Toxicity of cadmium and zinc to cercarial tail loss in *Diplostomum spathaceum* (Trematoda: Diplostomidae)

N. J. MORLEY*, M. CRANE *and* J. W. LEWIS

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

(*Received 5 February 2002; revised 11 April 2002; accepted 11 April 2002*)

SUMMARY

The effect of cadmium and zinc at concentrations ranging from 0·1 to 10000 μg/l on tail loss in cercariae of *Diplostomum spathaceum* was investigated at 3 temperatures (12, 20 and 25 °C) and 3 levels of water hardness (distilled water, soft water and hard water). Increasing tail loss over time was found to be linked with a parallel decrease in cercarial survival in controls. Exposure to the heavy metals induced, especially at high concentrations, a change in the relationship between cercarial tail loss and survival, causing either stimulation or inhibition of tail loss dependent on the individual toxic exposure. Under most environmental conditions the rate of tail loss over time was reduced by increasing metal concentrations. Inhibition of tail loss occurred in a limited number of both control and metal-exposed cercariae, with a number of low metal concentrations inducing greater inhibition than in controls. Stimulation of tail loss causing an increased tail loss rate above controls also occurred at certain high metal concentrations. Increasing water hardness and decreasing water temperature caused a reduced tail loss rate over time in both control and metal-exposed cercariae. However, with decreasing temperature a reduced rate of tail loss over time in metal-exposed cercariae compared to controls occurred at some low metal concentrations. When tail loss was compared against cercarial death of the experimental population toxic exposure induced changes in the parallel relationship of these parameters, dependent on individual metal concentration, water temperature and hardness. Differences in the relative effects of cadmium and zinc on cercariae were dependent on the environmental conditions of exposure. Both metals showed limited effects during the period of maximum cercarial infectivity (0–5 h). The mechanisms and importance of metal toxicity to cercarial tail loss are discussed.

Key words: cadmium, zinc, *Diplostomum spathaceum*, cercariae, tail loss.

INTRODUCTION

Free-living stages of digeneans, especially cercariae, have proved a popular choice for the study of pollutant toxicity. Historically, the main toxicity parameter studied prior to transmission has been survival (e.g. Holliman & Esham, 1977; Abd Allah, Wanas & Thompson, 1996; Pietrock *et al*. 2001). However, focusing on this one aspect may give misleading predictions of the effects of ultimate parasite establishment in the target host. Cercariae possess a number of distinct phases in their life-span which include dispersal, host location, host attachment, and host penetration (Haas, 1994). Throughout these phases the cercarial tail plays a pivotal role. Any premature tail loss would have adverse consequences for the cercariae, even though their immediate survival may be unimpaired.

Unfortunately tail loss in cercariae has been relatively poorly studied. Most available work has focused on *Schistosoma mansoni* cercariae and the role of tail loss in host penetration and associated parasite physiological changes (e.g. Howells *et al*. 1975). Rea & Irwin (1992) performed one of the few detailed studies of tail loss on the marine species *Cryptocotyle lingua* and found that as cercariae aged they tended to shed their tails, with higher temperatures inducing an increased separation rate. Several chemical substances induce tail loss in cercariae, including praziquantel (Coles, 1979) on *S. mansoni*, the insecticide Cartap hydrochloride (Oshima *et al*. 1992) on *Gigantobilharzia sturniae*, and the metals silver nitrate (Hara *et al*. 1993) and zinc (Asch & Dresden, 1977) on *S. mansoni*. Hara *et al*. (1993) considered that silver nitrate induced tail loss may be associated with the binding of silver to sensory papillae, as silver nitrate is commonly used for staining cercarial sensory receptors.

However, these toxicity studies neglected the influence of environmental parameters such as water temperature and hardness on the toxic action of metals on cercarial tail loss. Since cercariae can be shed by their snail host into a wide range of environmental aquatic conditions the present study investigates the influence of different water temperatures and hardness, as part of a larger multi-parameter study, on the toxicity of cadmium and zinc to *Diplostomum spathaceum* cercariae. An earlier study by Morley, Crane & Lewis (2001) measured cadmium and zinc toxicity to *D. spathaceum* cercarial survival using the same environmental conditions and metal concentrations used in the present study.

D. spathaceum is an ubiquitous parasite of fish

* Corresponding author: Tel: +01784 443266. Fax: +01784 470756. E-mail: n.morley@rhul.ac.uk

Parasitology (2002), **125**, 293–301.
DOI: 10.1017/S0031182002002007 Printed in the United Kingdom

throughout the UK and occurs in many different climate zones (Chubb, 1979). Temperatures of 10 °C or lower seem seriously to impede or prevent shedding of cercariae from the lymnaeid snail host (Lyholt & Buchmann, 1996; Sous, 1992). Experimental studies have demonstrated that 10 °C is also the minimum temperature for active transmission of *D. spathaceum* to both snail and fish hosts (Wootten, 1974; Stables & Chappell, 1986). Cercarial life-span is temperature dependent with maximum survival decreasing from 240 h at 4 °C to 72 h at 20 °C (Lyholt & Buchmann, 1996). Optimal infectivity occurs between 0 and 5 h post-emergence (Whyte, Secombes & Chappell, 1991).

The aims of the present study are to determine how exposure to cadmium and zinc affects tail loss of *D. spathaceum* cercariae at 3 temperatures (12, 20 and 25 °C) and 3 types of water hardness (distilled, soft and hard water). Cadmium and zinc are related heavy metals, almost always occurring together in pollution incidents. They are widely used in industry and are released into the environment as a by-product of ore smelting (Hellawell, 1986). In the UK concentrations of these metals chronically polluting the aquatic environment have been recorded as high as 160 μg/l for cadmium and 8800 μg/l for zinc (Vivian & Massie, 1977).

MATERIALS AND METHODS

Test solutions

Stock solutions of 100 mg/l 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 give the correct concentration of metal ions. Test solutions were obtained by diluting stock solutions in distilled water and adding to the test vessels at concentrations which gave final cercarial exposures of 0·1, 10, 100 and 10000 μg/l. Synthetic soft (25 mg/l $CaCO_3$, pH 7·85) and hard (250 mg/l $CaCO_3$, pH 8·00) waters were prepared using procedures described by HMSO (1969).

Water analysis

Samples of test solutions were analysed for metal loss from soft water incubated at either 25, 20 or 12 °C in the metal concentration range of 10–10000 μg/l (0·1 μg/l was below the limit of detection) after 0·5, 5 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 μg/l of cadmium and zinc in 1 % nitric acid. The accuracy of the data was assessed by analysing a certified reference material (NIST SRM 1643d) along with the samples and was calculated to have a relative error of 0·00037 % for cadmium and 0·00065 % for zinc.

Source of parasites

Specimens of *Lymnaea stagnalis* naturally infected with *D. spathaceum* were collected from Dinton Pastures, Reading, UK (National Grid Reference SU779724), maintained in aerated 8 litre Perspex tanks and fed on a diet of lettuce leaves. Recently emerged cercariae of *D. spathaceum* were identified according to morphological criteria described by Niewiadomska (1986). Infected snails and test solutions were acclimatized to each experimental temperature as required.

Cercarial tail loss toxicity test

Toxic effects were investigated by pipetting 36 cercariae (maximum age 25 min) individually into wells of flat-bottomed 96-well microtitre plates (Life Sciences International) using a wide-bore pipette tip to prevent cercarial damage. Each well contained a final solution volume of 300 μl of either distilled water, soft water, or hard water with sufficient test solution having been added, or a control addition of distilled water, to give the appropriate concentration exposure. Cercariae were maintained in a 12 h light/dark incubator set at either 25, 20 or 12 °C, and all experiments were set up at the same time of day. Three replicates of each treatment were set up and the number of cercariae shedding their tails was observed at intervals of 0·5–3 h, depending on the concentration of the test solution used. A Lee-Desu Comparison test was used to compare tail shedding through time and by Tukey's significant difference test for 5-h-old cercariae (the age limit of maximum cercarial infectivity) in different treatments. Statistical analysis was performed using UNISTAT 4.5 (Unistat Ltd, 1996).

RESULTS

Water analysis

Analysis of the test solution samples revealed that a loss of dissolved metals occurred within 0·5 h in most examples and concentrations generally continued to decline up to 24 h. The initial loss rate was greatest from the highest metal concentration (10000 μg/l). However, over the 24 h period there was no more than a 15 % concentration loss from any of the test solutions. There were no major differences in metal loss over the 3 experimental temperatures.

Cercarial tail loss

Under control conditions *D. spathaceum* cercarial tail loss increased over time in parallel with a decrease in cercarial survival. Most tail loss occurred a few hours prior to cercarial death but, in some

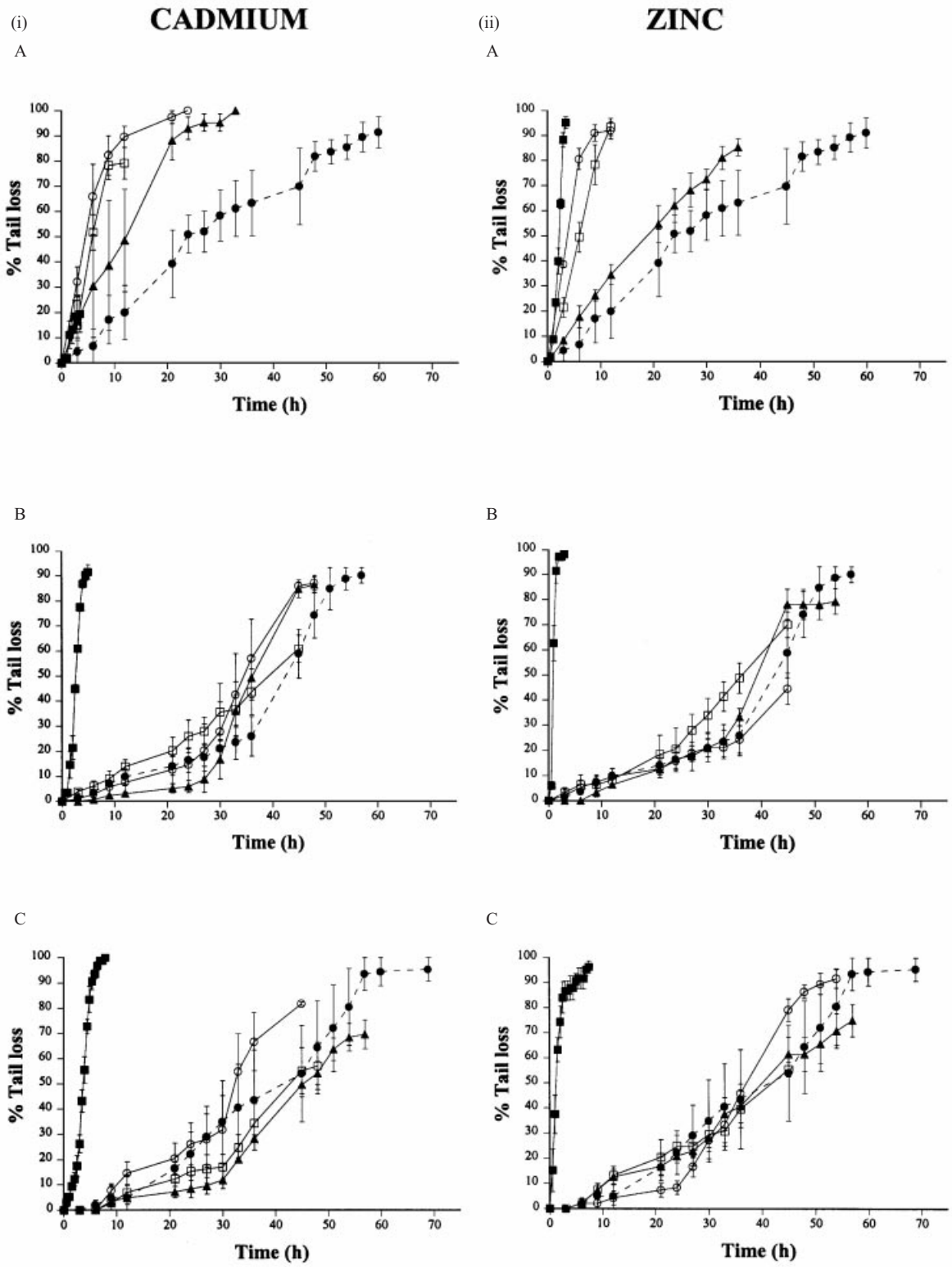

Fig. 1. Tail loss of cercariae of *Diplostomum spathaceum* at 25 °C exposed to (i) cadmium, (ii) zinc, in (A) distilled water, (B) soft water and (C) hard water. Error bars are standard errors. (●) Control, (▲) 0·1 μg/l, (○) 10 μg/l, (□) 100 μg/l, (■) 10000 μg/l.

cases, decaudized cercariae survived for up to 30 h, dependent on environmental conditions. Death of cercariae was pronounced when they failed to respond to mechanical stimulation with a fine needle. Inhibition of tail loss i.e. cercariae that died without shedding their tail, occurred in a small percentage of the cercarial population and was more common at lower temperatures and higher water hardness (Figs 1–3). Inhibition of tail loss in both controls and cercariae exposed to low metal concentrations usually only occurred in the latter half of the life-span of cercariae.

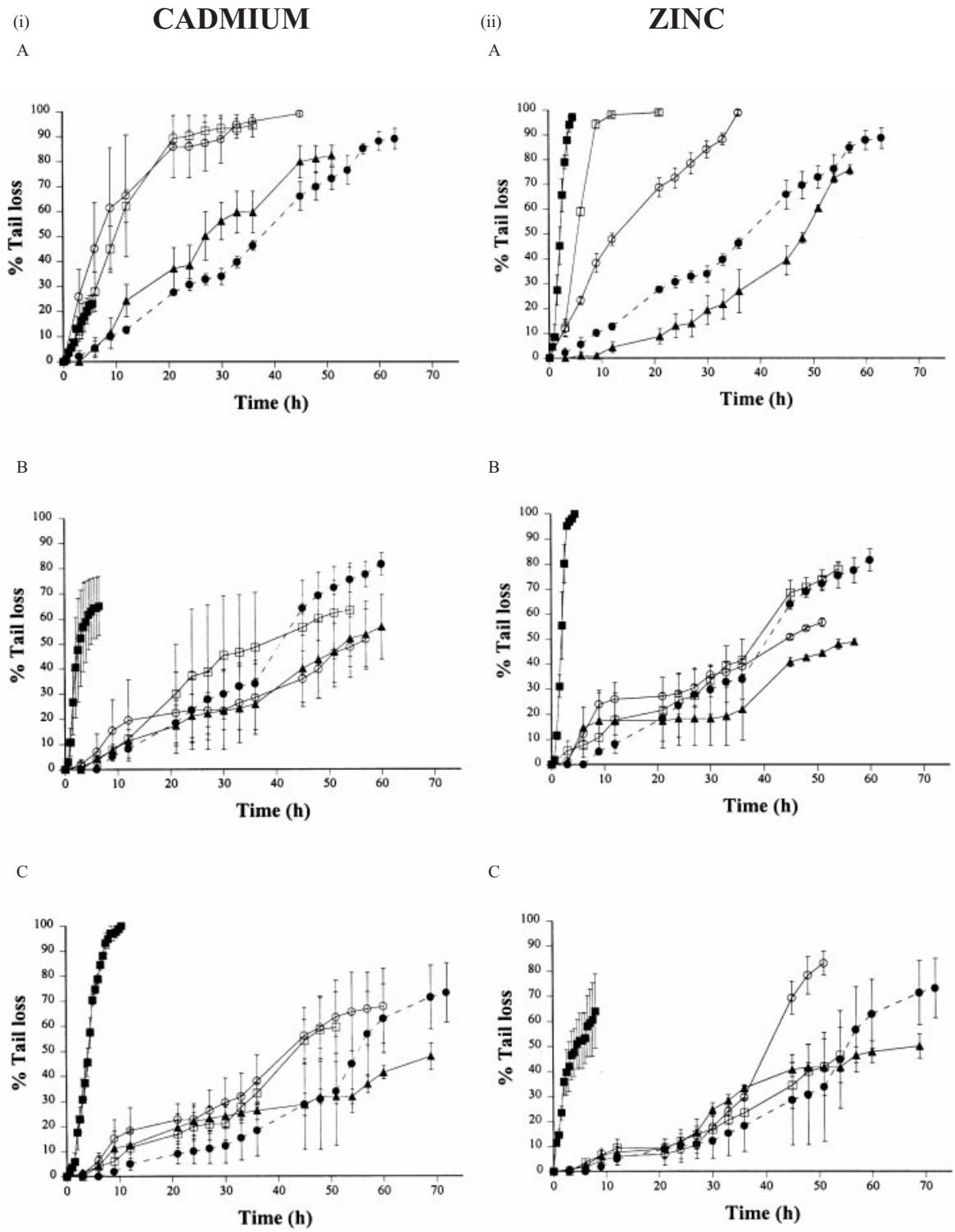

Fig. 2. Tail loss of cercariae of *Diplostomum spathaceum* at 20 °C exposed to (i) cadmium, (ii) zinc, in (A) distilled water, (B) soft water and (C) hard water. Error bars are standard errors. (●) Control, (▲) 0·1 μg/l, (○) 10 μg/l, (□) 100 μg/l, (■) 10000 μg/l.

Exposure to cadmium and zinc affected cercarial tail loss, with changes dependent upon metal concentration, water temperature and water hardness. Decreasing water temperature and increasing water hardness reduced the toxicity of the two metals. Statistical analysis demonstrated that metal toxicity at the different temperatures, with some individual exceptions, was significantly different (Lee-Desu, $P \leqslant 0{\cdot}0233$) between each metal concentration for both cadmium and zinc.

Water hardness also affected tail loss patterns. Significant differences generally occurred between distilled water and the harder waters (Lee-Desu, $P \leqslant 0{\cdot}0380$). However, significant differences in tail loss after exposure to metals in soft and hard water were less common with decreasing temperature.

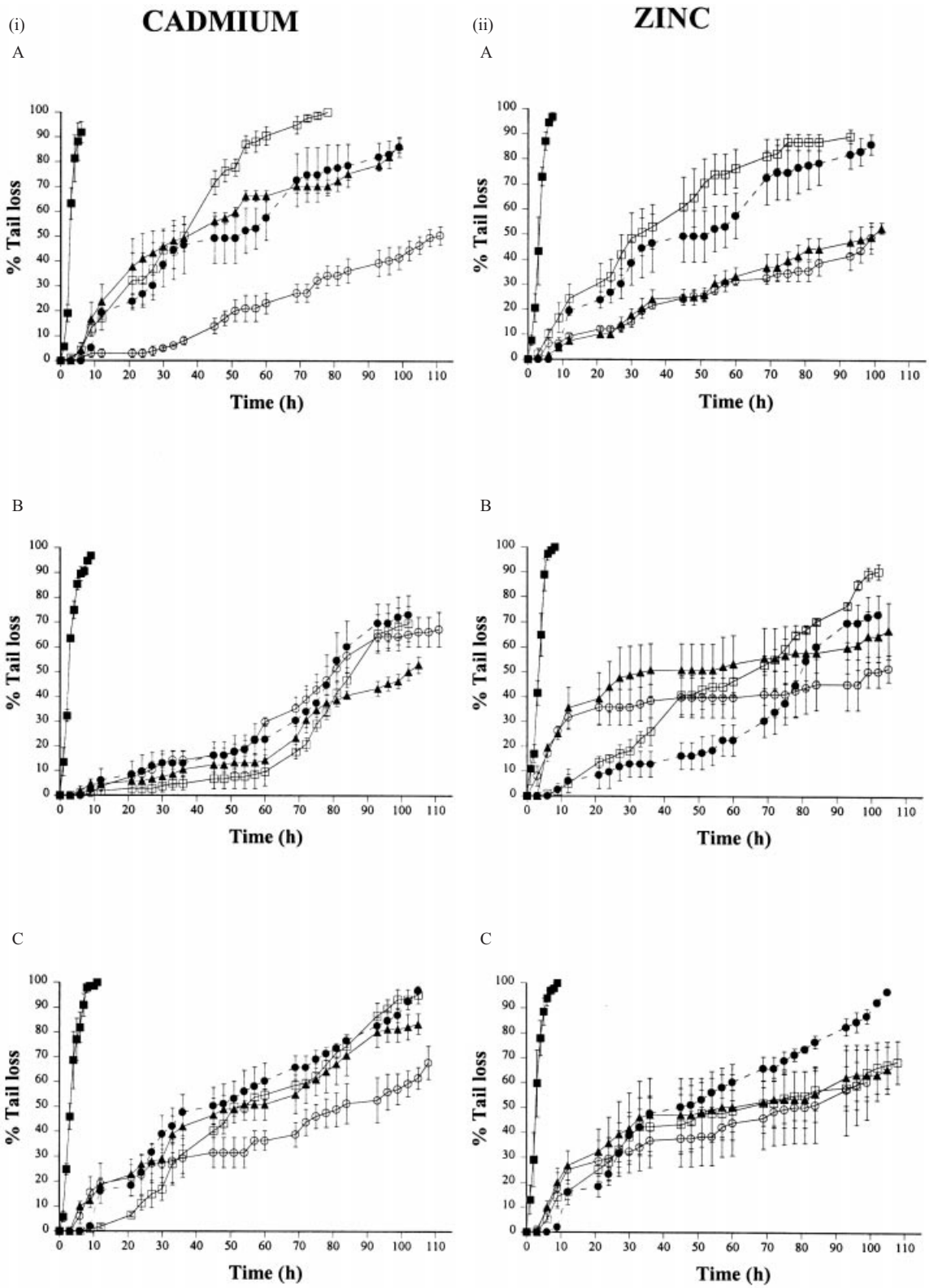

Fig. 3. Tail loss of cercariae of *Diplostomum spathaceum* at 12 °C exposed to (i) cadmium, (ii) zinc, in (A) distilled water, (B) soft water and (C) hard water. Error bars are standard errors. (●) Control, (▲) 0·1 μg/l, (○) 10 μg/l, (□) 100 μg/l, (■) 10000 μg/l.

Examination of the rate of tail loss over time under the different experimental parameters (Figs 1–3) generally demonstrated that increasing metal concentration was associated with more rapid tail loss (Lee-Desu, $P \leqslant 0{\cdot}0324$). However, unexpected tail loss responses occurred. A significantly slower rate of tail loss compared with controls occurred at 20 °C for 10 μg/l and 0·1 μg/l Cd in soft water (Lee-Desu, $P = 0{\cdot}0324$ and $P = 0{\cdot}0064$ respectively) and for zinc at 0·1 μg/l in both distilled and soft water (Lee-

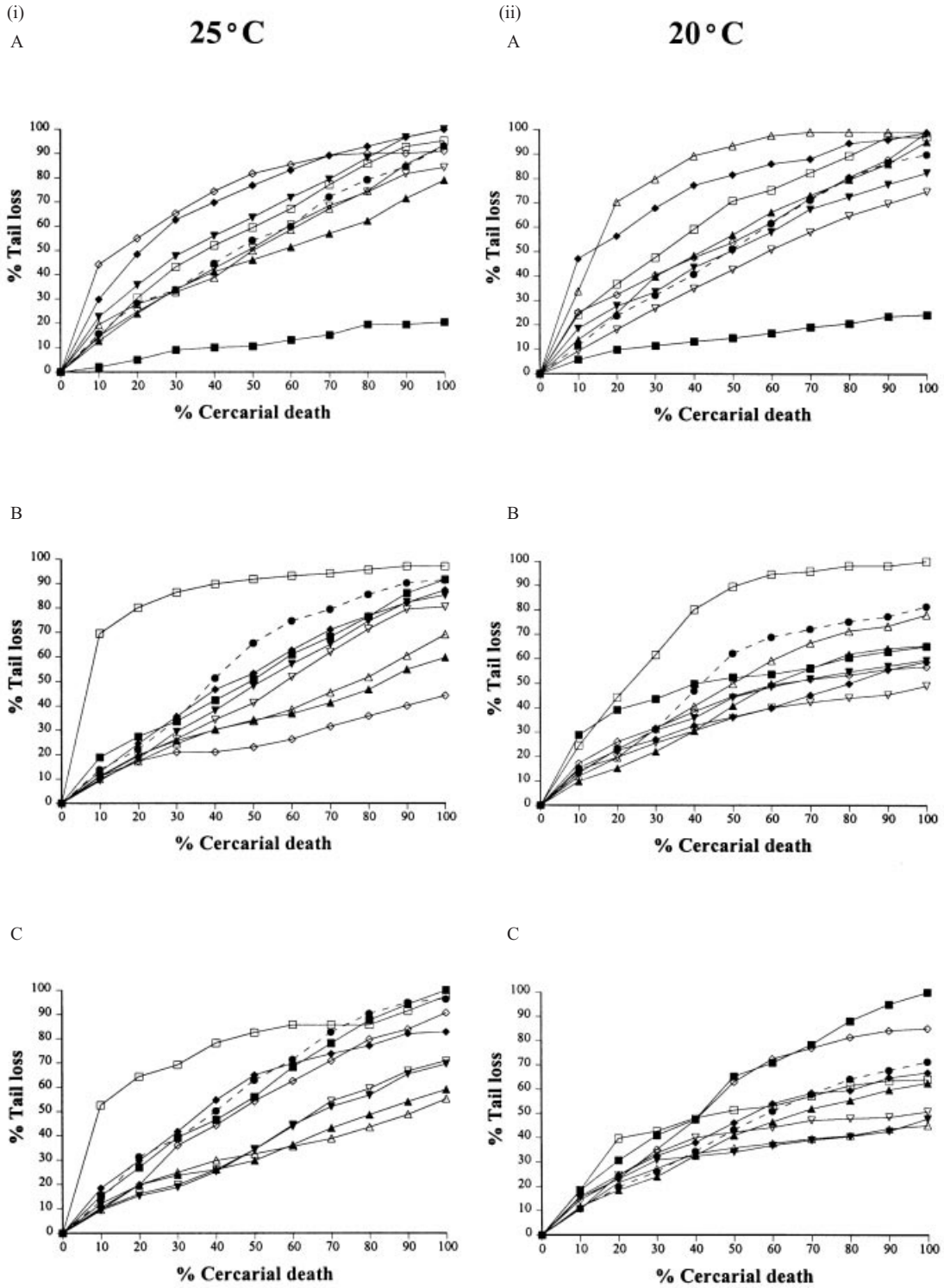

Fig. 4. Comparison of the relationship between cercarial tail loss and death in the experimental *Diplostomum spathaceum* population exposed to cadmium and zinc at (i) 25 °C and (ii) 20 °C, in (A) distilled water, (B) soft water and (C) hard water. (●) Control, (▼) 0·1 μg/l Cd, (▽) 0·1 μg/l Zn, (◆) 10 μg/l Cd, (◇) 10 μg/l Zn, (▲) 100 μg/l Cd, (△) 100 μg/l Zn, (■) 10000 μg/l Cd, (□) 10000 μg/l Zn.

Desu, $P = 0{\cdot}0011$ and $P = 0{\cdot}0119$ respectively). At 12 °C a similar pattern of tail loss occurred for 10 μg/l Cd in both distilled and hard water (Lee-Desu, $P < 0{\cdot}0001$ and $P = 0{\cdot}0017$ respectively) and for 10 μg/l and 0·1 μg/l Zn in distilled water (Lee-Desu, $P < 0{\cdot}0001$ for both metal concentrations). These reduced rates of tail loss corresponded to increased cercarial survival compared with controls for all metal concentrations apart from at 20 °C for 10 μg/l Cd and 0·1 μg/l Zn in soft water for tail loss and at 12 °C for 100 μg/l Cd in hard water for cercarial survival. At these particular concentrations there was a distinct change in the relationship between decreasing survival and increasing tail loss

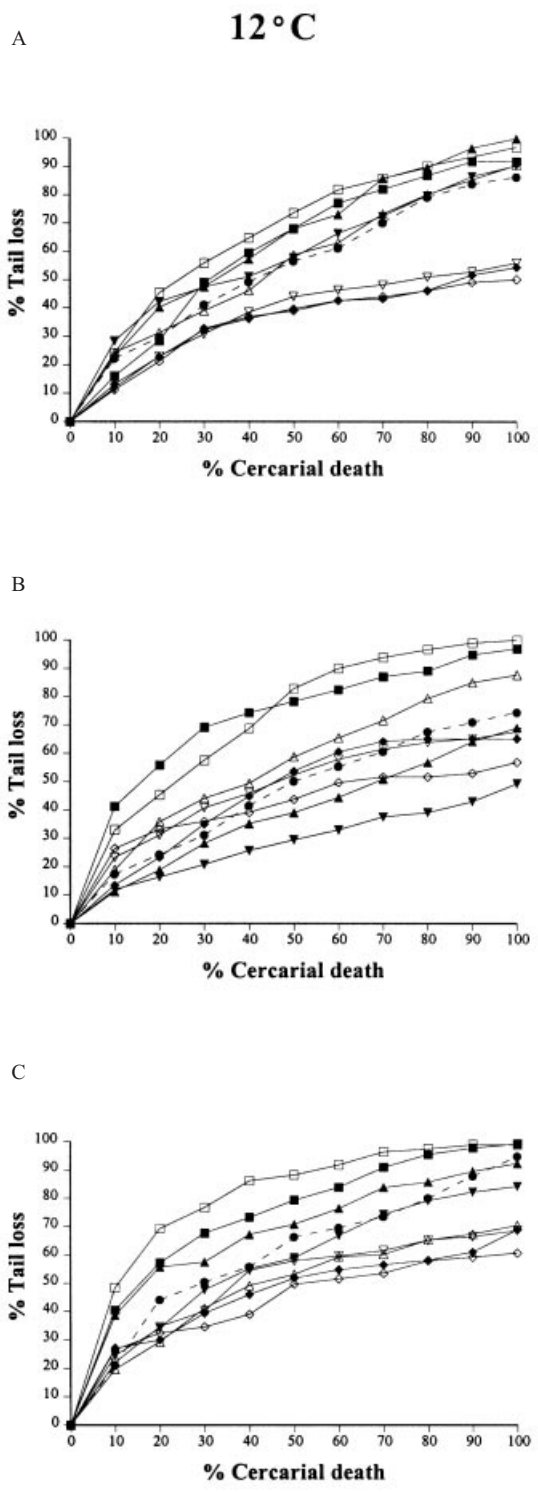

Fig. 5. Comparison of the relationship between cercarial tail loss and death in the experimental *Diplostomum spathaceum* population exposed to cadmium and zinc at 12 °C, in (A) distilled water, (B) soft water and (C) hard water. (●) Control, (▼) 0·1 μg/l Cd, (▽) 0·1 μg/l Zn, (◆) 10 μg/l Cd, (◇) 10 μg/l Zn, (▲) 100 μg/l Cd, (△) 100 μg/l Zn, (■) 10000 μg/l Cd, (□) 10000 μg/l Zn.

(Figs 4 and 5) (20 °C 10 μg/l Cd, Lee-Desu, $P = 0{\cdot}0149$; 0·1 μg/l Zn, Lee-Desu, $P = 0{\cdot}0033$; 12 °C 100 μg/l Cd, Lee-Desu, $P < 0{\cdot}0001$). Indeed, when increasing tail loss and decreasing survival within the cercarial population are compared directly against each (Figs 4 and 5) it is apparent that the parallel relationship can be significantly altered by abiotic changes in the water medium.

Under most control conditions changes in water temperature and hardness did not affect the tail loss/survival relationship. However, in hard water at 25 °C and 12 °C extended survival of the decaudized cercarial bodies induces a significant difference between control tail loss and eventual death (Lee-Desu, $P = 0{\cdot}0088$ and $P = 0{\cdot}0015$ respectively). Cadmium and zinc both caused changes in the tail loss/survival relationship across the range of water temperatures and hardnesses, inducing either stimulation or inhibition of tail loss compared to cercarial death with some trends apparent. Significant differences were recorded at all temperatures for 10000 μg/l Cd and 10 μg/l Cd in distilled water (Lee-Desu, $P \leqslant 0{\cdot}0332$), 100 μg/l Cd in hard water (Lee-Desu, $P \leqslant 0{\cdot}0238$) and 10000 μg/l Zn in soft water (Lee-Desu, $P < 0{\cdot}0001$); and for all water hardnesses at 12 °C for 10000 μg/l Cd and Zn (Lee-Desu, $P \leqslant 0{\cdot}0015$). In addition, significant differences occurred in hard water at 12 °C and 20 °C for 0·1 μg/l Cd and 20 °C and 25 °C for 100 μg/l Zn (Lee-Desu, $P \leqslant 0{\cdot}0372$). Increasing metal concentrations also induced significant changes at 12 °C for 10–10000 μg/l Cd in distilled water and 100–10000 μg/l Cd in hard water (Lee-Desu, $P \leqslant 0{\cdot}0332$) and for zinc at 25 °C in soft water (10–10000 μg/l Zn) and hard water (100–10000 μg/l Zn) (Lee-Desu, $P \leqslant 0{\cdot}0326$); at 20 °C in distilled water (100–10000 μg/l Zn, Lee-Desu, $P \leqslant 0{\cdot}0011$); and at 12 °C in soft water (100–10000 μg/l Zn, Lee-Desu, $P \leqslant 0{\cdot}0384$). In addition, significant changes in cercarial tail loss compared with death also occurred for individual exposures at 25 °C for 10000 μg/l Zn in distilled water (Lee-Desu, $P < 0{\cdot}0001$), 100 μg/l Cd in soft water (Lee-Desu, $P = 0{\cdot}0429$); at 20 °C for 0·1 μg/l Zn in hard water (Lee-Desu, $P = 0{\cdot}0450$); and at 12 °C for 10 μg/l Zn in distilled water (Lee-Desu, $P = 0{\cdot}0255$) and 0·1 μg/l Cd in soft water (Lee-Desu, $P = 0{\cdot}0031$).

Comparisons of the relative toxicity of cadmium and zinc to cercarial tail loss over time demonstrated that certain environmental conditions can induce significant differences between the two metals. At 25 °C in all water hardnesses cadmium and zinc induced variable but significantly different effects at all concentrations apart from 100 μg/l (Lee-Desu, $P \leqslant 0{\cdot}0386$). However, with decreasing temperature cadmium and zinc induced similar effects at many concentrations and water hardnesses.

The effect of the two metals on tail loss in 5-h-old cercariae was generally low and did not differ significantly across the range of environmental parameters (Tukey's test $P > 0{\cdot}05$). Generally only at the 10000 μg/l metal concentrations was there significant effect (Tukey's test $P < 0{\cdot}001$). How-

ever, significant effects at lower concentrations were in distilled water at 25 °C with concentrations of 10 μg/l or higher for both cadmium and zinc (Tukey's test $P \leqslant 0{\cdot}049$), at 20 °C with zinc concentrations of 10 μg/l or higher (Tukey's test $P \leqslant 0{\cdot}001$) and 12 °C with zinc concentrations of 100 μg/l or higher (Tukey's test $P \leqslant 0{\cdot}027$). Nevertheless, when the effects of these metals after 5 h are compared between tail loss and survival in cercariae, it was found that an increased significant effect occurred with tail loss in distilled water at 25 °C for 10 μg/l Cd, 20 °C for 10 μg/l Zn, and 12 °C for 100 μg/l Zn compared to that induced in cercarial survival.

DISCUSSION

This study has demonstrated that cercarial tail loss can be affected by both environmental conditions and heavy metal exposure. It is apparent that increasing tail loss over time is related to decreasing survival, and only under extreme conditions will tail loss deviate from this relationship. However, the pattern of tail loss in *D. spathaceum* cercariae may only be applicable to furcocercariae, which use their tails as an integral part of their transmission strategy. Similar patterns may not be found in cercarial types which demonstrate more passive transmission.

Both water temperature and hardness influenced tail loss through changes that these factors caused to cercarial survival and they do not appear, at least under most control conditions, to alter the relationship between cercarial tail loss and survival. Changes which do occur in this relationship may be a result of changes in the life-span of decaudized cercarial bodies. The modifying effects of tail loss caused by temperature and hardness in this study are similar to the findings of Rea & Irwin (1992) with the marine cercariae *C. lingua*, where temperature was highly influential to tail loss, a higher rate being recorded at higher temperatures. Rea & Irwin (1992) considered that this was related to increased cercarial activity at higher temperatures which could place an increased stress on the body–tail linkage, causing it to break. Such fatigue at the body–tail junction may explain why cercariae shed their tails as they age. Interestingly, Morley (unpublished observations) found that significantly reduced activity of *D. spathaceum* cercariae occurred over a range of cadmium and zinc concentrations at 20 °C, which may explain some of the altered tail loss patterns found in the present study. The influence of water temperature and hardness on metal toxicity and their influence on cercarial survival have previously been discussed by Morley *et al*. (2001), who found that increasing water hardness and decreasing temperature induce a reduced metal toxicity to cercarial survival, in a similar manner to heavy metal effects on other aquatic invertebrates.

Inhibition of tail loss, which was not reported by Rea & Irwin (1992) in *C. lingua*, was found in the present study in both control and metal-exposed cercariae of *D. spathaceum*. As inhibition only occurs in the latter period of the cercarial life-span it may be associated, in control conditions at least, with decreased activity of older cercariae (Anderson & Whitfield, 1975) which are unable to shake off the tail. Mohandas (1974) considered that tail loss close to death was a provision for reducing energy utilization. Therefore control cercariae that fail to shed their tails may do so only because they lack the energy reserves to initiate the action, whereas metal-exposed cercariae may retain their tails due to metal induced inhibition of their activity.

An important finding in the present study is the reduced rate of tail loss in some metal-exposed cercariae compared with controls. This is probably related to the increased survival of metal-exposed cercariae under the same conditions reported by Morley *et al*. (2001), which has been suggested may be related to both the inhibition of enzymes involved in glycogen utilization and a reduction in cercarial activity. Under normal conditions, glycogen in cercariae is used first of all from the tail reserves, prior to tail shedding (Ginetsinskaya, 1960). As tail loss patterns retain their relationship with survival under most conditions of reduced tail loss/increased survival, this possibly suggests that any inhibition of glycogen that may be induced by metal exposure does not appear to affect the overall pattern of cercariae glycogen utilization i.e. inducing glycogen uptake from the body reserves in preference to the tail reserves.

Hara *et al*. (1993) reported that 30 μM silver nitrate stimulated *S. mansoni* cercarial tail loss. Studies by Asch & Dresden (1977) found that *S. mansoni* cercariae exposed to zinc concentrations as low as 0·05 mM for 1·5 h also stimulated tail loss, although extensive alterations to cercarial morphology recorded by these authors were not found in the present investigation, probably due to the lower concentration range of metal used. Hara *et al*. (1993) considered that tail loss may be related to the selective binding of silver nitrate to cercariae sensory papillae. Cadmium and zinc can also selectively bind to areas associated with sensory receptors of *D. spathaceum* cercariae (Morley, unpublished observations) although there is little evidence to suggest that this may influence tail loss under the present experimental conditions. Nevertheless it may prove to be an important factor for cercariae in the presence of their target host. Silver nitrate is known to inhibit the penetration behaviour of *S. mansoni* cercariae (King & Higashi, 1992) and the present results have shown that stimulation or inhibition of tail loss can occur under a number of combinations of metal concentrations and environmental parameters, which in turn may influence successful transmission.

After 5 h, which is the limit of maximum cercarial infectivity (Whyte *et al*. 1991), cadmium and zinc demonstrated only limited effects on cercarial tail loss. However, these effects were greater than those reported on cercarial survival (Morley *et al*. 2001) suggesting that tail loss may be an important measurable parameter for future ecotoxicological studies on cercariae.

REFERENCES

ABD ALLAH, A. T., WANAS, M. Q. & THOMPSON, S. N. (1996). The effects of lead, cadmium, and mercury on the mortality and infectivity of *Schistosoma mansoni* cercariae. *Journal of Parasitology* **82**, 1024–1026.

ANDERSON, R. M. & WHITFIELD, P. J. (1975). Survival characteristics of the free-living cercarial population of the ectoparasitic digenean *Transversotrema patialensis*. *Parasitology* **70**, 295–310.

ASCH, H. L. & DRESDEN, M. H. (1977). *Schistosoma mansoni*: Effect of zinc on cercarial and schistosomule viability. *Journal of Parasitology* **63**, 80–86.

CHUBB, J. C. (1979). Seasonal occurrence of helminths in freshwater fish. II. Trematodes. *Advances in Parasitology* **17**, 141–313.

COLES, G. C. (1979). The effect of praziquantel on *Schistosoma mansoni*. *Journal of Helminthology* **53**, 31–33.

GINETSINSKAYA, T. A. (1960). The relationship between the distribution of glycogen in the bodies of different cercariae and their biological peculiarities. *Doklady Biological Sciences* **135**, 949–951.

HAAS, W. (1994). Physiological analysis of host-finding behaviour in trematode cercariae: adaptations for transmission success. *Parasitology* **109** (Suppl.) S15–S29.

HARA, I., HARA, S., SALAFSKY, B. & SHIBUYA, T. (1993). *Schistosoma mansoni*: fatty acid-induced cercarial tail loss does not involve eicosanoid production and chemoreceptors. *Experimental Parasitology* **77**, 484–488.

HELLAWELL, J. M. (1986). *Biological Indicators of Freshwater Pollution and Environmental Management*. Elsevier Applied Science Publishers, London.

H.M.S.O. (1969). *Fish Toxicity Tests*. Her Majesty's Stationary Office Leaflet, No. Dd. 139779 K36 12/69.

HOLLIMAN, R. B. & ESHAM, L. P. (1977). Toxicity of cadmium to *Schistosoma mansoni* cercariae: effects on vitality and developmental ability in white mice. *Hydrobiologia* **56**, 81–88.

HOWELLS, R. E., GERKEN, S. E., RAMALHO-PINTO, F. J., KAWAZOE, U., GAZZINELLI, G. & PELLEGRINO, J. (1975). *Schistosoma mansoni*: tail loss in relation to permeability changes during cercaria-schistosomulum transformation. *Parasitology* **71**, 9–18.

KING, C. L. & HIGASHI, G. I. (1992). *Schistosoma mansoni*: silver ion (Ag+) stimulates and reversibly inhibits lipid-induced cercarial penetration. *Experimental Parasitology* **75**, 31–39.

LYHOLT, H. C. K. & BUCHMANN, K. (1996). *Diplostomum spathaceum*: effects of temperature and light on cercarial shedding and infection of rainbow trout. *Diseases of Aquatic Organisms* **25**, 169–173.

MOHANDAS, A. (1974). Studies on the freshwater cercariae of Kerala. Biology II. Emergence, behaviour and viability of cercariae. *Proceedings of the National Academy of Science, India* **44**, 139–144.

MORLEY, N. J., CRANE, M. & LEWIS, J. W. (2001). Toxicity of cadmium and zinc to *Diplostomum spathaceum* (Trematoda: Diplostomidae) cercarial survival. *International Journal for Parasitology* **31**, 1211–1217.

NIEWIADOMSKA, K. (1986). Verification of the life-cycles of *Diplostomum spathaceum* (Rudolphi, 1819) and *D. pseudospathaceum* Niewiadomska, 1984 (*Trematoda, Diplostomidae*). *Systematic Parasitology* **7**, 23–31.

OSHIMA, T., KITAGUCHI, T., SAITO, K. & KANAYAMA, A. (1992). [Studies on the epidemiology of avian schistosome dermatitis caused by the cercariae of *Gigantobilharzia sturniae*.] *Japanese Journal of Parasitology* **41**, 194–201. (In Japanese.)

PIETROCK, M., MEINELT, T., MARCOGLIESE, D. J. & STEINBERG, C. E. W. (2001). Influence of aqueous sediment extracts from the Oder River (Germany/Poland) on survival of *Diplostomum* sp. (Trematoda: Diplostomidae) cercariae. *Archives of Environmental Contamination and Toxicology* **40**, 327–332.

REA, J. G. & IRWIN, S. W. B. (1992). The effect of age, temperature, light quantity and wavelength on the swimming behaviour of the cercariae of *Cryptocotyle lingua* (Digenea: Heterophyidae). *Parasitology* **105**, 131–137.

SOUS, S. M. (1992). Influence of abiotic factors on emission and survival of cercariae of *Diplostomum chromatophorum* (Brown, 1931) (Trematoda, Diplostomidae). *Ecological Parasitology* **1**, 154–159.

STABLES, J. N. & CHAPPELL, L. H. (1986). *Diplostomum spathaceum* (Rud. 1819): effects of physical factors on the infection of rainbow trout, *Salmo gairdneri*, to *Diplostomum spathaceum* infections. *Parasitology* **93**, 71–79.

VIVIAN, C. M. G. & MASSIE, K. S. (1977). Trace metals in waters and sediments of the River Tawe, South Wales, in relation to local sources. *Environmental Pollution* **14**, 47–61.

WHYTE, S. K., SECOMBES, C. J. & CHAPPELL, L. H. (1991). Studies on the infectivity of *Diplostomum spathaceum* in rainbow trout (*Oncorhynchus mykiss*). *Journal of Helminthology* **65**, 169–178.

WOOTTEN, R. (1974). Observations on strigeid metacercariae in the eyes of fish from Hanningfield Reservoir, Essex, England. *Journal of Helminthology* **48**, 73–83.