


No time to relax: Age-dependent infectivity of cercariae in marine coastal ecosystems

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Short Communication

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Abstract

Age dynamics of the ability of cercariae of two digenean species, *Himasthla elongata* (Himasthliidae) and *Renicola parvicaudatus* (Renicolidae), to infect the second intermediate host (SIH), mussels (*Mytilus edulis*), was investigated experimentally. This is the first study of this kind made on cercariae transmitted in the intertidal of the northern seas. The larvae of all tested ages (from 0.5 to 6 hr) were equally successful in infecting mussels. This finding disagrees with the literature data on cercariae of several freshwater digeneans, which are practically incapable of infecting the SIH during the first 1–3 hr of life. The presence of a time delay before the attainment of the maximum infectivity (TDMI) may be associated with the need for physiological maturation of cercariae in the very beginning of their life in the environment, the need for their broad dispersion, and the prevention of superinfection of the downstream host. The absence of TDMI in the cercariae examined in our study could be associated with the instability of environmental factors in the marine intertidal (wave impact, tidal currents). These factors promote a broad dispersion of cercariae in the intertidal biotope and prevent superinfection of potential SIHs. Biological and behavioural features may also play a role. We hypothesize that the presence or absence of TDMI does not depend on the taxonomic affiliation of the cercariae but is determined by the transmission conditions.

Introduction

In the complex life cycle of trematodes, an important stage of transmission is the endotrophic cercaria larva, which infects the second intermediate host (SIH) or the definitive host (DH). Cercariae have a complex set of behavioural reactions promoting dispersal and infection of a wide range of animal hosts, both invertebrate and vertebrate (reviewed in: Haas 2003). Many factors, exogenous as well as endogenous, influence the transmission of cercariae (reviewed in: Combes *et al.* 1994; Marcogliese 2001; Galaktionov and Dobrovolskij 2003; Pietrock and Marcogliese 2003; Lafferty and Kuris 2005). Their lifespan is limited, which was attributed to the depletion of glycogen reserves accumulated during the development in the molluscan first intermediate host (FIH) (reviewed in: Ginetsinskaya 1968; Morley 2011). The time during which cercariae remain infective is even more limited, ranging in the larvae of different species from a few hours (e.g. Miller and McCoy 1930; Anderson and Whitfield 1975; Lowenberger and Rau 1994; McCarthy 1999; Karvonen *et al.* 2003; de Montaudouin *et al.* 2016; Born-Torrijos *et al.* 2022) to several days (Pekkarinen 1987) or even weeks (Wetzel and Esch 1995). Age-related dynamics of infectivity differ significantly cercariae of all species studied in this respect.

Cercariae of some species reach maximal infectivity only after ca. 1–3 hr after leaving the molluscan host. An analysis of the literature on the age dynamics of cercarial infectivity (Table 1) showed that the methodological aspect, that is, the minimum age of cercariae used in the experiment, was often the key to the discovery of the time delay before the attainment of the maximum infectivity (TDMI). In most cases, the larvae were tested starting from the age of 1 hr after leaving the FIH (Table 1), which means that the period of their decreased infectivity might have already ended. At the same time, the minimum and the most frequently observed TDMI made up about 1 hr (McCarthy 1999; Toledo *et al.* 1999; Munoz-Antoli *et al.* 2002; Whitfield *et al.* 2003; Paller *et al.* 2007), and so could not be revealed in experiments with older cercariae (Stirewalt and Fregeau 1968; Malek 1977; Karvonen *et al.* 2003) (Table 1). Experiments with cercariae of *Schistosoma mansoni* are a case in point. Stirewalt and Fregeau (1968) tested them starting from the age of 1 hr and did not find any TDMI. However, as shown later by Whitfield *et al.* (2003), cercariae of *S. mansoni* do exhibit a decreased infectivity at an age of less than 1 hr, while at an age of 1 hr, the infectivity reaches the maximum (Table 1). Therefore, it cannot be ruled out that cercariae of other trematode species also have a decreased infectivity in the first minutes (before the age of 1 hr) of their life in the environment.

Table 1. Conditions and results of experiments on detection of time delay before the attainment of the maximum infectivity (TDMI) by cercariae of trematodes based on literature data and the data from this study

Trematode species (family)	FIH can be used as SIH	Downstream host	Time section	Age of attainment of maximal infectivity, hr	Presence of TDMI	References
<i>Schistosoma mansoni</i> (Schistosomatidae)	No	Mammalia (DH), Agile	1 hr–24 hr	1	–	Stirewalt and Fregeau 1968
— // —	No	Mammalia (DH), Agile	37 min–16 hr	1	+	Whitfield <i>et al.</i> 2003
<i>Schistosomatium douthitti</i> (Schistosomatidae)	No	Mammalia (DH), Agile	1 hr–36 hr	1	–	Malek 1977
<i>Transversotrema patialense</i> (Transversotrematidae)	No	Fish (DH), Agile	15 min–24 hr	0.25	–	Anderson and Whitfield 1975; Anderson <i>et al.</i> 1977
<i>Centrocestus armatus</i> (Heterophyidae)	No	Fish (SIH), Agile	14 min–32 hr	1	+	Paller <i>et al.</i> 2007
<i>Diplostomum spathaceum</i> (Diplostomidae)	No	Fish (SIH), Agile	3 hr–27 hr	3	–	Karvonen <i>et al.</i> 2003
<i>Echinoparyphium recurvatum</i> (Echinostomatidae)	Yes	Molluscs (SIH), slow-moving	10 min–25 hr	2	+	Evans and Gordon, 1983; McCarthy 1999
<i>Hypoderaeum conoideum</i> (Echinostomatidae)	Yes	Molluscs (SIH), slow-moving	0.5 hr–20 hr	3	+	Toledo <i>et al.</i> 1999
<i>Euparyphium albuferensis</i> (Echinostomatidae)	Yes	Molluscs (SIH), slow-moving	0.5 hr–20 hr	1	+	Toledo <i>et al.</i> 1999
<i>Echinostoma trivolvis</i> (Echinostomatidae)	Yes	Molluscs (SIH), slow-moving	0.75 hr–28 hr	0.75	–	Pechenik and Fried 1995
<i>Echinostoma friedi</i> (Echinostomatidae)	Yes	Molluscs (SIH), slow-moving	15 min–14 hr	1	+	Munoz-Antoli <i>et al.</i> 2002
<i>Plagiorchis elegans</i> (Plagiorchiidae)	No	Mosquito larva (SIH), intermittent swimming	0.5 hr–40 hr	4	+	Lowenberger and Rau 1994
<i>Himasthla elongata</i> (Himasthliidae)	Yes	Molluscs (SIH), slow-moving	0.5 hr–12 hr	0.5	–	This study
<i>Renicola parvicaudatus</i> (Renicolidae)	Yes	Molluscs (SIH), slow-moving	0.5 hr–12 hr	0.5	–	This study

TDMI may be due to the need for some morpho-physiological changes during the transition of the cercariae from the existence in the molluscan host to the life in the external environment (Whitfield *et al.* 2003). The adaptive significance of the delay is usually attributed mainly to the necessity of spatial dispersion of the larvae and the avoidance of superinfection of the SIH in the vicinity of the cercariae-shedding molluscan FIH (Evans and Gordon 1983; McCarthy 1999; Toledo *et al.* 1999; Lowenberger and Rau 1994; Whitfield *et al.* 2003). This is particularly important under conditions of low hydrodynamics (e.g. in lentic or slow-flowing freshwater bodies), if the SIH is sedentary and/or if the role of the SIH can be also played by the FIH. All these factors are characteristics of transmission of echinostomatid cercariae, which were found to exhibit TDMI of 1–3 hr (Lo and Cross 1975; Evans and Gordon 1983; McCarthy 1999; Toledo *et al.* 1999) (Table 1). To note, echinostomatid larvae are highly pathogenic for their molluscan SIH when the infection intensity is high (Kuris and Warren 1980; Fried *et al.* 1995; Ataev 2010). Cercariae of *Echinostoma trivolvis* may also have TDMI, but only at the first stages of their life in the

environment, which is why it was not recorded in the experiments of Pechenik and Fried (1995), who tested cercariae starting from the age of 45 min (Table 1).

Superinfection of an agile downstream host by cercariae shed by the mollusc is unlikely, which means that TDMI found in larvae of *S. mansoni* (Whitfield *et al.* 2003) and *Centrocestus armatus* (Paller *et al.* 2007) (Table 1) must be due to some other factors. The primary factor in this case is probably the need for a period of physiological maturation of the cercariae in the environment. To note, TDMI was not found in cercariae of *Transversotrema patialense* infecting agile fish hosts (Anderson and Whitfield 1975; Anderson *et al.* 1977), though these larvae were involved in the experiment as early as 15 min after emergence from the molluscan host. Information about the absence of TDMI in cercariae of *Diplostomum spathaceum* infecting fish requires verification since the minimum age of cercariae in the experiment was 3 hr (Karvonen *et al.* 2003).

The need for a period of maturation in the external environment after emergence from the molluscan host cannot be ruled out for

cercariae of other trematodes. All available data were obtained on freshwater species whose cercariae are usually transmitted under conditions of low hydrodynamics. There is no information on the age dynamics of infectivity of cercariae infecting SIHs in marine biotopes with an increased hydrodynamics such as the intertidal.

This lack of this information prompted our study of cercariae of two trematode species transmitted in the coastal ecosystems of the White Sea, *Himasthla elongata* (Mehlis, 1831) (Himasthliidae) and *Renicola parvicaudatus* (Stunkard & Shaw, 1931) (Renicolidae), which use mussels (*Mytilus edulis*) as the SIH. The FIH of these trematode species are snails *Littorina littorea*, while DH are sea-birds (Stunkard and Shaw 1931; Werding 1969; Galaktionov *et al.* 2021, 2023).

It should be noted that the family Himasthliidae and the family Echinostomatidae are very closely related. Previously, the himasthliids were even considered as a subfamily of the Echinostomatidae (Kostadinova 2005; Tkach *et al.* 2016). This means that our results obtained on *H. elongata* can be compared with those on freshwater echinostomatids.

Materials and methods

Sample collection

Periwinkles *Littorina littorea* (Linnaeus, 1758) were collected in the intertidal zone of the Chupa Inlet of the Kandalaksha Bay (the White Sea) in July 2023. Individuals of *L. littorea* shedding cercariae of *Himasthla elongata* (or *Renicola parvicaudatus*) were considered as infected and used as a source of cercariae. Mussels (*Mytilus edulis*) were taken from artificial substrates of the sea farm. The sea farms in the White Sea are situated at a distance of at least 50 m from the nearest shore, and mussels cultivated there are uninfected with renicolid and himasthliid larvae (Kulachkova 1985; Levakin *et al.* 2013).

Experiment

Experiments on the infection of mussels with cercariae of *H. elongata* and *R. parvicaudatus* were carried out in August 2023 at the White Sea Biological Station of the Zoological Institute of the Russian Academy of Science (Cape Kartesh, Kandalaksha Bay of the White Sea). In each experiment, a pool of cercariae was taken from 15 individuals of *L. littorea* infected with *H. elongata* and the same number of periwinkles was infected with *R. parvicaudatus*. In this way, possible interclonal differences in the infectivity of cercariae were levelled (Levakin *et al.* 2013). After that, the molluscs were exposed to diffused sunlight in 1 l jars with seawater. Cercariae were sampled for the experiment after 10 min of exposure of the snails to light (ca. 500 Lx). Groups of cercariae for the infection of one mussel (20 cercariae of *H. elongata* or 50 cercariae of *R. parvicaudatus*) were placed into separate Petri dishes with a volume of 5 ml filled with fresh seawater with a temperature of 20°C and a salinity of 24 ppt. The cercariae were kept in these Petri dishes at 20°C for 0.5; 1; 1.5; 2; 2.5; 3; 4; 5; and 6 hr and then used for mussel infection.

Uninfected mussels with a shell length of 18–20 mm were acclimated to 20°C for 2 days in aerated tanks with seawater. After that, they were placed in separate (100 ml) jars with fresh seawater with a salinity of 24 ppt. Then, either 20 cercariae of *H. elongata* or 50 cercariae of *R. parvicaudatus* aged 0.5; 1; 1.5; 2; 2.5; 3; 4; 5; and 6 hr were added to each jar with mussels. Taking into account the dependence of the pumping activity of the White Sea mussels

(E , l/hr) on the mean geometric length (l), height (h), and width (w) of their shell [$L = (l^3 h^2 w)^{1/3}$, mm], $E = 0.04L^{1.6}$ (Lezin *et al.* 2006), the volume of water pumped by a mussel of the size used in our experiments ($L = 9.2 \pm 0.2$ mm) during the time of incubation with the cercariae (0.25 hr) makes up 348 ± 12 ml. This volume is more than 3 times greater than that of the experimental jar (100 ml), which guarantees the host-parasite encounter and removes the possible influence of the behavioural differences between the larvae of the two trematode species involved in our experiments (Prokofiev 2002; Nikolaev *et al.* 2017) on the success of mussel infection.

A total of 180 mussels were infected (10 molluscs with each age gradation of cercariae). The molluscs were exposed to cercariae for 15 min at 20°C with regular room light (about 500 Lx), after which they were rinsed with fresh water to remove cercariae that had not penetrated during the exposure time and transferred to jars with fresh seawater (100 ml), where they were kept for 24 hr and then dissected. To detect the resulting infection, the mussels' soft tissues were squeezed and screened under a stereomicroscope, and the metacercariae were counted.

Statistics

Statistical treatment of the data followed standard recommendations (Sokal and Rohlf 1995; Underwood 1997). Infection success was estimated individually for each mussel as a ratio of the number of metacercariae found in a mussel to the number of cercariae used to infect this mussel (20 cercariae of *H. elongata* or 50 cercariae of *R. parvicaudatus*). The mean success of mussel infection with cercariae of the same age was considered as the infectivity of the larvae of this age. Statistical significance of the influence of cercarial age on their infectivity (mean infection success) was assessed with the help of one-way of Analysis of Variance (ANOVA). The data were arcsin-transformed before performing ANOVA. Means and confidence limits of means were back-transformed. Confidence intervals are given for a 95% significance level. The computations were carried out in R (<https://www.r-project.org/>).

Results and Discussion

Changes in the infectivity of cercariae of *H. elongata* and cercariae of *R. parvicaudatus* from 0.5 hr to 6 hr were insignificant (ANOVA; *H. elongata*: $F_{(8,81)} = 1.053$, $P > 0.05$, $\eta^2 = 9.4\%$; *R. parvicaudatus*: $F_{(8,81)} = 2.047$, $P > 0.05$, $\eta^2 = 16.8\%$) (Figure 1). This means that TDMI was absent in cercariae of *H. elongata* and *R. parvicaudatus* aged 0.5 hr and more.

At first sight, this finding seems strange. These larvae, especially those of *H. elongata*, can be highly pathogenic for SIHs, which are represented by sedentary bivalves such as mussels (Lauckner 1983). Besides, both *H. elongata* and *R. parvicaudatus* can use *Littorina* spp. (FIH) as SIHs (Lauckner 1983). Cercariae of echinostomatids, which are closely related to *Himasthla*, have precisely this combination of features and exhibit a pronounced TDMI (see the Introduction) (Table 1). It is not entirely impossible that the cercariae of the two species in our study do have TDMI in the first minutes of their life (up to 0.5 hr) and that they need it to reach full maturity, as, for example, larvae of *S. mansoni* (Whitfield *et al.* 2003). However, considering the biological features of cercariae of *H. elongata* and *R. parvicaudatus* and the fact that they are transmitted in marine intertidal biotopes, the adaptive character of

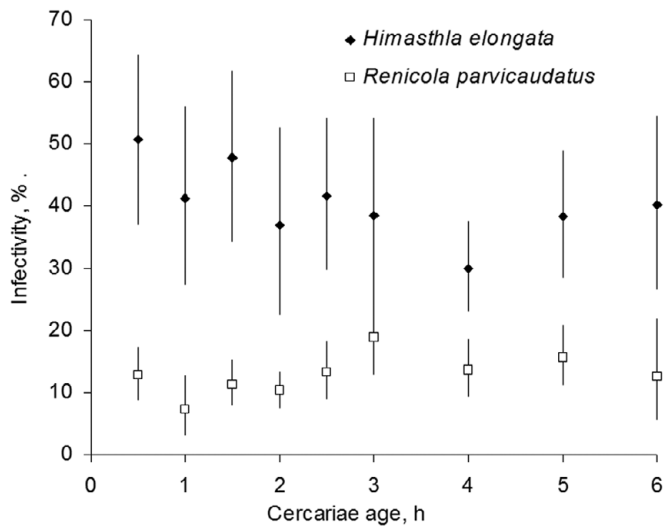


Figure 1. Age-dependent dynamics of infectivity of cercariae of *Himasthla elongata* and *Rencicola parvicaudatus*.

TDMI appears less important for them than for cercariae of freshwater digeneans in lentic and/or slow-flowing water bodies.

Under littoral conditions, passive transport by currents and microcurrents contributes significantly to cercarial dispersion (de Montaudouin *et al.* 1998; Fingerut *et al.* 2003; Zimmer *et al.* 2009). In addition, cercariae can be rapidly transported outside the host contact zone by wave action and tidal currents. These features considerably decrease the probability of superinfection of mussels in the vicinity of the mollusc that has emitted the larvae. The same is true of their FIH, periwinkles, which, not being specific SIH for cercariae of *H. elongata* and *R. parvicaudatus*, are infected with them with a very low efficiency. In the area of material (FIH) collection, where 100% prevalence and a high abundance of metacercariae of *H. elongata* and *R. parvicaudatus* in mussels is observed, periwinkles infected with these larvae are extremely rare (our observations).

Besides environmental factors, superinfection of SIHs may also be prevented by the features of cercarial emergence and biology. Cercariae of *H. elongata* have a relatively small average daily output, 707.9 ± 89.2 (438–1,225) (Prokofiev *et al.* 2016). Therefore, the absence of TDMI can increase the chances of an individual larva finding itself in the vicinity of a potential SIH and infecting it. On the other hand, a high hydrodynamics at the intertidal combined with the mobility of the FIH, *L. littorea*, decrease the risk of superinfection of sedentary SIHs (mussels). Renicolid metacercariae are less pathogenic for SIHs (Thieltges 2006), but the daily output of their cercariae is much higher than in *H. elongata*, $2,276.2 \pm 342.2$ (854–3,728) (Prokofiev *et al.* 2016). Having left the FIH, cercariae of *R. parvicaudatus*, which are already infective for SIHs, rise to the upper water layers, thus leaving the ‘host space’, the habitat of mussels on the sediment. They concentrate near the bottom, thus returning to the ‘host space’, only after 2–4 hr (Prokofiev 2002; Nikolaev *et al.* 2017). This ensures a more or less uniform distribution of the larvae within the littoral site, increasing the probability that they would find mussels in sparse settlements. This behavioural feature of cercariae of *R. parvicaudatus* can also be considered as a mechanism of preventing the superinfection of SIHs in the absence of TDMI.

The average encystment rate of cercariae of *H. elongata* in our experiments was $40.6 \pm 4\%$, while that of cercariae of *R. parvicaudatus*

did not exceed $12.7 \pm 1.7\%$. These differences are probably associated with the fact that the mussel is not the preferred host for renicolid larvae. The results of experimental infections (Thieltges and Rick 2006) confirmed that the preferred host for metacercariae of *R. parvicaudatus* is the mollusc *Cerastoderma edule* (Lauckner 1983). Therefore, an initial retreat of renicolid cercariae from the ‘host space’ is especially relevant in littoral biotopes of temperate seas inhabited by cockles *Cerastoderma edule*.

Conclusion

We would like to note that the presence or absence of TDMI in cercariae does not seem to depend on their taxonomic position. It appears to be determined by the transmission conditions for a particular species. In this regard, it would be interesting to study the age dynamics of the invasive capacity of echinostomatid cercariae that infect highly agile fish hosts and that are transmitted in a lentic freshwater biotope, such as *Isthmiophora melis* (Beaver 1941; Radev *et al.* 2009) and *Drepanocephalus spathans* (Alberson *et al.* 2022).

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Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional institutional guides on the care and use of laboratory animals.

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