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Effect of extreme temperature on egg development, larval and adult survival of *Chordodes nobilii* Camerano, 1901 (Gordiida, Nematomorpha)

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Abstract

On the basis of experimental studies we analyzed the effect of extreme temperatures on the aquatic free-living phases of the life cycle of *Chordodes nobilii* (Gordiida, Nematomorpha). Bioassays were performed with eggs, larvae, and adults. Eggs and larvae were exposed to 3°C below zero and 40.5°C; adults were exposed to 3°C below zero and to 38.5°C and 40.5°C. The results showed that egg development was inhibited in both tests. Exposed larvae showed a decrease in their infective capacity, which resulted almost null at 40.5°C. Adults exposed to 38.5°C showed mortality ≤10%, at 40.5°C the mortality was 100% and the adult's mortality at 3°C below zero was 89%. Results indicate that free living stages of *Chordodes nobilii* are susceptible to extreme temperatures.

Keywords

Nematomorpha, Gordiida, *Chordodes nobilii*, life cycle, extreme temperatures

Introduction

Many abiotic factors affect directly and indirectly freshwater organism causing no visible detrimental effects (Huggins and Harp 1983, Mason and Hominick 1995), temperature is one of them. Seasonal changes in freshwater environments such as ponds and streams, can reach extreme limits, from complete freezing to very hot temperatures in summer, like in Sierra de la Ventana (Argentina), where adults of *Chordodes nobilii* (Gordiida) have been collected during all seasons, the absolute maximum and minimum temperatures varies between 38.2–39.9°C (summer) and 7.7–9.8°C below zero (winter) (National Meteorological Services Information). These seasonal changes in temperature probably impacts on freshwater environments where free-life stages of Gordiida inhabit.

Although several studies have been developed on the complex life cycle of Nematomorpha over the past years (Schmidt-Rhaesa 2001, 2002; Bolek and Coggins 2002; Hanelt and Janovy 2003, 2004; De Villalobos *et al.* 2003; Zanca *et al.* 2007), the Gordiida is still a group that has received limited ecological or physiological study (Swanson 1982, Chandler 1985, Schmidt-Rhaesa 1997, Bolek and Coggins 2002). Tem-

perature is a principal factor influencing egg development and survival of free stages in Gordiida (Camerano 1897; Dorier 1925, 1930; Thorne 1940; Baer 1951; Cappucci 1976; Whittaker and Barker 1983 and Zanca *et al.* 2007). Camerano (1897) and Dorier (1925, 1930) analyzed, with different results, the thermal tolerances in different species of Gordiida. Taking into account the scarce data about the tolerance of Gordiida to extreme temperatures, the purpose of the present paper is to report results of experiments conducted with eggs, larvae and adults of *Chordodes nobilii* subjected to extreme temperatures. Using the information obtained from this study we hope to predict periods where gordiids may be susceptible to temperature related with their reproduction and mortality.

Materials and methods

Collection and maintenance of Chordodes nobilii

Adults of *Chordodes nobilii* were collected in late summer from El Negro stream (38°07'28"S, 61°45'32"W), Sierra de La Ventana, Argentina. The specimens were placed in 1000 ml plastic containers with well aerated water, at room tempera-

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ture ($23 \pm 1^\circ\text{C}$). After copulation the females were separated in individual containers. Egg strings were collected from each mated pair and rinsed in 1:250 Clorox bleach solution, as described previously (Hanelt and Janovy 1999) and placed into 50 ml plastic containers.

Bioassays were performed with eggs (mainly in blastula stage), larvae and adults. General bioassay conditions were selected based on preliminary trial (Achiorno *et al.* 2008).

Temperature test

Eggs: Twelve pieces of egg strings (3 mm in length each) from one of the females were stored in 12 ml plastic containers with well aerated water and observed with light microscope until most of them reached the blastula stage. Two exposure tests were carried out: one at $3 \pm 1^\circ\text{C}$ below zero and another at $40.5 \pm 1^\circ\text{C}$, for 96 hours. Both tests included control groups maintained at room temperature ($23 \pm 1^\circ\text{C}$). All tests were carried out in triplicate.

After the exposure time of each test the egg strings were maintained at room temperature with weekly observations of each replica ($n = 100$ eggs), during the following 30 days under light microscope (LM), in order to evaluate if the egg development was normal, retarded, interrupted or if the eggs were suffering some deterioration (e.g. collapse, wall rupture).

Larvae: Portions of egg strings from the selected female at room temperature ($23 \pm 1^\circ\text{C}$) were observed under LM during 20–25 days, until the larvae were completely developed (inside the egg shell as well as in hatched larvae). In order to determine the effect of extreme temperatures ($3 \pm 1^\circ\text{C}$ below zero and $40.5 \pm 1^\circ\text{C}$) during 48 hours on *Chordodes nobilii* larvae, the same test protocols used for the egg strings was followed. In order to evaluate the survival of *C. nobilii* larvae, taking into account that larvae do not show constant movement, once the exposure time ends, the larvae are placed at control temperature ($23 \pm 1^\circ\text{C}$). After 24 hours, experimental infections with larvae of *Aedes aegypti* (Diptera) were performed. Plastic capsules of 3 cm diameter each one containing 12 ml of well water, wheat germ as sediment and 30 larvae (L2 and L3 instars) of *A. aegypti* were used for this analysis. After being exposed for 72 hrs, mosquito larvae were fixed in 70% ethanol and then dissected under LM to search for *C. nobilii* larvae in the body cavity. This procedure was followed for each replicate.

The survival of *C. nobilii* larvae was determined by evaluation of their infective capacity with the Infection Index Mean Abundance (IIMA), as the total number of *C. nobilii* larvae divided by the total number of host (*A. aegypti* larvae) observed (Bush *et al.* 1997). These bioassays were considered valid when IIMA in the control group was ≥ 2 .

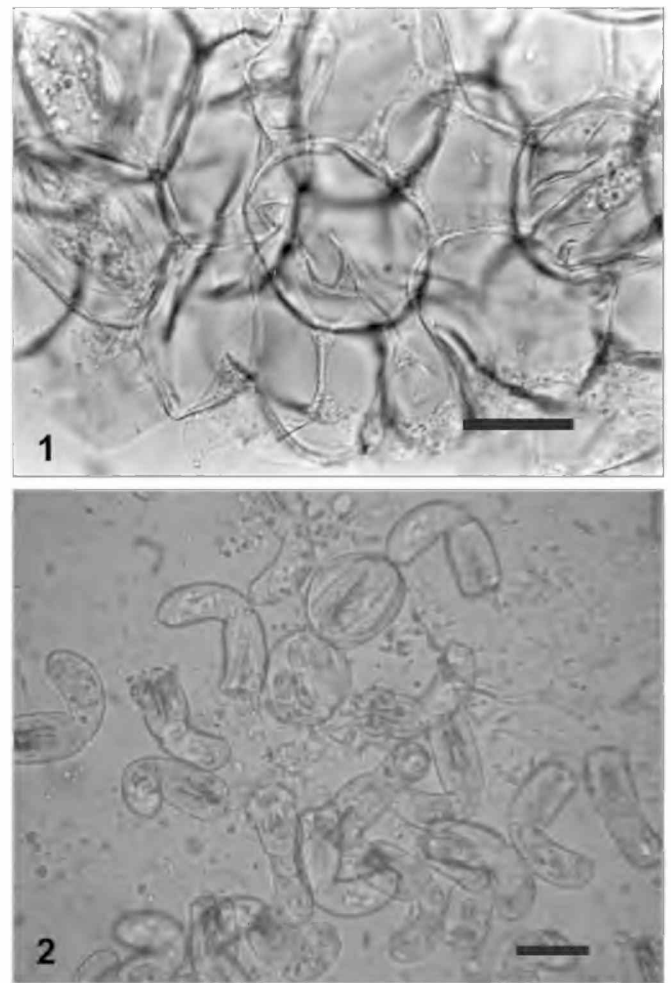
Adults: Two tests were carried out, at $3 \pm 1^\circ\text{C}$ below zero and at $38.5 \pm 1^\circ\text{C}$, during 96 hrs. The temperature chosen for the second test follows the results obtained by Dorier (1930) who observed that the movements of adults of *Gordius aquaticus*; *Gordionus violaceus* and *Parachordodes gemmatus* rapidly decreased when exposed at 37.5°C .

In both tests, forty males of *C. nobilii* were placed in four 500 ml glass containers each one with ten specimens. Two of the samples were taken as a control group and maintained at $23 \pm 1^\circ\text{C}$.

In order to test the survival at 38.5°C , adult specimens were observed four times, every 24 hrs, during 10–15 min. Viability was evaluated by their mobility and compared with the control groups. The adults that did not respond to mechanical stimulus were separated from the experimental units and placed at 23°C during 24 hrs. After this, the specimens that did not recover were considered dead to avoid confusion with sublethal effects.

As mortality in the specimens exposed during 96 hrs at 38.5°C was $\leq 10\%$, the survival specimens were exposed at $40.5 \pm 1^\circ\text{C}$, during other 96 hrs with daily observations to evaluate mobility.

In order to evaluate the survival of adults of *C. nobilii* exposed at 3°C below zero during 96 hrs, specimens were observed during 7 days post exposition at 23°C to determine whether they were recovering their normal activity (e.g.



Figs 1 and 2. 1. Non viable embryos in one of the samples from exposed eggs (30 days post exposition). Scale bar = $15 \mu\text{m}$. 2. Free living larvae and ready-to-hatch larvae within egg capsules in one of the samples from control eggs (30 days post exposition). Scale bar = $15 \mu\text{m}$

swimming, rolling). General bioassay conditions were selected based on a preliminary trial.

Data analysis

The Shapiro-Wilk test and the Levene median test were used to assess normality and homogeneity of variance of data, respectively.

For the assays with larvae the Student's t-test, $p < 0.05$ was used to check for significant differences between the control and treated group (Zar 1999). The null hypothesis (H_0) supposed that the IIMA media of the larvae exposed at 3°C below zero is equal to the IIMA media of the larvae of the control group.

Significant differences between the control and treated groups were determined for the tests with adults using one-way ANOVA with time as a covariable followed by Tukey's test after Ln transformation of the data (Zar 1999). The sig-

nificance level was set at $p < 0.05$. Statistical analyses were performed using Infostat software.

Results

From the results obtained during the observations of the eggs strings post exposition (30 days), both treatments at 3°C below zero and at 40.5°C interrupted their embryonic development and damaged eggs have been observed (egg membrane rupture and embryo retraction) (Fig. 1). The control group continued the egg development normally with over 50% proportion of free larvae after 30 days (Fig. 2).

The bioassays carried out with larvae showed that in spite of their exposure at 3°C below zero during 48 hrs, the *C. nobilii* larvae could infect the *A. aegypti* larvae, although a statistically significant decrease was registered with respect to

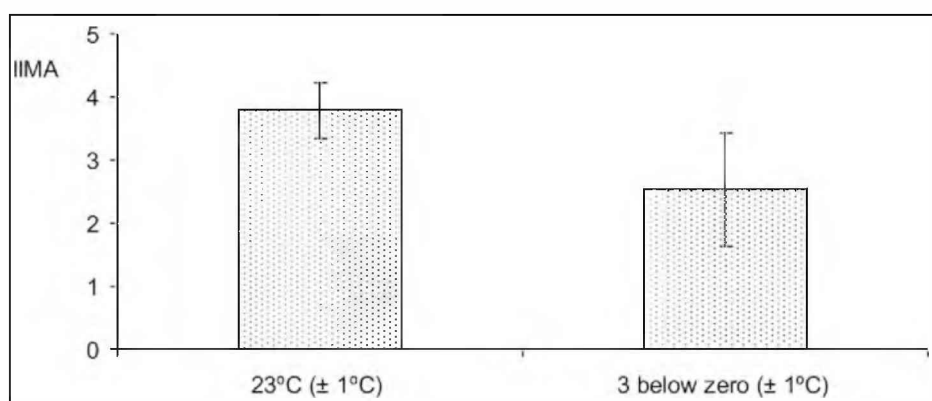


Fig. 3. Infection Index Mean Abundance (IIMA) \pm SD observed for the control and the $3^\circ\text{C} \pm 1^\circ\text{C}$ below zero in the larvae assays. Value expressed as arithmetic mean by treatment

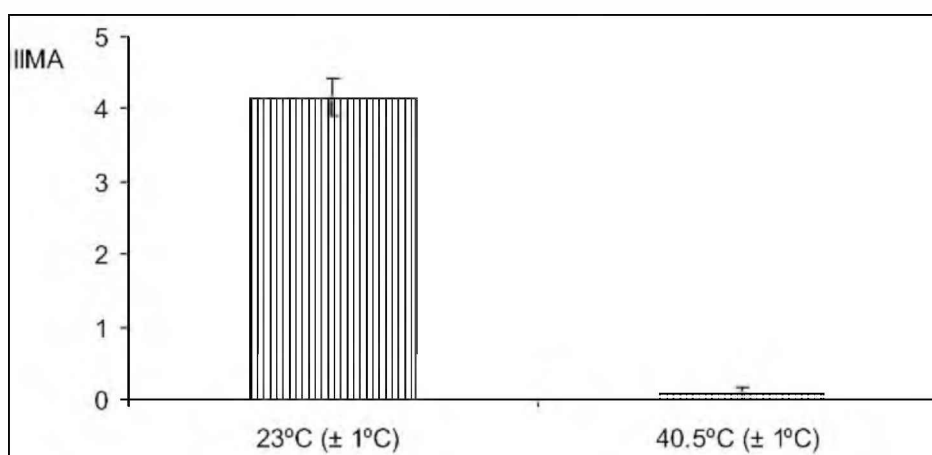


Fig. 4. Infection Index Mean Abundance (IIMA) \pm SD observed for the control and the $40^\circ\text{C} \pm 1^\circ\text{C}$ in the larvae assays. Value expressed as arithmetic mean by treatment

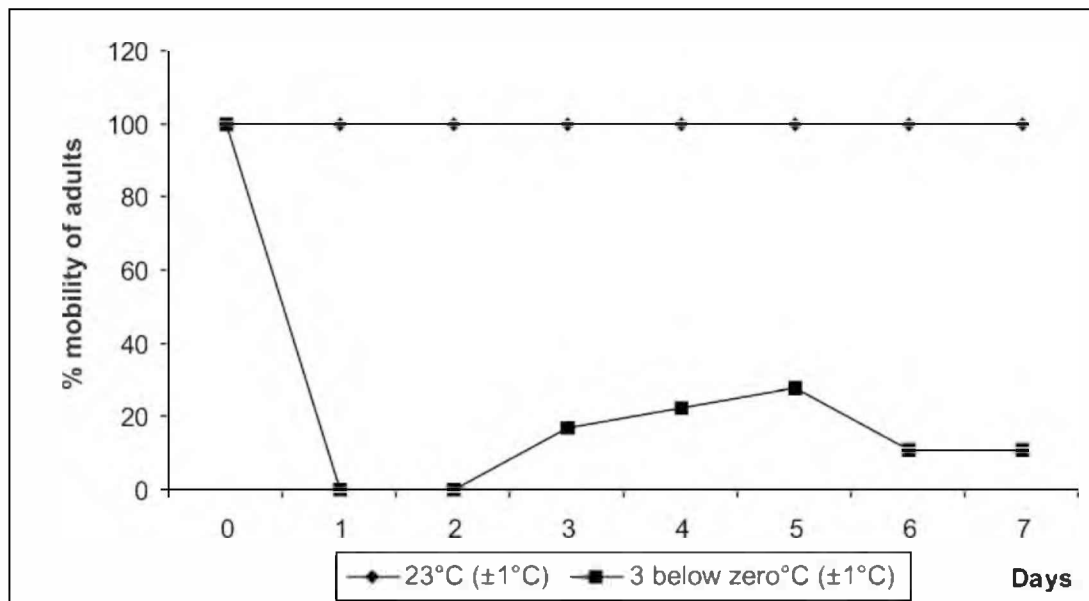


Fig. 5. Curve percentage of mobility in adults exposed to 3°C below zero (± 1°C) and the control 23°C (± 1°C), for the day 0 (pre-exposure) and for days after exposure (from day 1 to day 7)

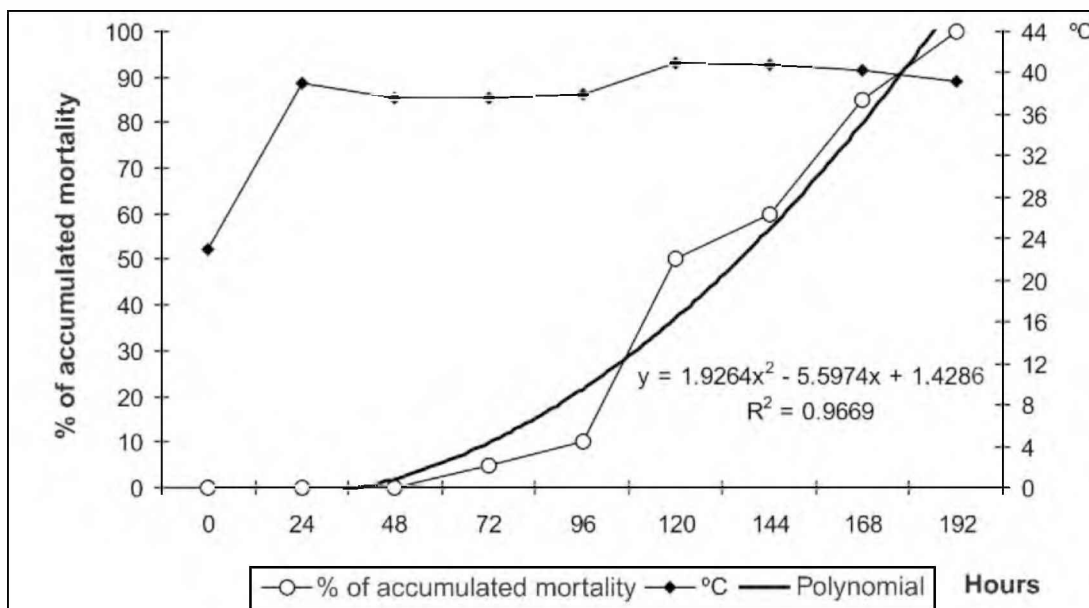


Fig. 6. Curve percentage of cumulative mortality for adults exposed to 38.5°C (± 1°C) and 40.5°C (± 1°C), respect to exposure time (in hours)

the control group (statistical $t_{0.05(2),2} = 4.53304148$) (Fig. 3). The larvae exposed at 40.5°C showed an important decrease in their infective capacity with a statistically significant difference (statistic $t_{0.05(2),2} = 31.38$) (Fig. 4).

The experimental studies with adults showed that 28% of the specimens exposed to 3°C below zero recovered their mobility 120 hrs post exposition. At day 7 only 11% of the specimens continued with mobility (Fig. 5). The control group showed normal movements. In the adult's bioassays at 38.5°C

during 96 hrs the specimens were found entangled in a knot with no mobility, but after 10 min worms recovered their normal activity. The mortality was 10%. with no significant differences with the control group. But when the temperature was increased in two grades (40.5°C), after the first 24 hrs an exponential increase of mortality of 50% could be observed, reaching 100% at the end of the exposure period (96 hrs) (Fig. 6). Median Lethal Temperature (TL50) occurs in the range between 38.5 and 40.5°C.

Discussion

The effect of low temperatures on the egg strings of *C. nobilii* was studied by Zanca *et al.* (2007) showing when maintained at 5°C they were capable of continuing their embryonic development. In our study we can prove for the first time that extreme temperature inhibits the embryonic development.

Dorier (1930) studied the effect of different temperatures on the mobility of *Gordius aquaticus* larvae. He observed that 50% of the larvae exposed at 37°C showed no mobility after two and a half hours and after thirty hours all of them were dead. Our observations of *C. nobilii* larvae exposed at 40.5°C showed that only a few larvae survived and could enter the corporal cavity of *A. aegypti* larvae.

There are previous reports on the effect of low temperatures on the survival of larvae. Hanel and Janovy (1999) found that *Gordius robustus* larvae stored in tap water at 4°C remained viable for 5 months. Zanca *et al.* (2007) reported that *C. nobilii* larvae were able to survive 6 months at 5°C. Our results are the first to evaluate the effect of the freezing temperature on *C. nobilii* larvae and allow us to determine that the larvae exposed at 3°C below zero can survive and are able to complete their life cycle in contrast to those exposed at 40.5°C.

The analyses carried out by Camerano (1897) on the thermal tolerances of adults in *Gordius aquaticus*, *Parachordodes tolosanus* and *P. pustulosus* showed that the mobility of the specimens decreases at 25°C, stops at 39°C, and at 44°C–46°C they die. Dorier (1930) considers that the adults of *Gordius violaceus* exposed at 37°C die after three days, while *G. aquaticus* and *Parachordodes gemmatus* die within 7–9 days.

In the adults of *C. nobilii* we observed that for the conditions tested the tolerance to temperatures exceeding 38.5°C significantly increased mortality of adults.

Our results indicate that aquatic free-living phases of the life cycle of *Chordodes nobilii* are susceptible to extreme temperatures, especially at 40.5°C.

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