

Survival and infectivity of *Hypoderaeum conoideum* and *Euparyphium albuferensis* cercariae under laboratory conditions

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Abstract

The survival characteristics of the cercariae of *Hypoderaeum conoideum* and *Euparyphium albuferensis* (Trematoda: Echinostomatidae) at 20°C and 30°C are described, and the age dependency of their infectivity at 20°C is studied to determine their respective transmission efficiencies. Cercarial survival was found to be age-dependent and was higher at 20°C. For both cercariae, the maximum life-span was 26 h at 20°C and 16 h at 30°C, and their respective times to 50% mortality were similar at each temperature. Both cercariae seem to be well adapted to transmission in their natural habitat, though cercarial infectivity of *H. conoideum* was higher than that of *E. albuferensis*, this being correlated with their prevalences in nature. The age-dependency of cercarial survival may be related to steadily diminishing endogenous energy levels, though the delay in attaining maximum infectivity suggests that other factors not related to energy considerations are involved in the delimitation of the cercarial infective period. However, this latter observation may constitute an adaptative mechanism allowing cercarial dissemination.

Introduction

During investigations on the helminth fauna of the Albufera Natural Park (Valencia, Spain), several members of the family Echinostomatidae were detected (Toledo *et al.*, 1996; Esteban *et al.*, 1997).

Hypoderaeum conoideum (Bloch, 1782), a widespread trematode parasite of fowl and mammals, utilizes *Lymnaea peregra* (Müller, 1774) (Gastropoda: Lymnaeidae) as first intermediate host in the Albufera Natural Park (Toledo *et al.*, 1998a), and this and other snail species as second intermediate hosts (Toledo *et al.*, 1998a,b). The life cycle has been described by Mathias (1925), though information on the cercarial biology has only been published by Haas *et al.* (1995). The taxonomy and life cycle of *Euparyphium albuferensis* Esteban *et al.*, 1997 have recently been described and discussed (Esteban *et al.*, 1997; Toledo *et al.*, 1998c). *Gyraulus chinensis* (Dunker,

1848) (Gastropoda: Planorbidae) acts as the first intermediate host in the Albufera Natural Park. This and several other freshwater snail species serve as second intermediate hosts, and rodents as definitive hosts but little data are available on its biology (Esteban *et al.*, 1997).

The transmission success of the cercariae is recognized as one of the most important steps in the life cycle of trematodes. The transmission of many digeneans depends on the ability of the cercariae to actively invade a spectrum of specific hosts (Haas, 1994). The biological characteristics of this larval stage, together with the environmental conditions, play a major role in their dissemination. Cercarial survival and infectivity are of extreme importance in the rate of trematode transmission. With few exceptions, cercariae have a short active life during which they do not feed but survive on accumulated reserves. In general, the cercarial life-span is temperature-dependent and species specific. However, the period during which cercariae are infective is often shorter than the maximum cercarial life-span, and several authors have also related the length of the infective period

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Table 1. Survival parameters of *Hypoderaeum conoideum* and *Euparyphium albuferensis* cercariae at 20°C and 30°C.

Cercarial species	Temperature (°C)	a	b	r ²	Mean instantaneous death rate	Time to 50% mortality	Maximum life-span
					$\bar{\mu}$ (Cercariae/h)	(h)	(h)
<i>H. conoideum</i>	20	0.0062	0.1365	0.99	0.23	20.35	26
	30	0.0092	0.2624	0.98	0.40	11.54	16
<i>E. albuferensis</i>	20	0.0048	0.1312	0.95	0.37	18.59	26
	30	0.0083	0.2350	0.92	0.87	12.88	16

a, b = constants; r² = adjusted correlation coefficient.

to the limitations imposed by finite energy stores. Nevertheless, some studies have shown that the cercarial infective period could also be delimited by other factors not related to the magnitude of the cercarial energy reserves (Lowenberger & Rau, 1994).

In this paper, we report on experimental studies on the dynamics of the cercarial survival of *H. conoideum* and *E. albuferensis* at 20°C and 30°C. In addition, the study of cercarial infectivity at 20°C allows us to establish the transmission efficiency of both cercariae at this temperature. The experimental findings are correlated with field data in order to assess trematode transmission in the natural habitat.

Materials and methods

Snail hosts and parasites

Cercariae of *H. conoideum* were obtained from a pool of naturally infected specimens of *L. peregra*, and cercariae of *E. albuferensis* were obtained from naturally infected specimens of *G. chinensis*, in the Albufera Natural Park of Valencia (Spain). Laboratory reared specimens of *Physa acuta* (Draparnaud, 1805) were used as experimental second intermediate hosts in the study on the infectivity of *E. albuferensis* cercariae, and *G. chinensis* for *H. conoideum*. These snail species were chosen as previous studies have shown them to be the most suitable second intermediate hosts for each trematode species (Esteban *et al.*, 1997; Toledo *et al.*, 1998b). All snails used were in the 0.3–0.5 cm size range.

Determination of cercarial survival

Survival of *H. conoideum* and *E. albuferensis* cercariae was investigated at 20°C and 30°C. Cercarial survival was monitored by introducing groups of 25 freshly emerged cercariae (maximum age 15 min) into glass dishes containing 10 ml of spring water and observing the proportion alive at intervals of 2 h. Cercariae were considered dead when they failed to respond to mechanical stimulation. Four hundred cercariae of *H. conoideum* and 300 of *E. albuferensis* were studied at each temperature.

Determination of age dependency of cercarial infectivity

The age dependency of cercarial infectivity for *H. conoideum* and *E. albuferensis* was investigated as follows.

Cercariae were collected within 15 min of their emergence and aged at a temperature of 20 ± 1°C. At 30-min intervals from 0 to 4 h after emergence and at 2 h intervals from 4 to 20 h, batches of 10 living cercariae were added to glass dishes containing 5 ml of spring water and an individual specimen of *G. chinensis* for *H. conoideum* cercariae, and *P. acuta* for *E. albuferensis* cercariae. The snails were exposed to infection for 15 min, and 24–48 h after exposure they were crushed and examined for metacercarial cysts. Twenty replicate exposures were conducted for each cercarial age.

Results

Cercarial survival

The survival characteristics of *H. conoideum* and *E. albuferensis* cercariae at 20°C and 30°C are illustrated in figs 1 and 2, respectively. At 20°C, most of the cercariae were active for the first 12–14 h post-emergence in both species. After this time, the number of living cercariae decreased over time, and at 26 h all were dead. At 30°C, the survival dynamics were similar, though cercarial mortality began at 6–8 h after emergence, and at 16 h no live cercariae were detected (figs 1A, 2A).

The instantaneous death rates (μ) over time were calculated and found to be age-dependent at any given temperature. The relationship is well described by the empirical model:

$$\mu(t) = a \exp(bt)$$

The value of the constants a, b and the adjusted correlation coefficient (r²) for each species and temperature are given in table 1.

At each temperature, the observed proportion of cercariae at successive points in time are in good agreement with those predicted by an age-dependent model (Anderson & Whitfield, 1975; Evans & Gordon, 1983), due to the fact that the instantaneous death rate of both cercariae increased exponentially with age (figs 1B, 2B).

The predicted proportion [P(t)] of cercariae surviving to age t with such an age dependency is given by Anderson & Whitfield (1975) as:

$$P(t) = \exp a/b [1 - \exp(bt)]$$

The predictions of the above model agree with the observed data for cercarial survival (figs 1A, 2A). The

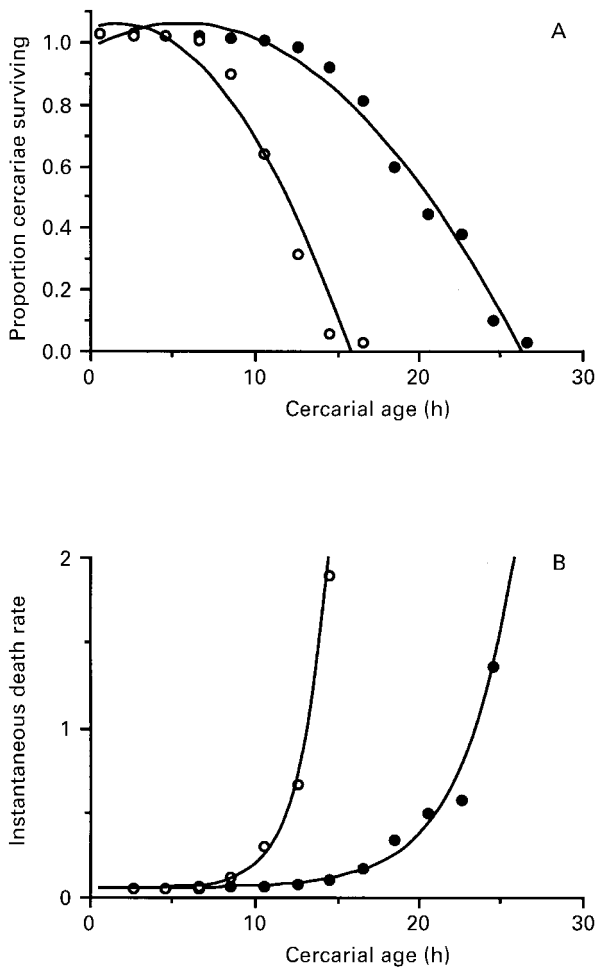


Fig. 1. Survival of *Hypoderaeum conoideum* cercariae: A, observed proportion of cercariae surviving at 20°C (●), at 30°C (○), and the expected proportion (-) according to an age-dependent survival model (Anderson & Whitfield, 1975); B, the observed instantaneous death rates at 20°C (●), at 30°C (○), and the expected rates (-) according to the empirical model.

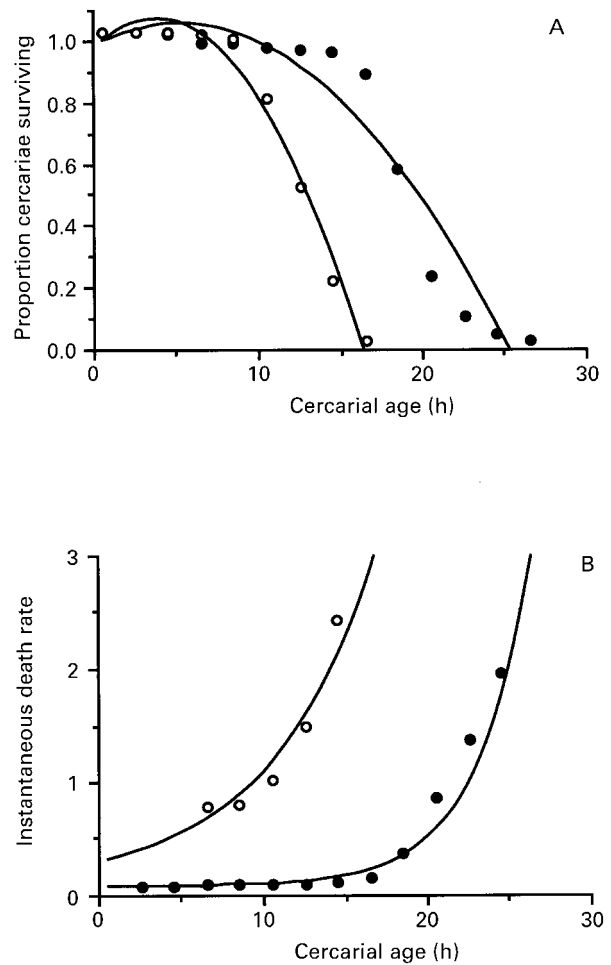


Fig. 2. The survival of *Euparyphium albuferensis* cercariae: A, observed proportions of cercariae surviving at 20°C (●), at 30°C (○), and the expected proportions (-) according to an age-dependent survival model (Anderson & Whitfield, 1975); B, the observed instantaneous death rates at 20°C (●), at 30°C (○), and the expected rates (-) according to the empirical model.

cercarial maximum life-span, average instantaneous death rate ($\bar{\mu}$) and the time to 50% mortality at each temperature are also reflected in table 1.

Age dependency of cercarial infectivity

The recovery of metacercariae from snails exposed to cercariae of different ages is illustrated in fig. 3A. Cercarial infectivity reached a peak at 3 h post-emergence from the first intermediate host for *H. conoideum*, and at 2.5 h for *E. albuferensis*. After this time, cercarial infectivity decreased steadily with increasing parasite age. Cercariae of both trematode species which had been aged 12 h were no longer infective. From the cercarial recovery data, the instantaneous rate of infection (α) according to Anderson (1978) can be calculated as:

$$\alpha = -\ln(1 - M/C_0)/t$$

where M is the number of metacercariae recovered/snail; C_0 is the number of cercariae present at $t=0$, and t is the exposure time. The instantaneous rates of infection for both cercariae are represented in fig. 3B, where α is the number of metacercariae formed per cercaria per snail per 5 ml water per hour.

The mean instantaneous rate of infection at 20°C ($\bar{\alpha}$) under the experimental conditions employed is 1.45 for *H. conoideum* and 0.22 for *E. albuferensis* cercariae.

Discussion

Our results support the idea that cercarial life-span is limited by rates of energy expenditure, since temperature affected the length of larval life. However, the duration of the infective period of the cercariae is also determined by other factors not directly related to energy considerations.

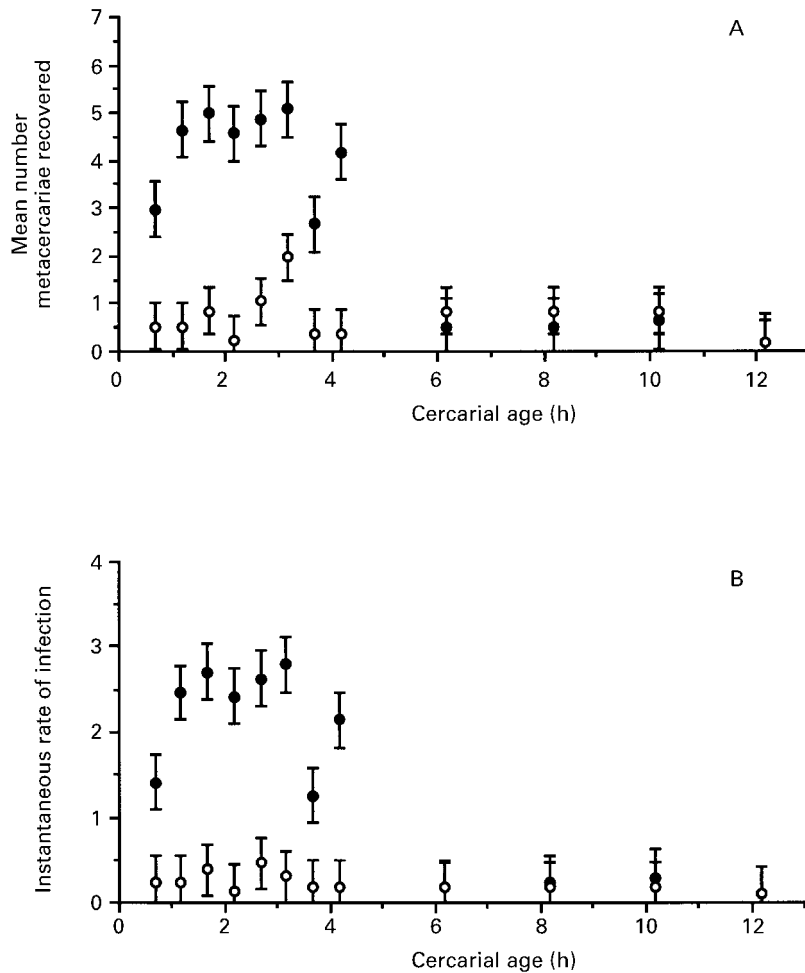


Fig. 3. Infectivity parameters of *Hypoderaeum conoideum* (●) and *Euparyphium albuferensis* (○) cercariae at 20°C: A, metacercarial cyst recovery from snails exposed to cercariae; B, values of the instantaneous infection rate at any cercarial age. Vertical bars represent 95% confidence limits of the mean.

The cercarial survival of *H. conoideum* and *E. albuferensis* is markedly age- and temperature-dependent. Their respective instantaneous death rates increase exponentially with cercarial age at any given temperature and it is likely to be generated by the progressive depletion of their limited energy reserves. For both cercariae, temperature has a remarkable effect on their survival. An increase in temperature results in a shortened larval life-span. The ratios for time to 50% mortality at 20°C and 30°C were 1:1.8 for *H. conoideum* and 1:1.5 for *E. albuferensis*. These findings agree with earlier studies on free-living larval stages of other echinostomatids (Evans & Gordon, 1983; Evans 1985; Meyrowitsch *et al.*, 1991; Pechenik & Fried, 1995; Idris & Fried, 1996), and seem to be due to the elevated cercarial activity observed as temperature (and hence also energy utilization rate) increases.

The cercarial life-spans of *H. conoideum* and *E. albuferensis* at 20°C were considerably shorter than those

reported for other echinostome cercariae at similar temperatures. The time to 50% mortality at 18–19°C observed for *Echinoparyphium recurvatum* (Evans & Gordon, 1983), *Echinostoma caproni* (Evans, 1985), and *E. trivolvis* (Pechenik & Fried, 1995) varied from 29 to 31 h, whereas in the present study we respectively recorded 20.4 h and 18.6 h for *H. conoideum* and *E. albuferensis* at 20°C. Despite this fact, the times to 50% mortality obtained in the present study at 30°C (11.5 h for *H. conoideum* and 12.9 h for *E. albuferensis*) were comparable with those reported for *E. caproni* (10.8 h at 30°C) and *E. trivolvis* (13 h at 32°C). Survival of *E. recurvatum* at 30°C was not studied by Evans & Gordon (1983). These observations suggest that although the life-spans of *H. conoideum* and *E. albuferensis* are shorter than other echinostome cercariae, they are less affected by temperature changes. This could constitute an ecological mechanism to compensate for the shorter life-span observed by these cercarial species at 20°C. Thus, they seem to be well

adapted to habitats involving exposure to a wide temperature range, as in the case of the temperate freshwater system in the Albufera Natural Park (Toledo *et al.*, 1998a).

It is widely recognized that survival time may overestimate the length of time for which cercariae are infective. This fact has often been related to the rates of energy expenditure (Anderson & Whitfield, 1975; Lawson & Wilson, 1980; Evans & Gordon, 1983; Evans, 1985; Meyrowitsch *et al.*, 1991; Lowenberger & Rau, 1994; Pechenik & Fried, 1995; Wetzel & Esch, 1995; Lo & Lee, 1996). However, our results show that the cercarial infectivity of *H. conoideum* and *E. albuferensis* gradually increases during the first few hours of life, reaching a peak following a prior ageing period. A delay in reaching maximum infectivity may represent a dispersal phase that aids cercariae dissemination, thus reducing superinfection and subsequent parasite-associated mortality of the first intermediate host. A similar situation has been previously reported for other cercarial species (Lo & Cross, 1975; Evans & Gordon, 1983; Lowenberger & Rau, 1994) and supports the idea that other factors, not related to energy considerations, are involved in the length of the cercarial infective period, especially as it is reasonable to assume that the larval energy contents are maximal during the first hours of life. Pechenik & Fried (1995) suggested that maximum cercarial life-span is limited by energy considerations, while the duration of the infective period is limited by shifts in gene expression such as the maintenance of surface chemoreceptors. Possibly, the rates of cercarial differentiation determine their infective period. Haas *et al.* (1995) found that *H. conoideum* cercariae at 18°C only show chemo-orientation in the life period between 1 and 5.5 h after shedding. Interestingly, this period approximately corresponds to the maximum cercarial infectivity observed in our study. This could indicate that cercariae continue to develop physiologically after being released and are only capable of locating and invading the snail hosts after a period of ageing, with the loss of their chemo-orientation capacity beyond a certain age. Unfortunately, the extent to which differentiation occurs during the cercarial stage has not been assessed for any trematode species.

With respect to other echinostome cercariae, the data published to date are confusing. Lo & Cross (1975) showed that cercariae of *Echinostoma revolutum* aged 4 h were more infective than when newly emerged, while Evans & Gordon (1983) found cercarial infectivity of *E. recurvatum* to reach a maximum after 2–3 h. Despite these observations, Pechenik & Fried (1995) detected no increased infectivity in the first hours of life of *E. trivolvis* cercariae. However, the cercariae used by the latter authors were at least 45 min old when first exposed to potential hosts, and cercarial infectivity may have increased before this time.

It is not yet clear why cercariae lose their ability to infect hosts, though the phenomenon has been thoroughly recorded. The situation could reflect an energy deficiency to locate, attach and/or invade the snail host, or even the existence of some post-penetration event (Pechenik & Fried, 1995). However, it should be considered that cercariae of *H. conoideum* lose their chemo-orientation capacity some time after being shed,

which could prevent the cercariae from locating the snails (Haas *et al.*, 1995).

From the experimental data obtained in the present study, a useful measure of cercarial transmission efficiency is provided either by the ratio $\bar{\mu}/\bar{\alpha}$ (H_0 , according to May & Anderson, 1978) which varies inversely with transmission efficiency, or by the ratio $\bar{\alpha}/\bar{\mu}$ ($= 1/H_0$, according to Evans, 1985). From our laboratory results, the cercarial transmission efficiency ($= 1/H_0$) of *H. conoideum* is 10.7-fold greater than for *E. albuferensis* (6.30 vs. 0.59) and this is probably due to the low infectivity shown by the latter cercarial species. A comparison of the transmission efficiency of *H. conoideum* and *E. albuferensis* with other echinostome cercariae is difficult, since the experimental conditions differ in each study, particularly with reference to the volume used in the infection experiences. However, our results show that *H. conoideum* and *E. albuferensis* cercariae display a lower experimental transmission efficiency than either *E. recurvatum* (9.69; with 20 ml of water at 18°C; Evans & Gordon, 1983) or *E. caproni* (16.80; with 3 ml of water at 19°C; Evans, 1985).

Although in nature the transmission efficiency obtained from laboratory data may be influenced in a number of ways, there could be a significant effect on the actual transmission efficiency of the cercariae. Thus, cercariae of *H. conoideum* seem to be better adapted to transmission than those of *E. albuferensis*. The high transmission efficiency displayed by *H. conoideum* cercariae may be decisive in establishing this cercarial species as the most frequently occurring in freshwater snails from the Albufera Natural Park (Toledo *et al.*, 1998a).

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