

Photoperiodic cercarial emergence patterns of the digeneans *Echinoparyphium recurvatum* and *Plagiorchis* sp. from a mixed infection in *Lymnaea peregra*

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Abstract

Photoperiodic emergence patterns of the cercariae of two digeneans, *Echinoparyphium recurvatum* (Echinostomatidae) and *Plagiorchis* sp. (Plagiorchiidae), were studied experimentally using a photoperiodic regime of 12 h light : 12 h dark at a constant 18°C. In single species infections of the first intermediate host snail *Lymnaea peregra*, the cercariae of *E. recurvatum* emerged exclusively during the light phase and those of *Plagiorchis* sp. emerged exclusively during the dark phase. In double infections, each species retained its own discrete photoperiodic emergence pattern. This result demonstrates the absence of interference between the mechanisms responsible for the photoperiodic emergence pattern of each species. A degree of interference between the two parasites in double infections of *L. peregra* was evident. The presence of *E. recurvatum* significantly reduced the number of *Plagiorchis* sp. cercariae emerging in a 24 h period and also delayed the mean peak emergence time of *Plagiorchis* sp. cercariae by 2 h. This result is consistent with the well documented antagonistic effect that the redial stages of echinostomes are known to have against the sporocysts of other digeneans within the same host mollusc.

Introduction

In the first study of its kind, Théron & Moné (1986) examined the photoperiodic cercarial emergence patterns of cercariae of *Schistosoma mansoni* and *Ribeiroia marini* in both single and mixed infections from the first intermediate host snail *Biomphalaria glabrata*. No previous study had dealt with the influence of double heterologous infections on the chronobiology of cercarial emergence. In single species infections, both parasites showed a marked photoperiodicity of cercarial emergence; *S. mansoni* peaking during light and *R. marini* peaking during dark. In mixed infections of the same snail, it was found that the

cercariae of both species retained their marked, discrete, photoperiodic emergence patterns with no overlap in emergence occurring.

The fact that the photoperiodic emergence patterns remained discrete in mixed infections demonstrated the absence of interference at the level of mechanisms directly responsible for the emergence pattern of each species. However, *S. mansoni* and *R. marini* occupy different microhabitats in *B. glabrata*; the digestive gland in the case of the daughter sporocysts of *S. mansoni*, and the genital gland in the case of the daughter rediae of *R. marini*. Théron & Moné (1986) suggested that it would be interesting to study a host–parasite system involving digeneans with distinct diurnal and nocturnal emergence patterns, but with more similar microhabitat niches within a single first intermediate host snail. It would be

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interesting to see if the photoperiodic emergence patterns remained discrete or whether a significant degree of overlap would occur, and also whether there would be evidence of any form of interference between the two species.

A host–parasite system which provides the required combination exists in the case of *Lymnaea peregra* snails carrying mixed, patent (cercariae-producing) infections of the echinostome *Echinoparyphium recurvatum* and the plagiorchiid *Plagiorchis* sp. The latter is a species of *Plagiorchis* which has been known to utilize *L. peregra* as first intermediate at Harting Pond, West Sussex, England, for several years (Evans & Higgs, 1982). The xiphidio-cercariae of *Plagiorchis* sp. possess a characteristically shaped stylet, are morphologically similar to those of the '*Plagiorchis elegans* group', (see Bock, 1984), and are known to utilize the aquatic larvae of the megalopteran insect *Sialis lutaria* as a second intermediate host. Pilot studies had previously indicated that the cercariae of *Plagiorchis* sp. exhibited a markedly nocturnal pattern of emergence from *L. peregra*.

The cercariae-producing stages of *E. recurvatum* and *Plagiorchis* sp., daughter rediae in the case of *E. recurvatum* and daughter sporocysts in *Plagiorchis* sp., utilize the same microhabitat niche (the digestive gland) in *L. peregra*. Both species show a marked photoperiodic pattern of cercarial emergence, with *E. recurvatum* emerging almost exclusively during light and *Plagiorchis* sp. during the dark. In addition, the cercariae of the two species are morphologically distinct and this would enable them to be easily distinguished in the event of an overlap in emergence occurring.

The discovery of specimens of *L. peregra* in early October 1987 at Harting Pond, West Sussex, carrying patent (cercaria-producing) double infections of both *E. recurvatum* and *Plagiorchis* sp. allowed an examination of the photoperiodic cercarial emergence patterns of the two species within single host individuals. It also allowed their comparison with the emergence patterns observed in single species infections of these digeneans.

Materials and methods

Naturally infected specimens of *L. peregra* carrying single and mixed patent infections of *E. recurvatum* and *Plagiorchis* sp. were obtained from Harting Pond, West Sussex, England in October 1987 by net sampling of the benthic mud. Three snails carrying double infections were obtained, these specimens measuring in shell length range 14–15.5 mm. Six specimens of *L. peregra*, within the same size range (14–15.5 mm), were obtained from the same net samples, three carrying single species infections of *E. recurvatum* and three carrying single species infections of *Plagiorchis* sp.. This enabled the analysis of cercarial emergence for three host–parasite combinations: (i) *E. recurvatum*/*L. peregra*; (ii) *Plagiorchis* sp./*L. peregra*; (iii) *E. recurvatum* + *Plagiorchis* sp./*L. peregra*.

Infected snails were isolated in clear polystyrene containers each containing 15 ml of synthetic hard water medium (HMSO, 1969), and were then transferred to a light-proof incubator in which both temperature and light-cycling were under automatic control. Cercarial emergence from each snail was monitored every hour,

over a period of 24 h of 12 h light : 12 h dark. Experimental light (intensity = 1600 Lux) began at 08.00 h and dark at 20.00 h. The ambient temperature was maintained at a constant 18°C.

The snails were acclimatized to the experimental conditions of light/dark and temperature for a period of three days prior to the commencement of the 24 h monitoring period. All snails were provided with an excess of clean boiled lettuce as food throughout the acclimatization and monitoring periods.

Results

The emergence of *E. recurvatum* cercariae from *L. peregra* carrying single infections of this digenean is shown in fig. 1A. The emergence pattern was of a circadian type, having a single peak in 24 h. Cercarial emergence began at the onset of experimental light at 08.00 h. Mean peak cercarial emergence occurred in the second hour after the onset of light in the period 09.00–10.00 h. The mean number of cercariae emerging from the three snails examined over the experimental period was 742 (\pm 84.5 S.E. (standard error)) cercariae per snail.

The emergence of *Plagiorchis* sp. cercariae from *L. peregra* carrying single infections of this digenean is shown in fig. 1B. The emergence pattern was also found to be of a circadian type with a single clearly defined emergence peak in the 24 h monitoring period. Cercarial emergence began with the onset of experimental dark at 20.00 h. Mean peak cercarial emergence occurred in the second hour after the onset of dark in the period 21.00–22.00 h. The mean number of cercariae emerging from the three snails examined over the experimental period was 1081.7 (\pm 74.7 S.E.) cercariae per snail.

In double infections, both *E. recurvatum* and *Plagiorchis* sp. cercariae were found to retain their markedly discrete respective light and dark patterns of emergence (see fig. 1C). *Echinoparyphium recurvatum* cercariae emerged exclusively during light and *Plagiorchis* sp. cercariae emerged exclusively during dark, with no overlap in emergence occurring.

Emergence of *E. recurvatum* cercariae began at the onset of light, but mean peak cercarial emergence did not occur until the third hour after the onset of light in the period 10.00–11.00 h. The mean number of *E. recurvatum* cercariae emitted per snail over the 24 h period was 575.3 (\pm 43.4 S.E.). Emergence of *Plagiorchis* sp. cercariae began at the onset of light, but mean peak cercarial emergence did not occur until the fourth hour after the onset of dark in the period 23.00–24.00 h. The mean number of *Plagiorchis* sp. cercariae emitted per snail over the 24 h period was 381.6 (\pm 60.5 S.E.).

Discussion

In single species infections of *L. peregra*, *E. recurvatum* exhibited an exclusively diurnal emergence pattern and the cercariae of *Plagiorchis* sp. exhibited an exclusively nocturnal pattern of emergence. For *E. recurvatum*, this finding is the same as that made by McCarthy (1989). The markedly nocturnal pattern of cercarial emergence exhibited by cercariae of *Plagiorchis* sp. is similar to that observed for the cercariae of a variety of species of the

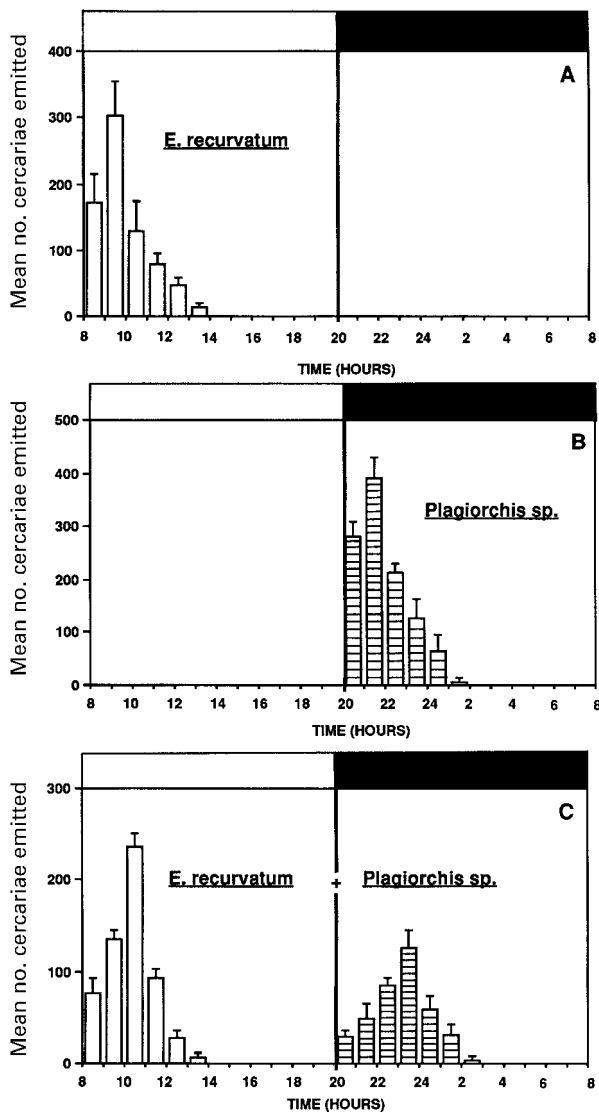


Fig. 1. The mean number of cercariae emitted per host snail each hour over the 24 h period of 12 h light : 12 h dark from each group of three *Lymnaea peregra* infected with: (A) *Echinoparyphium recurvatum* single species infection; (B) *Plagiorchis* sp. single species infection; (C) *E. recurvatum* + *Plagiorchis* sp. mixed infection. (Vertical error bars indicate standard errors of the means.)

genus *Plagiorchis* by several authors, e.g. *Plagiorchis vespertilionis parorchis* by Macy (1960), *Plagiorchis micranthos* by Wagenbach & Alldredge (1974), *Plagiorchis neomidis* by Théron 1976, and *Plagiorchis noblei* by Blankespoor (1977) and Webber *et al.* (1986).

The findings of the present study clearly show that in mixed infections of the first intermediate host *L. peregra* the cercariae of *E. recurvatum* and *Plagiorchis* sp. retain the markedly distinct photoperiodic patterns of emergence that they exhibit in single species infections. In mixed infections, *E. recurvatum* cercariae began to emerge at the onset of light and emerged exclusively in light. *Plagiorchis*

sp. cercariae began to emerge at the onset of dark and emerged exclusively in the dark. No overlap in cercarial emergence of the two species occurred. This finding is similar to that made by Théron & Moné (1986) who discovered that in mixed infections of the snail host *B. glabrata*, cercariae of *S. mansoni* and *R. marini* retained distinctly their marked respective light and dark emergence patterns without any observed overlap. Similar findings have also been made in experimental studies by Mouahid *et al.* (1991) on the emergence of cercariae of the congeneric species *Schistosoma bovis* and *S. haematobium* from mixed infections in *Bulinus truncatus*, and also by Théron *et al.* (1997) in the case of the emergence of cercariae of different chronobiological races of *Schistosoma mansoni* from mixed infections in *Biomphalaria glabrata*.

The fact that in the present study markedly separate diurnal and nocturnal cercarial emergence patterns were retained by the species involved in mixed infections, without overlap occurring, demonstrates the absence of interference at the level of the mechanisms directly responsible for stimulating cercarial emergence in each case. This is an important conclusion, and one also made by Théron & Moné (1986), as it provides a valuable insight to understanding what stimulates cercarial emergence from intermediate host molluscs. Anderson *et al.* (1976) suggested that it was possibly the locomotory behaviour of the snail host that was primarily responsible for producing cercarial emergence of *Trichobilharzia ocellata* from the snail host *Lymnaea stagnalis*; the cercariae being pushed to suitable exit points by muscular squeezing of cercariae along haemolymph channels. It would be difficult to explain the observed cercarial emergence patterns of *E. recurvatum* and *Plagiorchis* sp. in mixed infections as being simply a result of a diel activity pattern of *L. peregra*. If this were the case the locomotion of the snail could be expected to stimulate an emergence of cercariae of both species simultaneously. It would therefore be expected that at least some overlap in the emergence of cercariae of the different species would occur. The results of the present study show that this does not happen. At no time throughout the experiment did a snail carrying a mixed infection of *E. recurvatum* and *Plagiorchis* sp. emit cercariae of both species simultaneously. It therefore seems probable, as Théron & Moné (1986) implied for *R. marini* and *S. mansoni*, that *E. recurvatum* and *Plagiorchis* sp. cercariae are independently responding to photo-related emergence stimuli.

Although there was no overlap in the emergence patterns of *E. recurvatum* and *Plagiorchis* sp. in the present study, there was, nevertheless, some evidence that suggested a degree of 'interference' between the two in mixed infections. In mixed infections, mean peak cercarial emergence time occurred 1 h later in the case of *E. recurvatum* and 2 h later in the case of *Plagiorchis* sp., compared to the situation in single species infections. One possible explanation for the observed time shift in peak cercarial emergence may be that an accumulation of *E. recurvatum* cercariae occurring in the digestive gland of the host could delay the emergence of *Plagiorchis* sp. and vice versa. Some evidence of antagonism was also noted with respect to the numbers of *Plagiorchis* sp. cercariae emitted in mixed infections. In the case of *E. recurvatum*, a t-test on cercarial counts (transformed log (x+1)) revealed

no significant difference in the mean number of cercariae emitted per snail, whether the infection was *E. recurvatum* alone or mixed *E. recurvatum* + *Plagiorchis* sp., $P > 0.05$. However, in the case of *Plagiorchis* sp., the mean number of cercariae emitted from snails with mixed infections was significantly less than that from snails infected with *Plagiorchis* sp. alone, $P < 0.05$. This suggests that in a situation where it has to compete interspecifically with the echinostome *E. recurvatum*, *Plagiorchis* sp. produces many fewer cercariae per day than when it is the only parasite in the host mollusc. A similar type of interspecific competition effect was also noted by Théron & Moné (1986) who observed that in mixed infections with *R. marini*, *S. mansoni* produced significantly fewer cercariae than in single infections.

Conclusions from the above observation should be tempered inasmuch as it should be remembered that the present study deals with natural infections of *E. recurvatum* and *Plagiorchis* sp. of unknown age, and cercarial production may be influenced by the age of infection. However, the observation is interestingly consistent with the well documented strongly antagonistic influence that the rediae of echinostomes (and those of the cathaemasid *R. marini*) have on other digeneans within the same host mollusc; particularly those having only sporocyst generations. The markedly antagonistic effects of rediae of echinostomes such as *Paryphostomum radiatum* and *Echinostoma liei* on larval schistosomes within the same host mollusc have been noted in detail by Heyneman *et al.* (1972), Lie (1967) and Combes (1982). Køie (1987) has published excellent scanning electronmicrographs of a cannibalistic redia of the echinostome *Mesorchis denticulatus*. On the basis of their strongly antagonistic behaviour toward larval schistosomes, certain echinostomes have been seriously considered as potential biological control agents of the intramolluscan stages of human schistosomes. The fact that the rediae of *E. recurvatum* are predatory on cercariae of *Plagiorchis* sp. was affirmed in the current study by the discovery of stylets from *Plagiorchis* sp. cercariae in the saccate guts of some of the *E. recurvatum* daughter rediae recovered from the digestive glands of mixed infection snails when they were dissected. The probability that the rediae of *E. recurvatum* are antagonistic to the sporocysts and cercariae of *Plagiorchis* sp. in mixed infections provides a feasible explanation for the reduced cercarial output of *Plagiorchis* sp. in mixed infections with this echinostome.

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