SHORT NOTES

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THE EFFECTS OF NITRATE ON TADPOLES OF THE TREE FROG (*LITORIA CAERULEA*)

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Nitrate is relatively harmless to animal life, and is generally not considered to present a toxic hazard in surface waters (Russo, 1985). However, there is documented evidence of toxicity to fish (Russo, 1985; Tomasso & Carmichael, 1986) and freshwater invertebrates (Camargo & Ward, 1992). A comparison of the median lethal toxicity levels in these species suggests that fish are relatively tolerant of nitrate. For example, the 96 hr median lethal toxicity levels in fish (*Ictalurus punctatus*, *Lepomis macrochirus*, *Oncorhynchus tshawytscha*, *Poecilia reticulata*, *Salmo gairdneri* [review in Russo, 1985] and *Micropterus treculi* [Tomasso & Carmichael, 1986]), range from 800-8860 mg/l nitrate. However, some freshwater species may be more sensitive; for example, the equivalent toxicity levels for caddis larvae (*Cheumatopsyche pettiti* and *Hydropsyche occidentalis*) are 430-730 mg/l nitrate (Camargo & Ward, 1992). Hence, there is a need to establish the effects of nitrate on other freshwater species.

Preliminary work has shown that larvae of the common toad (*Bufo bufo*) subjected to sodium nitrate, in a laboratory situation, exhibit reduced growth and increased mortality (Baker & Waights, 1993). Such effects could be attributable to the presence of either sodium or nitrate ions, or a combination of both. The present experiment was designed to investigate the separate effects of sodium and nitrate ions on the larvae of an amphibian.

Larvae used in the experiment were taken from a single spawning of *Litoria caerulea*. When the external gills became covered and the larvae had begun to feed (stage 25 of Gosner, 1960), individuals were taken randomly from the spawning tank and measured, until 72 larvae measuring 7 mm had been obtained. These larvae were assigned to one of six treatments on a random basis. The six treatments consisted of concentrations of 40 mg/l and 100 mg/l of nitrate ion (made up by dissolving sodium nitrate in distilled water) and four controls. The lower concentration of nitrate was chosen because it is similar to the highest nitrate levels recorded from ponds on farm land in the eastern Midlands, England (n = 78, median = 6.6, maximum = 42). The high nitrate level was chosen arbitrarily, to magnify any effects too subtle to detect at a concentration of 40 mg/l nitrate. The four control treatments consisted of distilled water only, reconstituted soft water, and two concentrations of sodium chloride. The distilled water control was used to quantify larval growth in the absence of the sodium nitrate. Reconstituted soft water contains ions found in soft water and was used to test the validity of using distilled water as a control and as a 'dilution water'. The reconstituted soft water was made up by dissolving 48 mg $NaHCO_3$, 38 mg $MgCl_2$, 46.5 mg $CaCl_2$ and 2 mg KCl in each litre of distilled water, following Ireland's (1991) modification of Stephen's (1975) solution for toxicity testing. Sodium ion concentrations in the sodium chloride controls matched those of the two sodium nitrate treatments, and chloride ion concentrations did not exceed those of the reconstituted soft water.

Each larva was grown in a clear, food-quality, plastic beaker containing 250 ml of distilled water or solution, according to treatment. The beakers were arranged in 12 blocks on a laboratory bench. Indirect sunlight and a natural photoperiod was obtained via the laboratory window. Over the course of the experiment water temperature ranged from 22.5-26°C. Larvae were fed on JMC Aquatics cat fish pellet food; 0.015 g was provided on day 0, 0.02 g on day 5 and 0.035 g on day 9. On day 13 feeding was switched to an *ad libitum* regime, because by this stage the variation in larval food requirements was so great that a uniform *per capita* feeding rate would result in either pollution of the water of the smaller larvae, or prove to be insufficient to feed the larger larvae. The solutions were replaced every four days. pH was measured on three occasions during the course of the experiment. Nitrate levels in the nitrate treatments were recorded at the outset of the experiment and also immediately before and after the solutions were replaced.

Each larva was measured to the nearest 0.5 mm every four days, by placing the larva in a 'v'-shaped trough, marked with a mm scale. Growth of larvae in all six treatments is shown in Fig. 1. Any mortalities were noted each day, and, to record developmental rates, larvae were staged, without magnification, on day 16.

ANOVA of the size of larvae at 16 days was used to assess the effects of treatment and block on larval growth. There was no effect of block on larval growth, but there was a significant treatment effect, $F_{5,32}$ = 8.187, $P < 0.001$. Tukey multiple range tests showed that both of the nitrate treatments were significantly different from all four controls ($P < 0.05$), but not from each other, and that none of the controls differed from each other. By the end of the experiment, significantly more larvae had died in the nitrate solutions (13/24) than in the four controls (11/48), χ^2 (Yates' correction) = 5.70, df= 1, $P < 0.05$. There were also evident differences in development rates. Of the surviving larvae, significantly fewer had attained Gosner stage 27, in the nitrate treatments (1/11) than in the controls (28/37), χ^2 (Yates' correction) = 13.09, df = 1, $P < 0.001$.

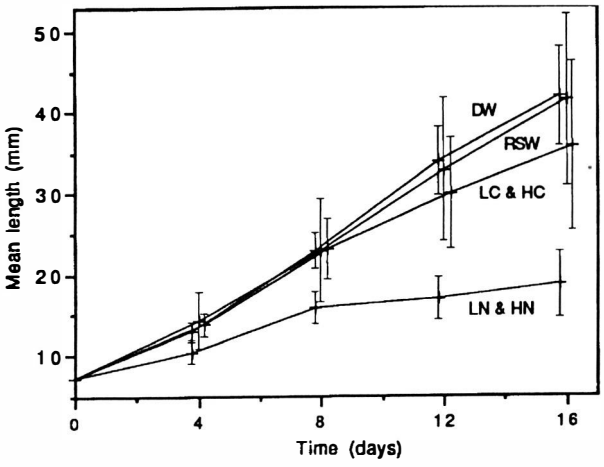

FIG. 1. Growth of larvae in experimental and control solutions. DW = distilled water, RSW = reconstituted soft water, LC & HC = low and high concentrations of sodium chloride, LN & HN = low and high concentrations of sodium nitrate. The data for the sodium chloride controls and sodium nitrate treatments have been combined, since growth curves for the individual treatments are indistinguishable.

pH varied between treatments. Lowest values were recorded in distilled water (pH 5.6-7.1) and highest values in nitrate solutions (pH 5.8-7.6) and reconstituted soft water (pH 7.0-7.3). Nitrate levels tended to decrease with time, falling from 40 to as low as 20 mg/l and from 100 to as low as 60 mg/l.

The presence of sodium nitrate in the growth media of the larvae of *Litoria caerulea* decreased rates of growth and differentiation and increased the rate of mortality. These adverse effects appear to be due to the presence of nitrate ions rather than sodium ions because the same concentrations of sodium ions were present in the sodium chloride controls, which had no statistically significant effect on the growth or survival of the larvae. Other studies have found sodium chloride to be toxic to anuran larvae (Beebee 1985; Padhye & Ghate, 1992), but this only occurs at much higher concentrations than those used in the present experiment. The higher of the two concentrations of sodium chloride used in the present experiment was 0.0094% (w/v) whereas Beebee (1985) found that mortality in *Bufo calamita* larvae occurred at approximately 0.8%, and Padhye & Ghate (1992) recorded mortality in *Microhyla ornata* at 0.4%. Sodium chloride may have adverse effects on *Litoria caerulea*, but these may be detectable only at concentrations much greater than those of the present experiment.

The adverse effects of nitrate seemed to be similar at both of the concentrations used in this experiment. Mortality was similar at both levels of nitrate (six of the twelve larvae died in the low nitrate treatment, and seven died in the high nitrate treatment). However, because of the small sample sizes it is difficult to draw conclusions concerning differences in mortality between these two nitrate concentrations. The growth trajectories of the larvae under the two different nitrate concentrations are similar, so it appears that the concentrations of nitrate used in this experiment had a similar adverse effect on the growth of larvae. It would be useful to examine the effects of lower nitrate concentrations on amphibian larvae to ascertain how much nitrate can be tolerated without affecting growth, development and survival.

There were pH differences between treatments, and it is known that pH can affect amphibian larvae. For example, low pH can cause mortalities in amphibian embryos and larvae (review in Freda, 1986) and, in addition, may decrease growth rates (review in Böhmer & Rahman, 1990). However, pH differences are unlikely to explain the mortality and growth differences observed between treatments in the present experiment, because lethal pH levels for amphibians are much lower (pH 3-4.5 [Freda, 1986]) than those recorded here, and because, although the nitrate solutions were the most inimical to the *Litoria caerulea* larvae, these solutions were less acidic than distilled water and no more basic than reconstituted soft water. Both of the latter supported healthy larvae.

Although the present study did not seek to establish median lethal toxicity concentrations of nitrate, it appears that *Litoria caerulea* may be more sensitive to nitrate than are fish. In fish the median lethal tolerance levels over a short time period (96 hr) were between 800-8860 mg/l nitrate (Russo, 1985; Tomasso & Carmichael, 1986). In the present study half of the larvae in the nitrate groups had died by day eight. This is a longer time scale than observed in the fish studies, but it should be noted that most larvae died within the first four days, and that the nitrate levels of the present study are one or two orders of magnitude smaller than those that resulted in deaths of half of the fish samples. If nitrate acts on amphibian larvae in nature in a similar way to that described here, in the laboratory, then the adverse effects on growth and development will have important consequences. Small larvae suffer a greater risk of predation (Travis, Keen & Julianna, 1985) and so poor larval growth rates presumably reduce an individual's probability of survival. Poor growth during the larval stage can also result in failure to escape from a deteriorating aquatic environment (Savage, 1961), prolongation of the time taken to attain maturity (Smith, 1987) and reduced body size at maturity (Berven & Gill, 1983; Semlitsch, 1987; Smith, 1987), a factor associated with low reproductive potential in amphibians (Halliday & Verrell, 1986).

The sensitivity of amphibian larvae to nitrate in the laboratory makes it desirable to assess this effect in the field. Effects observed in the laboratory may not be representative of events in the field, since toxicity to aquatic organisms varies with water quality and temperature (Mason, 1991). Particular attention needs to be paid to sub-lethal effects, such as reduced growth rates, which can be harmful to individuals: it is not sufficient to record data concerning mortality alone.

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