The Effect of Temperature on the Longevity of Cercariae of *Trichobilharzia* spp. Under Laboratory Conditions.

Eytan Shtull-Leber

University of Michigan Biological Station
General Ecology, EEB 381
8/13/09
Curt Blankespoor

ABSTRACT.

Cercarial dermatitis, or swimmer's itch, is a common inflammatory allergic reaction to the accidental attempted penetration of the human epidermis by the cercarial stage of avian schistosomes, most commonly of the genus *Trichobilharzia*. Longevity of cercariae of *Trichobilharzia* spp. was determined at the summer lake temperatures of 18.5°, 23.5°, and 28.5° C. Cercarial longevity was found to be negatively effected by high temperatures (28.5° C) but no significant difference was found between the two lower temperatures. Fifty percent (50 %) mortality occurred at 40, 44, and 18 hours for 18.5°, 23.5°, and 28.5° C, respectively. Because snails shed cercariae every morning, it is highly likely that high concentrations of swimmer's itch-causing cercariae are always present in the water. Increased mortality is likely due to a behavioral change caused by the high temperatures, where cercariae swim in bursts of rapid, sporadic shivering.

The Effect of Temperature on the Longevity of Cercariae of *Trichobilharzia* spp. Under Laboratory Conditions.

E. SHTULL-LEBER*, J. LIEBERT, H. ANDERSON-KNIGHT, Z. BOUMEDIENE
University of Michigan Biological Station, Pellston, Michigan

INTRODUCTION

Swimmer's itch, a common dermatological affliction in the Great Lakes region (Bakker and Blankespoor, 1995; Ridenour, 2003), is caused by the cercarial stage of certain schistosomes (parasitic trematodes), and can result in a week of intense itching (Horak et al., 2002; Verbrugge et al., 2004). Schistosomes of the genus *Trichobilharzia*, the most common cause swimmer's itch (Horak et al., 2002), have a 2-stage life cycle (Figure 1). On summer mornings, especially sunny ones (Anderson et al., 1976; Horak et al., 2002; Sheng et al., 2004), the intermediate molluscan hosts release large quantities of non-feeding cercariae into the water to find their avian definitive host (Bakker and Blankespor, 1995; Horak et al., 2002). When cercariae of *Trichobilharzia* spp. accidentally attempt to penetrate the human epidermis they die, causing an allergic reaction and inflammation of the skin conventionally called swimmer's itch (Bakker and Blankespoor, 1995; Haas and Roemer, 1998; Horak et al., 2002; Ridenour, 2003; Verbrugge et al., 2004).

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Figure 1. The Life cycle of *Trichobilharzia ocellata*. Source: http://www.biologie.uni-erlangen.de/parasit/contents/research/images/tricholc.jpg

Cercarial longevity, and how it is influenced by temperature, is an important factor in the consideration of risk factors and the development of preventative measures for swimmer's itch.

* Present address: Department of Ecology and Evolutionary Biology, University of Michigan, 830 North University, Ann Arbor, MI 48109.

Cercariae are non-feeding and must rely on internal energy reserves to find and penetrate their host (Bakker and Blankespoor, 1995). Cercariae thus have finite life spans that should be inversely proportional to the amount of energy they use during movement. Cercariae of *Trichobilharzia* spp. have been shown to swim in faster spurts, suggesting a faster depletion of energy reserves, at higher temperatures (Haas, 1994). Temperature has been shown to have a significant effect on cercarial survival; however there is much disagreement in the literature about how long cercariae actually live at various temperatures (Bakker and Blankespoor, 1995; Horak et al., 2002; Sheng et al., 2004). Horak et al. (2002) report that cercariae live between 1 and 1.5 days at 24° C, and Bakker and Blankespoor (1995) report that all cercariae died in under 40 hours at all temperatures above 17.5° C. However Sheng et al. (2004) report that only 50 % of cercariae had died after 41 hours, and some lived up to 52 hours. The purpose of this study was to determine the effects of lake temperatures on the longevity of cercariae of *Trichobilharzia* spp. Because temperature affects cercarial movement and energy consumption, cercarial longevity should be inversely proportional to water temperature.

METHODS AND MATERIALS

Methods closely resembling work done by Bakker and Blankespoor (1995) were developed independently. Naturally infected *Stagnicola emarginata* snails were collected from Burt Lake (Cheboygan County, MI). Snails were placed in individual communion cups with filtered lake water and examined for presence of cercariae of *Trichobilharzia* spp. under dissecting microscopes, after being exposed to light for two hours. Infected snails were kept in an enclosed minnow trap in Douglas Lake and fed ground fish food.

A heavily infected snail was shed under artificial light between 0600 and 1100 hours. Beginning at 0800 hours, ten cercariae were transferred with a plastic pipette into each of 22 plastic communion cups with 1-2 ml of filtered lake water. Cups were randomly separated into three different environment chambers (Precision Scientific, 818 Low Temperature Illuminated Incubator) set at the minimum (18.5° C), mean (23.5° C), and maximum (28.5° C) temperatures (all ±2° C) of the source of the Maple River at Douglas Lake (Keller, 2008). The river temperatures were chosen as they accurately reflect the mean lake temperature and account for an increased variance, reflecting extremes that may be seen in warmer or colder years than the data were collected. The environment chambers were set on a 16:8 light:dark cycle, synchronized to the natural light patterns of summers at Great Lakes latitudes.

The number of cercariae that remained alive in each communion cup was recorded every 3 hours during the 16 hour light cycle. Cercariae were considered alive if they showed any signs of movement during the 2-3 minutes they were under the dissecting microscope. Cercariae were counted in each cup until none were found alive in two consecutive counts. To account for human error in counting, if the number of cercariae tallied as being alive was less than two later tallies, the former was increased to the lesser of the two later numbers. After a count of zero living cercariae, if a single later count revealed cercarial movement, the former was increased to the value of the latter.

The experiment was repeated with multiple snails collected from a lake near Traverse City, Michigan. Snails were shed between 0600 and 0900 hours. All additional experimental conditions were kept constant. Chi-Square tests were used on the data where appropriate. Best-fit lines were used on the data to calculate the amount of time it took 50 % of the cercariae to die (LD_{50}) at each temperature.

RESULTS

Despite significant differences in the percent of cercariae that had died at given times between the first and second experimental trials (χ^2=29, p=0.004; χ^2=85, p<0.001; χ^2=22, p=0.005 for 18.5°, 23.5° and 28.5° C, respectively), the data were combined.

Cercarial longevity of *Trichobilharzia* spp. is negatively affected by high temperatures (Figure 2). There were significant differences between the percent of cercariae that had died at given times between cercariae kept at 28.5° C and both other temperatures (χ^2=81, p<0.001;

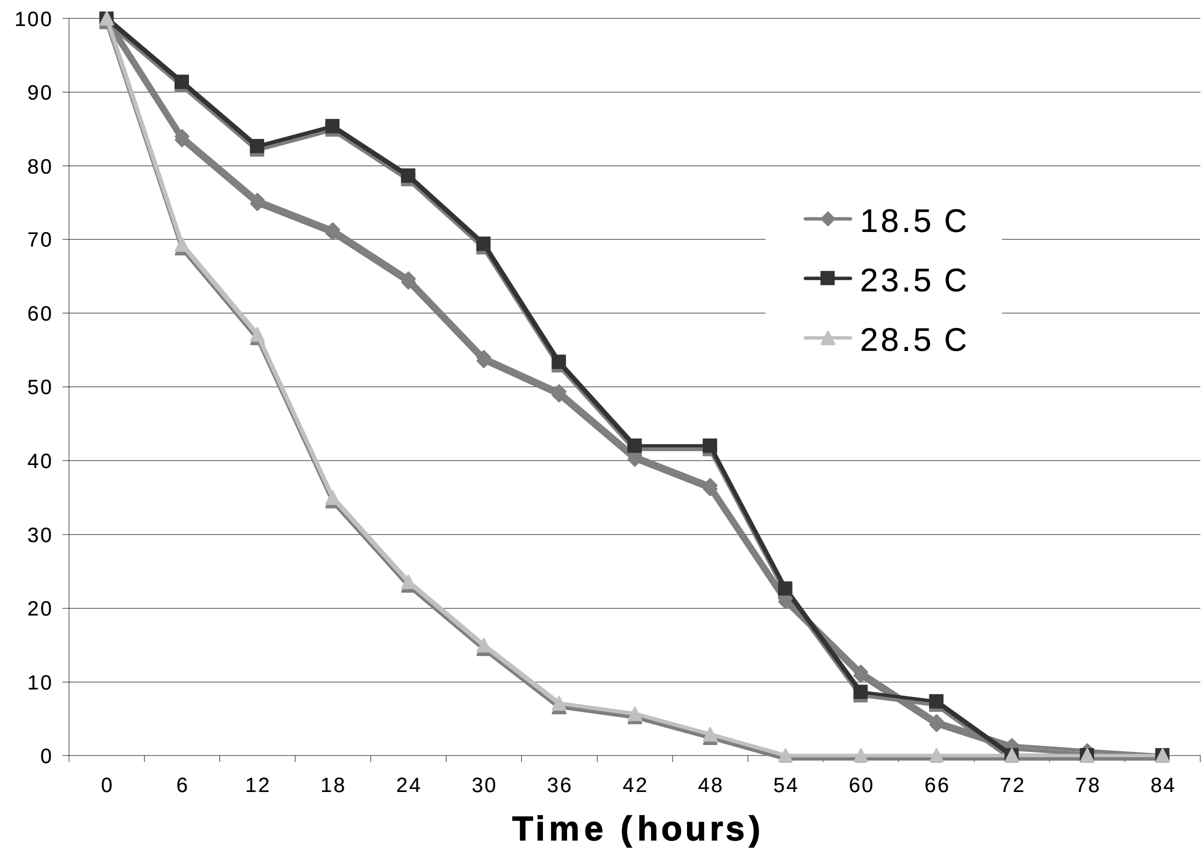

Figure 2. Survivorship of cercariaae of *Trichobilharzia* spp. at various constant temperatures.

χ^2=149, p<0.001 compared to 18.5° and 23.5° C, respectively). However, no significant difference was found between cercariae kept at 18.5° and 23.5° C (χ^2=15, p=0.24). At 28.5° C all cercariae were dead by the 53rd hour after shedding. At 18.5° and 23.5° C all cercariae had died by the 77th and 71st hour, respectively. The LD_{50}'s for cercariae kept at 18.5° and 23.5° C were 40 (linear, R^2=0.99) and 44 hours (linear, R^2=0.97) respectively, while the LD_{50} for the cercariae kept at 28.5° C was 18 hours (exponential, R^2=0.99). Thirty-eight percent (38 %) of cercariae kept at 18.5° C lived for 48 hours, and 44 % of cercariae kept at 23.5° C lived for 48 hours.

DISCUSSION

Summer water temperatures for the source of the Maple River in Douglas Lake range from 18.5° to 28.5° C, with a mean value of 23.5° C. With the exception of the highest lake temperatures, the vast majority of cercariae live well beyond 24 hours, and about 40 % of cercariae lived 48 hours in water at or below the mean lake temperature. Because snails release cercariae every morning (Anderson et al., 1976; Horak et al., 2002; Sheng et al., 2004), except for the hottest days, there is likely to always be relatively high concentrations of living cercariae in the lake. Furthermore, even at 28.5° C half of the cercariae were still alive after 18 hours, which is well after dark. At night, there are no differential light and dark stimuli to cause the cercariae to initiate host-finding movements (Wright, 1974; Feiler and Haas, 1988; Haas, 1994), and so risk of infection is greatly reduced. It is therefore highly probable that throughout the day, at all water temperatures, the majority of shed cercariae will be present in the water with the potential to find and penetrate their definitive host.

The reason for higher cercarial mortality at 28.5° C than the lower temperatures is likely to due differences in swimming behavior at different temperatures. Cercariae of *Trichobilharzia* spp. have been shown to swim in faster spurts at higher temperatures (Haas, 1994; Bakker and Blankespoor 1995), suggesting a faster depletion of energy reserves. These swimming patterns were observed while counting cercariae. In the lower temperatures, especially early in the experiment, cercariae moved at a relatively constant and slow rate. At 28.5° C cercariae alternated between floating in a resting position and bursts of rapid and stochastic shivering. These differences in cercarial behavior are likely linked to differences in the rate of depletion of energy reserves and thus mortality rates.

Data from the two experimental trials were combined because the reasons for the differences in the data are likely due to a variety of factors that may have significant influences on the variance of cercarial longevity in natural conditions. The trials used different snails, collected form different lakes, and with different levels of infectivity, all of which could have an effect on the amount of nutrients the parasite can derive from the snail. Additionally, it is possible that different species of cercariae were used in the two trials, and even within the second trial. Two different people identified the cercariae as *Trichobilharzia ocellata*, however species identification in the cercarial stage has historically been, and continues to be considered nearly impossible under a dissecting microscope (Anderson et al., 1976; Horak et al., 2002). Furthermore, all species in the genus *Trichobilharzia* can cause swimmer's itch in humans (Horak et al., 2002), so the presence of different species in the two trials may better reflect the average longevity of all swimmer's itch-causing cercariae.

Cercarial longevity was found to be consistent with the results of Sheng et al. (2004), where 50 % mortality was determined to occur after 41 hours, in temperatures between 18° and 24° C. They report, however, that all cercariae were dead within 52 hours, while at these temperatures some cercariae lived more than 70 hours. Our results are not consistent with the reports of Bakker and Blankespoor (1995) and Horak et al. (2002), both of whom report maximum cercarial longevity as approximately 1.5 days near 23.5° C. Possible reasons for the difference between this study and that of Bakker and Blankespoor are that our study had a higher density of cercariae in each communion cup, with 10 cercariae to 1-2 ml, compared to 5-6 cercariae to 2.5 ml. Because cercariae respond to touch stimuli (which are often induced by contact with other cercariae in the communion cups) with shivering movements and swimming (Wright, 1974; Feiler and Haas, 1988), density should be proportional to swimming and energy consumption. Cercariae may have died earlier in the Bakker and Blankespoor experiment because they were kept in complete darkness, being exposed to light for counting every three hours (8 times a day), thus inducing swimming responses. In our study, cercariae were only counted during the light portion of the cycle, and so the change in lighting was minimal in comparison, and so the energy-consuming response was also likely to be less. Additionally, *Trichobilharzia stagnicolae* was used for that experiment, while *T. ocellata* was used for the present one. However both species of cercariae were identified under dissecting microscopes, the soundness of this method being highly questionable (see above).

The applicability of the present results to an understanding of cercarial longevity under field conditions is not fully known. As noted above, cercariae respond to changes in illumination with various swimming behaviors that increase the probability of contact with their host. Cercariae were kept in clear water, under unchanging, uniform light (the 16:8 light:dark cycle can be ignored here as it mimics an identical pattern in nature) so differential illumination-induced swimming probably only occurred during the counting, every 3 hours. Swimming (and therefore energy consumption) as a result of changes in lighting was therefore more common in the lab than it would be in nature if the frequency of the induced response in nature were less than once per 3 hours (a frequency that is presently unknown). Additionally, as noted above, cercariae respond to touch stimuli with shivering movements and swimming (Wright, 1974; Feiler and Haas, 1988). Knowledge of the frequency of this induced response in nature would also indicate if the present data represent an over- or under-estimate.

Acknowledgements—The authors would like to thank Curt Blankespoor and Jen Mills for their assistance in all aspects of the experiment, from cercarial identification to data analysis. Dave Karowe graciously lent us his environment chambers and lab space. Harvey Blankespoor gave us many infected snails and helped sort cercariae into communion cups.

REFERENCES

ANDERSON, P.A., NOWOSIELSKI J.W., CROLL. N.A. 1976. The emergence of cercariae of Trichobilharzia ocellata and its relationship to the activity of its snail host Lymnaea stagnalis. Can. J. Zool. 54:1481-1487.

BAKKER, D. and BLANKESPOR, H.D. 1995. Host-parasite relationships of cercariae of Trichobilharzia stagnicolae. http://hdl.handle.net/2027.42/54569.

FEILER, W. and HAAS, W. 1988. Host-finding in Trichobilharzia ocellata cercariae: swimming and attachment to the host. Parasitology 96:493-505.

HAAS. W. 1994. Physiological analyses of host-finding behaviour in trematode cercariae: adaptations for transmission success. Parasitology 109:S15-S29.

HAAS, W. and VAN DE ROEMER, A. 1998. Invasion of the vertebrate skin by cercariae of Trichobilharzia ocellata: penetration processes and stimulating host signals. Parasitol. Res. 84:787-795.

HORAK, P., KOLAROVA, L., ADEMA, C.M. 2002. Biology of Trichobilharzia. Adv. Parasit. 52:155-233.

KELLER, T. 2008. Personal communication.

RIDENOUR, N. 2003. Prevention key to managing swimmer's itch. Nurs. Pract. 28:54-55.

SHENG, S., QIN, Z., ZHANG, M., TAI, Y., NI, S., WEN, J. 2004. Preliminary study on the ecology of Trichobilharzia cercariae in the Huaihe river system. Chin. J. Parasitol. Parasit. Dis. 22:349-352.

VERBRUGGE, L.M., RAINEY, J.J., REIMINK, R.L., BLANKESPOOR, H.D. 2004. Swimmer's itch: incidence and risk factors. Am. J. Public Health. 94:738-741.

WRIGHT, D.G.S. 1974. Responses of cercariae of Trichobilharzia ocellata to white light, monochromatic light, and irradiance reduction. Can. J. Zool. 52:575-579.