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# Effects of Nitrite on Bullfrog (*Rana catesbeiana*) Tadpoles from Central Ohio, USA

G. R. Smith, D. A. Vaala, H. A. Dingfelder, K. G. Temple

Department of Biology, Denison University, Granville, OH 43023, USA

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Nitrogenous pollution from a variety of sources (e.g., agricultural runoff, urban wastewater, atmospheric deposition; see Carpenter et al. 1998; Naiman and Turner 2000) has the potential to contribute to amphibian declines by altering the water chemistry of amphibian breeding sites (review in Rouse et al. 1999). Much of the research on the effect of nitrogenous pollution on amphibians has focused on nitrates. Although nitrite is not very common in the field (Marco et al. 1999; Rouse et al. 1999), it may be even more toxic and damaging to amphibians than nitrate. For example, Marco et al. (1999) found that it takes a lower concentration of nitrite than nitrate to have a negative impact on amphibian performance. The tolerance for nitrite appears to vary among the few species of amphibians studied to date, although they all seem to be fairly sensitive to nitrite (e.g., Huey and Beitinger 1980*b*; Marco et al. 1999). Given that the effects of nitrite on amphibians has been studied in so few species, and that most of those species studied thus far are from the Pacific Northwest USA, additional information on amphibian tolerance of nitrite is needed from other species and other geographic regions, particularly those with a long history of agriculture (e.g., Midwestern USA), and higher ambient levels of nitrogen pollution (see Rouse et al. 1999) To this end, we studied the effect of nitrite on the growth, survival, and behavior of bullfrog (*Rana catesbeiana*) tadpoles from an area with a history of agriculture (central Ohio, USA).

## MATERIALS AND METHODS

*Rana catesbeiana* egg masses were collected from a small seasonal pond within the Denison University Biological Reserve located in Granville, Licking Co., Ohio, USA. Egg masses were brought back to the laboratory where they were incubated in aged tapwater (dechlorinated; nitrite < 0.05 mg $L^{-1}$) at 17 - 19°C. Upon hatching, tadpoles were maintained in plastic containers and fed daily. At the start of the experiment, tadpoles were Gosner stage 26, and the mean body mass ($\pm$ 1 SE) of 10 randomly selected *R. catesbeiana* tadpoles was 0.018 $\pm$ 0.003 g (these tadpoles were not used in the experiment).

Nominal nitrite concentrations of 0, 0.375, 0.75, 1.5, 3.0, 6.0 mg $L^{-1}$ N-$NO_2^-$ were created using sodium nitrite (Fisher certified A.C.S., 99.6%). These

*Correspondence to:* G. R. Smith

concentrations are within the range of nitrite-N concentrations in cental Ohio rivers and streams (0.02 to 1.90 mg $L^{-1}$; Ohio EPA 1995) and nitrate-nitrite concentrations in Licking County wetlands (1.63 to 12.5 mg $L^{-1}$; Spieles and Mitsch 2000*a,b*). Four randomly selected tadpoles were placed into 3 L of the test solution. Each concentration was replicated 4 times. Plastic experimental containers (20 cm x 32 cm x 11 cm) were marked with a grid dividing the underside into eight equally-sized sections (6 cm x 5 cm).

Tadpoles were maintained in their treatments for 15 days, and mortality checked daily. Tadpoles were fed ground Purina Rabbit Chow, and feces and excess food removed every third day. Water and nitrite treatments were refreshed after 7 days, and thus this is a "pulse" experiment (see Hatch and Blaustein 2000). At the end of the experiment, surviving tadpoles were weighed to the nearest 0.001 g after blotting dry.

Six days after nitrite exposure had begun; tadpoles were videotaped for 10 minutes, and activity levels were estimated by counting the mean number of times the tadpoles in each container crossed the grid lines.

Separate univariate ANOVAs were performed on the proportion of tadpoles surviving to the end of the experiment (arcsine square root transformed) and on final mass, with nominal nitrite concentration as the independent variable. We used a Kruskal-Wallis test to assess the effect of nominal nitrite concentration on activity levels.

## RESULTS AND DISCUSSION

Tadpole final mass was marginally affected by nitrite concentration (Table 1; $F_{5,18}$=2.71, P= .054). Bullfrog tadpoles exposed to intermediate levels of nitrite had higher final masses than other levels of nitrite and approached or exceeded the values for the control treatment. Why this is the case is unclear; however, there have been some other studies on the responses of anuran tadpoles to nitrogenous compounds that have found that tadpoles exposed to intermediate levels perform better than those exposed to low or high levels (e.g., Hecnar 1995; Xu and Oldham 1997; Johansson et al. 2001). Perhaps these results occur as an extrapolation of an inverse dose response or hormesis. In an inverse dose response, performance increases with stressor concentration due to a stress response in the individual that overcompensates for the effects of the stressor (see Davis and Svendsgaard 1990; Calabrese and Baldwin 2001, 2003). In our case, and maybe others, the highest levels of nitrite may have been too high for the stress response to overcompensate, and so the intermediate concentrations, where the stress response was able to overcompensate, performed best.

Bullfrog tadpoles exposed to all nitrite concentrations had relatively high survivorship (> 80%), and nitrite concentration did not affect tadpole survival

**Table 1**. Nominal nitrite concentration (mg $L^{-1}$ N-$NO_2^-$) effects on survivorship (proportion), final mass (g), and activity levels of *Rana catesbeiana* tadpoles.

| Concentration | Survivorship | Final Mass | Activity* |
|---|---|---|---|
| Control | $0.938 \pm 0.062$ | $0.034 \pm 0.003$ | $5.75 \pm 3.49$ |
| 0.75 | $0.812 \pm 0.062$ | $0.025 \pm 0.002$ | $2.88 \pm 0.72$ |
| 1.25 | $0.875 \pm 0.072$ | $0.034 \pm 0.002$ | $6.69 \pm 4.26$ |
| 2.5 | 1.000 | $0.026 \pm 0.003$ | $4.12 \pm 2.16$ |
| 5 | 1.000 | $0.029 \pm 0.002$ | $2.06 \pm 0.76$ |
| 10 | $0.875 \pm 0.125$ | $0.029 \pm 0.003$ | $2.27 \pm 0.77$ |

(Means are given $\pm$ 1 SE, N = 4 in all cases.)
*mean moves per 10 minutes

(Table 1; $F_{5,18}$= 1.20, P= 0.35). Nitrite also did not affect tadpole activity (Table 1; $H_5$ = 1.0, P = 0.96).

In general, the bullfrog tadpoles in our experiments were much more tolerant than the tadpoles of *Bufo boreas*, *Hyla regilla*, *R. aurora*, and *R. pretiosa* tested by Marco and Blaustein (1998) and Marco et al. (1999). Marco et al. (1999) found nearly 100% mortality in several species of amphibian larvae (exposure started on 7-d old larvae; *Ambystoma gracile*, *Bufo boreas*, *Hyla regilla*, *Rana aurora*, and *Rana pretiosa*) after 15-d exposure to nitrite concentrations $\leq 3.5$ mg $L^{-1}$ N-$NO_2^-$. Marco et al. (1999) also found that elevated levels of nitrite decreased tadpole activity in *Bufo boreas*, *Hyla regilla*, *R. aurora*, and *R. pretiosa*, and Marco and Blaustein (1998) observed that tadpoles of *Rana cascadae* exposed to nitrite spent more time in shallow water and with their heads at the surface. Our results support the suggestion of Marco et al. (1999) that one possible reason for the success of bullfrogs as invaders is their ability to tolerate chemical contaminants.

One possible explanation for the difference in tolerance between the bullfrogs we studied, and the species studied by Marco and Blaustein (1998) and Marco et al. (1999) is that bullfrogs may have a more effective physiological mechanism for dealing with nitrite than do the other species. For example, Huey and Beitinger (1980*a*) also found that "late larval" stage bullfrog tadpoles (15 – 26 g) are relatively tolerant of short-term (24 h) nitrite exposure (up to 50 mg $L^{-1}$) and its physiological consequences (e.g., methemoglobinemia), possibly due to lower ion uptake or a relatively efficient methemoglobin reductase system. It is tempting to speculate that the general tolerance for nitrite observed in bullfrogs may be the result of longer, historical exposure to nitrogenous pollution; however, additional studies on more species from agricultural regions are needed. In addition, further studies on the physiological mechanisms underlying any observed tolerance for nitrite exposure are needed.

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