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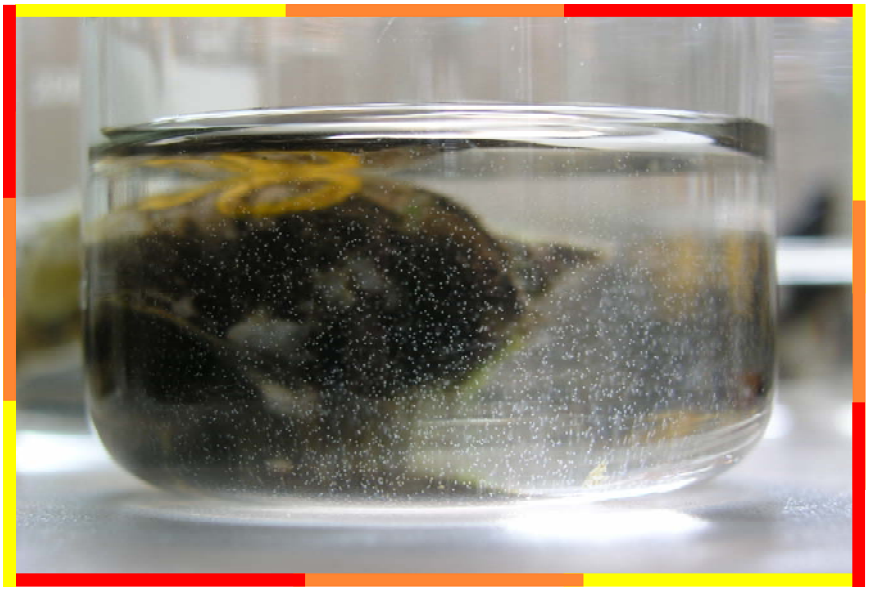

EFFECT OF TEMPERATURE ON EMERGENCE OF CERCARIAE OF MODEL FRESHWATER TREMATODES

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ANNOTATION

The influence of water temperature on cercarial emission of three trematode species parasitising freshwater first intermediate hosts, was studied in laboratory conditions: *Echinoparyphium aconiatum* from *Lymnaea stagnalis*; *Neoglyphe locellus* from *Planorbarius corneus*; and *Echinostoma miyagawai* from *Planorbis planorbis*. The study provided evidence for increase in cercarial emission with temperature increase and revealed both interspecific and intraspecific variations.

DECLARATION

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České Budějovice, 29th April, 2011 .....

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1. INTRODUCTION

There is no doubt that the weather and climate of Earth is changing towards global warming that influences the functioning of natural ecosystems and thus life on our planet. A considerable debate centres around the effect of climate change associated with the global warming on the distribution and abundance of individual organisms, their populations and communities. The temperature of living environments is a very important abiotic factor to all organisms in the world. With rising temperature, environmental conditions change and this would affect not only free-living organisms but also parasites utilizing these animals as hosts that provide supplies of energy and habitats necessary for living and/or reproducing to successfully complete parasite life-cycles. Thus, changes in temperature can affect the geographical distribution of parasitic diseases with a severe impact on their hosts. Unfortunately, rising temperature generally favours parasites rather than their hosts by leading to expansion of parasite areals which is important especially for species with medical and veterinary importance such as *Schistosoma* spp. (causative agent of schistosomiasis), *Trypanosoma brucei* (causative agent of African trypanosomiasis, sleeping sickness), *Trypanosoma cruzi* (causative agent of American trypanosomiasis, Chagas disease) and other parasites (Lafferty, 2009).

The intimate relationship between the trematodes and their first intermediate snail hostss, involving complex life-cycles in which they are functionally coupled with all their hosts through trophic interactions, parasite-mediated regulation of host populations, reflection of environmental fluctuation *via* changes in hosts composition and abundance makes snail-trematode system a suitable model to monitor the impact of climate change. Trematodes are represented in all sorts of ecosystems, their distribution is practically cosmopolitan. Their life-cycles are complicated and generally include two intermediate hosts. Trematodes, especially their developmental stages and their first intermediate hosts are easy to collect in nature, so this system is very good for studies focusing on effect of climate changes. Molluscs are obligate first intermediate hosts for trematodes and their occurrence in the nature is restricted to their aquatic habitats making their movement ability from site to site very low. Cercariae, resulting from reproduction by asexual process within the snail, are released to the external, usually aquatic environment where they are easily detectable and allow species identification of the parasite without killing the snail host.

Moreover, temperature is very important factor for trematode parasites, for their development, distribution and dispersion in the external environment. When the temperature rises, the cercarial output increases as well (Poulin, 2006; Poulin & Mouritsen, 2006).

Temperature increase raises the speed of development of intramolluscan stages and this leads to increased abundance of cercariae shed into the external environment which rises parasite chances for infection of more hosts and completing its life-cycle. Therefore, it is very important to examine how rising temperature can affect the emergence of cercariae from their first intermediate hosts because of the geographical range expansion that would expand the areas of trematode infections of wildlife and livestock. However, although the number of experimental studies on the effects of increased temperature on cercarial emergence is growing rapidly, most concern marine trematodes (Finergut et al., 2003; Koprivnikar & Poulin, 2009a,b; Prinz et al., 2010; Thieltges & Rick, 2006) while very few have used freshwater species as experimental models (Lyholt, & Buchmann, 1996; Morley et al., 2010)

2. AIM AND OBJECTIVES

This study is designed to investigate the influence of water temperature on the emergence of cercariae from the first freshwater intermediate hosts by means of laboratory experiments under different temperature regimes.

OBJECTIVES

(i) To review the methodological aproaches to assessing the effect of temperature on rates of cercarial production in trematodes.

(ii) To perform laboratory experiments at three temperatures with three trematode species representing different types of intramolluscan development: *Echinoparyphium aconiatum* from *Lymnaea stagnalis*, *Neoglyphe locellus* from *Planorbarius corneus* and *Echinostoma miyagawai* from *Planorbis planorbis*.

(ii) To evaluate statistically the effect of temperature changes on rates of cercarial emergence in the studied species.

3. LITERATURE REVIEW

3.1. GENERAL CHARACTERISTICS OF TREMATODES

Trematodes (Digenea) are large, exclusively parasitic group of helminths (Plathelmintes: Neodermata) which includes more than 25,000 described species (Esch et al. 2002). Trematodes are represented in all sorts of ecosystems, their distribution is practically cosmopolitan. They are obligate parasites of vertebrates, mostly fish, birds and mammals including humans. Lots of them are veterinary important species such as *Fasciola hepatica* (liver-fluke infecting cattle and sheep which can cause anaemia, weight loss, diarrhoea and can lead to host death) or *Fascioloides magna* (also liver trematode species of wild and domestic ruminants with similar symptoms like *F. hepatica*), and medically important species such as *Clonorchis sinensis* (liver trematode species of mammals including man and using as second intermediate hosts fish; this parasite occurs in Asia where people eat insufficiently cooked fish; it can cause cancer of bile duct), *Opisthorchis viverriny* (also liver trematode species occurred in Asia invading fish-eating mammals such as dogs and cats including man; it can also caused cancer of bile duct), *Paragonimus westermani* (lung trematode species of mammals including man which can cause bronchitis, blood in sputum and can lead to death) and especially species of the genus *Schistosoma* (causing schistosomiasis which is among the ten most important parasite infections worldwide; Hotez et al., 2007). These include *Schistosoma haematobium* (parasites in urinary system of man which can cause acute inflammation of the urinary bladder and participate in bladder cancer); *S. mansoni* (intestinal trematode in mammals, including man which can cause intestinal schistosomiasis with symptoms such as diarrhoea, fever, coughs or gland enlargement) and *S. japonicum* (which can parasite in whole mammal/human body and can cause for example liver fibrosis and cirrhosis and splenomegaly).

Trematodes underwent long co-evolution with their hosts and possible due to this they have developed morphological and biological adaptations leading to their complex life-cycles (Galaktionov & Dobrovolskij, 2003). The basic type of trematode life-cycle involves three hosts but trematodes can have one, two or even four hosts (Esch et al., 2002) (Fig. 3.1). First intermediate hosts are generally snails (only some marine trematode species use polychaets), the second intermediate hosts can be vertebrates, invertebrates, and the definitive hosts are vertebrates. Trematodes in the definitive host are sexually mature adults which produce eggs by sexual reproduction. From the egg hatches a miracidium, a free-living ciliated stage with

active locomotion. The function of the miracidium is finding the first intermediate host (Galaktionov & Dobrovolskij, 2003).

After penetrating the mollusc miracidium transforms into an asexually reproducing mother sporocyst. The role of the mother sporocyst is to produce daughter sporocysts or mother rediae which produce daughter rediae, it depends on species. Sporocysts are some type of sac, they take in feed only with entire body surface and daughter sporocysts usually produce only cercariae, the next larval stage of trematodes. In contrast, rediae have pharynx and primitive gut and are able to feed actively. Rediae are also capable to produce rediae of the next generation or cercariae or both together (Galaktionov & Dobrovolskij, 2003; Esch et al., 2001). At this stage of development trematodes reproduce asexually as well. Asexual reproduction of trematodes within the snail host (sporocysts and/or rediae) takes less time than sexual which means that sporocysts or rediae can produce more cercariae and that ensures more infected second intermediate hosts and later more chances to complete the life-cycle.

The final stage resulting from the reproduction in the first intermediate host is the cercaria. This larval stage is free-living, non-feeding and with active locomotion. Cercariae are dispersed in the water; they are actively searching the second invertebrate or vertebrate intermediate hosts. The trematode life-cycle is completed when the second intermediate host is eaten by the definitive host. In the definitive host trematodes mature and the whole life-cycle starts again with sexual reproduction.

3.2. CERCARIAL LARVAL STAGES OF TREMATODES

Cercariae are free-living, active moving and non-feeding developmental stages of trematodes. They possess tails which they use for swimming and moving in water column and which serves as reserve of energy (glycogen) (Esch et al., 2001). Cercariae have very variable morphologies and because of that there are lot of types of cercariae (*e.g.* echinostome cercariae, monostome cercariae, furcocercariae or xiphidiocercariae). Because of the limited energy reserves cercarial life span is very short, ranging from several hours to days, depending on trematode species (e.g. McCarthy (1999) recorded life span of *Echinoparyphium recurvatum* to range from 12 to 68 hours at different temperatures) so as soon as possible they must find, recognize and penetrate the next host in order to enhance the probability of successful life-cycle completion (Haas, 1994, 1995; Galaktionov & Dobrovolskij, 2003).

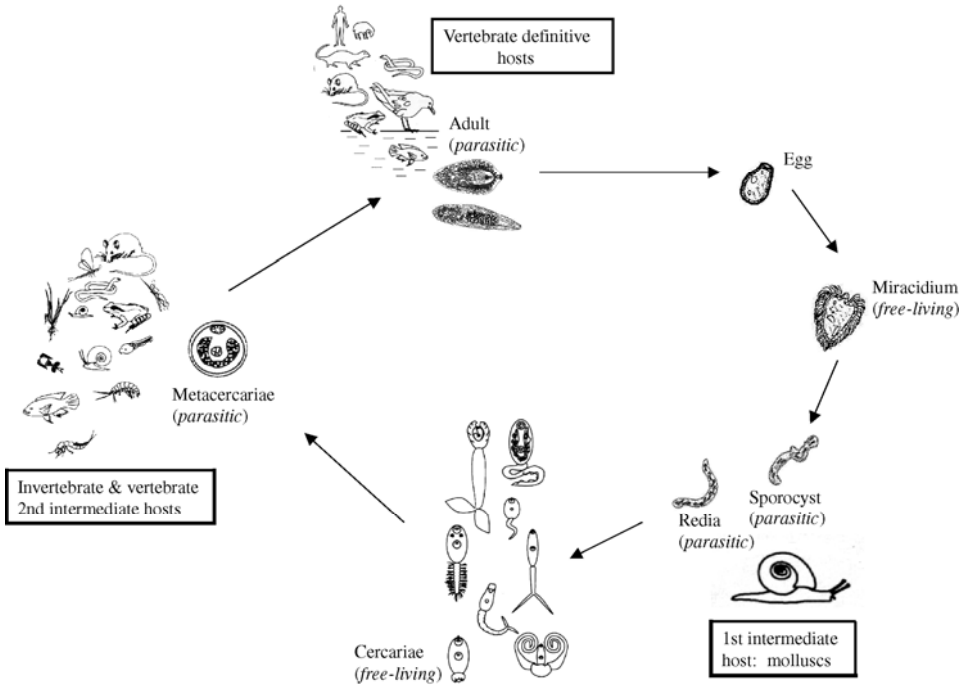

Fig. 3.1. Schematic illustration of a trematode life-cycle (modified after Combes, 2002).

As free-living stages, cercariae are exposed to influences of the external environment such as temperature, light, water currents, gravity as well as to influences from possible hosts, such as shadows, water turbulences and chemical compounds (Haas, 1994). Due to that they have developed many adaptations, both morphological and behavioural. First of the morphological adaptations is the tail which is a structure used for active movement (after penetration into the host the cercaria looses the tail). Next morphologically adapted organ is the ventral sucker which serves for attachment to the substrate or water surface during the resting period of swimming. Another adapted organs are penetration structures which cercariae use for penetrating the hosts (if they are actively penetrating), *i.e.* penetration spines or stylet (Galaktionov & Dobrovolskij, 2003).

A basic function of cercariae in the trematode life-cycle is dispersing in the water column followed by host-finding, host-recognition and host-penetration. Trematodes developed a number of behavioural strategies for this (Galaktionov & Dobrovolskij, 2003; Haas, 1994). An example of behavioural adaptation is cercarial swimming. Cercariae have many styles of swimming movements. The patterns of movement differ among the species but movement cycles are repeated in defined periods. The speed and intensity of movement can be influenced by many factors, *i.e.* temperature, gravity, chemicals, direction and intensity of light radiation (Haas, 1992). Cercariae can swim actively or only rest in water column or change these two strategies, it depends on energy reserves, hosts accessibility and environment conditions.

Cercariae use photo- or chemo-receptors for orientation, host-finding and host-recognition. They can orientate by intensity and beam of light using photoreceptors and swim to the light (*Cryptocotyla lingua*) or away from light (*Trichobilharzia szidati*); this depends on the next host (fast- or slow-moving, benthic or living near the water surface or ashore) (Haas, 1992, 1994). Very little is known about chemical receptors. Cercariae recognize specific host molecules in the water and move towards the host. For example cercariae of some *Schistosoma spp.* react to presence of unsaturated fatty acids (Haas, 1994). This strategy is used by species which have slowly moving hosts (*e.g. Echinostoma trivolvis*, Haas, 1992). For penetrating hosts' surface epithelium cercariae also use chemical compounds like peptide enzymes (especially serine and cysteine based) which degrade compounds of individual tissues (Haas, 1992).

Another behavioural adaptation of cercariae is the variations in cercarial emission and different periods of cercarial production. Production of cercariae in the first intermediate host is variable among different groups or species of trematodes. They can produce in specific periods of day or all the time (Esch et al., 2001; Galaktionov & Dobrovolskij, 2003). In many trematode species cercariae emerge randomly but there are several species with cercarial emergence occurring in exact periods of the day and timed very precisely. It depends on the photoperiod and synchronisation of cercarial emission with the occurrence of the next hosts in the life-cycle. The most famous described examples are those on *Schistosoma* species in Africa which have circadian rhythm with two peaks of cercarial output, one at the break of day (when humans are in contact with water at higher level) and other at dusk (when wild baboons and some rodents occurred at the water) (Esch et al., 2001). Cercariae can be emitted in limited abundance or during the entire life of the snail correlating with occurrence of hosts and depending on the species and the life span of snail (Sukhdeo & Sukhdeo, 2004).

3.3. INFLUENCE OF TEMPERATURE ON CERCARIAL DEVELOPMENT

Cercarial development within the snail can be directly influenced by the temperature of the external environment. Several studies have shown that increasing temperature can accelerate the development and accumulation of the cercariae in the host (Poulin, 2006; Poulin & Mouritsen, 2006). The production of cercariae is influenced by many factors, for example the temperature, light, host size, water level and salinity (Koprivnikar & Poulin 2009a), but temperature is probably the most important factor (Poulin, 2006). With increasing temperature the cercarial emergence demonstrably increases. Also when temperature increases, the speed of cercarial development in snail will be increased which leads to more cercarial production

into external environment which elevates chances to infect more hosts thus leading to higher plausibility to complete the life-cycle of trematode species (Poulin, 2006). If the temperature decreases at specific limiting level, the cercarial production would be decreased to very low levels or definitely stopped but accumulation of sporocysts and rediae in snail still continues, even at very low temperatures (Galaktionov & Dobrovolskij, 2003). The temperature of maximal or minimal production is specific for each trematode or trematode group. If the snail is returned at higher temperature (for example 20°C), there will be a significant increase of cercarial emission. This increase of cercarial production is consequence of extensive accumulation of sporocysts/rediae in the snail, and not of direct influence of higher temperature (Galaktionov & Dobrovolskij, 2003; Poulin, 2006).

An increase of temperature of water and air for several degrees of Celsius is expected in next few decades. Environmental temperature is very important factor which determines the success of parasites. Global changes could influence the geographical distribution and infection dynamics of cercariae in many trematode species which have direct effects on geographical distribution of parasite diseases and can have potential serious consequences for the hosts of these trematode species (Poulin, 2006; Poulin & Mouritsen, 2006; Lafferty, 2009).

3.4. EFFECT OF TEMPERATURE ON CERCARIAL OUTPUT

The influence of temperature on cercarial output was studied in both freshwater and marine systems because the temperature is probably the most important factor in cercarial development and production (ref). Temperature can increase or decrease the speed of cercarial development in the first intermediate hosts and also increase or decrease cercarial emission into the external environment. This review will focus on approaches to assessing the effects of temperature on cercarial emission in two marine and two freshwater systems: trematodes *Maritrema novaezealandensis*, *Acanthoparyphium* sp. and *Philophtalmus* sp. parasitising the marine snail *Zeacumantus subcarinatus* in New Zealand (Koprivnikar & Poulin, 2009a,b) and *Diplostomum spathaceum* in the freshwater gastropod *Lymnaea stagnalis*, (Lyholt & Buchmann, 1996) and *Echinoparyphium recurvatum* in the freshwater snail *Lymnaea peregra* (Morley et al., 2010) in Europe.

Koprivnikar & Poulin (2009a) examined interspecific (between trematode species) and intraspecific (among populations of each species) variations in cercarial release of *Maritrema novaezealandensis* (family Microphallidae) and *Acanthoparyphium* sp. (family Echinostomatidae) which both use the intertidal mudsnail, *Zeacumantus subcarinatus* as first

intermediate host, in response to increased temperature. Infected snails were collected from different places with different latitudes on the South Island of New Zealand in order to assess the effect of elevated temperature on cercarial emission. The authors used environmental chamber and two incubators with temperatures 20°C and 25°C. The experiment was conducted separately for each trematode species in 3 periods of the year. For *M. novaezealandensis* snails were kept at 15°C for 24 hours. After this period snails were transferred to pre-warmed water at 20°C and cercariae in previous plate were counted. After 24 hours snails were transferred to pre-warmed water at 25°C and counting procedure was performed in the same way. The same process was applied for experiments with *Acanthoparyphium* sp.

Koprivnikar & Poulin (2009a) found no correlation between host size and cercarial output at all three experimental temperatures for *M. novaezealandensis* whereas for *Acanthoparyphium* sp. they found relationship between host size and the first temperature. *M. novaezealandensis* exhibited decreased cercarial emergence with increased temperature which also differed among study sites, cercariae from lowest latitudes were emitted in higher abundances. In contrast, the numbers of *Acanthoparyphium* sp. cercariae increased in warmer temperature, as generally expected from other studies. In addition to this interspecific variation, intraspecific variation was aslo observed in *Acanthoparyphium* sp. In summary, Koprivnikar & Poulin (2009a) have shown interspecific variation between *M. novaezealandensis* and *Acanthoparyphium* sp. and in both of these species intraspecific variation influenced by temperature and concluded tha climate changes would not influence all parasite trematode species in the same way.

In a second study, Koprivnikar & Poulin (2009b) investigated the effects of temperature, salinity and water level fluctuation (environmental factors affected by the global climate change; see Marcogliese 2001) on the emergence of cercariae of two trematodes: *Maritrema novaezealandensis* (Microphallidae) and *Philophthalmus* sp. (Philophthalmidae) infecting the same first intermediate snail host, *Zeacumantus subcarinatus* which occur in intertidal zones of the marine environment. The life-cycle of *Philophthalmus* sp. is unclear, however most probably without second intermediate host (cercariae encyst in the external environment) and using an avian vertebrate as a definitive host. *M. novaezealandensis* uses as second intermediate hosts various species of crabs and amphipods, and gulls as definitive hosts. Snails were collected from Lower Portobello Bay on the South Island of New Zealand, subsequently transported to the laboratory where they were placed into the containers with artificial sea water and examined for patent infections.

The salinity and water level experiments associated with the effect of temperature were performed in environmental chamber with two incubators; in the first the temperature was set up to 20°C and in the second to 25°C. The number of emitted cercariae of each species was counted after 24 hours at these two temperatures. For salinity and temperature experiment 36 infected snails for each trematode species were used in 3 salinity levels (12 for each) simulating the fluctuation in concentrations in natural conditions. For water level and temperature experiment 24 snails infected with each trematode species were used to examine cercarial output in two water levels, *i.e.* at which snails were partially or completely submerged (12 for each water level).

Koprivnikar & Poulin (2009b) found that temperature affected cercarial emergence in both trematode species in a different manner. Whereas *M. novaezealandensis* emergence exhibited decrease at 25°C, a result corresponding with the previous experiment conducted by these authors (Koprivnikar & Poulin, 2006a), cercarial output of *Philophthalmus* sp. increased at this warmer temperature which reflected the general emergence pattern observed e.g. by Lyholt & Buchman (1996; see below). Similar discrepancies between trematode species under study were recorded in relation to the water level fluctuation. The increase in cercarial emergence of *M. novaezealandensis* when snails were partially submerged and of *Philophthalmus* sp. when snails were completely submerged (low *vs* high water level experiment, respectively) was attributed to the different transmission strategies of each trematode species to infect next hosts in their life-cycle.

The passive transmission *via* encysted metacercariae of *Philophthalmus* sp. more likely occur at higher water levels when snails are submerged and cercariae are able to disperse within the outside environment. The authors suggested that in contrast, the diverse life-cycle involving active transmission of *M. novaezealandensis* to the second intermediate hosts benefits from partially submerged snails carrying infection with this species since these hosts occur in the intertidial zones and cercariae are more concentrated in low water levels. Both trematode species showed an increased cercarial emergence at the lowest salinity concentration used in the experiment. This result contrasted to the conclusion of Mouritsen (2002b) who found positive correlation between cercarial emergence and host activity due to the higher salinity levels. Although Koprivnikar & Poulin (2006a) did not monitor such relationships, they suggested that this contradicting observation may be due to less stressful conditions for hosts at low salinity concentrations rather than responses of trematodes to the host physiological changes associated with oxygen consumption and movement rates. In summary, these authors concluded that trematode parasitism in intertidal zones is influenced by climate changes which lead to different cercarial production strategies.

Lyholt & Buchmann (1996) experimentally investigated the effect of temperature and light on cercarial shedding of *Diplostomum spathaceum* infecting *Lymnaea stagnalis* as first intermediate host and the ability of cercariae to penetrate and successfully establish infection (to develop metacercariae) in eye lenses of the second fish intermediate host, the rainbow trout *Oncorhyncus mykiss*, with respect to the temperature. Naturally infected snails were collected in a Danish trout farm and incubated separately in a thermostat. Fish were taken from a parasite-free trout-rearing environment. The period of light in the incubator was set up to the same part of the day at which snails were sampled, i.e. 15 hours of light and 9 hours of dark and after few days the light-dark cycle was reversed in order to examine its effect on the emergence of cercariae. Lyholt & Buchmann (1996) used temperatures from 3°C to 20°C to monitor daily cercarial output from snails. The water was changed every day and 3 homogenized subsamples were taken from each snail to count the number of cercariae. Infection experiments of fish were conducted in laboratory using 700 to 900 cercariae released at the temperature of 7 and 20°C and the number of metacercariae was counted.

Lyholt & Buchmann (1996) found that cercarial shedding is strongly temperature dependent. Up to 58,000 cercariae were shed from snail per day at temperature of 20°C and only up to 10,000 cercariae from snail per day at temperature of 10°C. These authors revealed that the lowest level of temperatures at which cercariae of *D. spathaceum* were shed ranged between 4°C and 6°C, and this was in contrast to the previous investigations of Berrie (1960), Brassard et al. (1982) and Stables & Chappel (1986a, b) who worked with the same trematode species and recorded the minimum temperature for its transmission between snail and fish hosts as low as 9 to 10°C. Furthermore, Lyholt & Buchmann (1996) found cercariae shedding to be independent on the light and dark and concluded that *D. spathaceum* do not need to synchronize cercarial output in a particular day period since the infection of fish intermediate hosts is also light independent. Additionally, cercariae emitted at two different temperatures and used to experimental infections of trout succeeded to penetrate and infect fish eye lenses, although they exhibited 4-5 times lower infectivity than at higher temperature. This result proved that fishes might be able to become infected even at temperatures lower than 10°C (7°C in this study) compared to the statement of Stables & Chappel (1986b) who observed a failure of migrating cercariae to reach their final location within the fish and suggested that infection with *D. spathaceum* does not take place below 10°C. Lyholt & Buchmann (1996) attributed the successful infection of fish by trematode larvae at 7°C to their relatively longer life span at lower temperatures. In summary, this study provided new information on the strong effect of temperature on cercarial emission and fish host infection by *D. pseudospathaceum*.

Morley et al. (2010) investigated the effects of host size and temperature on the emergence of the trematode species *Echinoparyphium recurvatum* from its first intermediate host *Lymnaea peregra* under natural sunlight conditions. They used naturally infected snails of different size (10-17 mm) and experimental temperatures from 10°C to 29°C. Naturally infected snails were collected in Bushy Park (Middleesex, UK). Snails were placed in tanks at 20°C with filtered stream water and fed on lettuce and Tetramin fish flake food. Cercarial production was investigated in room with nature sunlight conditions (snails were placed near a window). Morley et al. (2010) used 118 snails in total. In the first experiment they investigated pattern of hourly periods of cercarial emergence over 24-hour period for 72 hours at temperature 21°C. Snails were separated according the size into 7 classes. Afterwards they observed snails for 3 weeks with samples in days 10, 17 and 24 and observations were undertaken between 06:00 and 00:00.

In another experiment Morley et al. (2010) investigated long-term daily and weekly cyclic variations in cercarial emission for 3 weeks within three snail size categories and water changing every 2 hours. Their last experiment examined the effects of temperature on cercarial output between 10°C and 29°C according to previous studies showing no cercarial emergence below 10°C for *E. recurvatum*. They used snails from three size groups, each group separated into groups of three and each snail individually placed into 50 ml of water. Groups were acclimatized at different temperatures (10°C, 14°C, 17°C, 21°C, 25°C and 29°C) for three days.

Morley et al. (2010) found that host size influenced the emergence of cercariae with more cercariae emitted from large snails at each temperature. They observed only few evidences of cyclic emergence patterns in the three-week period due to the high mortality of snails. Finally, they observed high dependence of cercarial output upon temperature with an optimum from 17°C to 25°C and a limited output at 10°C. Poulin (1996) suggested using Q_{10} rates to analyse the effect of temperature on cercarial production and used these rates in his meta-analysis study (Poulin, 2006). Morley et al. (2010) used this approach but found, in contrast with Poulin's (2006) findings, that cercarial emergence does not increase with elevated temperature, and that long-standing high temperatures lead to decrease in cercarial production. In summary, Morley et al. (2010) proved the dependence between host size and cercarial emission in *E. recurvatum* from *L. peregra* and reduced production of cercariae at temperatures above 25°C. They also have shown that very high temperatures (above 25°C) influence cercarial production in a negative way, in contrast with general reasoning of Poulin (2006).

4. MATERIALS AND METHODS

4.1. TREMATODE SPECIES USED

We examined cercarial emission by three trematode species which are common and widespread parasites of their three first intermediate hosts: *Echinoparyphium aconiatum* (Echinostomatidae) from *Lymnaea stagnalis* (Lymnaeidae), *Neoglyphe locellus* (Plagiorchiidae) from *Planorbarius corneus* (Planorbidae) and *Echinostoma miyagawai* (Echinostomatidae) from *Planorbis planorbis* (Planorbidae). The two echinostomatids use molluscs as second intermediate hosts and develop in rediae within the first intermediate hosts whereas *N. locellus* uses molluscs and larval insects and develop in sporocysts only within the first intermediate hosts (Brown et al., 2011).

All naturally infected snails emitted cercariae when collected (summer 2009) at three localities: Bohdanečský pond, Vlkovský pond and pond Loužek (Table 4.1). *L. stagnalis* and *P. corneus* were measured (shell width and height in mm, see Table 1. All *P. planorbis* were of similar size (c. 10 mm shell width). Snails were kept until the experiment (1-10 November 2010) in individual containers (0.5l) with dechlorinated water in a cool room at constant temperature (17.5°C) and natural photoperiod, and fed lettuce. Water was changed every 4 days.

Table 1. Trematode species used in experiments, their host codes, sizes, localities and dates of collection.

Species/Host	Snail code	Replicate code	Shell width	Shell height	Locality	Date of collection
N. locellus ex *P. corneus*	2B2PC37NL	NL7	35.67	13.45	Bohdanečský pond	6.07.2010
	2B2PC34NL	NL8	34.44	13.66	Bohdanečský pond	6.07.2010
	2B2PC40NL	NL1	34.4	12.28	Bohdanečský pond	6.07.2010
	2B2PC31NL	NL5	33.85	13.33	Bohdanečský pond	6.07.2010
	2B2PC29NL	NL6	30.28	10.81	Bohdanečský pond	6.07.2010
	2B2PC32NL	NL9	33.89	13.43	Bohdanečský pond	6.07.2010
	2B2PC61NL	NL4	21.41	9.36	Bohdanečský pond	6.07.2010
	1V1PC25NL	NL2	28.86	12.66	Vlkovský pond	6.06.2010
	1V1PC95NL	NL3	29.48	12.8	Vlkovský pond	6.06.2010
E. aconiatum ex *L. stagnalis*	3B4LS37EA	EA2	31.16	56.98	Bohdanečský pond	6.09.2010
	3B3LS93EA	EA3	24.13	48.22	Bohdanečský pond	6.09.2010
	2B1LS72EA	EA4	30.21	53.29	Bohdanečský pond	6.07.2010
	3B2LS8EA	EA5	27.59	53.23	Bohdanečský pond	6.09.2010
	3B4LS20EA	EA6	28.66	55.08	Bohdanečský pond	6.09.2010
	2B1LS97EA	EA7	28.28	57.91	Bohdanečský pond	6.07.2010
	3B1LS57EA	EA8	25.62	50.19	Bohdanečský pond	6.09.2010
	3B2LS64EA	EA9	24.41	47.12	Bohdanečský pond	6.09.2010
E. miyagawai ex *P. planorbis*	1LPP12EM	EM1	-	-	Pond Loužek	2.06.2010
	1LPP7EM	EM2	-	-	Pond Loužek	2.06.2010
	1LPP3EM	EM3	-	-	Pond Loužek	2.06.2010
	1LPP4EM	EM4	-	-	Pond Loužek	2.06.2010
	1LPP8EM	EM5	-	-	Pond Loužek	2.06.2010
	1LPP6EM	EM6	-	-	Pond Loužek	2.06.2010
	1LPP58EM	EM7	-	-	Pond Loužek	2.06.2010
	1LPP57EM	EM8	-	-	Pond Loužek	2.06.2010
	1LPP17EM	EM9	-	-	Pond Loužek	2.06.2010
	1LPP55EM	EM10	-	-	Pond Loužek	2.06.2010
	1LPP21EM	EM11	-	-	Pond Loužek	2.06.2010
	1LPP65EM	EM12	-	-	Pond Loužek	2.06.2010
	1LPP11EM	EM13	-	-	Pond Loužek	2.06.2010
	1LPP22EM	EM14	-	-	Pond Loužek	2.06.2010
	1LPP9EM	EM16	-	-	Pond Loužek	2.06.2010
	1LPP18EM	EM15	-	-	Pond Loužek	2.06.2010

4.2. EXPERIMENTAL PROCEDURE

The experiments were conducted with all three species simultaneously in an incubator with a glass door at natural light:dark conditions. Experimental scheme is provided in Table 4.2. Each experimental treatment lasted 72 h (3 days). Within the first three days cercarial emission was examined for snails kept in the cool room at 17.5°C. On Day 4 they were transferred to the incubator at 22.5°C for 72 h (3 days). On Day 7 of the experiment the snails were transferred to 27.5°C for 72 h (3 days). Thus, cercarial emission was measured at 5°C intervals within the temperature range in natural conditions.

Table 4.2. Experimental scheme followed for the three trematode species studied.

Time of count	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**
T°C	17.5	17.5	17.5	22.5	22.5	22.5	27.5	27.5	27.5
08:00 h/8:00 h									
12:00 h/14:00 h									
16:00 h/20:00 h									
20:00 h									

A total of 31 snails were used in the experiments. There were 9, 8 and 16 replicates for *N. locellus*, *E. aconiatum* and *E. miyagawai*, respectively. *P. corneus* infected with *N. locellus* and *L. stagnalis* infected with *E. aconiatum* were placed into individual 400 ml beakers containing 300 ml dechlorinated water. *P. planorbis* infected with *E. miyagawai* was placed in small pots in 40 ml water.

Cercarial counts were performed every 4 hours during the first day of experiment when it was decided that the period can be extended to 6 hours. Overnight emission was counted at 8:00 h on the following day. After each 6 h period, snails were transferred in new beakers/pots with pre-heated dechlorinated water. Emitted cercariae were counted from 1 ml aliquots for *N. locellus* and *E. aconiatum* whereas for *E. miyagawai* the cercariae from the entire volume of water were counted. For the former two species, 10 subsamples of 1 ml each were taken from each beaker with a micropipette while vigorously mixing the water containing swimming cercariae and transferred into cell-well plates. Cercariae were then killed by adding a drop of 10% formaldehyde solution and counted under stereomicroscope.

4.3. STATISTICAL ANALYSES

Raw data (*i.e.* counts per snail per unit time) for each temperature/replicate combination were converted into daily output rates (*i.e.* no. of cercariae emitted $snail^{-1}$ day^{-1}) used to assess the effect of increased temperature on cercarial emission. Using the data for daily output rates at each temperature treatment we calculated the temperature coefficient (Q_{10}) for cercarial emission rates using the equation:

$$\mathbf{Q_{10} = (R_2/R_1)^{10/(T2 - T1)}}$$

where R_1 and R_2 are rates of cercarial emission (no. cercariae $snail^{-1}day^{-1}$) at the first (T_1) and second (T_2) temperature treatments, respectively.

Q_{10} rates were used to assess the influence of temperature on cercarial emission rates at any two different temperatures (*i.e.* 17.5-22.5^{o}C; 22.5-27.5^{o}C and 17.5-27.5^{o}C in our experiment). In general, Q_{10} rate is the relative change in a physiological rate over a ten degree change in temperature and thus describes the temperature sensitivity of physiological rates. Thus, a Q_{10} value of 1 indicates no change in cercarial emission rate, a value lower than 1 indicates a reduction in the emission rate, a value of 2 indicates a 2-fold increase, etc.) (Poulin, 2006).

Since data distributions did not agree with normal distribution, nonparamethic tests were carried out: correlations were tested with Spearman's correlation coefficient (r_s) and Kruskal-Wallis nonparametric ANOVA (abbreviated in the text as K-W test) was used for statistical comparisons of multiple samples.

5. RESULTS

5.1. CERCARIAL EMISSION OF *NEOGLYPHE LOCELLUS*

Using nonparametric Spearman's rank, no correlation between snail size of *Planorbarius corneus* and cercarial production of *Neoglyphe locellus* was detected at all three experimental temperatures (all $P > 0.05$). Estimated total number of emerged cercariae for each day of experiment at different temperatures showed variable cercarial emission of the individual replicates (Fig. 5.1.1.). When snails were transferred from 17.5°C to 22.5°C, i.e. the first day of temperature change (at Day 4 of experiment), a rapid increase in the emission of cercariae in all tested snails was observed. These extreme production levels were followed by a sharp emergence decrease at the subsequent Day 5 and similar pattern in cercarial output occurred at the last day (Day 6) of experiment at this temperature. Although, the number of emerged cercariae was lower at the first day at the highest temperature (27.5°C, Day 7), a higher emission started at Day 8, i.e. the day after last temperature increase.

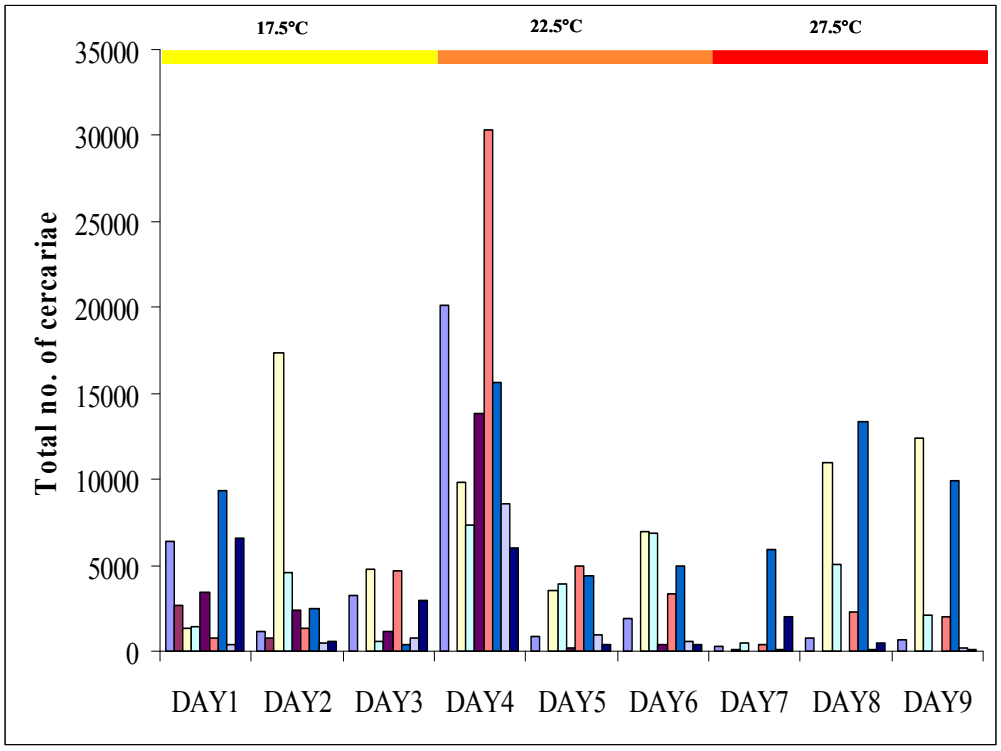

Fig. 5.1.1. Daily output rates of cercarial emission of *N. locellus* at the level of the individual snail hosts.

The plot representing the mean number of emerged cercariae per day and standard deviation at three temperatures (Fig. 5.1.2.) pinpoints the same sharp increase at Day 4 (day of the transfer to the second temperature) which reached the maximum of emitted cercariae of *N. locellus* per day in the entire experiment (mean, 13,965; see also Table 5.1.1.). The highest variability in cercarial output was observed on this day as well. In the following days the production reached the levels similar to those in the previous experimental temperature (Fig. 5.1.2. and Table 5.1.1.). In contrast, the lowest emission level was observed after transferring the snails to the highest temperature in the study (Day 7, see also Table 5.1.1.).

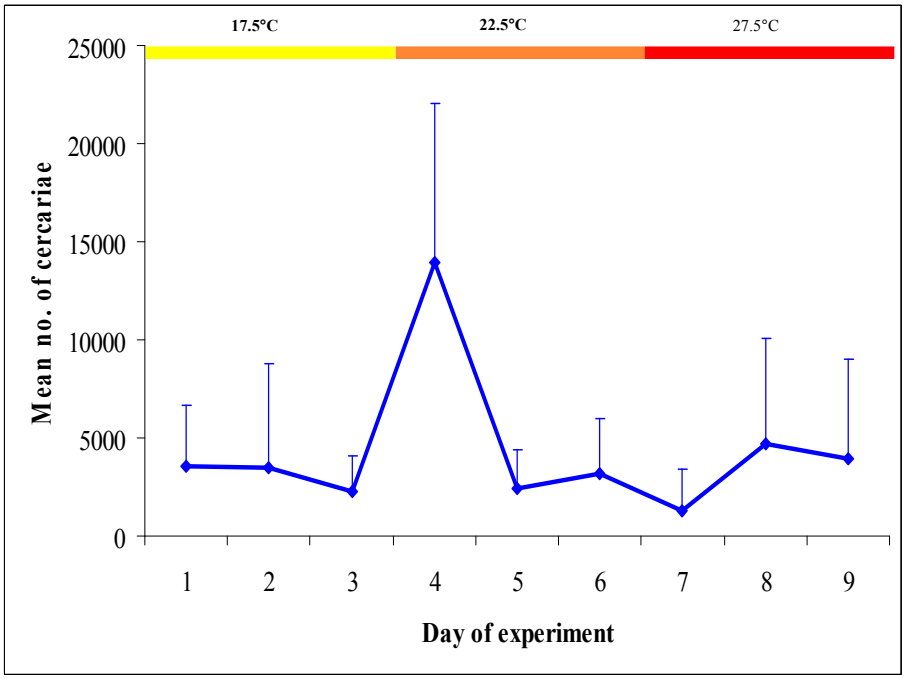

Fig. 5.1.2. Mean daily rates of cercarial emission of *N. locellus* during the experiment.

Table 5.1.1. Mean (±standard deviation, SD) and median numbers of cercariae of *N. locellus* per snail.

Species	Day of experiment	Mean ± SD	Median
T1 (17.5 °C)	Day 1	3,593 ± 3,110	2,670
	Day 2	3,457 ± 5,364	1,380
	Day 3	2,306 ± 1,822	2,010
T2 (22.5 °C)	Day 4	13,965 ± 8,116	11,850
	Day 5	2,400 ± 1,966	2,265
	Day 6	3,169 ± 2,810	2,640
T3 (27.5 °C)	Day 7	1,307 ± 2,130	420
	Day 8	4,710 ± 5,401	2,310
	Day 9	3,909 ± 5,081	1,980

Although the mean number of emitted cercariae estimated for each temperature tended to increase at second temperature, there was a substantial variability (Fig.5.1.3.). This resulted in the lack of significant differences in emergence among all three temperatures used in experiments (K-W $H_{(2,24}$=5.28, p=0.072).

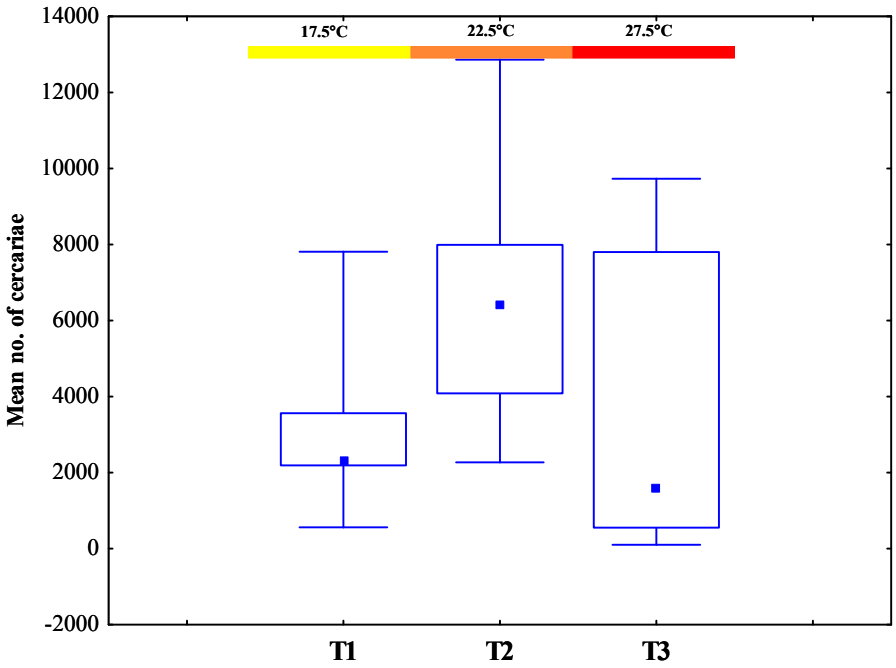

Fig. 5.1.3. Mean rates of cercarial emission of *N. locellus* at each of the three temperature regimes.

Q_{10} rates revealed a highly significant effect of temperature on cercarial emergence of *Neoglyphe locellus* (K-W $H_{(2,22)}$=10.41, p=0.006) (Fig. 5.1.4.). The 5°C difference between the first and second temperature (17.5 and 22.5°C) resulted in an increase rate at which cercariae were emitted up to 36 times within this temperature range (Table 5.1.2.). The mean Q_{10} values for the other 5°C change (22.5-27.5°C) and for the 10°C change (17.5-27.5°C) were less than one indicating a strongly reduced rate of cercarial output (Table 5.1.2.).

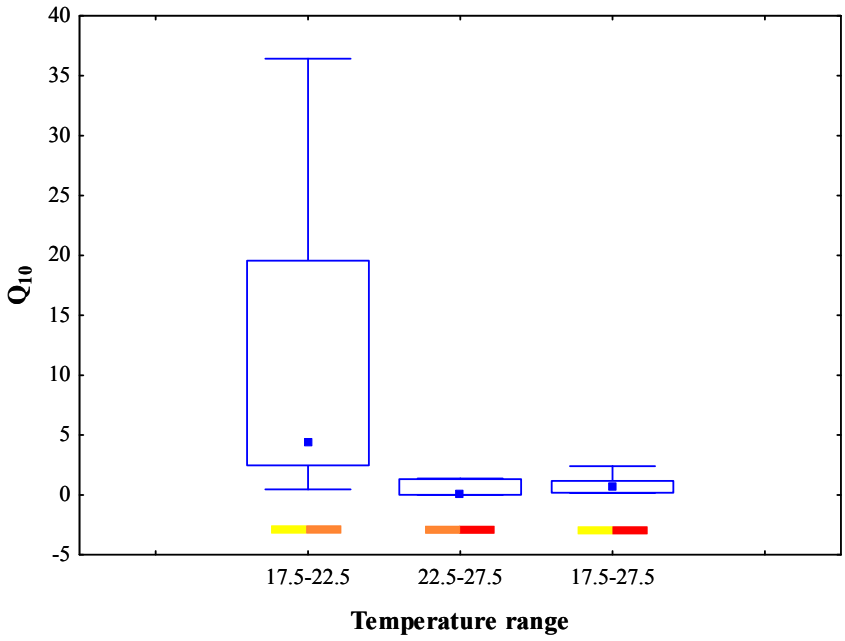

Fig. 5.1.4. Box-and-whisker plots (range, median and interquartile range) for Q_{10} rates of cercarial emission of *N. locellus*.

Table 5.1.2. Ranges, means and standard deviations (SD) for Q10 rates for the cercarial production of *N. locellus*.

Temperature increase (^{o}C)	**Temperature range**	**n**	**Minimum**	**Maximum**	**Mean**	**SD**
5 ^{o}C	T1 - T2 (17.5 - 22.5 ^{o}C)	8	0.46	36.43	11.23	14.28
5 ^{o}C	T2 - T3 (22.5-27.5 ^{o}C)	7	0.0009	1.37	0.43	0.63
10 ^{o}C	T1-T3 (17.5-27.5 ^{o}C)	7	0.15	2.40	0.83	0.80

5.2. CERCARIAL EMISSION OF *ECHINOPARYPHIUM ACONIATUM*

The mean size of *Lymnaea stagnalis* showed no effect on cercarial production of *Echinoparyphium aconiatum* monitored at 17.5 and 22.5 °C ($P > 0.05$). However, there was a significant negative correlation between these parameters at the highest experimental temperature ($-r_s = -0.94$; $P < 0.05$) indicating that cercariae were released in higher abundances from smaller snail individuals.

Daily emergence of the total number of cercariae resulted in similar cercarial output with a slight gradual increase throughout the three days of experiment at 17.5°C (Fig. 5.2.1.). Temperature change to 22.5°C led to enhanced emission which increased gradually up to a notable peak on Day 7 when replicates were transferred to the highest temperature (27.5°C). In the subsequent days, cercarial output rapidly decreased reaching the emergence levels of the experimental period at the second temperature. Two snail individuals exhibited distinctly higher production of cercariae accounting for the observed overall increases in emission rates (Fig. 5.2.1.).

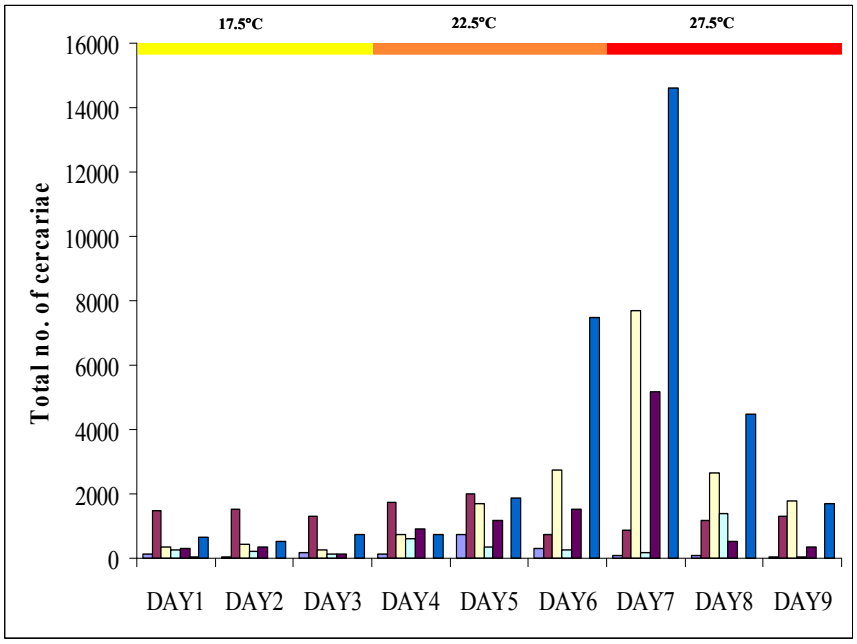

Fig. 5.2.1. Daily output rates of cercarial emission of *E. aconiatum* at the level of the individual snail hosts.

Mean cercarial emission of *E. aconiatum* in the "cold" temperature (T_1) was very low Days 1-3 reaching levels of 450 cercariae per day; these were the lowest values recorded throughout the experiment (Fig. 5.2.2. and Table 5.2.1.). After the first increase of the

temperature (Day 4) cercariae emerged from snails in gradually increasing numbers in the subsequent days ranging from around 800-2,200 within this temperature treatment. The maximum level with high variability of emitted cercariae was observed at Day 1 of experiment at 27.5°C reaching 4,800 on average (Table 5.2.1.). The drop in the mean cercarial output on Day 8 (1,725) and Day 9 (870) approached the levels of mean cercarial emission on Day 4 (810) and Day 6 (2,180) at the second temperature (22.5°C) (Table 5.2.1.)

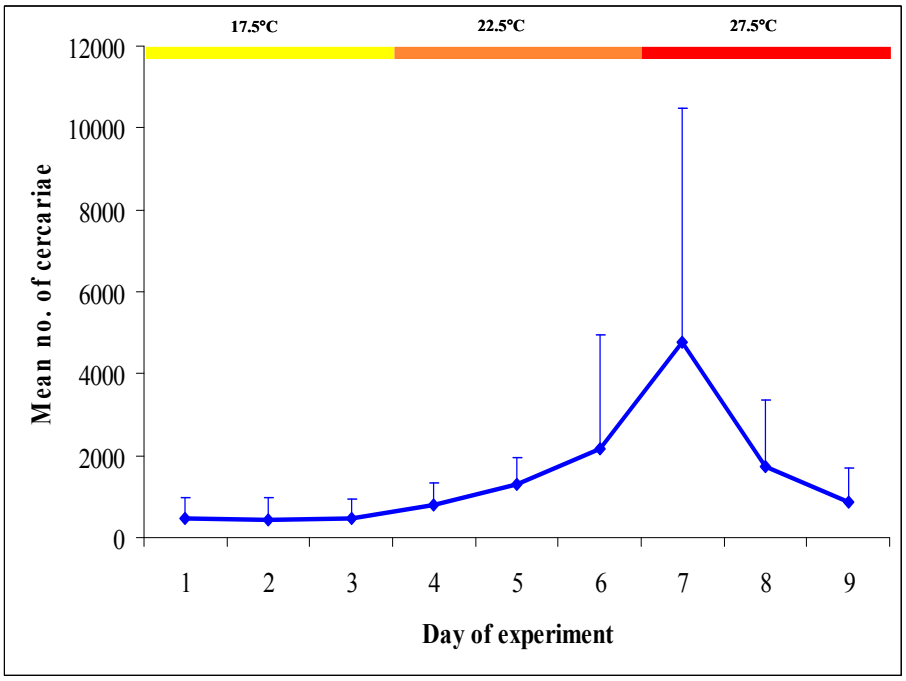

Fig. 5.2.2. Mean daily rates of cercarial emission of *E. aconiatum* during the experiment.

Table 5.2.1. Mean (±standard deviation, SD) and median numbers of cercariae of *E. aconiatum* per snail per day.

Species	Day of experiment	Mean ± SD	Median
T1 (17.5 °C)	Day 1	463 ± 497	360
	Day 2	441 ± 520	420
	Day 3	455 ± 486	348
T2 (22.5 °C)	Day 4	810 ± 531	735
	Day 5	1,295 ± 660	1,233
	Day 6	2,180 ± 2,771	1,855
T3 (27.5 °C)	Day 7	4,770 ± 5,721	4,980
	Day 8	1,725 ± 1,622	1,516
	Day 9	870 ± 822	846

Despite the higher cercarial emergence at the third temperature and because of the larger ranges of variation at this treatment, there were no significant differences in cercarial emission among the three experimental temperatures (K-W $H_{(2,19)}$=0.28, p=0.87) (Fig. 5.2.3.).

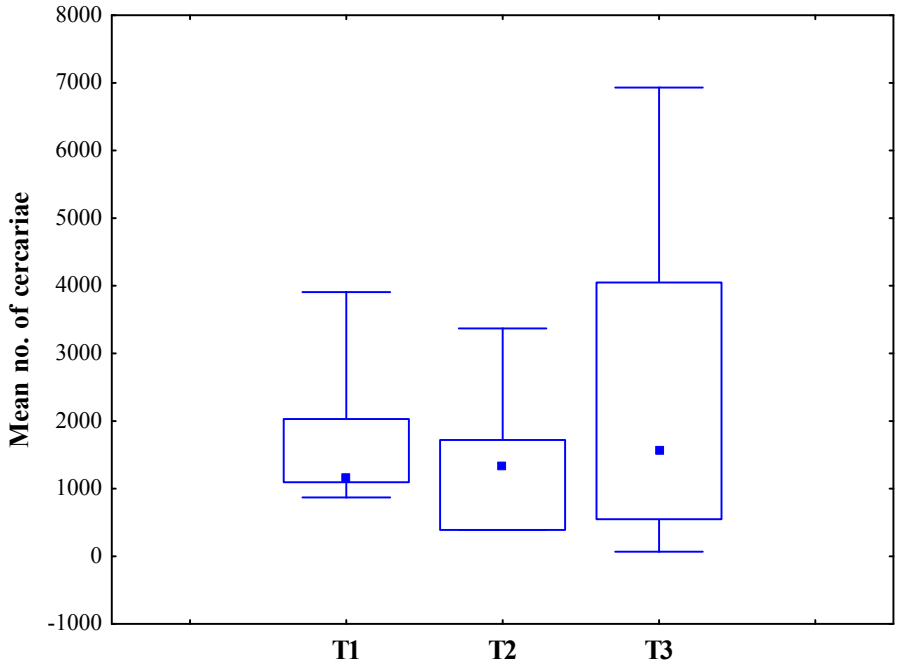

Fig. 5.2.3. Mean rates of cercarial emission of *E. aconiatum* at each of the three temperature regimes.

Because of high variation in rates of cercarial emergence at 17.5°C no marked differences in Q_{10} values among three temperature changes was detected (K-W $H_{(2,18)}$=5.48, p=0.064) (Fig. 5.2.4.). As for *N. locellus*, the tendency was in direction of increasing cercarial production of *E. aconiatum* up to 27 times at the first 5°C temperature change (Fig. 5.2.4. and Table 5.2.2.) however not significantly. Little change in cercarial output rates was recorded for the other 5°C increase in temperature (2.52 times higher on average) as well as for the 10oC range (5.77 times higher on average) (Table 5.2.2.).

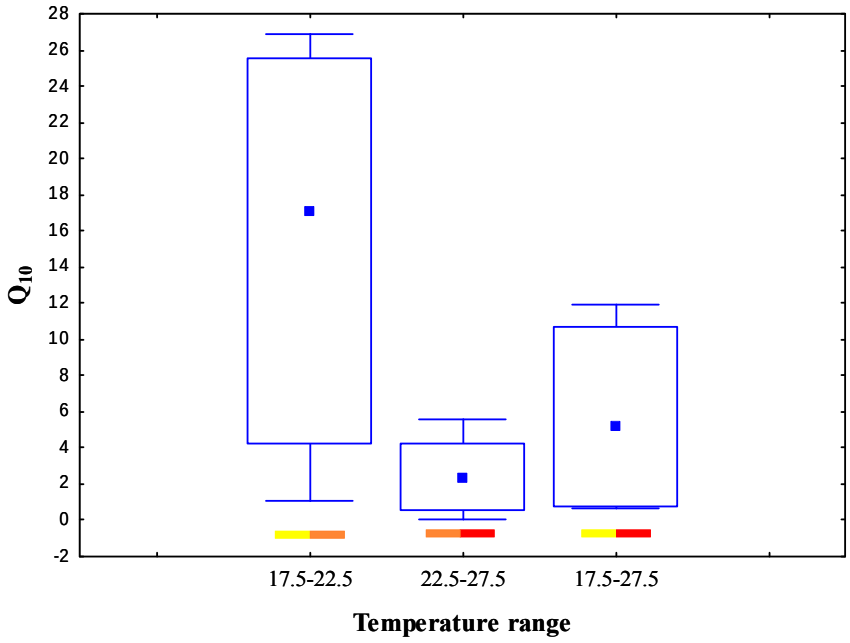

Fig. 5.2.4. Box-and-whisker plots (range, median and interquartile range) for Q_{10} rates of cercarial emission of *E. aconiatum*.

Table 5.2.2. Ranges, means and standard deviations (SD) for Q10 rates for the cercarial production of *E. aconiatum*.

Temperature increase (^{o}C)	**Temperature range** (^{o}C)	**n**	**Minimum**	**Maximum**	**Mean**	**SD**
5 ^{o}C	T1 - T2 (17.5 - 22.5 ^{o}C)	6	1.06	26.88	15.33	11.08
5 ^{o}C	T2 - T3 (22.5 - 27.5 ^{o}C)	6	0.03	5.54	2.52	2.12
10 ^{o}C	T1 - T3 (17.5 - 27.5 ^{o}C)	6	0.64	11.91	5.77	5.00

5.3. CERCARIAL EMISSION OF *ECHINOSTOMA MIYAGAWAI*

Because exact snail sizes of *P. planorbis* were not measured, Spearman's rank correlation was not estimated. However, all snail individuals used in experiment were of a similar size, i.e. around 10 mm in width. The total number of emitted cercariae of *Echinostoma miyagawai* showed a large variation among replicates (Fig. 5.3.1. A). There was one snail individual exhibiting enormously high cercarial production throughout the entire experiment, especially at Days 2, 3, 8 and 9 (indicated by a star in Fig. 5.3.1. A). When this snail was excluded from the analysis, the only notable peak in cercarial production levels was detected at the first day after snails' transfer to the second experimental temperature (i.e. Day 4 of experiment see Fig.

5.3.1. B). Such a rapid emergence increase was caused by the markedly enhanced production from the other replicates.

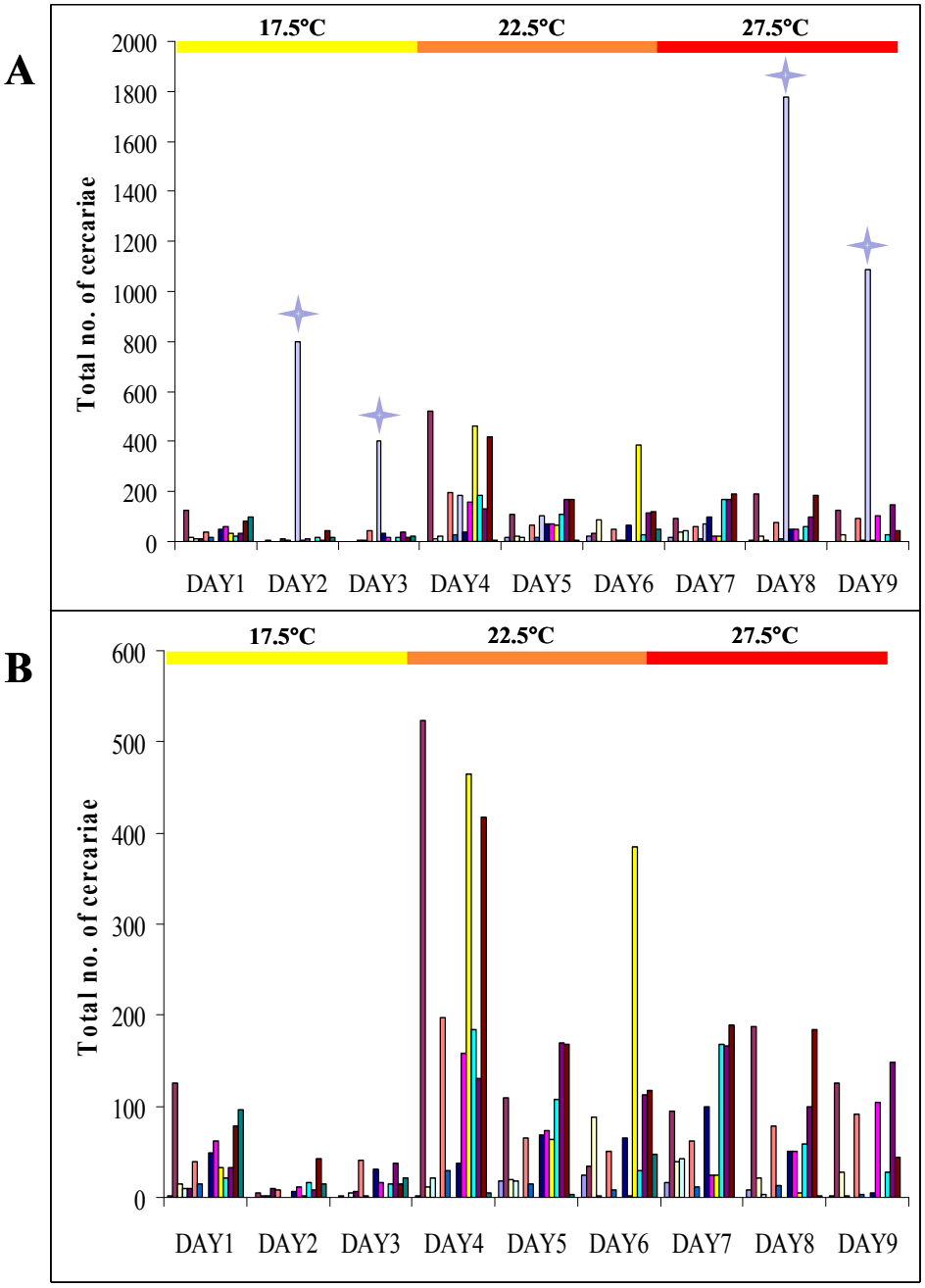

Fig. 5.3.1. Daily output rates of cercarial emission of *E. miyagawai* at the level of the individual snail hosts.

The plot showing the daily mean number of cercariae also depicted the increased levels, and more importantly high variation, introduced by the single outlier (Fig. 5.3.2. A), especially at Day 2 (17.5°C), and Days 8-9 (27.5°C). In contrast, exclusion of this replicate from the analysis revealed a different pattern in mean cercarial production at these days of experiment depicting an overall decline after the pronounced peak at Day 4 (Fig. 5.3.2. B). A

generally low cercarial output was observed during the first three days at 17.5°C with a decrease in mean counts on Days 2-3 thus reaching the lowest levels throughout the experiment (means 9 and 14 cercariae, respectively; see also Table 5.3.1.). A similar trend as in the case of *N. locellus* with mean cercarial emission rapidly increasing after placing snails at the higher temperature (22.5°C) was observed for cercarial output of *E. miyagawai* (Fig. 5.3.2. B). In the following two days (Days 5 and 6) at this temperature, the emergence declined reaching a similar mean number of cercariae (69 and 74, respectively; see Table 5.3.1.). There was no notable change in cercarial emission after the transfer of the snails from the second (22.5°C) to the highest (27.5°C) temperature on Day 7 of the experiment. An overall slight gradual decrease was observed in following days at this temperature the mean counts at the last day reaching levels comparable to Day 1 of the experiment (*i.e.* the initial temperature, 17.5°C) (Fig. 5.3.2. B and Table 5.3.1.). The remaining analyses on cercarial emission in *E. miyagaway* were performed using data without the single replicate exhibiting a high cercarial production (Fig. 5.3.1. A and Fig. 5.3.2. A) in order to avoid its substantial effect on the overall results.

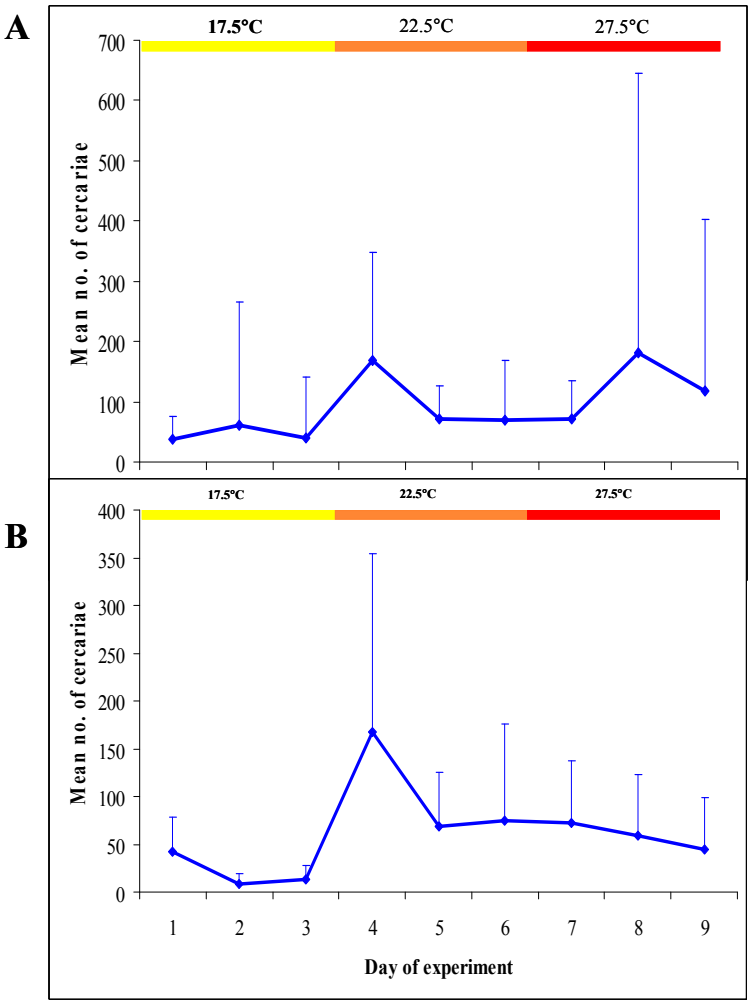

Fig. 5.3.2. Mean daily rates of cercarial emission of *E. miyagawai* during the experiment.

Table 5.3.1. Mean (±standard deviation, SD) and median numbers of cercariae of *E. miyagawai* per snail per day.

Species	Day of experiment	Mean ± SD	Median
T1 (17.5 °C)	Day 1	42 ± 37	33
	Day 2	9 ± 11	7
	Day 3	14 ± 14	10
T2 (22.5 °C)	Day 4	168 ± 186	130
	Day 5	69 ± 56	65
	Day 6	74 ± 101	47
T3 (27.5 °C)	Day 7	72 ± 66	42
	Day 8	59 ± 65	50
	Day 9	45 ± 54	28

There were significant differences in the mean cercarial production of *E. miyagawai* measured at three temperatures (K-W $H_{(2,40)}$=8.68, p=0.013) (Fig. 5.3.3.). Multiple comparisons revealed marked differences between the first and second temperature due to the substantial increase in cercarial abundance released from replicates at the latter (22.5°C; Fig. 5.3.3).

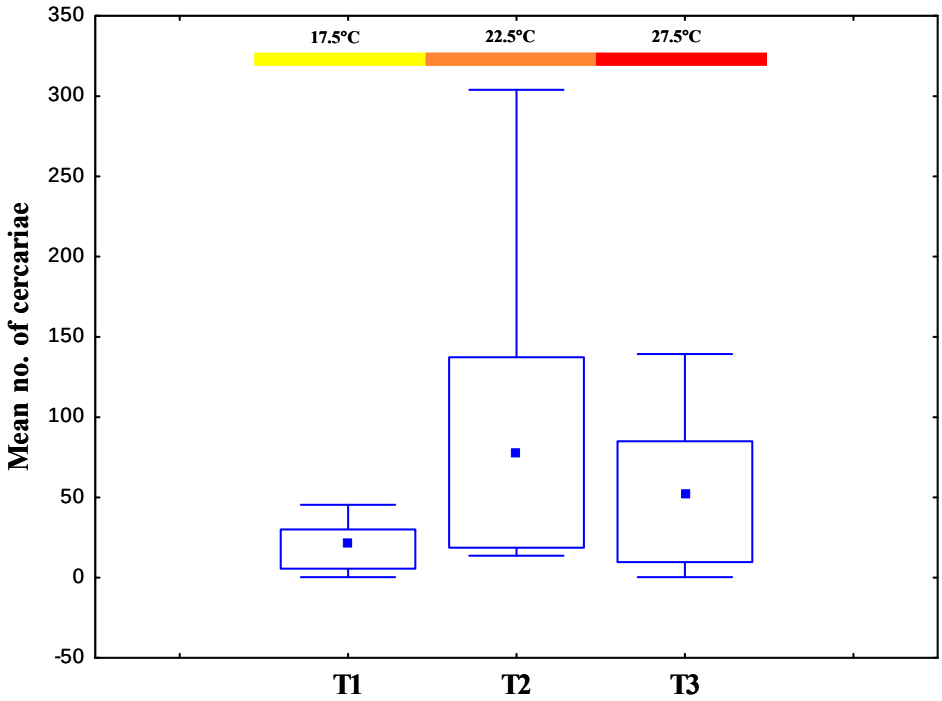

Fig. 5.3.3. Mean rates of cercarial emission of *E. miyagawai* at each of the three temperature regimes.

The values of Q_{10} coefficient showing the rates of cercarial production highly significantly differed among three ranges of temperature (K-W $H_{(2,38)}$=22.45, p=0.0001) (Fig. 5.3.4.). The first 5°C temperature change at the lower range (17.5°C–22.5°C) resulted into a sharp increase in cercarial emission rates, *i.e*. around 720 times, which were in contrast to the second 5°C change indicating reduced cercarial output rates and slight change at 10°C increase in temperature (Table 5.3.2.). However, the Q_{10} rates of cercarial emergence exhibited extremely high variation at this lower temperature range change (*i.e.* 17.5-22.5, see Fig. 5.3.4.).

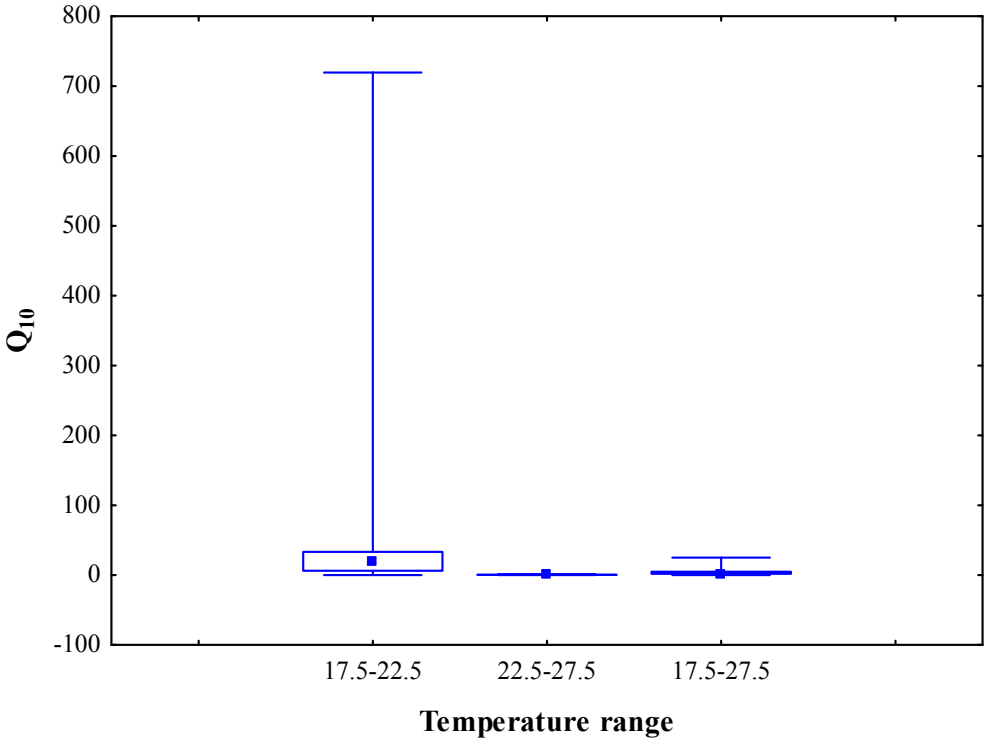

Fig. 5.3.4. Box-and-whisker plots (range, median and interquartile range) for Q_{10} rates of cercarial emission of *E. miyagawai*.

Table 5.3.2. Ranges, means and standard deviations (SD) for Q10 rates for the cercarial production of *E. miyagawai*.

Temperature increase (°C)	**Temperature range**	**n**	**Minimum**	**Maximum**	**Mean**	**SD**
5 °C	T1 - T2 (17.5 - 22.5 °C)	12	0.18	719.50	78.65	202.61
5 °C	T2 - T3 (22.5-27.5 °C)	13	0.0003	1.37	0.53	0.38
10 °C	T1-T3 (17.5-27.5 °C)	13	0.0076	25.00	4.56	6.37

6. DISCUSSION

The influence of host size on cercarial output is possible so we examined the dependence of production on host size. In *Neoglyphe locellus* there was no correlation between cercarial emergence and snail size, cercarial output is independent on the snail size in this case. Otherwise, for *Echinoparyphium aconiatum* there was no correlation between cercarial production and host size at the first two temperatures but there was negative correlation at the third temperature which means that smaller snails produced more cercariae than larger. The cercarial output is dependent on snail size at the highest temperature. There were no size measurements for *Planorbis planorbis* but *Echinostoma miyagawai* belongs to the same group like *E. aconiatum* which offered hypothesis that the negative correlation could be valid for this species at higher temperatures too. In contrast, Morley et al. (2010) observed greater numbers of cercarial output in larger snails for *Echinoparyphium recurvatum*, which is the same trematode group like *E. aconiatum* (Echinostomatidae), but they used smaller size snails in total (10-17 mm) which may have caused the differences with our data.

As expected from the patterns observed in other snail-trematode systems studied to date, the increase in temperature influenced cercarial emergence in all three freshwater trematode species examined. However, there were interspecific differences in the patterns of increase. Thus, a similar trend in increase at the first transfer to higher temperature (Day 4 of the experiment) was observed for *N. locellus* and *E. miyagawai* whereas the highest cercarial output for *E. aconiatum* was observed at the second transfer to the highest temperature (Day 7 of the experiment). The following decrease in emission suggests that the increase in cercarial emission in both cases could represent a shock reaction due to high temperature followed by quick adaptation to the higher temperature. On the other hand, the delayed reaction of E. aconiatum could be due to better adaptation of this species to the warmer range or to a prolonged development of mature cercariae inside the snail.

High intraspecific variation was a characteristic feature of the response to increased temperature of all three species studied although they were developing in snail hosts differing in size and other biological features. Thus, there was one snail infected with *E. miyagawai* which was excluded from comparisons due to the extremely high cercarial output. This fact shows that some individual snails have potential to produce huge abundance of cercariae while the rest of snails produce similar abundance at lower levels. The high variability in cercarial production among individual snails indicates that probably many other factors are involved in trematode response to temperature changes.

N. locellus and *E. miyagawai* (with the outlier replicate excluded) showed similar trend in mean cercarial output per day at the three temperatures with the highest production at Day 4. In *N. locellus* increased production was followed by similar production decrease maybe because of the absence of ready cercariae for emission or probably they were stressed by the high temperature. Cercarial output of *E. miyagawai* had very low levels at first three days of experiment probably due to stress caused with low level of temperature (17.5°C). *E. aconiatum* had very low cercarial production at first three days which can be caused by low level of the temperature. The general increase at the seventh day can be caused by shock due to high temperature. Morley et al. (2010) investigated freshwater host-parasite system with *Echinoparyphium recurvatum* (Echinostomatidae) infecting the snail *Lymnaea peregra*. They observed the same trend seen in our study (*i.e.* cercarial production increased with increasing temperature). In contrast, Koprivnikar and Poulin (2009a) examined marine trematodes *Acanthoparyphium* sp. (Echinostomatidae), nevertheless they observed the same trend. Generally, tendencies in cercarial output are increasing production with increasing temperature but there is exception in the reviewed papers that is presented by Koprivnikar & Poulin (2009a; 2009b) who observed cercarial production decrease with increasing temperature in marine trematode species *Maritrema novaezealandensis* (Microphallidae).

The mean cercarial production at each temperature showed similar trends for *N. locellus* and *E. aconiatum*, there were no significant differences in production. For *N. locellus* mean production increased at second temperature and for *E. aconiatum* increased at third temperature. In contrast, there were significant differences between three temperatures in *E. miyagawai* due to the notable cercarial output increase at the second temperature. The extreme productive snail in *E. miyagawai* was excluded from this analysis. Researches from other studies investigated different temperatures than in this study.

The Q_{10} rate analyses for *N. locellus* and *E. miyagawai* confirmed the strong increase in the rates of cercarial emergence within the lower temperature range, at the first 5°C change. Due to this there was highly significant effect of temperature on cercarial production. The extreme productive snail in *E. miyagawai* was excluded from this analysis again. In *E. aconiatum* we observed increase rates of cercarial production at the lower 5°C change as well as in *N. locellus*. Morley et al. (2010) used Q_{10} rate analysis for *Echinoparyphium recurvatum* and found that cercarial production significantly increase within the temperature range 15°C - 25°C, which is similar to present investigation of E. *aconiatum*.

In my opinion it is very important to continue studying the influences of temperature on cercarial production which can be caused by global warming and other external factors. It can help us to control the environmental changes.

7. CONCLUSIONS

7.1. The increase in temperature influences cercarial emergence of the freshwater trematode species examined. However, the patterns are difficult to establish due to the high intraspecific variation. Therefore, much higher number of replicates should be used in the experiments.

7.2. In spite of the intraspecific variation, the data revealed interspecific differences in the patterns of increase. It is difficult at this early stage of experiments to judge how the different types of intramolluscan development affect the response of cercarial emission to temperature changes.

7.3. The present pilot experiments revealed a shock effect in the first days at higher temperatures. The lowering of the rates of cercarial emission following the stress response may reflect a longer time needed for maturation cercariae for subsequent emissions. To test this suggestion cercarial emission should be followed for much longer periods.

7.4. Statistical assessment of the data should involve more sophisticated methods.

8. REFERENCES

Berrie, A.D. (1960) Two *Diplostomum* larvae (Strigeida: Trematoda) in the eyes of sticklebacks (*Gasterosteus aculeatus* L.). *Journal of Helminthology*, 34, 211-216.

Brassard, P., Curtis, M.A. & Rau, M.E. (1982) Seasonality of *Diplostomum spathaceum* (Trematoda: Strigeidae) transmission to brook trout (*Salvelinus fontinalis*) in northern Quebec, Canada. *Canadian Journal of Zoology*, 60, 2258-2263.

Brown, R., Soldánová, M., Barrett, J. & Kostadinova, A. (2011) Small-scale to large-scale and back: Larval trematodes in *Lymnaea stagnalis* and *Planorbarius corneus* in Central Europe. *Parasitology Research*, 108, 137-150.

Esch, G.W., Curtis, L.A., & Barger, M.A. (2001) A perspective on ecology of trematode communities in snail. *Parasitology*, 123, 57-75.

Esch, G.W., Barger, M.A., & Fellis, K.J. (2002) The transmission of digenetic trematodes: style, elegance, complexity. *Integrative & Comparative Biology*, 42, 304-312.

Fingerut, T.J., Zimmer, C.A., Zimmer, R.K. (2003) Patterns and processes of larval emergence in an estuarine parasite system. *Biological Bulletin*, 205, 110 – 120.

Galaktionov, K.V. & Dobrovolskij, A.A. (2003) *The biology and evolution of trematodes. An essay on the biology, morphology, life cycles, transmissions, and evolution of digenetic trematodes.* Kluwer Academic Publishers, Dordrecht, The Netherlands.

Haas, W. (1992) Physiological analysis of cercarial behavior. *Journal of Parasitology*, 78, 243-255.

Haas, W. (1994) Physiological analyses of host-finding behaviour in trematode cercariae: adaptations for transmission success. *Parasitology*, 109, 15-29.

Haas, W., Haberl, B., Kalbe, M., & Kömer, M. (1995) Snail-host-finding by miracidia and cercariae: chemical host cues. *Parasitology Today*, 11, 468-472.

Koprivnikar, J., & Poulin, R. (2009a) Interspecific and intraspecific variation in cercariae release. *Journal of Parasitology*, 95, 14-19.

Koprivnikar, J., & Poulin, R. (2009b) Effects of temperature, salinity, and water level on emergence of marine cercariae. *Parasitology Research*, 105, 957-965.

Lafferty, K.D. (2009) The ecology of climate change and infectious diseases. *Ecology*, 90, 888-900.

Lyholt, H.C., & Buchmann, K. (1996) *Diplostomum spathaceum*: effects of temperature and light on cercarial shedding and infection of rainbow trout. *Diseases of Aquatic Organisms*, 25, 169-173.

Macrogliese, D.J. (2001) Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology*, 79, 1331-1352.

McCarthy, A.M. (1999) The influence of temperature on the survival and infectivity of the cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology*, 118, 383-388.

Morley, N.J., Adam, M.E., & Lewis, J.W. (2010) The effect of host size and temperature on the emergence of *Echinoparyphium recurvatum* cercariae from *Lymnaea peregra* under natural light conditions. *Journal of Helminthology*, 84, 317-326.

Mouritsen, K.N. (2002b) The *Hydrobia ulvae – Maritrema subdolum* association: cercarial emergence controlled by host activity. *Journal of Helminthology*, 76, 349-353.

Poulin, R. (1996) The evolution of life history strategies in parasitic animals. *Advances in Parasitology*, 37, 107-134.

Poulin, R. (2006) Global warming and temperature-mediated increases in cercrial emergence in trematode parasites. *Parasitology*, 132, 143-151.

Poulin, R., & Mouritsen, K.N. (2006) Climate change, parasitism and the structure of intertidal ecosystems. *Journal of Helminthology*, 80, 183-191.

Prinz, K., Kelly, T.C., O'Riordan, R.M. & Culloty, S.C. (2010) Infection of Mytilus edulis by the trematode Echinostephilla patellae (Digenea: Philophthalmidae). *J. Helminthol* 84, 193-198.

Stables, J.N., & Chappell, L.H. (1986a) The epidemiology of diplostomiasis in farmed rainbow trout from north-east Scotland. *Parasitology*, 92, 699-710.

Stables, J.N., & Chappell, L.H. (1986b*) Diplostomum spathaceum* (Rud. 1819): effects of physical factors on the infection of rainbow trout (*Salmo gairdneri*) by cercariae. *Parasitology*, 93, 71-79.

Sukhdeo, M.V.K., & Sukhdeo, S.C. (2004) Trematode behaviours and the perceptual worlds of parasites. *Canadian Journal of Zoology*, 82, 292-315.

Thieltges, D.W. & Rick, J. (2006) Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode Renicola roscovita (Digenea: Renicolidae). *Diseases of Aquatic Organisms*, 73, 63-68.