

THE EFFECT OF TEMPERATURE ON THE LONGEVITY OF *Trichobilharzia ocellata* CERCARIAE IN LABORATORY CONDITIONS

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Abstract- Our experiment investigated the effect of temperature on the longevity of *Trichobilharzia ocellata* cercariae. Since cercariae emerge from their intermediate snail host in search of their definitive avian hosts, cercarial longevity affects trematode transmission success, which in turn influences abundance and distribution of host populations. Recently emerged cercariae were placed in three temperature intervals, 18.5°C, 23.5°C, and 28.5°C, representing low, intermediate, and high temperatures of Douglas Lake, Michigan; cercariae were counted in time intervals until death. Cercariae in high temperatures experienced significantly lower survivorship than low and intermediate temperatures. Cercariae in the 28.5°C temperature setting lived to a maximum of approximately 45 hours post-emergence, while cercariae in the 23.5°C and 18.5°C temperature settings lived approximately 70 hours post-emergence (at maximum). Observed trends were consistent with results from previous studies.

Key Words- *Trichobilharzia ocellata*, Swimmers' itch, Temperature, Cercarial longevity.

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INTRODUCTION

Due to its complex life cycle, *Trichobilharzia ocellata*—a common bird schistosome found in North America, Europe, and Asia (McMullen and Beaver, 1945)—employs multiple hosts, resulting in a broad interaction with species of differing trophic levels in various ecological communities (Verbrugge et al., 2004). Since the success of *T. ocellata* depends heavily on the effectiveness of its methods of emergence and transmission from one host to the next, the larval trematode is influenced by environmental factors that both directly affect its longevity and indirectly affect its transmission success by altering the abundance and distribution of its host, making it a useful bioindicator of environmental change (Keas and Blankespoor, 1997). *T. ocellata* experiences a two-host life cycle, with an avian definitive host, normally ducks, and an intermediate snail host, *Lymnaea stagnalis* (Anderson et al., 1976). In its definitive host, the schistosome matures and reproduces, dispensing their eggs through host feces into the water. The eggs release miracidia into the water, which enters the snail host; after two generations of sporocyst-stage, cercariae are shed from the snail and emerge into the water (Figure 1, Verbrugge et al, 2004). During the cercarial stage, *T. ocellata* seeks out its definitive host in which it can continue its life cycle (Leighton et al., 2000).

Since the cercarial stage may be highly adapted for the transmission success (Haas, 1992), the pattern of larval behavior, especially regarding the influence of environmental and host signals, had been reviewed for several trematode species, including *T. ocellata* (Anderson et al., 1976; Haas, 1992, 1994; Lo and Lee, 1996; Schmidt and Fried, 1996; Wolmarans et al., 2002). Comprehending these patterns provides insight on the adaptive benefits of cercarial behavior and corresponding effects on fitness (Haas, 1992). Such behavior also impacts human health, since cercariae may mistake humans for their definitive hosts and burrow into the skin, causing Schistosome Dermatitis, or swimmers' itch (Cort, 1950; Leighton et al., 2000).

Considering the onset of global climate change and its alteration of lake mean temperatures, the effect of temperature on the free-swimming lotic cercarial population is particularly relevant to the study of parasite and host populations in fresh water systems, although few studies have focused on this topic. This study concentrates on the effect of different temperatures on the longevity of *T. ocellata*. While no correlation was observed

between temperature and *T. ocellata* emergence from its snail host (Anderson et al., 1976), the cercarial species has shown high sensitivity to warmth. As an adaptation for directing cercariae toward their final duck hosts, this sensitivity allows for detection of warm-blooded vertebrates when the target substrate is only 1°C warmer than ambient water temperature, suggesting that *T. ocellata* is highly responsive to their thermal environment (Haas, 1992, 1994). Based on previous studies involving temperature and free-swimming longevity in cercariae of other species and *T. ocellata*, significant deviation from the mean ambient temperature to which *T. ocellata* are adapted should negatively affect cercarial longevity; an inverse relationship between longevity and temperature, in which lower survivorship results at higher temperatures, is predicted (Bakker and Blankespoor, 1995; Lo and Lee, 1996).

METHODS AND MATERIALS

Emergence and Collection of Cercariae- In order to test the longevity of *T. ocellata* in different temperature settings, the cercariae were placed in the trial temperature immediately after emerging from *L. stagnalis*. Snail hosts were collected from Douglas Lake (Pellston, MI, Bert Lake (Cheboygan, MI) and other lakes in Northern Michigan, and kept in minnow traps in South Fishtail Bay, Douglas Lake. At 6:00 am, 50 infected snails were removed from the lake and isolated in 2 cm x 3 cm clear plastic communion cups filled with Douglas Lake water. The cups were placed into holes in a circular Lexan tray and exposed to fluorescent light for approximately two hours to induce cercarial emergence (Blankespoor and Reimink, 1998; Keas and Blankespoor, 1997). This method was selected to obtain cercariae due to the well-documented responsiveness of *T. ocellata* to light as stimulation for emergence behavior; since peak emergence activity occurs in the first four hours of illumination, allowing *L. stagnalis* to shed for two hours yielded enough cercariae to compose the desired number of samples (Anderson et al., 1976). During the first trial, cercariae were collected from a single snail with a high-emergence yield, while in the second trial, cercariae were collected from ten different snails and were randomly placed in different temperature intervals, ensuring that all the cercariae in a given temperature level were not from a single snail, in an attempt to account for any inter-host variation in parasite load.

Samples and temperature intervals- After the two[-hour waiting period, the tray of communion cups was placed into a glass-bottom pizza pan and examined under the dissecting microscope in order to identify infected snails (Keas and Blankespoor, 1997). After collecting cups that contained cercariae, the snails were removed and placed back in the lake. Ten cercariae were pipetted out with a Pasteur pipette and deposited into clean communion cups with 2-3ml of Douglas Lake water (Bakker and Blankespoor, 1995). Twenty-two samples were used in the first trial and twenty-three samples were used in the second, with seven to eight samples of ten cercariae each tested per temperature level. This allowed us to test the longevity of 150 cercariae at each temperature level.

We tested three temperature levels, representing a) the average summer (June-August) temperature of Douglas Lake at the mouth of the east branch of the Maple River, 23.5°C, b) the maximum temperature, 28.5°C, and c) the minimum temperature, 18.5°C (Personal Communication with Troy Keller, Stream Lab, University of Michigan Biological Station) in order to test cercariae longevity in a situation representative of natural habitat. The three temperature levels were simulated by three *Pearson Scientific* (818 Low Temp Illumination) Illuminated Incubator environmental chambers. The environmental chambers were set on a 16 hours of light: 8 hours of darkness cycle to mimic the natural light conditions experienced by *T. ocellata* in Douglas Lake; since the cercarial were previously exposed to this pattern when they inhabited their snail hosts, imposing a different light-dark cycle would inflict an additional, confounding stress on cercariae longevity. We set the environmental chambers to being the life cycle at 6:00 am and the stark cycle at 11:00 pm. Haas (1994) reported that *T. ocellata* experiences an active phase at a mean temperature of 25°C and can maintain activity at a range of 15°C to 40°C, suggesting that the proposed trial temperatures, which are well within this range, will allow the cercariae to maintain activity in the opening hours of the experiment and will not impose a fatal thermal shock upon placement into the chambers.

Data Collection- Cercariae were examined at three-hour time intervals using a dissecting microscope; cercariae that exhibited any bodily movement were considered alive, and were thus counted as dead only when rendered completely immobile (Lo and Lee, 1996).

Cercariae were examined in three-hour time intervals from 7:00am to 10:00pm. Counting was continued until all the cercariae in each trial were considered dead, which required two consecutive time-interval counts of 0 cercariae per sample. Samples were removed from the environmental chambers and counted one at a time to prevent temperature changes due to exposure to room temperature environments, which may add a confounding variable. Counts began on the hour and lasted fifteen to twenty minutes; samples were counted in a consistent order, with the intention of maintaining the same three-hour time interval between counts for each sample. Some samples, particularly in the 28.5°C temperature, evaporated water after twenty-four hours into the experiment. To prevent the lack of water from influencing cercarial longevity, Douglas lake water was added drop wise to samples so that each sustained a consistent water level. The data from both trials was combined to increase sample size before statistical tests were applied.

Data Correction- To account for human error, in which counts of the number of live cercariae appeared to increase across consecutive time intervals, a data correction method was applied. If a count in a given time interval was followed by two consecutive higher counts, the count from the first time interval was changed to reflect the smaller of the two subsequent higher counts.

RESULTS

While the data from the two trials were found to deviate significantly, (Comparison of number of cercariae in 24-hour time intervals; Chi-squared test for 18.5°C: $\chi^2 = 10.340$, $df = 2$, $p = .005$; Chi-squared test for 23.5°C: $\chi^2 = 14.810$, $df = 2$, $p < .001$; Chi-squared test for 28.5°C: $\chi^2 = 7.618$, $df = 1$, $p = .005$), differences were expected to reflect natural variation in *T. ocellata* inhabiting Northern Michigan. Thus, we chose to combine the data for statistical analyses.

In comparing the survival rate of *T. ocellata* for in different temperature settings, we found that temperature variation affected cercarial longevity, as Figure 2 shows (Comparison of number of cercariae in 9-hour intervals; Chi-squared test: $\chi^2 = 87.335$, $df = 12$, $p < .001$). Additional Post-hoc tests revealed that cercariae in high (28.5°C)

temperature settings exhibited significant decreased longevity when compared to intermediate (23.5°C) temperature settings (Chi-squared test: $\chi^2 = 41.084$, $df = 6$, $p < .001$) and low (18.5°C) temperature settings (Chi-squared test: $\chi^2 = 25.311$, $df = 5$, $p < .001$). Cercarial longevity in low and intermediate temperature settings were not significantly different (Chi-squared test: $\chi^2 = 5.939$, $df = 6$, $p = .430$) as Figure 2 suggests.

Table 1 shows that in the highest temperature setting, all of the cercariae were dead by 45th-hour interval post-emergence, compared to 69th-hour interval post-emergence in the intermediate temperature setting and 75th-hour interval post-emergence in the lowest temperature setting. The time it took for 100% of the cercariae in each temperature setting to die is contrasted by the results of the LD-50, or lethal dose-50, which predicts the amount of time required for 50% of the population to die based on a regression analysis; we compared exponential and linear regression analyses (R^2 values) for each temperature interval to identify the model of best fit. An exponential regression analysis for the highest temperature setting ($R^2 = 0.88295$) predicted that 50% of the cercariae in the 28.5°C temperature interval would die approximately 8 hours post-emergence (Figure 3). A linear regression analysis for the intermediate temperature setting ($R^2 = 0.96553$) estimated that 50% of cercariae in the 23.5°C temperature interval would die after 31 hours (Figure 4), while a linear regression analysis for the low temperature setting ($R^2 = 0.98257$) estimated that 50% of cercariae in the 18.5°C temperature interval would die after 34 hours (Figure 5).

DISCUSSION

The significant differences between trials were due to a number of factors: the first trial only used one snail, compared to multiple snails used in the second trial; the first trial snail was from Douglas Lakes, while the snails from the second trial were from various Northern Michigan lakes; during the first trial, counting was less precise due to less practice. However, since these differences are not the result of some experimental confounding variable, combining data from both trials should only help to account for the variation between *T. ocellata* in the Northern Michigan region.

As hypothesized, variation in temperature has a significant effect on cercarial survivorship (Figure 2). Selective pressures due to the importance of host-finding as a life-cycle determinant may impose selection on the complex host-finding and host-recognition patterns and highly sensitized responses to external environments in actively-host-invading cercariae such as *T. ocellata* (Haas, 1994). If *T. ocellata* have highly specific adaptations to their Northern Michigan environment, including average lake temperatures, deviations from normal conditions may significantly alter their behavior. Such responses to temperature extremes (both extreme cold and heat) were seen in other cercariae species in previous studies (Lo and Lee, 1996). However, our results indicated that cercarial longevity was negatively affected only by the higher temperature setting, and was not significantly affected by the low temperature environment (Figure 2). Bakker and Blankespoor (1995) also observed this inverse relationship between longevity and temperature, finding that in even colder temperatures (6°C), cercariae lived longer than 80 hours. This distinction may be explained by species-specific swimming behavior: *T. ocellata* are intermittent swimmers, meaning that instead of swimming continuously, a *T. ocellata* individual has an active phase in which it engages in an upward directed swimming burst, alternated with a passive phase in which it sinks (Haas, 1994). Haas (1994) suggested that such intermittent swimming behavior could be interpreted as an adaptation to minimize the energy expenditure. As non-feeding larvae, individuals in the cercarial stage have a fixed energy budget based on energy acquired in other life stages. Thus, if dramatic changes in temperature are expected to interfere with the developed cercarial adaptations to preserve energy, such temperature changes will necessarily, negatively impact cercarial longevity by imposing additional, unbalanced energetic costs. In similar intermittently swimming cercarial species, high temperatures prompt an increased frequency of swimming bursts that cover shorter distances than passive sinking behavior (Haas, 1994); this increased effort to move to lower (and thereby cooler) water temperatures requires increased energy expenditure—since cercarial energy reserves are depleted in proportion to the rate and duration of movement (Bakker and Blankespoor, 1995)—which may translate into diminished survivorship. Lower temperatures may not provoke such energy-expensive swimming behavior. Our observations of cercarial behavior, as well as observations by Bakker and Blankespoor (1995), support this

interpretation: as hours past emergence increased, cercariae appeared to be resting and moved only in response to shaking or tapping on the cup, at which point they launched into rapid swimming bursts.

The results of the LD-50 suggest that while temperature may negatively influence longevity, the experimental temperatures were not extreme enough to cause immediate shock, since at least 50% of all cercariae were living 8 hours post-emergence. We would expect at least limited survival in all temperature levels, since *T. ocellata* can maintain an active swimming phase in 15°C to 40°C. The LD-50 results from the intermediate and lower temperature settings, both exceeding 30 hours, suggest that the range of 18.5°C to 23.5°C provides a relatively favorable environment for cercarial survival. Similar survivorship, in which individuals were able to survive over 50 hours in relatively favorable temperatures, were observed in other cercarial species (Lo and Lee, 1996).

The outcome of this experiment has significant implications for controlling swimmers' itch. The results suggest that half of the population of *T. ocellata* can survive between 30 and 70 hours in free-swimming cercarial stage. If this can be extrapolated to a natural environment, then under normal thermal conditions, cercariae will live multiple days. This suggests that the higher rate of infection observed in the morning and lower rate of infection observed in the evening does not result from cercarial longevity differences, but must instead be influenced by factors such as currents and onshore winds (Verbugge et al., 2004). Additionally, these results should be considered when examining *T. ocellata* in the context of ecological significance; patchy host distributions may decrease cercarial transmission success more significantly in environments experiencing considerable temperature increases, perhaps due to global warming (Keas and Blankespoor, 1997). Deeper understanding of other factors effecting cercarial behavior, as well as a more detailed mechanistic explanation of cercarial responses to temperature, can enhance our knowledge in both ecological and public health contexts.

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APPENDIX

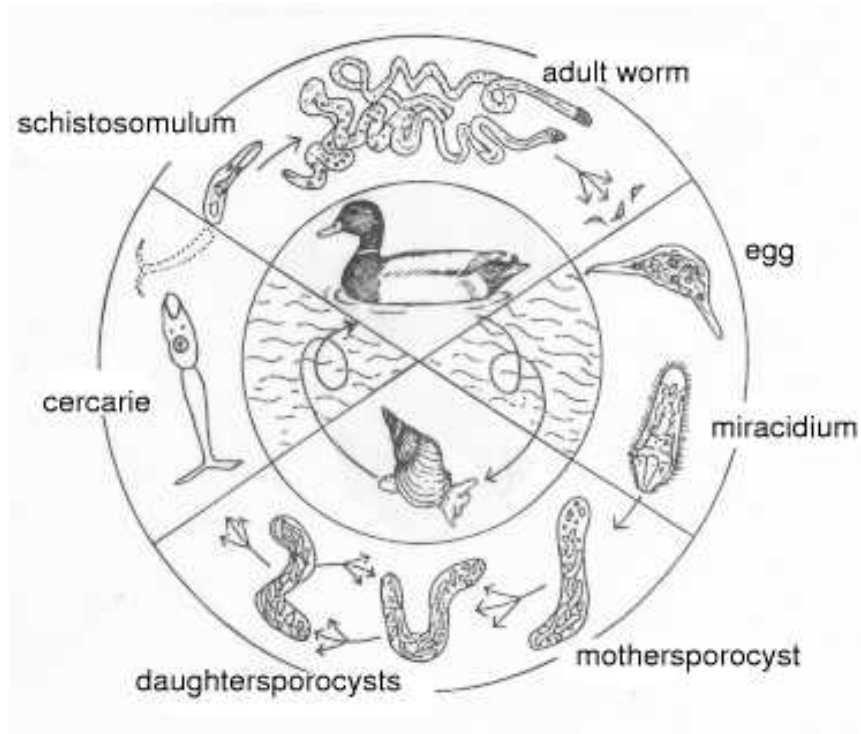


FIGURE 1. LIFE CYCLE OF *Trichobilharzia ocellata* (Haas et al., 2003)

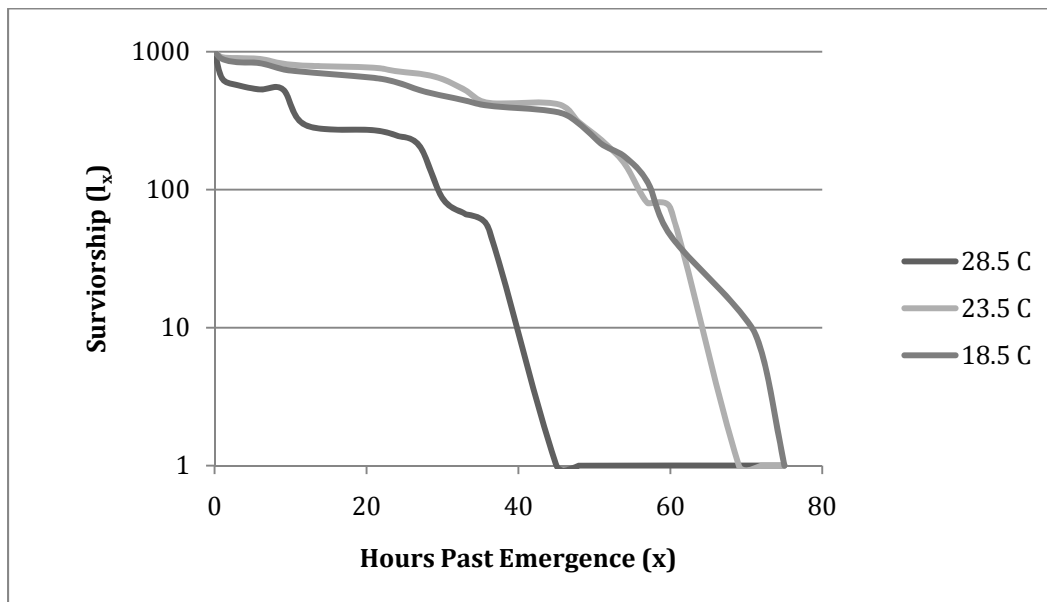


FIGURE 2. SURVIVORSHIP CURVE OF *T. ocellata* CERCARIAE IN DIFFERENT TEMPERATURE SETTINGS

TABLE 1. LIFE TABLE OF *T. ocellata* IN DIFFERENT TEMPERATURE SETTINGS

NOTE: n_x = # cercariae alive in each interval; l_x = survivorship of cercariae (n_x/n_0)

Hours since emergence (x)	28.5°C		23.5°C		18.5°C	
	n_x	l_x	n_x	l_x	n_x	l_x
0	150	1.000	150	1.000	150	1.000
1	96	0.640	137	0.913	133	0.887
3	86	0.573	135	0.900	126	0.840
6	80	0.533	133	0.886	124	0.827
9	80	0.533	123	0.820	112	0.747
12	44	0.293	119*	0.793	107	0.713
21	30	0.270	115	0.767	97	0.647
24	37	0.246	108	0.720	90	0.600
27	31	0.206	104	0.693	79	0.527
30	13	0.086	95	0.633	72	0.480
33	10	0.066	79	0.527	65*	0.443
36	8	0.053	64	0.427	61	0.407
45	0	0.000	63	0.420	55	0.367
48	-	-	46	0.307	45	0.300
51	-	-	34	0.227	32	0.213
54	-	-	23*	0.153	26	0.173
57	-	-	12	0.080	17	0.113
60	-	-	11	0.073	7	0.047
69	-	-	0	0.000	2	0.013
72	-	-	-	-	1	0.067
75	-	-	-	-	0	0.000

*Corrected data still indicated an increase in number of live cercariae during these time intervals. To construct a coherent life table, n_x for these intervals was instead calculated by averaging n_{x-1} and n_{x+1} .

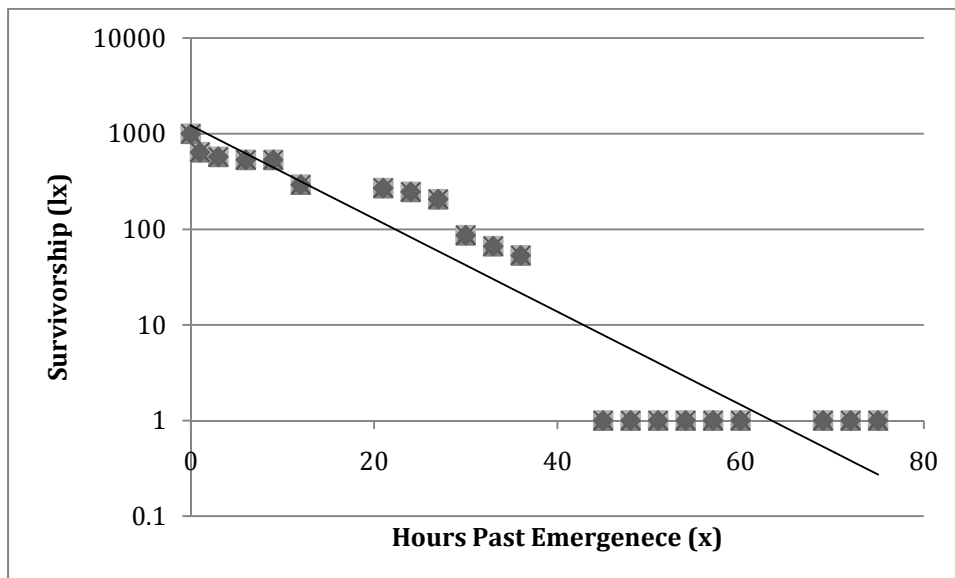


FIGURE 3. LD-50 FOR CERCARIAE IN 28.5°C: EXPONENTIAL REGRESSION ANALYSIS

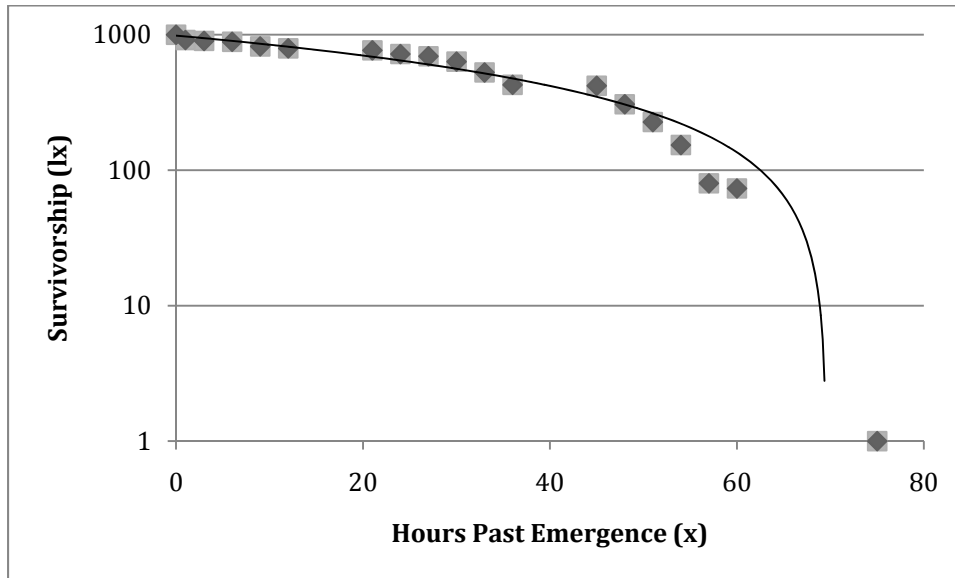


FIGURE 4. LD-50 FOR CERCARIAE IN 23.5°C: LINEAR REGRESSION ANALYSIS

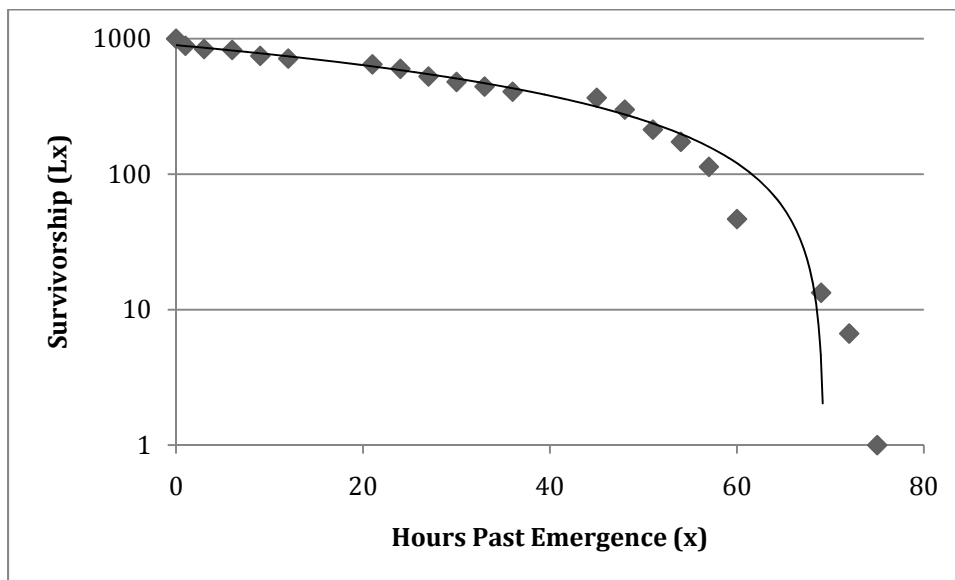


FIGURE 5. LD-50 FOR CERCARIAE IN 18.5°C: LINEAR REGRESSION ANALYSIS