The effect of constant low temperatures on the survival of *Bulinus africanus* (Krauss), *Bulinus globosus* (Morelet) and *Biomphalaria pfeifferi* (Krauss)

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The tolerance to low temperature of the freshwater snail species *Bulinus africanus, Bulinus globosus* and *Biomphalaria pfeifferi* was experimentally investigated. Snails were exposed to temperatures ranging from 0 °C to 8 °C and their survival was noted daily. The results showed that the survival of the cohorts of all three species decreased as the temperature was lowered in the range investigated. Evidence is presented which demonstrates that *B. africanus* can withstand cold conditions better than *B. globosus*, and that *B. globosus* is better equipped than *B. pfeifferi* in withstanding low water temperatures.

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Die temperatuurverdraagsaamheid van die varswaterslakspesies *Bulinus africanus, Bulinus globosus* en *Biomphalaria pfeifferi* is eksperimenteel ondersoek. Slakke is aan temperature wat vanaf 0 °C tot 8 °C gestrek het, blootgestel en hulle oorlewing is daagliks aangeteken. Die resultate toon dat die oorlewing van die kohorte van al die slakspesies afgeneem het namate die temperatuur in die ondersoekte reeks verlaag is. Getuienis word voorgelê wat toon dat *B. africanus* beter toegerus is om koue toestande te oorleef as *B. globosus*, wat in hierdie verband op sy beurt beter toegerus is as *B. pfeifferi*.

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Regional temperatures exert a fundamental influence on the geographical distribution of certain freshwater snail species (Brown 1980). Whereas water temperatures are largely dependent on climate, local water temperatures can also be influenced by other factors which are not dependent on climate, such as the differences that occur in climatic transitional areas which coincide with the boundaries of the distribution of intermediate host snails of *Schistosoma* spp. In these transitional areas habitats may occur which, in an otherwise unfavourable climate, create localized areas favourable for snail infestation. In the upper reaches of the Harts and Mooi Rivers, such habitats exist and two of the three snail species discussed in this paper are from that region.

Studies have been carried out on the temperature tolerance of freshwater snail species at temperatures corresponding to their optimum temperature (Shiff 1962, 1964, 1966; De Kock 1973). However, survival of local snail species at low temperatures had not been investigated and any attempt to establish the likelihood of colonization of new areas must take such a potential habitat extreme into consideration. In this study, survival at constant low temperatures ranging from 0 °C to 8 °C for *Bulinus africanus* (Krauss), *Bulinus globosus* (Morelet) and *Biomphalaria pfeifferi* (Krauss) was investigated.

Method

Eggs laid in the laboratory by snails brought in from a veld habitat were collected, hatched and reared to maturity for experimental use. The parental snails of *B. africanus* originated from the 'Bovenste Oog' (uppermost source) of the Mooi River in the district of Ventersdorp (26°12′S/27°10′E). The parental snails of *B. globosus* originated from the outdoor holding tanks of the Bilharzia Field Research Unit of the South African Medical Research Council at Nelspruit (25°28′S/30°58′E) while those of *B. pfeifferi* were obtained from a pond at the Game Breeding Farm of the National Zoological Gardens of South Africa which is situated in the municipal area of Lichtenburg (26°08′S/26°10′E).

An aquarium system with continuously circulating water, similar to the one described by De Kock & Van Eeden (1980), was used for the breeding and housing of experimental animals. The temperature in the aquaria was kept constant by Lauda ultra thermostats each equipped with a heater and a cooling spiral. During the experiments, cohorts of 15 snails in the case of *B. globosus* and 25 snails of the other two species were kept in the aquaria in compartments approximately 10 cm in depth, containing about 4 400 cm^3 water. The daily changing of water prevented possible negative effects of over-

population (DeWit 1954; Chernin & Michelson 1957; Coles 1973; Liang 1974).

Borehole water that had been biologically conditioned in a pond in a nearby botanical garden was filtered through a fine gauze net and used in all the aquaria. The water in the aquaria was continuously aerated and the electrical conductivity kept at a level of 350 – 500 μS by the periodic addition of the conditioned borehole water. This range of electrical conductivity was found to be favourable for these three snail species by De Kock (1973) and Jennings (1976). The snails were kept, maintained and fed as described by Jennings, De Kock & Van Eeden (1970) and De Kock & Van Eeden (1981).

During the experiments a control cohort of snails was kept at a higher, more favourable temperature of 24 °C to ensure that deaths were the result of lowered temperature and no other variable.

Snails were put into a 500 ml glass beaker filled just beforehand with water from the holding aquarium. The beaker with snails was then put in water at the experimental temperature where it assumed that temperature over a period of about 2 h. The snails were then released in the experimental aquarium. By following this procedure the physiological shock of a sudden transfer at the start of an experiment was lessened.

Criterium of death was the lack of response to mechanical stimuli. This was observed under the microscope. If examination revealed movement then the snail was returned to the experimental regime. Snails evaluated to be dead were subsequently placed in optimal conditions (24 °C) and observed. This procedure was used to confirm initial observations.

Results and Discussion

The survival of *B. africanus, B. globosus* and *B. pfeifferi*, kept at the various low temperatures, is indicated in survival graphs in Figure 1. The most important characteristics of survival are the following: (i) the time elapsed before any of the specimens died, for example in the case of *B. africanus* which survived for 102 h at 8 °C without any of the snails dying; (ii) the time elapsed until all the members of the cohort were dead, as in the case of *B. africanus* where the last members died after about eight days at 8 °C; (iii) the pattern followed by survival between these two extremes.

The survival of the cohorts of all three snail species became poorer as the temperature decreased. Although all three species showed the same tendency, it is clear that the experimental temperatures were almost always more unfavourable for *B. pfeifferi* than for either *B. africanus* or *B. globosus*. At 6 °C, the time elapsed until the first deaths occurred, and the time elapsed until the whole cohort died out, were shorter by nearly half for *B. pfeifferi* than for the other two species.

Survival of individual snails in a cohort differed even though all members of a cohort were of the same age, were descended from the same parental snails and were subjected to strictly standardized experimental procedures. For example, some snails survived for shorter or longer periods than the survival tendency, such as *B. globosus* at 2 °C, *B. pfeifferi* at 4 °C and, to a lesser degree, *B. africanus* at 4 °C and 6 °C (Figure 1). To lessen the effect of this inherent variation on the interpretation of the data, the 50% survival time (LT_{50}) was plotted for every experimental temperature and snail species. Regression lines drawn through these co-ordinate points and their equations are given in Figure 2. The good fit ($100R^2 > 88\%$) shows that these regressions adequately describe the survival of snails at low temperatures.

No deaths were recorded in any of the control cohorts in any of the experiments. Consequently, it is assumed that the increasingly poorer survival that was observed can be attributed to the experimental temperatures. It appears that none of the three snail species could survive in nature at these temperatures. In fact *B. africanus, B. globosus* and *B. pfeifferi* are known to thrive in relatively warm water. Although the habitat from which *B. africanus* originated, is more temperate than the typical Highveld habitat in which this snail species sporadically occurs, the results indicate that *B. africanus* is nevertheless

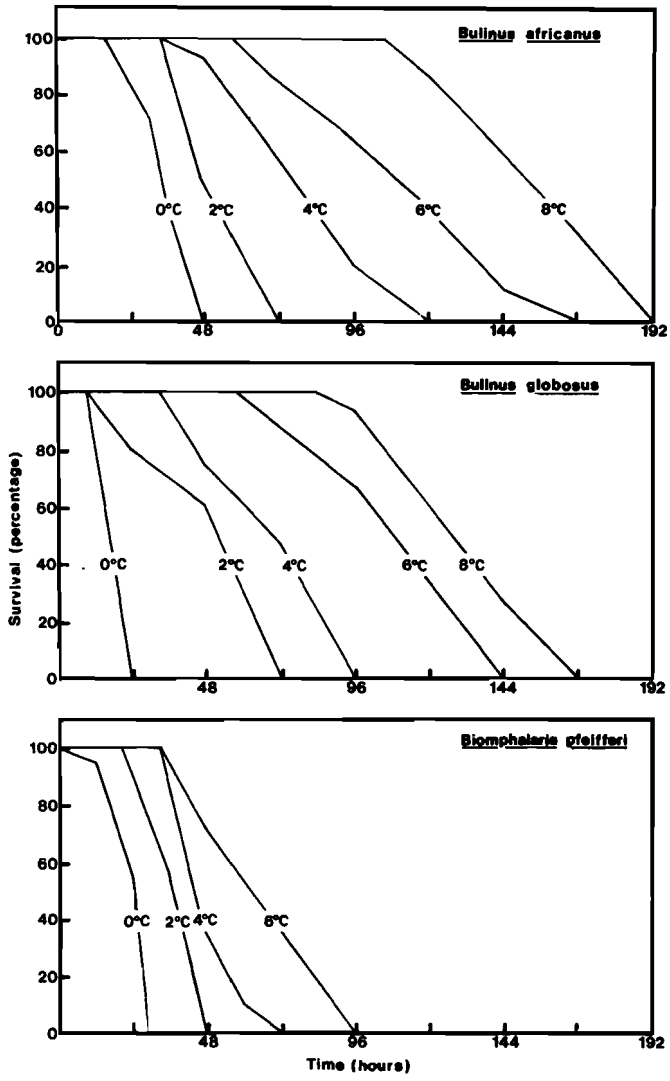

Figure 1 Percentage survival of *B. africanus, B. globosus* and *B. pfeifferi* after sustained exposure to various low temperatures.

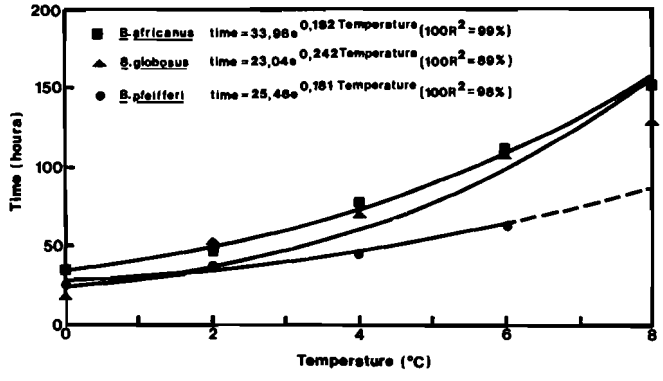

Figure 2 The relation between the time it took for 50% of the snails to die (LT_{50}) and the temperatures to which they were continually subjected.

better equipped to survive low temperatures than the other two species studied. In contrast, *B. pfeifferi* originated from a habitat which is colder on average than the habitats where this species is normally found. It is therefore not unrealistic to presume that the snails used in these experiments had been more acclimatized than usual to cold in the case of *B. pfeifferi*, and more acclimatized than usual to heat in the case of *B. africanus*. The difference in survival would probably have been larger had the parental snails originated from their more endemic habitats.

It is unlikely that the low temperatures used in this study occur for extended periods in the Republic of South Africa. The possibility is not excluded, however, that water temperatures may drop to 0 °C during cold winter nights on the Highveld. Winter also coincides with the dry season in these areas, with the result that pools of water where the snails exist may become so shallow that refuge at warmer depths no longer exists. Water surfaces may freeze over occasionally during winter. It is therefore possible that the absence of snails in otherwise suitable habitats in those areas of the Highveld where they have not yet occurred, is due to the low temperatures that prevail during some winter nights.

The theoretical survival of *B. africanus* and *B. globosus* between 0 °C and 8 °C and of *B. pfeifferi* between 0 °C and 6 °C can be calculated from the information in Figure 2. These findings now make it possible to determine the survival of these three very important freshwater snail species at low temperatures. Should these snails spread from their endemic areas to other unoccupied areas where low temperatures may occur for part of the year, their chances of permanent occupation can be evaluated from the information obtained in this study.

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