

Effect of temperature changes on the cercarial-shedding rate of two trematodes

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ABSTRACT. Since temperature is a key condition in the initiation of cercarial emergence, the objective of the work reported here was to evaluate the effect of that variable on the shedding of two morphologic and taxonomic types of cercariae. Bioassays were accordingly performed with snails naturally infected with either monostome or pleurolophocercous cercariae because those two cercarial types were found to be the most prevalent in the environment under study. The snails were placed in 6-ml circular plastic dishes with soft plastic stoppers containing 3 ml of reconstituted water and then sequentially exposed for 24 h to a 14:10-h light:dark cycle at 20, 25, or 30 °C. The data were analyzed at a significance level of $p < 0.05$ by means of the InfoStat program. The optimum temperature for cercarial emergence was typically in the range of 25 °C and decreased significantly at 30 °C. We observed no significant difference in the number of cercariae emerging between the two morphologic types.

KEYWORDS. Parasites, cercarial emergence, freshwater, temperature, bioassay.

RESUMEN. Efecto de cambios en la temperatura sobre la emergencia de cercarias de dos trematodos. Considerando que la temperatura es un factor clave en la emergencia de cercarias, el objetivo de este trabajo fue evaluar el efecto de ésta variable sobre la emergencia de dos tipos morfológicos de cercarias. Los bioensayos se realizaron con caracoles naturalmente infectados con cercarias de los tipos monostoma o pleurolofocerca, debido a que estos dos tipos fueron los más prevalentes en el ambiente en estudio. Los caracoles fueron ubicados en cápsulas plásticas de 6 ml conteniendo 3 ml de agua reconstituida, y entonces fueron secuencialmente expuestos por 24 h con un ciclo 14:10 h luz:oscuridad a 20, 25 y 30 °C. Los datos fueron analizados a un nivel de significación de $p < 0.05$ con el programa InfoStat. Los resultados mostraron que la temperatura óptima de la emergencia de cercarias estuvo en el rango de 25 °C, y disminuyó significativamente a 30 °C. Se observó también que no existía diferencia significativa en el número de cercarias emergentes entre los dos tipos morfológicos.

PALABRAS-CLAVE. Parásitos, emergencia de cercarias, agua dulce, temperatura, bioensayo.

Parasites are one of the main ecologic groups involved in the organization of faunal communities (THOMAS *et al.*, 2006) and therefore data on the dynamics of the different parasitic developmental stages is fundamental. For example, cercarial emergence from the first intermediate host is a key phase in the reproductive success of trematodes; and this step is directly related to the life-span of the cercariae (at *ca.* 24 h), within which period emergence needs to occur at an optimal time (ESCH *et al.*, KARVONEN *et al.*, 2004). Therefore, a determination of the environmental conditions that influence cercarial emergence becomes imperative for gaining an understanding of trematode transmission since the latter often begins in response to abiotic changes. Temperature, in addition to light and water conditions, is one of the main drivers of cercarial emergence through, either directly or indirectly, an acceleration of both the maturation and the liberation of the cercariae. Cercarial emergence from the snail host can be stimulated by a change in temperature, with the effect of this influence being often trematode-specific (MOURITSEN, 2002; FINGERUT *et al.*, 2003;

AHMED *et al.*, 2006; POULIN, 2006; KOPRIVNIKAR & POULIN, 2009a,b; VIGNOLES *et al.*, 2014). Consequently, in order to initiate ecological studies on a given trematode group, a determination of the optimum temperature must be one of the first steps to be carried out before other parameters can be reasonably investigated.

In Argentina the evaluation of freshwater trematodes has focused on morphological descriptions and on the study of the life cycles of different species, whereas considerations of environmental variables affecting cercarial emergence have been restricted principally to the influence of light – for example, the number of cercariae emerging per h after exposure to the light (MARTORELLI, 1984, 1989, 1990; OSTROWSKI DE NUÑEZ, 1995; OSTROWSKI DE NUÑEZ *et al.*, 2003; DAVIES & OSTROWSKI DE NUÑEZ, 2012; FERNANDEZ *et al.*, 2013a,b). According to several authors, cercarial emergence from the snail is stimulated by light by means of a larval positive phototropism occurring at the time when the snail tissues emerge – a timing that is, in turn, governed by the circadian light-dark cycle along with temperature

differences as Zeitgebers (MORLEY *et al.*, 2010).

Since the environmental characteristics promoting the development of trematode life cycles within this region are still not understood, a determination of the optimum temperature for the progression of these organisms through the different stages of their life cycle becomes crucial for an elucidation of their dynamics. The objective of the experiments reported here was thus to evaluate the effect of temperature on the emergence of two cercarial morphologic types, with an aim at determining both the optimal temperature and whether or not significant differences in the degree of cercarial shedding resulted between the two types.

MATERIALS AND METHODS

We examined the two cercarial morphologic types found to be the most common in prior screenings of infected snails, the monostome and the pleurolophocercous (SCHELL, 1985; MARTORELLI & ETCHEGOIN, 1996; ETCHEGOIN & MARTORELLI, 1998). Monostome cercariae typically have three eyespots, lack a ventral sucker, and contain abundant cystogenous glands along with dorsal adhesive organs. The pleurolophocercous cercariae have a tail with anterior cuticular thickening and lateral and/or dorsoventral fins and/or fin folds. These two cercarial types belong to the Notocotyliidae and Cryptogonimidae families, respectively. The life cycle of these trematode types has not yet been elucidated, but both use the snail *Heleobia parchappei* (Orbigny, 1835) as the first intermediate host.

The bioassays were made with naturally infected *H. parchappei* because the latter are more suitable for the investigation of the levels of cercarial emergence (MORLEY *et al.*, 2010). Approximately 1,300 snail individuals were collected from the Martín Stream (Buenos Aires province, Argentina) during December 2014. The mean annual temperature for this location is 16.2 °C, with January being the hottest month (mean temperature, 22.8 °C) and July the coldest (mean temperature, 9.9 °C; <http://www.estadistica.laplata.gov.ar/paginas/climasueloLP.htm>).

The snails were collected randomly by hand and after transportation to the laboratory were kept in 2-L glass containers filled with reconstituted water (hardness 108 mg Ca/L) at an ambient temperature of 23 ± 2 °C and in the dark and fed with flaked fish food.

To screen for infected snails for use in the bioassays, individuals from six groups of 240 snails each were placed in 6-ml wells of microtest plates containing 3 ml of reconstituted water and exposed to light stimulation before being examined periodically for cercarial emergence. Individuals found to be infected were then transferred to 2-L glass containers under the same conditions as described above. For the bioassays 10 snails each parasitized by either cercarial type were used for the abrupt-exposure test, and were placed in 6-ml wells of microtest plates containing 3 ml of reconstituted water.

To evaluate the effect of three temperatures, 20, 25, and 30 °C on the emergence of cercariae, the experiments were conducted separately for the two cercarial morphologic

types in the form of abrupt bioassays (*cf.* Fig. 1). The experiments were initiated in an incubator set at 20 °C with a 14:10-h light:dark cycle, where all the plates were kept at this temperature for 24 h. A closed thermostatic bath used within the incubator was set at the next temperature (KOPRIVNIKAR & POULIN, 2009a). After 24 h at 20 °C, all the snails were individually transferred into new dishes (prewarmed at 25 °C within the thermostatic bath) for abrupt exposure before being placed in the incubator now reset at 25 °C. The cercariae remaining in the dishes at the lower temperature were then directly counted after transfer of the snails to the new temperature. The thermostatic bath was then set at 30 °C. After 24 h at 25 °C in the incubator, all the snails were then individually transferred into new dishes (prewarmed to 30 °C within the thermostatic bath) for a second abrupt exposure before being placed in the incubator reset at 30 °C. The cercariae remaining in the dishes at the lower temperature (25 °C) were then counted after transfer of the snails to the new temperature. At the end of the assay, the cercariae remaining in the wells of the third set of dishes were counted and the snails measured with calipers before being returned to their original wells. The protocol followed in this experiment was based on the work of KOPRIVNIKAR & POULIN (2009a) and previous bioassays performed by us to maximize the methodology.

Statistical analyses were initially conducted for each separate bioassay in order to determine the differences between the cercarial numbers with respect to temperature. The normality of cercarial-emergence values was analyzed by the Shapiro-Wilk test. When that distribution was nonnormal, the Kruskal-Wallis test was used to establish the optimal temperature.

The second step – expressing the mean values registered for the total numbers of emerged cercariae per morphologic type per day – involved comparisons made between the two cercarial types (*i. e.*, monostome and

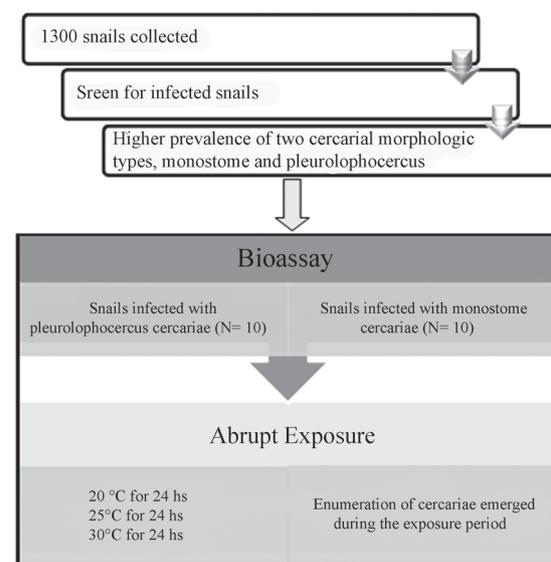


Fig. 1. Flow diagram of experimental procedure for cercariae emergence assays.

pleurolophocercous) for each temperature. When comparisons were made, the data were subjected to a Wilcoxon test because the data distribution was nonnormal.

As snail size could potentially affect the number of cercariae emerging (KOPRIVNIKAR & POULIN 2009a,b), a correlation analysis was also made by calculating the Spearman coefficient. Because, however, that last test indicated that the parameters snail size and cercarial number emerging were not correlated, no further transformations of the data were performed.

All statistical analyses were carried out with InfoStat software (<http://www.infostat.com.ar>) at a significance level of 0.05.

RESULTS

The Fig. 2 summarizes the results of the experiments testing cercarial emergence as a function of ambient temperature. The optimum temperature for the shedding of the monostome type appeared to be from 20 °C (at a mean value of 30.0 ± 25.7 cercariae emerging in 24 h) to 25 °C (at a mean value of 31.5 ± 41.2 individuals per 24 h) since the emergence diminished markedly at 30 °C (down to a mean value of only 6.80 ± 9.11). Likewise, with the pleurolophocercous cercariae, the optimum temperature was 25 °C (at a mean value of 20.0 ± 10.6 individuals per 24 h) in contrast to a lower average shedding at 20 °C (at a mean value of 12.0 ± 15.9) and a still lower level at 30 °C (at a mean value of only 3.30 ± 9.41). Accordingly, the statistical treatments of the data for both cercarial types verified that the emergences at 30 °C were significantly lower than those at either of the two lower temperatures – with H values for the two respective trematode types at 7.88 ($p = 0.0192$) and 10.4 ($p = 0.0046$).

From the data for the average daily cercarial emergence shown in Fig. 2, the maximum values occurred with the shedding from snails exposed to a temperature of 25 °C while the minima were recorded pleurolophocercous (3.30 ± 9.41) after an abrupt shift to 30 °C with both types of trematode cercariae. From these differences in the data, we might have concluded *prima facie* that emergence was greater with the monostome cercariae at both 25 and 30 °C;

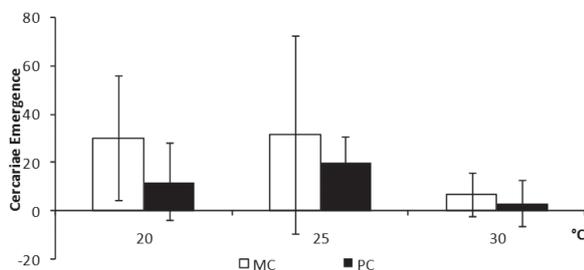


Fig. 2. Average daily emergence of cercariae at three temperatures (MC, monostome cercariae; PE, pleurolophocercous cercariae). The bars correspond to the standard deviation. In the figure the *ordinate* indicates the mean number of cercariae shed per 24 h at each of the temperatures shown on the *abscissa*.

but since the only comparison manifesting a statistically significant difference in emergence between the two cercarial types occurred at the basal temperature of 20 °C, statistical analysis of the data (Tab. I) indicated that cercarial emergence in general was not unilaterally greater for the monostome than for the pleurolophocercous trematodes.

Tab. I. Comparison of the shed data between the monostome and pleurolophocercous morphologic cercarial types at the different temperatures tested (*, significantly different; NS, not significantly different)

Temperature	p value	Test	Evaluation
20	0.0444	Wilcoxon	*
25	0.9698	Wilcoxon	NS
30	0.0822	Wilcoxon	NS

DISCUSSION

The ambient temperature – recognized as one of the conditions that influences snail-trematode interactions – could have different effects on either the host or the parasite. The developmental state of trematodes in mollusks is influenced by temperature since an increase leads to a reduction in the cercarial developmental time (MORLEY *et al.*, 2010). Temperature – either directly or indirectly – also triggers cercarial release (KOPRIVNIKAR & POULIN, 2009b). The results of our study demonstrated that, as expected, the emergence of cercariae from snails infected with the monostome and pleurolophocercous cercarial types was significantly affected by temperature and indicated an optimal temperature for the emergence of those types as being within the range of 25 °C.

Even though emergence was somewhat greater with the monostome than with the pleurolophocercous cercariae, that difference was statistically significant at only an ambient temperature of 20 °C. At that temperature the monostome cercariae would presumably have a higher probability of emergence for encysting within the environment than the pleurolophocercous.

The results upon testing the effect of temperature on the emergence of monostome and pleurolophocercous cercariae in the present work were similar to those reported for other trematode groups (Tab. II), although a direct comparison of these results with those obtained by other investigators was difficult to realize: first because the temperatures encountered in the bioassays described in the literature were often not the same and second since the parameters evaluated by other authors did not permit a conclusive comparison with the conditions examined by us – namely, the protocol followed, the data obtained with respect to the time of exposure to the experimental conditions, and especially the form of expression of the values (*i.e.*, as the maximum, minimum, and mean emergence along with the range). The following two examples illustrate the wide variability in the expression of experimental details and results that occurs within the literature (for these examples and three others *cf.* Tab. II): emergence per h (SCHMIDT & FRIED, 1996; Tab. II [2]) or “daily emergence was subsequently observed on 3 variable days over a 6-day period at each temperature” (MORLEY *et al.*, 2010; Tab. II [5]). For their part, certain investigators used

Tab. II. Reports of cercariae emergence at temperatures comparable to those used in the present work [Refs. (References): 1, LO & LEE, 1996; 2, SCHMIDT & FRIED, 1996; 3, LYHOLT & BUCHMANN, 1996; 4, TERHUNE *et al.*, 2002; 5, MORLEY *et al.*, 2010].

Temperature (°C)	Species	Emergence			Observations	Refs.
		Average	Maximum	Range		
25	<i>Centrocestus formosanus</i>	1.64	63,400	-	Emergence/day	1
12	<i>Echinostoma trivolvis</i>	13	-	-	Emergence/h	2
22–24		29	-	-		
37		82	-	-		
20	<i>Diplostomum spathaceum</i>	-	58,000	-	Cercaria/snail/day	3
25	<i>Bolbophorus confusus</i>	-	-	86.3–350	Average number of cercariae shed over a 24-h period	4
21	<i>Echinoparyphium recurvatum</i>	-	-	741–2,214	Each group of 3 snails was acclimatized to a different temperature for 3 days. Daily emergence was subsequently observed on 3 variable days over a 6-day period at each temperature.	5
25		-	-	798–1,752		

a gradual exposure of the snails to a change in temperature (LO & LEE, 1996; Tab. II [1]; LYHOLT & BUCHMANN, 1996; Tab. II [3]; TERHUNE *et al.*, 2002; Tab. II [4]; MORLEY *et al.*, 2007, 2010), while others exposed the host individuals abruptly to different temperatures (SCHMIDT & FRIED, 1996; FRIED *et al.*, 2002; KOPRIVNIKAR & POULIN, 2009a; VIGNOLES *et al.*, 2014; PAULL *et al.*, 2015).

Despite differences in the expression of results or the protocol for evaluating the effect of temperature, the examples listed in Tab. II show that cercarial emergence usually increases with elevations in temperature up to an optimum level. In addition to the those studies, we would cite results obtained by VIGNOLES *et al.* (2014) with the *Fasciola hepatica* – *Galba truncatula* model since with that paradigm a decrease in temperature by an abrupt change into colder water, in fact, stimulated emergence. By contrast, MORLEY *et al.* (2010) found that the shedding of *Echinoparyphium recurvatum*, after first increasing with elevations in temperature, stabilized within the range of 17 to 25 °C, and after that plateau decreased at temperatures above 25 °C. This latter decline in emergence is consistent with our own observations but differs from the findings of SCHMIDT & FRIED (1996), whose results coincided with those of POULIN (2006) – namely, that “an increase of a few degrees in environmental temperatures should lead to marked increases in cercarial emergence from the snail’s first intermediate hosts”, but, we must advise that these studies have been indicated by MORLEY & LEWIS (2013) to be likely associated with inadequate acclimation of snails to experimental conditions. Our results and the comparable findings of MORLEY *et al.* (2010) would indicate that the emergence and resulting abundance of freshwater cercariae in temperate climates are not necessarily increased by an elevation in temperature above 25 °C.

This conclusion coincides with the proposal in the review of MORLEY & LEWIS (2013) that midlatitude species (*i. e.*, from between 36 and 60°), such as those studied here, exhibit an optimum temperature of emergence from 15 to 25 °C. Therefore, from the results of the present study and the observations of the earlier work cited above, an elevation

in ambient temperature cannot be said to enhance cercarial shedding in general; rather, the effect of temperature on emergence is a trematode-specific phenomenon.

Finally, since information on the influence of temperature is needed to gain a better understanding of cercarial emergence in Argentina, the results reported here will provide valuable data on this question since these findings enable a greater understanding of the ecology of the South-American trematodes of temperate climates. Because temperature would be one of the main abiotic conditions affecting cercarial shedding, the use of an optimum temperature with these trematode types will facilitate further studies for determining the effect of additional influences – both environmental and anthropic – on the life cycle of these parasites.

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