INFLUENCE OF LOW TEMPERATURES ON THE CERCARIAL SHEDDING OF *PARAMPHISTOMUM DAUBNEYI* FROM THE SNAIL *LYMNAEA TRUNCATULA*

ABROUS M.*,**, RONDELAUD D.* & DREYFUSS G.**

Summary :

Lymnaeids in nature are subjected to temperature fluctuations that may have an influence on the shedding of cercariae. Thus, experimental infections of *Lymnaea truncatula* by *Paramphistomum daubneyi* were performed to determine whether a sudden fall in temperature – daily dipping of infected snails into spring water at 14-15 °C, or at 6-8 °C – followed by its increase at 20 °C in the subsequent hour, had an influence on the characteristics of snail infection and cercarial production. The immersion of infected snails in cold water during a short period delayed the first cercarial shedding, at day 66 on average, in the 6-8 °C group vs at day 57 in the 14-15 °C group, or at day 49 in the 20 °C group. The percentage of cercaria-shedding snails was greater in the 6-8 °C group than in the 14-15 °C and the 20 °C ones : 41.8 % vs 17.3 % and 7.1 %, respectively. The total number of cercariae given by each infected snail was also higher in the 6-8 °C group. A fall in the temperature of water during daily change, followed by its increase at 20 °C within the subsequent hour, stimulated the cercarial shedding of *P. daubneyi*.

KEY WORDS : cercarial shedding, *Paramphistomum daubneyi*, *Lymnaea truncatula*, temperature.

Résumé : INFLUENCE DE TEMPÉRATURES BASSES SUR LES ÉMISSIONS CERCARIENNES DE *PARAMPHISTOMUM DAUBNEYI* À PARTIR DU MOLLUSQUE *LYMNAEA TRUNCATULA*

Dans la nature, les limnées sont soumises à des fluctuations de température et ceci pose le problème de savoir si ces dernières ont une influence sur l'émission des cercaires. C'est la raison pour laquelle des infestations expérimentales de Lymnaea truncatula *par* Paramphistomum daubneyi *ont été réalisées afin de déterminer si une chute brutale de la température – immersion quotidienne des mollusques dans de l'eau à 14-15 °C ou à 6-8 °C – suivie de sa remontée à 20 °C dans l'heure qui suit, ont une influence sur les caractéristiques de l'infestation et la production cercarienne. L'immersion des mollusques infestés dans une eau froide pendant une courte période entraîne un retard de la première émission cercarienne, au 66e jour en moyenne dans le groupe 6-8 °C au lieu du 57e jour dans le groupe 14-15 °C, ou du 49e jour dans le lot 20 °C. Le pourcentage des mollusques émettant des cercaires est plus élevé dans le groupe 6-8 °C : 41,8 % au lieu de 17,3 % dans le groupe 14-15 °C et de 7,1 % dans le lot 20 °C). Les cercaires produites par chaque mollusque infesté sont, de même, plus nombreuses dans le groupe 6-8 °C. Une chute dans la température de l'eau lors du changement quotidien, suivie de sa remontée à 20 °C dans l'heure qui suit, stimulent les émissions cercariennes de* P. daubneyi.

MOTS CLÉS : *émissions cercariennes*, Paramphistomum daubneyi, Lymnaea truncatula, *temperature*.

The snail *Lymnaea truncatula* acts as an intermediate host in the life cycle of *Paramphistomum daubneyi* and *Fasciola hepatica*. Several differences can be noted in the larval development of these trematodes. Under controlled conditions, the first cercarial shedding of *P. daubneyi* often occurs during the course of week 7 postexposure (at 20 °C), however, cercariae are few in number and numerous infected snails often die without emission (Sey, 1979; Szmidt-Adjidé, 1996). In the case of *F. hepatica*, cercariae do not emerge from the snail at temperatures below 9 °C. Above 9 °C, cercarial release occurs indiscriminately throughout a wide range of temperatures up to 26 °C and under conditions of rising or of failing temperatures (Kendall & McCullough, 1951). The scarcity or absence of shedding in snails infected by *P. daubneyi* and maintained under constant conditions at 20 °C is surprising and necessitates experimental studies to understand this phenomenon. As the emergence of cercariae for many trematodes is associated with changes in the temperature of the environment of the snail (Smyth & Halton, 1983), it was interesting to determine whether the fall in temperature, which occurs during the night in the field, might have an influence on the exit of *P. daubneyi* cercariae from *L. truncatula*. To answer this question, snails infected by *P. daubneyi* were immersed each day in cold spring water (at 14-15 °C, or at 6-8 °C) before the placing of breeding petri dishes at the constant temperature of 20 °C. The present note groups the results on the characteristics of infection in these snails and cercarial production.

* Laboratoire d'Histopathologie Parasitaire, Faculté de Médecine,
** Laboratoire de Parasitologie, Faculté de Pharmacie,
2, rue du Docteur-Raymond-Marcland, F-87025 Limoges Cedex.
Correspondence: Dr. D. Rondelaud
Tel: (33) 5.55.43.58.33 – Fax: (33) 5.55.43.58.93.

MATERIALS AND METHODS

The population of *L. truncatula* used in this study was living in a road ditch at Beauloup, commune of Saint-Ours, department of Puy-de-Dôme (Massif Central, France). Snails were free of any natural trematode infections following regular sampling in May and June in this site, and from dissections of 50 adult snails on each date. A total of 550 snails measuring 4 mm in height were collected in this site at the end of June and were acclimatized to laboratory conditions for 48 hours. Adult paramphistomes were collected in the rumen of cattle and placed in a saline solution (NaCl, 0.9 %; glucose, 0.45 %) at 40°C for four hours. Eggs of *P. daubneyi* were washed in tap water and were incubated for 20 days at 20°C in the dark. The 150 first snails were used as controls. The 400 others were each exposed to a single *P. daubneyi* miracidium for four hours. Controls and exposed snails were subsequently raised for 30 days in open boxes (1 m by 60 cm and 15 cm deep), with a density of 50 snails per recipient. Each box contained small boulders and a constantly aerated, 2 cm deep water layer. Natural water coming from the original environment was weekly added to replace water lost by evaporation. Snails were fed decayed lettuce, and maintained in an air-conditioned room, under the following conditions: a constant temperature of 20°C, a diurnal photophase of 12 hours with a 3,000-4,000 lux light intensity over the boxes.

At day 30 post-exposure (p.e.), the surviving snails from the control group were counted and divided into three subgroups, as shown in Table I. A similar protocol was used for exposed snails. Each surviving snail was individually placed in a 35 mm diameter petri dish, containing 2-3 ml of spring water and a piece of decayed lettuce. These dishes were maintained in the same air-conditioned room at 20°C as the breeding boxes. Every day a cercarial count was performed (between 2 p.m. and 4 p.m.) and the water in the dish was changed until the snail's death. In two subgroups (one for controls and one for exposed snails, see Table I), the temperature of water after change was the same (20°C). In two other subgroups (one for controls and one for exposed snails), the temperature of water after change was 14-15°C and subsequently increased at 20°C in the minutes following placing of petri dishes in the air-conditioned room. In the two last subgroups, the temperature of water was 6-8°C and subsequently increased at 20°C in the subsequent hour. Routine post-mortem dissection of snail cadavers was performed to recognize uninfected snails from infected snails that died without shedding.

The parameters studied were the survival rate of snails at day 90 p.e., the percentage of cercaria-shedding snails (CS snails), the percentage of infected snails that died without cercarial shedding (NCS snails), the growth of NCS and CS snails between exposure and day 90 p.e., the length of time between exposure and the first cercarial shedding, the duration of the shedding period, the total number of cercariae counted, and the global production of cercariae by each snail isolated at day 30 p.e. The values of the three first parameters were estimated in relation to the number of surviving snails at day 30 p.e. The value of the last parameter was calculated in each subgroup using the ratio between the total number of cercariae given by infected snails and the number of snails isolated at day 30 p.e. Comparison test of experimental frequencies and one-way analysis of variance (Stat-Itcf, 1988) were used to establish levels of significance.

RESULTS

Snail survival of controls at day 90 p.e. (Table I) was significantly higher ($P < 0.001$) than that of exposed snails, whatever water temperature. In controls, the fall in water temperature did not induce a significant variation in survival rates. In contrast, in exposed snails, the survival rate in the 6-8°C subgroup was significantly lower ($P < 0.001$) than that noted in the 20°C subgroup. The percentage of NCS snails (Table I) decreased as the temperature of water: from

Snail group	**Controls**		**Snails exposed to miracidia of *P. daubneyi***			
Number of snails at the onset of experiment	150			400		
Number of surviving snails at day 30 p.e.	145			294		
Constitution of subgroups at day 30 p.e.	A	B	C	D	E	F
Temperature of water just after daily water change	20 °C	14-15 °C	6-8 °C	20 °C	14-15 °C	6-8 °C
Number of snails per subgroup	48	48	49	98	98	98
Survival rate (%) of snails at day 90 p.e.	93.7	95.8	83.6	76.5	58.1	50.0
Percentage of:						
– infected snails, but not shedding cercariae	0	0	0	44.8	22.4	7.1
– cercaria-shedding snails	0	0	0	7.1	17.3	41.8

Table I. – The experimental protocol used to study the effect of a thermal stress (snail dipping into cold water at 14-15 °C, or at 6-8 °C) during a short period on the cercarial production of *Paramphistomum daubneyi*.

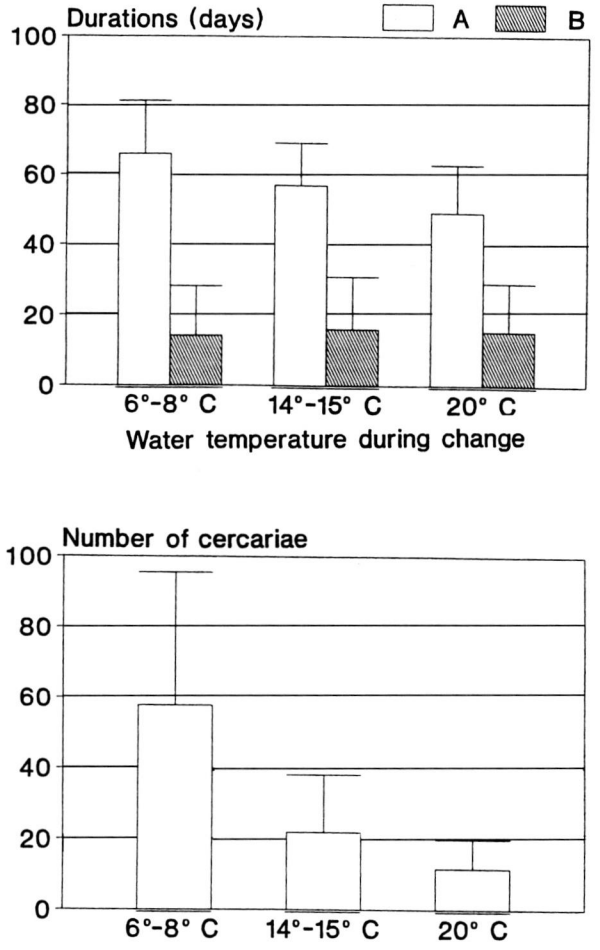

Fig. 1. – Characteristics of *Paramphistomum daubneyi* infection in *Lymnaea truncatula* : the time between exposure and the first cercarial shedding (symbol A), the duration of cercarial shedding (symbol B), and the total number of cercariae recorded for each cercaria-shedding snail. Mean values are given with their standard deviations.

44.8 % (in the 20°C subgroup) to 7.1 % (in the 6-8°C subgroup). Inversely, the percentage of CS snails increased: from 7.1 % (20°C) to 41.8 % (6-8°C). Significant differences between these percentages were noted, for NCS snails ($P < 0.001$) as well as for CS snails ($P < 0.001$). The growth of NCS and CS snails between miracidial exposure and day 90 ranged from 2.6 ± 0.6 mm in the 20°C subgroup to 2.9 ± 0.7 mm in the 6-8°C subgroup (data not shown).

The mean time between miracidial exposure and the first cercarial shedding (Fig. 1) was 49.2 days (20°C), 57 days (14-15°C), and 66.0 days (6-8°C), however, the difference between these mean durations was not significant. The duration of the shedding period (Fig. 1) ranged from 14.1 to 16.2 days and no significant difference between these durations was noted. The total number of cercariae recorded in the 6-8°C subgroup (Fig. 1) was significantly higher ($F = 4.57$, $P < 0.05$) than those from the two others (a mean of 57.7 cercariae versus 21.8 in the 14-15°C subgroup and 11.6 in the 20°C subgroup). The maximum number of cercariae was 146 for a single snail from the 6-8°C subgroup. Lastly, the ratio between the total number of cercariae in each group and the number of snails alive at day 30 p.e. was 0.8 cercaria in the 20°C subgroup, 3.7 cercariae in the 14°-15°C subgroup, and 24.1 in the last subgroup (data not shown).

DISCUSSION

Despite a longer time between exposure and the first cercarial shedding, our results demonstrated a significant increase in the percentage of CS snails when the *L. truncatula* were immersed each day in 6-8°C spring water before being subjected to a constant temperature of 20°C. As the protocol was the same in the six subgroups of our experiment except for the temperature of water at daily change, it is logical to think that removing infected *L. truncatula* from watery petri dishes and placing them in 6-8°C spring water would stress the snails and induce the exit of *P. daubneyi* cercariae within a very few hours after the subsequent increase of water temperature to 20°C. Thus, the fall in the temperature of water when changed might be considered as a factor stimulating the cercarial shedding of this trematode. The number of *P. daubneyi* cercariae released from CS snails was greater in the 6-8°C subgroup than in the 14-15°C subgroup. This finding contrasts with the reports by several authors. According to Kendall & McCullough (1951), there was no evidence of any significant differences in the numbers of *F. hepatica* cercariae which emerged from snails kept between 9°C and 26°C. Inversely, in another parasite snail-system such as that studied by Schmidt & Fried (1996), the number of *Echinostoma trivolvis* cercariae, which emerged from naturally-infected *Helisoma trivolvis*, was significantly lower in snails kept at a constant temperature of 12°C compared to the controls at 28-29°C. This behavioural pattern in cercarial shedding noted for each trematode species is so much interesting to understand when parasites, such as *P. daubneyi* and *F. hepatica*, have the same definitive host and the same snail host. A possible explanation is to consider that in the field, the periods of shedding for the former trematode would be different from those existing for the latter species. Release of *P. daubneyi* cercariae would be earlier in spring or in summer than that of *F. hepatica* cercariae, for overwintering snails as well as for spring-born *L. truncatula*. To verify this hypothesis, further studies will be necessary to determine the precise periods of cercarial shedding in the field in relation to the altitude of sites in which snails lived.

REFERENCES

KENDALL S.B. & MCCULLOUGH F.S. The emergence of cercariae of *Fasciola hepatica* from the snail *Limnaea truncatula*. *Journal of Helminthology*, 1951, *25*, 77-92.

SCHMIDT K.A. & FRIED B. Emergence of cercariae of *Echinostoma trivolvis* from *Helisoma trivolvis* under different conditions. *Journal of Parasitology*, 1996, *82*, 674-676.

SEY O. Life-cycle and geographical distribution of *Paramphistomum daubneyi* Dinnik, 1962 (Trematoda : Paramphistomata). *Acta Veterinaria Academiae Scientarum Hungaricae*, 1979, *27*, 115-130.

SMYTH J.D. & HALTON D.W. The physiology of trematodes. Cambridge University Press, Cambridge, U.K., 1983, 446 p.

STAT-ITCF. Manuel d'utilisation. Institut Technique des Céréales et des Fourrages, Service des Études Statistiques, Boigneville, 1988, 210 p.

SZMIDT-ADJIDÉ V. Étude de relations entre *Paramphistomum daubneyi* Dinnik (Trématodes) et *Lymnaea truncatula* Müller (Mollusques). Etude comparative avec *Fasciola hepatica* Linné. Thèse de Doctorat, Univ. Limoges, 1996, n° 302B, 141 p.

Reçu le 14 janvier 1998
Accepté le 21 octobre 1998