SHORT NOTES

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THE EFFECT OF SODIUM NITRATE ON THE GROWTH AND SURVIVAL OF TOAD TADPOLES (*BUFO BUFO*) IN THE LABORATORY

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Amphibians have been proposed as potential biological indicators of pollutants, because of their biphasic lifestyles and permeable skins (Blaustein & Wake, 1990), and yet there is relatively little information available regarding their susceptibility to chemical hazards (Hall & Henry, 1992). Nitrate in water can contribute towards eutrophication (Mason, 1991), and laboratory tests have shown that it can also be toxic to invertebrates (Camargo & Ward, 1992) and fish (Westin, 1974). It is not known whether nitrate has an effect on amphibians, but it has been suggested that anions may adversely affect larval growth (Ireland,1991). The present experiment investigated the effect of a nitrate salt on the growth and survival of tadpoles of the common toad (*Bufo bufo*).

Sections were taken from six spawn strings deposited in a pond in Northamptonshire, England. These sections were allowed to hatch in a plastic tub, filled with tap water, and placed outdoors. After hatching, larvae were captured on a random basis, and total length (TL) was measured. Thirty-nine larvae, each measuring 10 mm TL, were then selected from the random sample. At this time all larvae had attained Gosner stage 25. The selected larvae were assigned randomly to one of three groups, each group being reared in different water conditions. The first group was grown in distilled water only, as a control. The remaining experimental groups were grown in two different concentrations of sodium nitrate solution. These two treatments were made up by dissolving sodium nitrate in distilled water to make solutions of 40 and 100 ppm of nitrate ion. The 40 ppm level was chosen because this is similar to the maximum recorded levels in ponds on agricultural land in Northamptonshire (unpubl. data) at times of the year when amphibian larvae can be found in the water. The higher level of 100 ppm was chosen in order to magnify any effects that may have been too subtle to detect at lower levels. The pH of the distilled water and sodium nitrate solutions was measured, to ensure that variation in levels of sodium nitrate was not confounded by a pH effect on larval growth. The pH fluctuated between 5.57 and 7.47. Distilled water had the lowest pH values, whilst adding sodium nitrate increased pH. The maximum difference between the pH of distilled water and the high nitrate condition on any single recording period was 1.0.

The tadpoles were reared individually. Each tadpole was grown, in a clear, food-quality, plastic beaker, containing 500 ml of either distilled water or nitrate solution. The beakers were placed on plastic trays in a blocked arrangement to ensure that any positional effects were evenly spread over all three treatments. Water temperature fluctuated between 19°C and 24°C. The solutions or water were replaced after seven and thirteen days. The plastic beakers were also changed at these intervals to prevent algal growth. The total length (TL) of each tadpole was measured on these days. Tadpoles were fed a food pellet (0.02 g JMC Aquatics cat fish pellet food) on days 0, 7 and 13. There was always some food left in the beakers at the time of replacing the beakers, and so growth was assumed not to be limited by food. The tadpoles were exposed to a natural photoperiod via the laboratory windows.

The growth of tadpoles in the three groups is shown in Fig. 1a. An analysis of variance of total length at seven days was carried out using Genstat (Genstat 5 Committee, 1987). Block effects are significant ($F_{12,15}=2.98$, $P<0.05$), and when these are removed, there is a significant treatment effect, $F_{2,15}=79.71$, $P<0.001$. Tukey tests, as described in Zar (1984), show that tadpoles in the control group are significantly bigger than those in the 40 ppm nitrate group ($q=9.177$, $P< 0.05$) and that tadpoles in the 40 ppm nitrate group are significantly bigger than those in the 100 ppm nitrate group ($q=6.557$, $P< 0.05$). Survival of tadpoles in the three groups is shown in Fig. 1b. To test whether a greater proportion of tadpoles in the nitrate solutions had died compared to those in distilled water, a chi-square analysis of survivors and mortalities on day 13 (the day on which the last larva in the 100 ppm nitrate solution died) was performed. Data from the two nitrate concentrations were pooled. Mortalities were significantly more frequent in the nitrate solutions than in distilled water, $\chi^2=23.4$, $P<0.001$ (with Yates' correction).

Sodium nitrate solution appears to have clear, adverse effects on the growth and survival of *Bufo bufo* tadpoles grown in the laboratory. Sodium nitrate caused a decrease in the rate of growth and caused higher mortality than was found in tadpoles grown in distilled water. The magnitude of the effect of sodium nitrate on growth may have been exaggerated by the growth of a *Saprolegnia* fungus on uneaten food pellets. The tadpoles in the nitrate solutions tended to feed less than those in distilled water, allowing the fungus to grow on food that

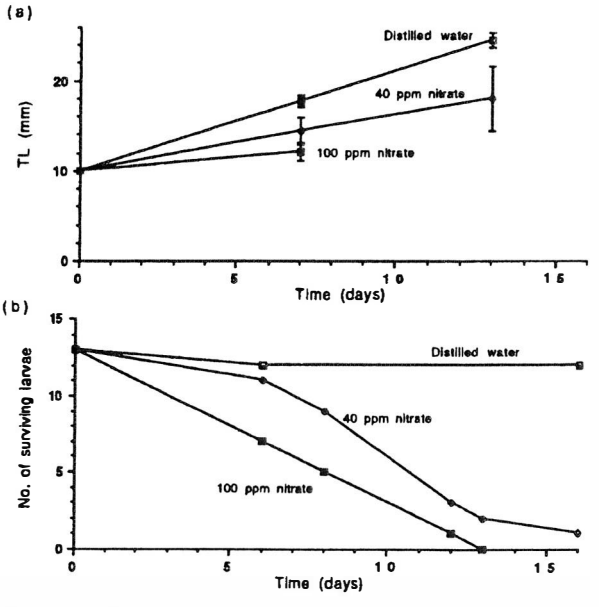

Fig. 1. (a) Growth of larvae of *Bufo bufo* in distilled water and two sodium nitrate solutions. Error bars represent one standard deviation. (b) Survival of larvae of *Bufo bufo* in distilled water and two sodium nitrate solutions.

was undisturbed by tadpole feeding. Hence it is possible that the low rate of feeding by tadpoles in the nitrate solutions allowed the *Saprolegnia* to grow, and this in turn may have further reduced the feeding activity of the tadpoles. The fungus did not appear to affect the tadpoles directly.

From this study it is not possible to conclude which ions are responsible for the observed effects on the growth and survival of toad tadpoles, since both nitrate and sodium ions co-vary between the treatments. Ireland (1991) has found that sodium ions may reduce growth rates in the larvae of *A. maculatum* and so it is quite possible that sodium ions were also responsible for the effects observed in the present experiment. We are currently seeking to separate the effects that sodium and nitrate ions may have on amphibian larvae (Baker & Waights, in prep.)

The present results should be treated with some caution. Firstly, dissolving sodium nitrate in distilled water causes a shift in pH from slightly acidic to slightly alkaline. pH can affect the growth of anuran larvae; it is generally observed that low pH reduces growth (Freda, 1986; Böhmer & Rahman, 1990). However, the lower pH of the distilled water in the present experiment was in fact associated with enhanced growth. Therefore, it is unlikely that the pH shift in the present experiment is sufficient to explain the mortalities and reduced growth that were recorded. It should also be noted that toads are frequently found breeding in ponds with an alkaline pH (pH 7-9 unpubl. data). The second note of caution is that effects detectable in a laboratory situation do not always translate to the field. Toxicity to aquatic organisms varies with temperature and water quality (Mason, 1991). The present experiment was carried out in a laboratory, rearing tadpoles at a relatively constant, high temperature. These conditions differ from those of a pond, which may either increase or decrease the magnitude of the effect of nitrate on toad larvae. Finally, it should be noted that the nitrate concentrations used in this experiment are nominal. Actual nitrate levels may change over time, due to the presence of the food pellets and due to the activities of the toad tadpoles.

It is desirable to find out whether nitrates in natural breeding sites can cause adverse effects on toad tadpoles, either directly through mortality or through reduced growth rate. There is a body of work that has shown that rapid growth or attainment of large body size during the larval phase reduces the risk of predation (Cooke, 1974; Heyer, McDiarmid, & Weigman, 1975; Caldwell, Thorpe & Jervey, 1980; Travis, Keen & Julianna, 1985) and is critical to the survival and reproductive success of individuals during later stages of the life cycle (Berven & Gill, 1983; Smith, 1987; Semlitsch, Scott and Pechmann, 1988). Hence the response of reduced growth observed in the laboratory may have important consequences for larvae in the natural situation.

In nature, any adverse effect of nitrates may be attenuated due to the timing of the amphibian breeding and seasonal fluctuations in nitrate levels. Amphibian populations most likely to be exposed to high levels of solutions of nitrate salts are those breeding in ponds on agricultural land. Nitrate levels may become high due to fertilizer run-off from surrounding land. However, the amphibian larval stage may avoid the peak levels which will occur during the winter months. During the winter there is little plant growth to assimilate the nitrate fertilizer, and water run-off is at its highest due to the lack of plant transpiration and respiration (Mason, 1991). Hence it remains to be seen whether amphibian larval populations on agricultural land can be adversely affected by the application of nitrate fertilizers. However, the clear effects of sodium nitrate demonstrated in the present study suggest that the subject of amphibian sensitivity to nitrates is worthy of future investigation. We are carrying out further work in this area, using more realistic and carefully-monitored growth conditions.

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