Effect of temperature on growth, survival and reproduction of *Bulinus nyassanus* (Smith, 1877) (Mollusca: Gastropoda) from Lake Malawi

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***Bulinus nyassanus,* endemic to Lake Malawi, is an intermediate host for *Schistosoma haematobium* and plays an important role in transmission of this parasite along some shorelines on the Nankumba Peninsula in the southern part of the lake. Density of *B. nyassanus* in shallow water is usually low from January to April and a major population increase takes place during May to August when water temperatures are relatively low (about 22°C) and supposedly suboptimal for *B. nyassanus*. This study was designed to compare performance (growth, reproduction, survival, and hatchability of egg-masses) of *B. nyassanus* at four constant temperatures (22, 25, 28 and 31°C). Survival and reproduction were optimal at 25°C while growth and egg development were faster at the higher temperatures. Findings are discussed in view of the population dynamics of *B. nyassanus* at Cape Maclear.**

Key words: ***Schistosoma haematobium, Bulinus nyassanus,* survival, reproductive performance, Lake Malawi.**

INTRODUCTION

At Cape Maclear, on Nankumba Peninsula, in the southern part of Lake Malawi, urinary schistosomiasis due to *Schistosoma haematobium* is highly prevalent among local people and many tourists are infected with this parasite each year (Cetron *et al.* 1996; Madsen *et al.* 2004). Transmission of schistosomes in the lake along open shorelines is a relatively new development (Stauffer *et al.* 1997) with the primary intermediate host being *Bulinus nyassanus* (Madsen *et al.* 2001; 2004). This development is possibly the result of over-fishing of snail-eating cichlids (Stauffer *et al.* 1997).

Populations of *B. nyassanus* undergo great fluctuations in density over the year in the southern part of the Lake, especially in shallow (water depth <2 m) water (Phiri *et al.* 1999; Madsen *et al.* 2004). Populations increase from about April/May to reach a peak in September/October, but density can remain high until December/January when great reductions in density usually occur in shallow water, possibly as a result of strong wave action from the north-northwest (Madsen *et al.* 2004). With variation from year to year, population density can be very low during the period January–April especially in the shallow waters (Madsen & Stauffer unpublished). During this period water temperature is 28–29°C, which should be acceptable for breeding as judged from studies on other *Bulinus* species (Shiff 1964; O'Keeffe 1985; Joubert *et al.* 1986). It is interesting therefore that the most marked population growth in *B. nyassanus* occurs in June/July when water temperatures are relatively low, i.e. 22–23°C (Eccles 1974; Halfman 1993; Chavula *et al.* 2009).

This study was designed to test if *B. nyassanus* differs in its temperature preferences from those of other *Bulinus* species. Hence, the performance (survival, growth, hatchability and reproduction) of *B. nyassanus* at four constant temperatures (22, 25, 28 and 31°C) was compared.

MATERIALS & METHODS

Snails

Snails were collected from Cape Maclear on the Nankumba Peninsula, close to the southern end of

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Lake Malawi (see map in Madsen *et al.* 2004), at a water depth of 2–3 m to minimize the risk of capturing *S. haematobium* infected snails. Snails were transported to Bunda College, University of Malawi, where they were checked for patent trematode infections by the shedding procedure (Frandsen & Christensen 1984) and uninfected snails were placed in 30 l fibreglass tanks containing dechlorinated tap water at 20–23°C for one week. Water was continuously aerated and changed every second day. Snails were fed on a locally formulated feed containing 30% crude protein (Lundeba *et al.* 2006).

Experimental tanks and setup

The experiments were conducted in four waterbaths set in fibreglass tanks at 22, 25, 28 and 31°C. Aerators were placed in each waterbath to ensure homogeneous temperatures throughout each bath. Since waterbath temperature was affected by ambient temperature, temperatures were checked twice daily and heaters adjusted to ensure relatively constant temperatures.

Growth, survival and reproduction

Snails with shell heights of 5.31–5.64 mm and live body mass of 40.0–42.0 mg were used for growth studies; however, larger snails (live mass 200–310 mg; shell height 7.34–8.76 mm) were used for reproductive studies. Prior to each experiment, 100 snails were acclimatized to each of the four temperatures (20 snails in each of five beakers with 2 l of water at each temperature) for one week. From the surviving snails at each temperature, 75 snails were randomly selected and distributed among five beakers (15 snails in each).

Snails were fed daily in excess of consumption. The amount given per snail was the same for all groups, thus the amount of feed was adjusted relative to the number of surviving snails. Excess food from the previous day was removed by siphoning before feeding.

Prior to stocking, body mass to the nearest 0.1 mg and shell height to the nearest 0.01 mm for individual snails were determined using an electronic balance and vernier callipers, respectively. For the growth experiment, shell height and body mass were measured every two weeks for 12 weeks. In the reproduction trial, egg-masses were removed at three-day intervals for a total of 33 days, and the eggs in each were counted.

Water temperature, pH, dissolved oxygen, and conductivity were measured daily throughout the study period, while total ammonia-nitrogen was monitored twice a week using standard methods (Tucker 1993).

Egg hatchability and development

Five 1 l beakers were placed in each of the four waterbaths. Variable numbers of eggs collected within 30-minute intervals after deposition were incubated at the corresponding temperatures. Three to five egg-masses were kept in each beaker. Water volume was maintained constant at 100 ml/egg-mass. Beakers were then monitored daily to establish the time taken for the eggs to hatch. Hatched and unhatched eggs were counted using a stereo-loupe for each beaker. In a separate but similar study, embryo size was measured at intervals of two hours for the first 10 h and then at 24-h intervals until hatching. From each of egg-masses in a beaker, two randomly chosen embryos were measured at each time point, using an ocular scale in a stereo-loupe.

Data analysis

Reproductive output as a 'partial net-reproductive rate' (R_0) for each group of snails was estimated as $\sum l_x \times m_x$ where l_x is the proportion of snails still alive after three-day periods (x) and m_x is the number of eggs laid per snail during the three-day period. Embryo size, hatchability (arcsine transformation of proportion hatched), time at first hatch, live mass, and reproduction in *B. nyassanus* were compared among temperatures using one-way analysis of variance (for embryo size and snail mass one analysis was made for each time point). Distribution of these variables was checked graphically for normality. Pairwise comparisons were made with the Bonferoni post-hoc test. Survival functions were analysed following procedures described in Kalbfleisch & Prentice (2002). *P*-values <0.05 were taken to indicate significant differences.

RESULTS

Growth, survival and reproduction

Growth was slowest at 22°C, while differences between the three other temperatures were not significant (Fig. 1). Survival, however, was optimal at 25°C ($P < 0.001$) and did not differ significantly among the other three temperatures. Median times to reach 25% mortality were 42, 56, 14 and 28 days at 22, 25, 28 and 31°C, respectively. During the first two weeks, mortality was high at 28°C but

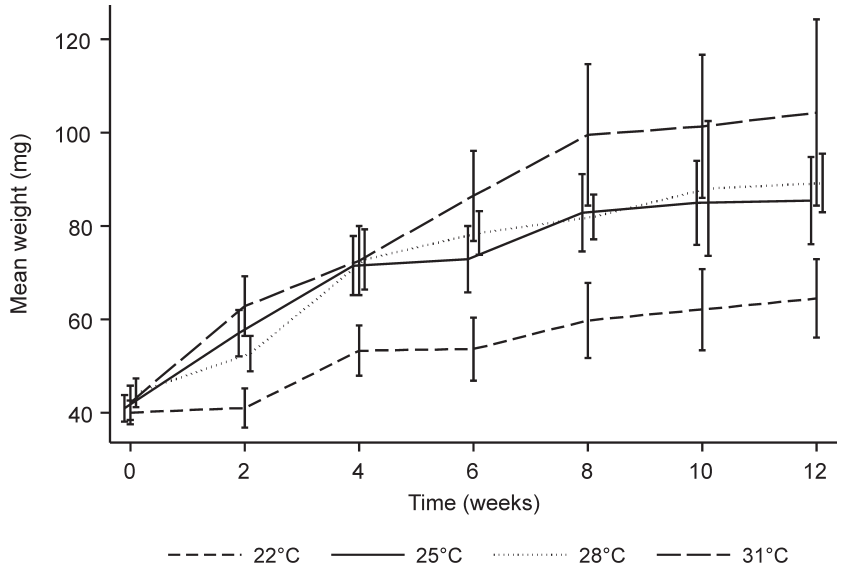

Fig. 1. Changes in mean body mass (mg ± 95% CL) of *Bulinus nyassanus* at two-week interval at four constant temperatures.

for the remaining period, survival was similar to that at 31°C. The mean pH (±1 S.E.) decreased with temperature and ranged from 6.38 ± 0.02 at 31°C to 7.21 ± 0.02 at 22°C, while mean conductivity (±1 S.E.) increased with temperature from 173 ± 3 μs/cm at 22°C to 244± 8 μs/cm at 31°C. Mean total ammonia increased (±1 S.E.) with temperature from 2.14 ± 0.13 mg/l at 22°C to 3.78 ± 0.22 mg/l at 31°C. Mean oxygen concentration (% saturation ± 1 S.E.) was above saturation across treatments and ranged from 109.60 ± 2.22 % at 25°C to 115.40 ± 2.08 % at 31°C. While these water parameters differed significantly ($P < 0.05$) across temperatures, oxygen saturation did not.

Snails laid less than one egg-mass per three-day period irrespective of the temperature (Table 1). The mean number of eggs per snail (Table 1) did not differ significantly across temperatures. Net reproductive rate was lower ($P < 0.01$) at 22°C than at the other three temperatures. Survival of these snails was best at 25°C ($P < 0.001$) while differences between the other three temperatures were not significant. Oxygen saturation (mean 124–125%) and pH (mean: 8.13–8.19) did not vary significantly across treatments, while mean conductivity (±1 S.E.) increased ($P < 0.001$) with temperature, i.e. 190 ± 10 μs/cm at 22°C and 850 ± 40 μs/cm at 31°C. Similarly, mean total ammonia (±1 S.E.) increased with temperature up to 28°C, i.e. 0.05 ± 0.01 mg/l at 22°C and 0.23 ± 0.03 mg/l at 28 and 31°C, respectively.

Hatchability and development of eggs

Hatchability (% ± 1S.E.) was 91.12 ± 2.09% at 31°C, 88.96 ± 2.17% at 28°C, 88.65 ± 1.24% at 25 °C and 67.11 ± 3.71% at 22°C. Hatchability of eggs at

Table 1. Mean number of eggs/egg-mass (±1 S.E.), egg-mass/three-day period, eggs/snail/three-day period, 'net reproductive rate' (no. of eggs) and mortality (days until 25% mortality) of adult *Bulinus nyassanus* kept at different constant temperatures for 33 days. Means with different superscripts in the same column are significantly different at $P < 0.05$.

Water temperature (°C)	No. of eggs/egg-mass	No. of egg-masses/ snail per 3-day period	No. of eggs/snail per 3-day period	'Net reproductive rate'	Mortality (days for 25% mortality)
22	12.71 ± 1.04^{ab}	0.688 ± 0.077	8.54 ± 1.18	58.67 ± 10.51^{a}	9
25	15.33 ± 0.79^{b}	0.630 ± 0.054	10.01 ± 1.33	92.85 ± 11.24^{b}	30
28	12.90 ± 0.72^{ab}	0.891 ± 0.167	12.68 ± 3.10	89.59 ± 19.68^{b}	9
31	10.69 ± 1.01^{a}	0.857 ± 0.064	11.20 ± 1.15	90.91 ± 13.19^{b}	12

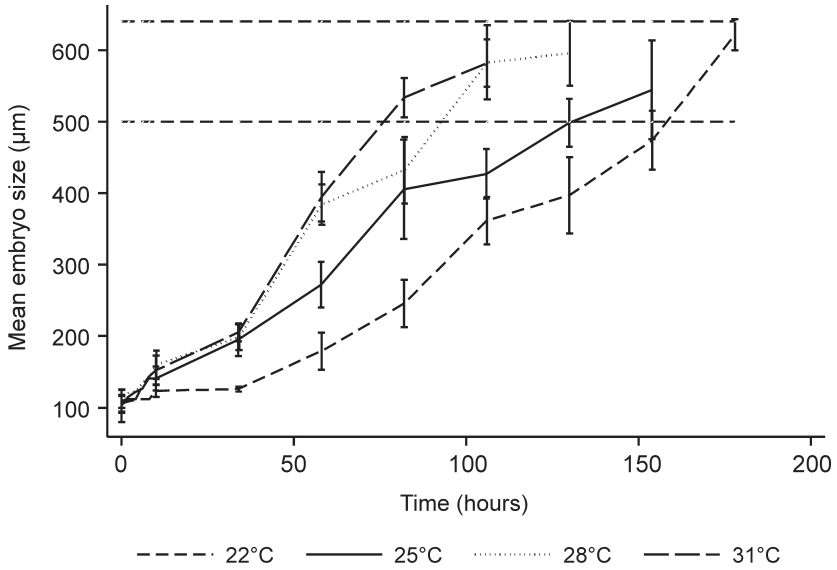

Fig. 2. Mean embryo size (µm ± 95% CL) of *Bulinus nyassanus* kept at four different temperatures over time. The horizontal dashed lines show the potential size range at hatching (size range of all newly hatched snails from all temperatures combined).

22°C was significantly lower than that observed at the three higher temperatures ($P < 0.05$) while differences among the three highest temperatures were not significant. Mean pH values (±1 S.E.) ranged from 7.75 ± 0.06 at 22 and 25°C to 8.35± 0.17 at 28 and 31°C and were significantly higher ($P < 0.05$) at 28 and 31°C than at the two lower temperatures. Mean conductivity (±1 S.E.) increased with temperature from 223 ± 17 μs/cm at 22°C to 395 ± 32 μs/cm at 28°C. Conductivity values were significantly lower at 22°C ($P < 0.05$) than at the three higher temperatures, while differences among the three higher temperatures were not significant.

The increase in embryo size was in the order 22 < 25 < 28 < 31°C, with a considerably lower rate of development at 22°C (Fig. 2). Significant differences appeared after 6 h with embryos kept at 22°C being smaller than those kept at 28°C and after 58 h all pairwise comparisons except 28 compared with 31°C were significantly different. Mean time to first hatch (±1 S.E.) of *B. nyassanus* eggs decreased with increasing temperature as follows: 12 ± 0.23 days at 22°C, 6.80 ± 0.24 days at 25°C, 5.85 ± 0.17 days at 28°C, and 5.0 ± 0.18 days at 31°C.

Eggs with two embryos were commonly deposited by *B. nyassanus* at all experimental temperatures. Most egg-masses contained only eggs with a single embryo, while some egg-masses with a total of 12–36 eggs could contain two to four eggs with two embryos. A few exceptionally big egg-masses (with 56–60 eggs) contained 7–12 eggs with two embryos. Interestingly, when these eggs were incubated and monitored at the respective temperatures, the two embryos developed and eventually hatched into two separate snails of which the shell height at day zero ranged from 570 μm and 630 μm which is within the range of snails hatching from eggs with a single embryo.

DISCUSSION

The optimal temperature for population increase of *B. nyassanus* is 25°C. Although growth and egg production are higher and egg development faster at higher temperature, survival is reduced at those temperatures and therefore the 'net reproductive rate' is optimal at 25°C. The results therefore do not provide evidence that *B. nyassanus* should differ in its temperature preference from those of other *Bulinus* species (Shiff 1964; O'Keeffe 1985; Joubert *et al.* 1986). The reproductive output of *Bulinus nyassanus* at 31°C was similar to that observed at 25 and 28°C and is in contrast to other studies. Chernin (1967) associated poor egg laying in *Biomphalaria glabrata* at 30°C to pathological changes (gonadal atrophy) in the snails' reproductive system. Our study, however, differs in that we used field-collected adult snails which had acclimatized to the experimental temperatures for only one week before we started quantifying reproduc-

tive output. It is likely, therefore, that differences would be more pronounced if snails had been maintained at these temperatures throughout their life. Transferring snails to the laboratory may have stressed them and induced them to devote more energy to egg laying within the first days of exposure (especially at the high temperatures). In fact, we observed that body composition of snails at the end of the study had changed compared to that of field-collected snails; especially lipid content had declined (data not shown).

Hatchability of *B. nyassanus* eggs increase with temperature, while the time taken to first hatch decrease with increasing temperature. These results agree with findings from several other authors (de Kock & van Eeden 1986; Aziz & Raut 1996). Hatchability was not affected by the high temperature (31°C) and a similar observation was made by Appleton (1977) for *Biomphalaria pfeifferi.* Bayomy & Joose (1987) also found that egg-masses of *Bulinus truncatus* tolerated a temperature of 30°C. Although eggs with two egg cells have been reported for other pulmonate snail species (Bondesen 1950), it is interesting that for *B. nyassanus* both egg cells in a single egg developed into embryos and eventually hatched into viable snails (we did not, however, follow longer-term survival of these snails).

In Lake Malawi, transmission of *S. haematobium* may occur when the density of *B. nyassanus* is high in shallow water (water depth less than about 1.5 m) close to the shore and this occurs primarily (perhaps exclusively) in the southern part of the lake (Nankumba peninsula) along shorelines facing north or northwest (Madsen *et al.* 2004). These shorelines are well protected from wave action during the winter months (June–August) when the winds are predominately southerly. This is the period when *B. nyassanus* density increases markedly in the shallow water and population density remains high until November/December when great reductions can occur possibly due to wave action from the north (Madsen *et al.* 2001). During the beginning of the rainy season, size distribution of *B. nyassanus* indicates that reproduction occurs in deeper water, but in the last part of the rainy season population density declines in the shallower water (Madsen *et al.*, unpubl.). This could be because of storms but possibly also other factors related to the season, e.g. increased turbidity, local changes in water chemistry. After the rainy season, snail breeding takes place in the deeper water first and later in the shallow water as snails migrate into that area. During the cold-dry season young snails are commonly collected (Madsen *et al.* unpubl. data). During the hot-dry season relatively few young snails are collected, perhaps because temperature is above optimal or survival of young snails is reduced although this is not supported by our current findings.

In conclusion, *B. nyassanus* responds to temperature in a similar manner as reported for other *Bulinus* species and therefore other factors must be more responsible for population fluctuations in Lake Malawi and should be further explored.

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