water					
Control		5.33 (0.56)	5.33 (0.56)	100	53.3
Cd	10 000	3.25 (0.75)	3.90 (0.74)	83.3	32.5
	1000	5.08 (0.62)	5.08 (0.62)	100	50.8
	100	6.17 (0.82)	6.73 (0.65)	91.7	61.7
Zn	10 000	5.75 (0.69)	5.75 (0.69)	100	57.5
	1000	5.50 (0.74)	5.50 (0.74)	100	55.0
	100	5.17 (0.82)	5.17 (0.82)	100	51.7
Cd/Zn mixture	10 000	3.25 (0.59)	3.55 (0.56)	91.7	32.5
	1000	2.75 (0.76)	3.67 (0.80)	75.0	27.5
	100	6.00 (0.70)	6.00 (0.70)	100	60.0

differences are only statistically significant for hard water (soft water $F = 3.374$, $P = 0.0798$; hard water $F = 4.656$, $P = 0.0421$). Nevertheless heavy metals had a differential effect on parasite transmission into the two snail hosts (tables 1, 2; fig. 1).

Metal exposure of parasites caused a differing response between the two snail species (fig. 1) dependent on metal concentration and water hardness. Analysis by three-way ANOVA (snail species/water hardness/metal concentration) showed these differences to be significant for cadmium ($F = 3.612$, $P = 0.0145$), non-significant for zinc ($F = 1.111$, $P = 0.3461$), and significant for Cd/Zn mixtures ($F = 3.449$, $P = 0.0179$).

However, two-way interactions (snail species/water hardness, snail species/metal concentration, water hardness/metal concentration) were not significant for metal

Table 2. Transmission of metal-exposed *Echinoparyphium recurvatum* cercariae into *Physa fontinalis*.

Test solution ($\mu g\,l^{-1}$)		Mean no. cysts per snail (± S.E.)	Mean no. cysts per infected snail (± S.E.)	% snails infected	Transmission success
a. Soft water					
Control		3.00 (0.90)	4.00 (0.99)	75.0	30.0
Cd	10 000	1.50 (0.56)	3.00 (0.68)	50.0	15.0
	1000	3.58 (0.63)	3.91 (0.60)	91.7	35.8
	100	3.42 (0.85)	3.73 (0.86)	91.7	34.2
Zn	10 000	2.92 (0.78)	3.50 (0.82)	83.3	28.3
	1000	4.00 (0.84)	4.80 (0.77)	83.3	40.0
	100	3.25 (0.69)	3.55 (0.68)	91.7	32.5
Cd/Zn mixture	10 000	0.50 (0.29)	2.00 (0.58)	25.0	5.0
	1000	2.17 (0.84)	3.25 (1.08)	66.7	21.7
	100	4.08 (0.70)	4.46 (0.65)	91.7	40.8
b. Hard water					
Control		3.58 (0.67)	3.91 (0.64)	91.7	33.3
Cd	10 000	2.67 (0.72)	4.00 (0.68)	58.3	23.3
	1000	2.92 (0.65)	3.50 (0.62)	91.7	29.2
	100	5.67 (0.67)	5.67 (0.67)	100	56.6
Zn	10 000	2.33 (0.48)	3.11 (0.35)	75.0	23.3
	1000	2.83 (0.72)	3.40 (0.73)	83.3	28.3
	100	4.33 (0.77)	4.73 (0.73)	91.7	43.3
Cd/Zn mixture	10 000	1.75 (0.58)	2.33 (0.67)	75.0	17.5
	1000	5.33 (1.10)	5.33 (1.10)	100	53.3
	100	4.42 (0.68)	4.42 (0.68)	100	44.2

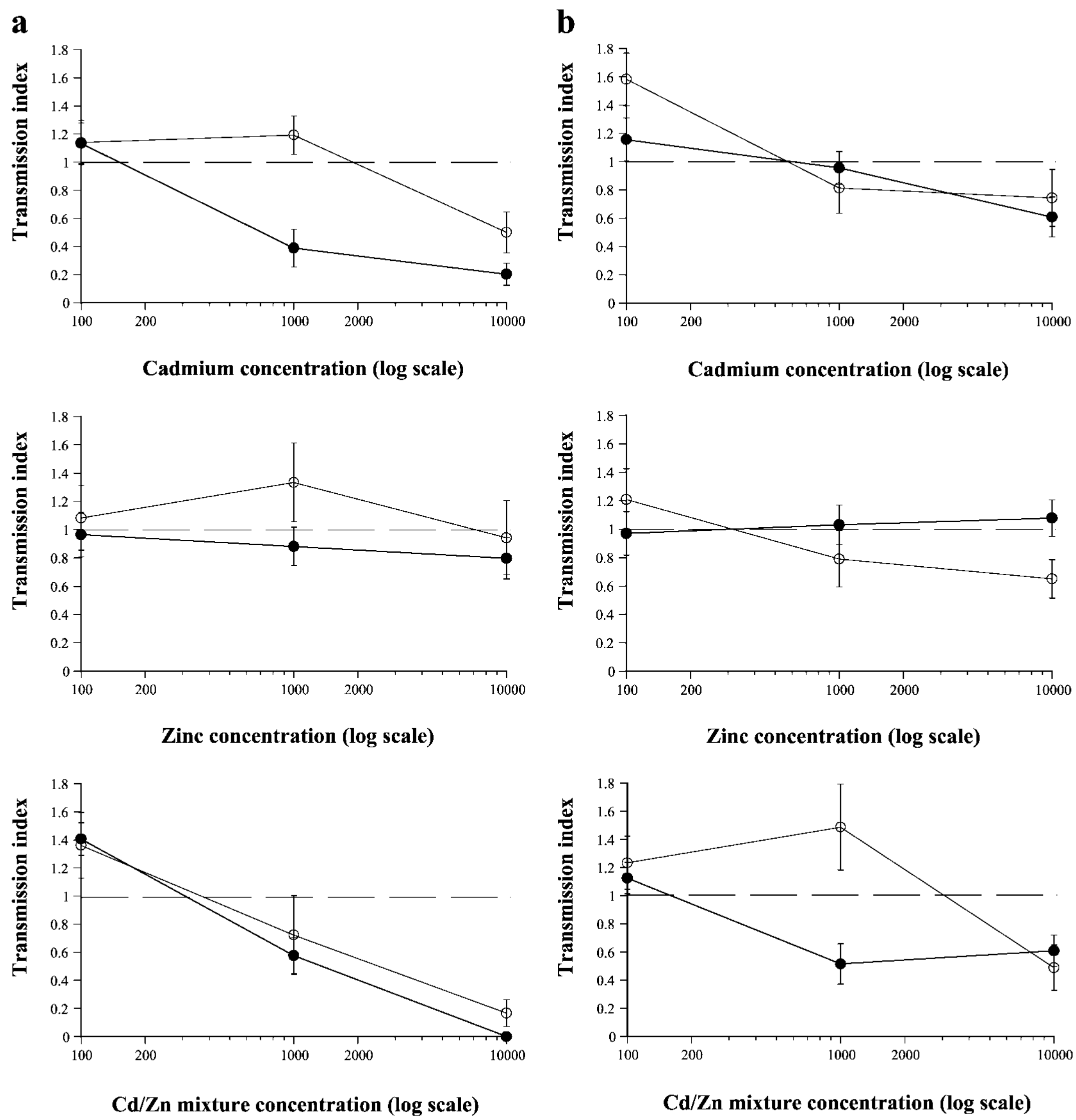

Fig. 1. Transmission of metal (Cd, Zn, Cd/Zn mixture) exposed (100 $\mu g\,l^{-1}$, 1000 $\mu g\,l^{-1}$, and 10 000 $\mu g\,l^{-1}$ metal concentration) *Echinoparyphium recurvatum* cercariae into *Lymnaea peregra* (●) and *P. fontinalis* (○) in soft water (a) and hard water (b). Note T.I. > 1 (---) is equivalent to increased transmission whilst T.I. < 1 (---) is reduced transmission. Error bars are standard errors.

exposures except for Cd/Zn mixtures where differences were significant for snail species/metal concentration ($F = 3.132$, $P = 0.0270$) and water hardness/metal concentration ($F = 3.945$, $P = 0.0094$). Analyses of individual differences by one-way ANOVA showed significant differences for cadmium between species ($F = 5.454$, $P = 0.0207$), water hardness ($F = 14.763$, $P = 0.0002$), and metal concentration ($F = 15.453$, $P < 0.0001$); for zinc between species ($F = 22.056$, $P < 0.0001$); for Cd/Zn mixtures between species ($F = 7.086$, $P = 0.0085$), water hardness ($F = 14.157$, $P = 0.0002$), and metal concentration ($F = 29.298$, $P < 0.0001$).

Exposure of the snail hosts *L. peregra*, *P. fontinalis* and *L. stagnalis* to heavy metals had a variable effect depending on the snail species being exposed (table 3, fig. 2). A two-way ANOVA revealed that there was a significant difference between the interactions of snail species and metal exposure ($F = 2.173$, $P = 0.0470$), whilst a one-way ANOVA demonstrated differences between snail species ($F = 90.675$, $P < 0.0001$) and metal exposure ($F = 4.451$, $P = 0.0047$).

Analyses of responses of individual snail species showed that exposure of *L. peregra* to metals had no significant effect on parasite transmission (table 3, fig. 2). However examination of cyst establishment compared to snail size revealed that changes in susceptibility occurred

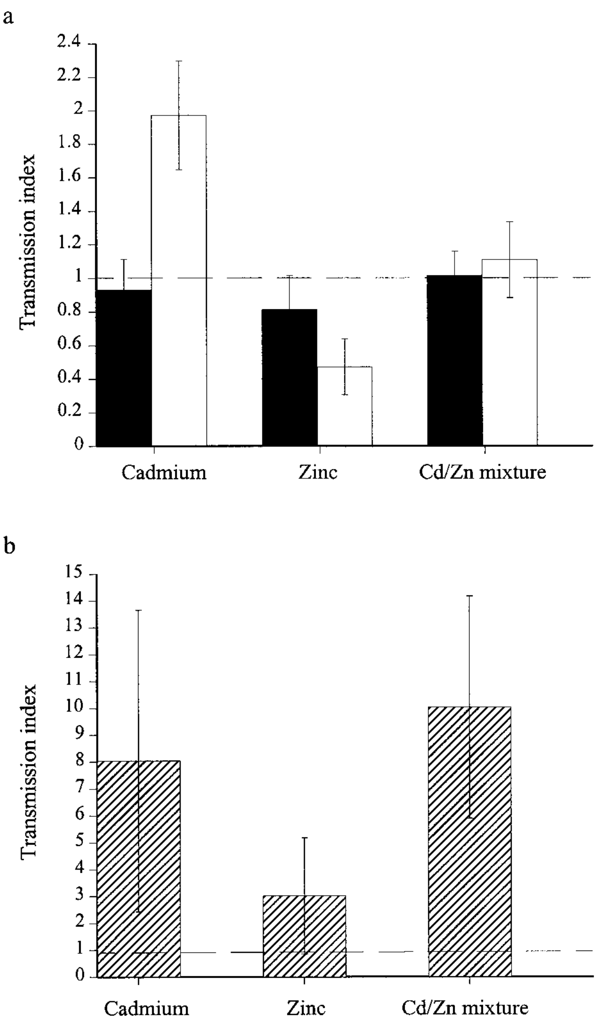

Fig. 2. Transmission of *Echinoparyphium recurvatum* cercariae into metal (Cd, Zn, Cd/Zn mixture) exposed ($100\,\mu g\,l^{-1}$ metal concentration) snails (mean ± S.E.). Note T.I. > 1 (---) is equivalent to increased transmission whilst T.I. < 1 (---) is reduced transmission. a. (■) *L. peregra*; (□) *P. fontinalis*., b. (▨) *L. stagnalis*. Error bars are standard errors.

between smaller (3–4 mm) and larger snails (5–6 mm), which did not occur in the other two snail species.

Physa fontinalis demonstrated an increased susceptibility to infection when exposed to cadmium and a decreased susceptibility when exposed to zinc (table 3, fig. 2). Mixtures of Cd/Zn showed the two metals to be antagonistic to each other which gave an infectivity little different from controls. However, statistical analyses revealed that only cadmium exposure showed a significant difference in infectivity ($F = 4.793$, $P = 0.0395$).

Lymnaea stagnalis exposed to cadmium, zinc, or a Cd/Zn mixture showed an increase in mean susceptibility to *E. recurvatum* many times in excess of control levels (table 3, fig. 2). However, only Cd/Zn mixtures were found to be significantly different from controls ($F = 5.414$, $P = 0.0296$).

Discussion

The present study has demonstrated that parasite transmission is influenced by interactions between host species, water hardness and metal concentrations.

It has been shown that transmission differences occur between two snail species infected with metal-exposed cercariae. Under control conditions, a significant difference between the transmission of the two species is dependent on water hardness, but when cercariae are exposed to metals, differences between the two snail species are more apparent. Such differences may be related to a number of factors including the effects of metal toxicity on cercarial host-finding or the ability to encyst. Korner & Haas (1998) found that chemo-orientation of *Pseudoechinoparyphium echinatum* and *Echinostoma revolutum* towards their snail host was inhibited by exposure to 0.45 μM silver nitrate which the authors attributed to the binding of metal to ciliated papillae on the cercarial surface. A similar conclusion was also reached by King & Higashi (1992) who showed that a sub-lethal dose of silver nitrate (0.9 μM) significantly inhibited *Schistosoma mansoni* cercarial penetration. This inhibition was reversed when cercariae were washed free of the metal. However, in the present study immunological differences between the two host species are likely to be a more influential factor in their variable susceptibility. It is worthwhile noting that Dragneva & Kanev (1983) observed that cercariae of the closely related *Echinoparyphium aconiatum* possess antigens in common with the musculature of their second intermediate host *Lymnaea stagnalis*, and this reduces the chance of a host-tissue reaction occurring. Morley (unpublished observations) demonstrated that heavy metals selectively bind to the cercarial surface of *E. recurvatum* in areas associated with sensory receptors. Such metal binding may partially interfere with cercarial defence mechanisms against host rejection or, alternatively, interfere with the host response where increased transmission success occurs with metal-exposed cercariae.

Although the present study has demonstrated a limited impact of metal toxicity on the establishment of the parasite within the host, an examination of infection rates in the snail population suggests that host susceptibility can show both increasing and decreasing trends of parasite establishment. Although the snail population was regarded as an entire population unit for this study, Evans (1982a) suggested that observing metal effects on transmission in a number of 'groups' of snail populations demonstrates a better resolution of metal toxicity to *E. recurvatum* transmission.

Exposure of three snail hosts to heavy metals induced a different response in each species, indicating differing degrees of physiological tolerance. The interference of snail resistance to the establishment of *E. recurvatum* metacercarial cysts may be indicative of the compatibility of the individual snail species to infection and the subsequent extent of any host-tissue reaction against the parasite. *Lymnaea peregra*, regarded as a highly compatible species (Evans & Gordon, 1983a) shows no difference in infection levels from those in controls, although variation in susceptibility between small and large snails was apparent. This may be indicative of some effect on

Table 3. Transmission of *E. recurvatum* cercariae into metal-exposed snails (*Lymnaea peregra*, *Physa fontinalis*, *Lymnaea stagnalis*).

Snail species	Exposure	Mean no. cysts per snail (± S.E.)	Mean no. cysts per infected snail (± S.E.)	% snails infected	Transmission success
L. peregra	Control	4.92 (0.76)	4.92 (0.76)	100	49.2
	100 $\mu g\,l^{-1}$ Cd	4.58 (0.90)	4.58 (0.90)	100	45.8
	100 $\mu g\,l^{-1}$ Zn	4.00 (0.99)	5.33 (0.96)	75.0	40.0
	100 $\mu g\,l^{-1}$ Cd/Zn mix	5.00 (0.72)	5.00 (0.72)	100	50.0
P. fontinalis	Control	3.00 (0.90)	4.00 (0.99)	75.0	30.0
	100 $\mu g\,l^{-1}$ Cd	5.92 (0.98)	6.46 (0.90)	91.7	59.2
	100 $\mu g\,l^{-1}$ Zn	1.42 (0.50)	2.43 (0.61)	58.3	14.2
	100 $\mu g\,l^{-1}$ Cd/Zn mix	3.33 (0.68)	3.33 (0.68)	100	33.3
L. stagnalis	Control	0.08 (0.00)	1.00 (0.00)	8.3	0.83
	100 $\mu g\,l^{-1}$ Cd	0.67 (0.47)	4.00 (1.00)	16.7	6.7
	100 $\mu g\,l^{-1}$ Zn	0.25 (0.18)	1.50 (0.50)	16.7	2.5
	100 $\mu g\,l^{-1}$ Cd/Zn mix	0.83 (0.34)	1.67 (0.49)	50.0	8.3

parasite transmission, although larger sample sizes are needed before more precise conclusions can be drawn. Nevertheless, the influence of host size on susceptibility is worthy of further investigation.

Exposure of *P. fontinalis* causes a metal specific response, with cadmium leading to increased transmission, possibly due to immunosuppression, zinc inducing a reduced transmission, possibly due to immunostimulation, and Cd/Zn mixtures producing an antagonistic effect, causing little alteration in parasite infectivity. *Lymnaea stagnalis*, a host species of low compatibility, shows an increase in transmission success, possibly because of immunosuppression, in all three toxic media, with Cd/Zn mixture inducing the greatest increase in *E. recurvatum* transmission. Differing responses to heavy metals by different snail species has previously been demonstrated (Ravera, 1991; Gomot, 1998) although the degree of toxicity varies widely, depending on the individual metal (Ravera, 1991).

The susceptibility of control *L. peregra* and *P. fontinalis* to *E. recurvatum* cercariae in the present study, although comparable with the work of Adam (1991), is much lower than that recorded in previous studies (e.g. Evans & Gordon, 1983b; McCarthy, 1990b, 1999b) and may be related to differences in snail host susceptibility or parasite infectivity. The majority of previous studies on *E. recurvatum* transmission can be split into three main groups – those that utilized Harting Pond, Sussex, UK for parasite material and mature snails for laboratory breeding of experimental animals (Evans *et al.*, 1981; McCarthy, 1990a,b, 1999a,b); those that utilized a tributary of the River Great Ouse, Buckinghamshire, UK and Kelsey Park Lake, Kent, UK for material (Evans, 1982a; Evans & Gordon, 1983a,b); and those that utilized Bushy Park, Greater London, UK for material (Adam, 1991; Adam & Lewis, 1992 and the present study). Comparison of results from these geographically distinct sites must take into consideration the differing aquatic habitats as a factor influencing levels of parasite transmission. It has been demonstrated that different geographical sites may produce considerable variation in snail populations that are correlated with different habitat types, for example Calow (1981) found that *L. peregra* cultured from populations in 'exposed' and 'sheltered' habitats demonstrated different growth patterns that were associated with differences in reproductive pattern. Such differences may cause fluctuations in snail susceptibility to *E. recurvatum*.

Alternatively, transmission differences may be due to variations in infectivity between parasite populations at each site and to the occurrence of different strains of *E. recurvatum*. Parasite strains, differing in their infectivity, have been known to exist in many species primarily due to their self-fertilization, polyembryony, asexual reproduction, clone formation and isolation (Kennedy, 1975).

These potential transmission differences, caused by either differing snail susceptibility or parasite infectivity need to be taken into consideration when studying the toxicity of pollutants to parasite transmission. Host/parasite populations are likely to react differently to identical toxin incidents. This has been demonstrated when comparing the present study to that of Evans (1982a) on the effects of copper and zinc toxicity on *E. recurvatum* transmission to *L. peregra*. Evans (1982a), utilizing the same synthetic water medium devised by HMSO (1969), reported a much higher toxicity of zinc, after a 30 min exposure, than shown in the present study where zinc toxicity was negligible. This is most likely to be related to host/parasite strain differences, e.g. an increase in transmission of control snails derived from a more 'exposed' habitat in a tributary of the River Great Ouse (Evans, 1982a) contrasts with that in the present study where snails were collected from a shallow slow-moving stream connecting two leisure ponds at Bushy Park.

The present study has therefore demonstrated that the relationship of transmission success in *E. recurvatum* and metal toxicity is highly complex and is dependent upon at least water hardness, the snail host species and host/parasite strains. Predicting the impact of metal pollution on parasite transmission in natural systems is likely to be difficult especially when stimulation or suppression of infectivity may depend on potential interactions of any of the combinations of abiotic and biotic factors considered in this investigation. Indeed both cercariae and their snail hosts can be exposed to pollutants concurrently and therefore future studies need to concentrate on investigating such cumulative effects on parasite transmission.

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