Contaminant Report Number: R6/719C/02

U.S. FISH & WILDLIFE SERVICE
REGION 6

CONTAMINANTS PROGRAM

Determination of Impacts on the Endangered Wyoming Toad (*Bufo baxteri*) at Mortenson National Wildlife Refuge from Ammonium Nitrate Concentrations

By
Edward E. Little[1], Robin D. Calfee[1], and Kimberly Dickerson[2]

Project #: 99-6-6N41-GC

[1]U.S. GEOLOGICAL SURVEY
Biological Resource Division
Columbia Environmental Research Center
4200 New Haven Road
Columbia, Missouri 65201

[2]U.S. FISH AND WILDLIFE SERVICE
Ecological Services
Wyoming Field Office
4000 Airport Parkway
Cheyenne, Wyoming 82001

March 2002

Executive Summary

The endangered Wyoming toad (*Bufo baxteri*) is found only as a reintroduced population at Mortenson National Wildlife Refuge (NWR) in the Laramie Plains of southeast Wyoming. Reasons for the decline of this amphibian are unknown. Data on predation, habitat modification, soil and water conditions (Stone 1991), and water quality (Ramirez 1992; Ramirez and Armstrong 1992), are available; but, none of these parameters are documented as posing serious threats to the toad.

One threat that has not been investigated is the potential for agricultural fertilizers containing nitrates to enter surface water at Mortenson NWR. Nitrates are transported by irrigation runoff and snowmelt during the same time frame as the growth and development period of the Wyoming toad (Stone 1991); and, although little information exists on the effects of nitrates on amphibians, nitrates are suspected of playing a role in the decline of some amphibian populations. Hecnar (1995) suggests that nitrates may pose a risk to amphibians that is equivalent to that of pesticides.

The current water quality guideline for nitrates is 10 mg/L NO_3-N (EPA 1986). This guideline is set to protect human health but is not protective of some amphibians species (Hecnar 1995). In acute toxicity tests with the American toad (*B. americanus*), chorus frog (*Pseudacris triseriata*), leopard frog (*Rana pipiens*), and green frog (*R. clamitans*) exposed to ammonium nitrate fertilizer, the amphibians exhibited reduced activity, weight loss, and physical abnormalities. The toxic effects were observed at “concentrations that are commonly exceeded in agricultural areas” (Hecnar 1995) and frequently occurred during the amphibian larvae development period (Berger 1989). Additionally, toad (*Bufo spp*.) tadpoles were more sensitive to nitrates than were water frogs (*Rana spp*.) (Berger 1989). When exposed to nitrates, the amphibians often showed a sluggish behavior or appeared somewhat paralyzed when prodded. This behavior impairs the ability of the tadpoles to acquire food or avoid predation (Hecnar 1995). Both of these activities can ultimately result in poor survivability and low reproductive success for the Wyoming toad.

The purpose of this study was to determine the concentrations of nitrates at Mortenson NWR and whether these concentrations are potentially affecting the survival of the Wyoming toad. Water samples were collected pre-, during, and post-irrigation at Mortenson NWR and submitted for general water quality analyses and analysis of nitrite, nitrate, and ammonia. Frog embryo teratogenesis assays, using Woodhouse’s toad (*B. woodhousii*) embryos as surrogates for the Wyoming toad, were conducted at the Midwest Science Center at Columbia, Missouri. Test solutions were prepared using a standard dilution series with one dilution representative of nitrate concentrations present in the field. Percent mortality, timing of metamorphosis, and presence of deformities were measured.

Survival and metamorphosis were significantly reduced by the ammonia nitrate concentrations at the two highest treatments tested in the assay. The treatment representative of ammonia nitrate concentrations found at Mortenson NWR did not impact tadpole survival or metamorphosis. Deformities in the tadpoles were observed in all treatments including controls. The deformities did not appear to be caused by the ammonia nitrate concentrations and may be the result of the tadpoles’ diet.

Results of this study show that ammonia nitrate concentrations are not currently elevated to concentrations that would adversely affect the Wyoming toad. Increases in nitrogen input, such as what might occur with changes in land use, could increase the risk for adverse affects to the toad, particularly because ammonia nitrate concentrations may act synergistically with other environmental factors or may serve as a stressor for increasing the toads' susceptibility to disease. Periodical sampling of water from Mortenson NWR will ensure that nitrogen input does not increase to concentrations exceeding the tolerance level of Wyoming toads.

Table of Contents

Figure

Appendices

Introduction

The endangered Wyoming toad (*Bufo baxteri*) is a glacial relict species confined to the Laramie Plains of southeastern Wyoming. Once common in the area, it declined dramatically in the 1970's and was subsequently listed under the Endangered Species Act in 1984 (Lewis et al. 1985). This prompted the development of a recovery plan and captive breeding program to establish viable toad populations at Mortenson and Hutton Lake National Wildlife Refuges (NWRs). In order for the effort to be successful, the effects of several environmental stressors (e.g. red-leg disease, predation, habitat alteration) have been explored. Because none of the variables were considered a serious threat, toads were reintroduced to both refuges in 1995. However, it remains of concern that Wyoming toad breeding sites may be contaminated with agricultural chemicals, which could lower fecundity and jeopardize the establishment of viable populations. Amphibian eggs and larvae are the most sensitive stages to chemical contamination and reproductive success may be reduced if exposure results in slower growth or increased mortality. Aquatic habitat at Mortenson NWR is fed by the Laramie River, but also receives snowmelt and irrigation runoff from nearby agriculture fields and pastures. The timing of this input coincides with the primary growth and development period of pre-metamorphic toads (Stone 1991).

Fertilizer runoff is a major source of nitrogen pollution in aquatic habitat. Common ingredients include ammonium sulfate, sodium nitrate, and ammonium nitrate. Reduced feeding, weight loss, low activity, and physical abnormalities are among the reported effects of nitrogen toxicity (Baker & Waights 1993; Hecnar 1995; Xu & Oldham 1997). The use of nitrogen fertilizers in the United States has increased dramatically in the last fifty years and currently more than 9.98 million metric tons are applied annually (Lanyon 1996). Application typically occurs in the late spring and early summer when watershed runoff from snowmelt and rainfall may cause pulses of elevated nitrate and ammonia concentrations that can degrade the water quality and pose a risk to aquatic species. Nitrogen pollution of aquatic habitat has been linked to the decline or disappearance of some amphibian populations (Wederkinch 1988; Berger 1989). Exceedences of the current United States drinking water guideline of 10 mg/L NO_3-N (EPA 1986) have been observed in the field (Schuytema & Nebeker 1999) and may not adequately protect some organisms (Kincheloe et al. 1979; Hecnar 1995).

A laboratory study was performed using ammonium nitrate to assess the effects of aquatic nitrogen pollution on the reestablishment of *B. baxteri* at Mortenson NWR. The Woodhouse's toad (*B. woodhousii*) was tested as a surrogate because of the endangered status of the Wyoming toad. Water chemistry conditions modeled those found at Mortenson NWR during the *B. baxteri* breeding season (late May – early July). Because of a limited supply of toad eggs, a single chronic study was conducted to model a worst-case scenario.

Methods

Sample collection

We collected water samples from Mortenson NWR, the Laramie River, and Hutton NWR sites pre-, beginning, mid-, and post-irrigation (Figure 1). Water samples were collected in chemically-cleaned polyethylene jars using standard methods (USFWS 1996) for basic water chemisty analysis and analysis of nitrite, nitrate, and ammonia. We also collected sediment samples from each site for analysis of nitrite, nitrate, and ammonia pre-, beginning, mid-, and post-irrigation. Sediment was placed in whirl-pak bags using standard methods (USFWS 1996) and frozen. Chemical analyses was conducted by the Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado.

Test organisms

A single clutch of *B. woodhousii* eggs was obtained July 10, 2000 from a captive female at the Saratoga National Fish Hatchery in Saratoga, Wyoming. Spawning was induced by injection with human chorionic gonadotropin, then the eggs were shipped to the USGS Columbia Environmental Research Center (CERC). The eggs were hatched in aquaria containing aerated, room temperature ($23 \pm 2^{o}C$) well water. Although hatching success was low, surviving larvae appeared healthy and were fed ground fish flakes *ad libitum* until 24 hours prior to testing. The exposure began on July 24, when the tadpoles were at approximately stage 25 (Gosner 1960). During testing, tadpoles were fed ground fish flakes *ad libitum* in the 24-hour period prior to each water renewal.

Test water

Test water of two different hardnesses (nominal 200 and 800 mg/L as $CaCO_3$) was made according to ASTM standards (ASTM 2000). Dissolved oxygen (DO), temperature, and pH were measured daily and alkalinity and hardness were measured every Monday and Friday. Samples were taken from twelve test jars selected at random. Averages and ranges for these parameters are reported in Table 1.

Organisms were exposed to six concentrations of ammonium nitrate (nominal 0, 12.5, 25, 50, 100, 200 mg/L NH_4NO_3) made from aqueous stock solutions of ammonium nitrate (99.5% purity; Sigma Chemical Company, St. Louis, MO, USA) and two hardnesses, for a total of twelve treatments. All treatments were replicated four times. One ammonium nitrate stock was prepared for each hardness using the ASTM water. When not in use, the stock solutions were wrapped in foil and refrigerated at $4^{o}C$.

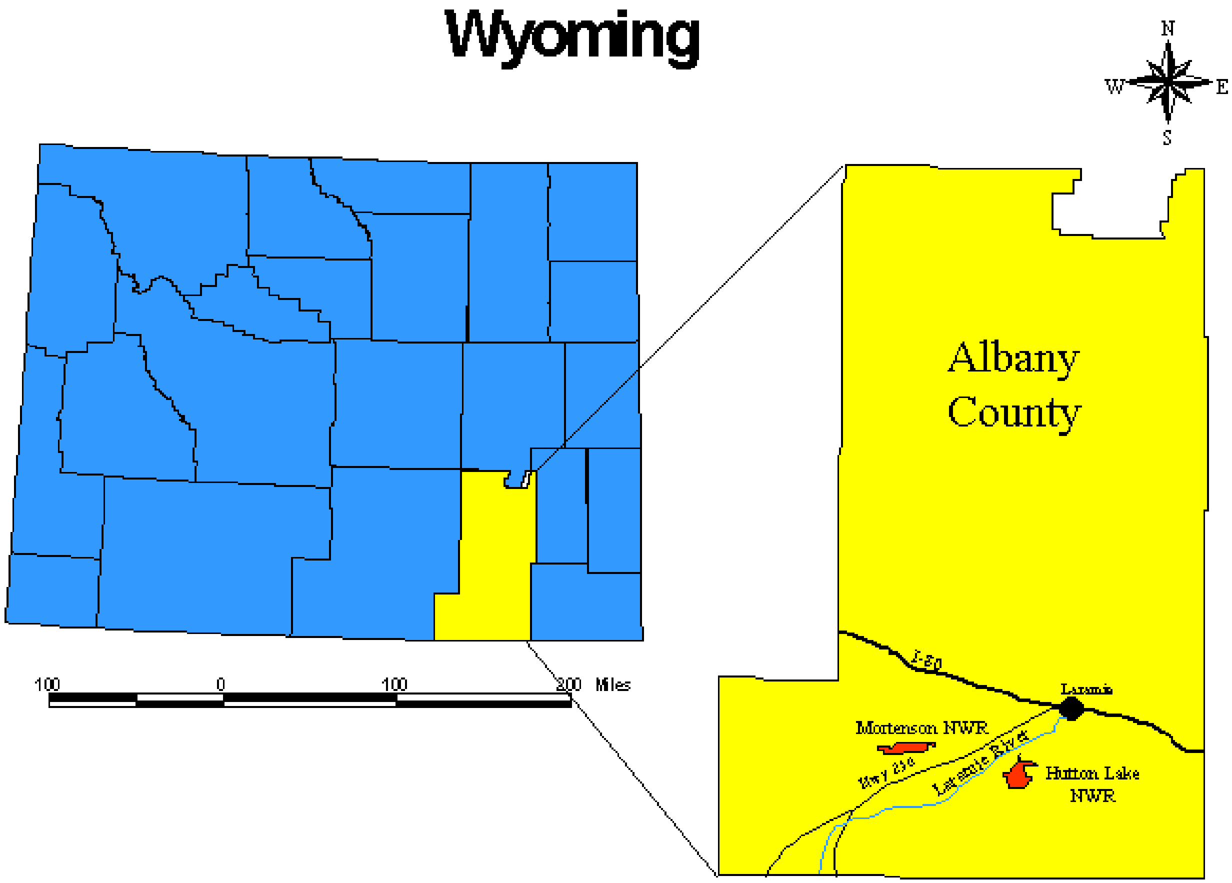

Figure 1. General location of Mortenson and Hutton National Wildlife Refuges and the Laramie River, Albany County, Wyoming.

Table 1. Water quality parameters measured during *Bufo woodhousii* exposure to ammonium nitrate.

Parameter	200 Hardness Mean	Range	800 Hardness Mean	Range
Alkalinity (mg/L as $CaCO_3$)	119.6	100 - 169	103.3	84 - 126
Dissolved oxygen (mg/L)	7.0	3.5 - 9.0	7.1	3.3 - 9.1
Hardness (mg/L as $CaCO_3$)	210.1	170 - 333	726.8	620 - 964
pH	8.1	7.5 - 8.9	8.0	7.3 - 8.6
Temperature (°C)	21.3	18.8 - 23.7	21.3	18.7 - 23.3

Nitrogen as nitrate (NO_3-N) and total ammonia (NH_4-N) was verified by taking samples from twelve randomly selected jars every Monday and Friday. Ammonia samples were preserved with concentrated sulfuric acid and all nitrogen samples were kept at 4°C until analyzed. Concentrations were determined with a Technicon II Autoanalyzer, which had a detection limit of 0.05mg/L for both ammonia and nitrate. All check standards were within 10% of target value and all blanks were less than 5% of the range. Un-ionized ammonia (NH_3-N) was determined based on NH_4-N, pH, and temperature (EPA 1998). Mean values of nitrogen as nitrate, total ammonia, and ammonia are given in Table 2.

Table 2. Mean nitrogen as nitrate (NO_3-N), total ammonia (NH_4-N), and un-ionized ammonia (NH3-N) for each concentration of ammonium nitrate (NH_4NO_3). All values are in mg/L as N.

Nominal NH_4NO_3	Measured NH_4NO_3	NO_3-N	NH_4-N	NH_3-N
0	0.54	0.03	0.32	0.03
12.5	11.62	1.99	2.19	0.15
25	24.58	4.25	4.49	0.32
50	45.56	8.02	7.84	0.44
100	90.56	15.94	15.58	0.76
200	210.04	36.44	37.97	1.67

Test procedure

Testing was conducted according to ASTM standards (ASTM 2000). The experiment took place in a wet lab dilutor at the USGS Columbia Environmental Research Center, Columbia, MO. On July 24, six *B. woodhousii* tadpoles were added to each of 48 glass chambers that contained 3.5 L of test solution. Care was taken to only include individuals that appeared healthy and of similar size. The chambers were placed in a 4 x 12 block and treatment locations were determined randomly. Water was renewed every three days while mortality and physical and behavioral abnormalities were recorded daily. On August 14, the tadpoles were transferred to larger glass chambers containing 6 L of solution to avoid overcrowding. Tadpoles began metamorphosing on September 6 and all metamorphs were transferred to tilted plastic containers that held well water at one end and a moist paper towel at the elevated end. The water was changed daily and organisms were not fed.

Statistical analyses

Two-factor analysis of variance (ANOVA) was performed to determine the effects of ammonium nitrate and hardness on the proportion of individuals that survived or metamorphosed, as well as time and mass at metamorphosis and tadpole stage and mass. All proportional data were arcsine transformed and mass and time to metamorphosis were log transformed. Stages were ranked prior to ANOVA analysis. Dunnett's t tests for *a posteriori* multiple comparisons were performed when ANOVA results were significant. Type III SS were used in all ANOVAs because of unequal sample size.

Results

Tadpole survival

Mortality among the controls was less than 10% through 56 days of exposure in the low hardness water. Mortality was observed in the two highest ammonium nitrate concentrations, 100 and 200 mg/L, after 7 days of exposure and likely reflects the acute toxicity of this substance (Table 3a). By 21 days of exposure mortality was nearly 100% in these concentrations. Although mortality was evident among tadpoles in the 50-mg/L group, it was not significantly different from the controls.

Mortality among controls was within an acceptable range throughout 60 days of exposure in the high hardness water. Mortality was limited to the highest concentrations of ammonium nitrate (Table 3b) and was evident within 7 days of exposure.

Table 3a. Cumulative percent mortality of *Bufo woodhousii* exposed to five concentrations of ammonium nitrate at 200 mg/L $CaCO_3$ water hardness. Standard deviations are in parentheses.

Concentration (mg/L)	**200 $CaCO_3$ mg/L hardness**								
	7d	**14d**	**21d**	**28d**	**35d**	**42d**	**49d**	**56d**	**66d**
0	0 (0)	0 (0)	0 (0)	0 (0)	8.33 (0.57)	8.33 (0.57)	8.33 (0.57)	8.33 (0.57)	29.1 (1.54)
12.5	0 (0)	4.16 (0.50)	4.16 (0.50)	8.33 (1.0)	12.5 (0.96)	12.5 (0.96)	20.83 (0.50)	33.33 (1.83)	37.5 (2.03)
25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.16 (0.50)
50	0 (0)	8.33 (0.58)	8.33 (0.58)	12.5 (0.96)	16.67 (1.15)	20.83 (0.96)	20.83 (0.96)	25.0 (0.58)	37.5 (0.96)
100	50.0 (0.82)	75.0 (1.29)	91.67 (0.58)	91.67 (0.58)	91.67 (0.58)	91.67 (0.58)	91.67 (0.58)	91.67 (0.58)	95.83* (0.50)
200	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100* (0)

*Represents a significant difference from the control at 66 days.

Table 3b. Cumulative percent mortality of *Bufo woodhousii* exposed to five concentrations of ammonium nitrate at 800 mg/L $CaCO_3$ water hardness. Standard deviations are in parentheses.

Concentration (mg/L)	**800 $CaCO_3$ mg/L hardness**								
	7d	**14d**	**21d**	**28d**	**35d**	**42d**	**49d**	**56d**	**66d**
0	5.6 (0.58)	5.6 (0.58)	5.6 (0.58)	11.11 (0.58)	11.11 (0.58)	22.22 (1.15)	22.22 (1.15)	22.22 (1.15)	22.22 (1.15)
12.5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.16 (0.50)	4.16 (0.50)
25	0 (0)	0 (0)	0 (0)	4.16 (0.50)	8.33 (0.58)	8.33 (0.58)	8.33 (0.58)	8.33 (0.58)	12.5 (0.50)
50	0 (0)	0 (0)	0 (0)	4.16 (0.50)	4.16 (0.50)	4.16 (0.50)	4.16 (0.50)	4.16 (0.50)	4.16 (0.50)
100	12.5 (1.50)	16.67 (1.41)	16.67 (1.41)	25.0 (1.73)	25.0 (1.73)	25.0 (1.73)	25.0 (1.73)	25.0 (1.73)	25.0 (1.73)
200	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100* (0)

*Represents a significant difference from the control at 66 days.

Metamorphosis

Among tadpoles exposed to control conditions under low water hardness conditions, 66.7% metamorphosed over an average of 56 days, whereas a significantly lower number of tadpoles (30.8%) exposed to 50 mg/L metamorphosed over an average of 63 days (Table 4). The time to metamorphosis was not significantly different.

Even though mortality was limited in the 100 mg/L treatment, metamorphosis was significantly reduced under high water hardness conditions. Only 23.8 and 13.3 % of the tadpoles exposed to 50 and 100 mg/L ammonium nitrate metamorphosed (Table 4), in contrast 60% of the controls metamorphosed.

Table 4. Percent of surviving individual *Bufo woodhousii* tadpoles that went on to metamorphosis and the average time it took for them to undergo metamorphosis. Standard errors are in parentheses.

Ammonium Nitrate (mg/L)	200 mg/L $CaCO_3$ hardness		800 mg/L $CaCO_3$ hardness	
	Percent metamorphosed	**Days to metamorphosis**	**Percent metamorphosed**	**Days to metamorphosis**
0	66.67 (0.06)	56.0 (5.24)	60.0 (0.09)	58.39 (6.32)
12.5	46.67 (0.13)	57.89 (4.48)	56.52 (0.08)	56.40 (5.55)
25	47.83 (0.14)	57.69 (6.59)	52.38 (0.18)	58.07 (5.87
50	30.77* (0.07)	63.33 (2.83)	23.81* (0.04)	60.75 (4.04)
100	--	--	13.33* (0.08)	62.0 (0.00)

*represents a significant difference from the controls for each water hardness.

Deformities

Deformities were rare among the test organisms and unrelated to any exposure condition. Nearly all metamorphs (including controls) had very thin and weak forelimbs, which prevented them from maintaining an upright posture and caused some to drown.

Ammonia nitrate caused significant reductions in survival and metamorphosis over the range of concentrations tested. However, comparison of these results to concentrations measured at Mortenson NWR indicate that current levels of aquatic nitrate and ammonia contamination do not pose a threat to the Wyoming toad at Mortenson NWR (Table 5).

Table 5. Environmental variables at Mortenson NWR from water sampling done in 1999. Nitrate (NO_3-N) and total ammonia (NH_4+-N) were analyzed by the Colorado State University Soil, Water, and Plant Testing Laboratory, and unionized ammonia (NH_3-N) was measured with a Hach kit in the field.

Date	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	NO_3-N (mg/L)	NH_4^+-N (mg/L)	NH_3-N (mg/L)
May 12	8.4	218	283	<0.01	<0.01	<0.01
June 2	7.7	218	782	0.55	0.15	<0.01
July 7	7.8	180	204	0.60	<0.01	<0.01
August 25	7.9	318	409	0.23	<0.01	0.01

Discussion

Ammonia nitrate caused significant reductions in survival and metamorphosis over the range of concentrations tested. However comparison of these results to concentrations measured at Mortenson NWR indicate that current levels of aquatic nitrate and ammonia contamination do not pose a threat to the Wyoming toad at Mortenson NWR (Table 5). In the present study, tadpole survival and metamorphosis were only impaired in the two highest treatments (100 and 200 mg/L NH_4NO_3) having nitrate (NO_3-N) concentrations of 15.94 mg/L and unionized ammonia concentrations of 0.76 mg/L (NH_3-N). In contrast, the maximum concentrations of nitrate and unionized ammonia observed in aquatic habitats at Mortenson NWR during the breeding season of 1999 were approximately two orders of magnitude lower at 0.6 mg/L NO_3-N and 0.1 mg/L NH_3-N (Table 3; NH_4^+-N is nitrogen as ionized ammonia).

Unionized ammonia is the most toxic form of nitrogen to aquatic life with a threshold for acute toxicity to amphibians ranging between 0.2 and 0.88 mg/L (Schuytema and Nebeker 1999). Unionized ammonia exists in equilibrium with the hydroxide ion and the ammonium ion (NH4) Thurston et al. 1977), and increases with increasing temperature and pH (EPA 1998). Higher pHs associated with increasing water hardness could increase the proportion of ammonia in the toxic unionized form. Therefore, total ammonia could increase to toxic levels under low hardness conditions. Nitrate concentrations observed during this study were not likely the cause of mortality or impaired metamorphosis. Reported LC50 values for the Pacific tree frog and African clawed frog are over 1500 mg/L (Schuytema and Nebeker 1999). In our study, the greater mortality occurred in soft water and was likely caused by the toxicity of the total ammonia levels. The 96 h LC50 for the American toad (*B. americanus*) larvae exposed to ammonium nitrate ranged from 13.6 to 39.3 NH4-N, varying by population tested (Hecnar 1995).

The results of our study may represent a worse case estimate of ammonium nitrate toxicity because the test organisms may not have been in good vigor. Many eggs failed to hatch, high mortality occurred in the control treatments, and the metamorphs in all treatments had weak forelimbs that prevented normal movement. The poor condition of the test organisms may reflect poor genetic stock since all eggs were obtained from a single captive female. The low survival of control tadpoles in this experiment may have also been caused by diet. Feeding studies conducted at the Saratoga National Fish Hatchery determined that *B. woodhousii* metamorphs reared on fish flakes (Tetramin) had a higher frequency of weak forelimbs than those were reared on algae (Deedee Roberts, pers. comm.).

The results of our study using *B. woodhousii* as a surrogate may serve as a useful guideline for *B. baxteri*. Research on various *Bufo* species indicates that toads are relatively tolerant of nitrogen pollution. For example, *B. americanus* hatching success and rate of deformities was found to be unaffected by un-ionized ammonia levels as high as 0.9 mg/L (Jofre & Karasov 1999).

Other nitrogen-based fertilizers appear to be similarly toxic to amphibian larvae. Mortality among *B. bufo* tadpoles occurred after a thirteen-day exposure to sodium nitrate, 22.7 mg/L NO_3-N (Baker & Waights 1993). Ammonium sulfate-based fire retardant chemicals were toxic to *Rana* *sphenocephala* when unionize ammonia concentrations reached 0.37 to 0.96 mg/L (Little and Calfee, 2000).

In conclusion, the low environmental nitrogen values suggest that Mortenson NWR is not polluted by fertilizer input from surrounding agricultural fields or more distant input sources. However, if land use practices change, with a subsequent increase in nitrogen input, sampling should be conducted to ensure tolerance levels are not exceeded. While nitrogen contamination alone is not likely to affect the *B. baxteri* population, ammonia and nitrate could serve as sublethal stressors or even act synergistically with other environmental variables.

References

ASTM (American Society for Testing and Materials). 2000. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. E 729-96. In *Annual Book of ASTM Standards*, Vol 11.05. West Conshohocken, PA. pp. 213-233.

Baker, J. and V. Waights. 1993. The effect of sodium nitrate on the growth and survival of toad tadpoles (*Bufo bufo*) in the laboratory. Herpetological Journal 3:147-148.

Berger, L. 1989. Disappearance of amphibian larvae in the agricultural landscape. Ecology International Bulletin 17:65-73.

Gosner, K. L. 1960. A simplified table for staging embryos and larvae with notes on identification. Herpetologica 16:183-190.

Hecnar, S. J. 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. Environmental Toxicology and Chemistry 14:2131-2137.

Jofre, M.B. and W.H. Karasov. 1999. Direct effect of amonia on three species of North American anuran amphibians. Environmental Toxicology and Chemistry 18: 1806-1812.

Kincheloe, J. W., G. A. Wedemeyer, and D. I. Koch. 1979. Tolerance of developing salmonid eggs and fry to nitrate exposure. Bulletin of Environmental Contamination and Toxicology 23:575-578.

Lanyon, L. E. 1996. Does nitrogen cycle? Ag Consultant 52:17.

Lewis, D. L., G. T. Baxter, K. M. Johnson, and M. D. Stone. 1985. Possible extinction of the Wyoming Toad (*Bufo hemiophrys baxteri*). Journal of Herpetology 19:166-168.

Little, E.E. and R.D. Calfee. 2000. Photoenhanced toxicity of fire-fighting chemicals. Report to the U.S. Forest Service. March 2000.

Ramirez, Jr., P. 1992. Trace elements in water, sediments, and biota from wetlands in the Laramie Basin and their relationship to the recovery of the endangered Wyoming toad (Bufo hemiophyrs baxteri). U.S. Fish and Wildlife Contaminant Report. No. R6/704C/92. Cheyenne, WY.

Ramirez, Jr., P. and J. Armstrong. 1992. Environmental contaminant surveys in three national wildlife refuges in Wyoming. U.S. and Wildlife Contaminant Report. No. R6/702C/92. Cheyenne, WY.

Schuytema, G. S., and A. V. Nebeker. 1999. Comparative toxicity of ammonium and nitrate compounds to Pacific treefrog and African clawed frog tadpoles. Environmental Toxicology and Chemistry 18:2251-2257.

Stone, M. D. 1991. Wyoming Toad Recovery Plan. U.S. Fish and Wildlife Service. Denver, CO. 28 pp.

Thurston, R. V., R. C. Russo, and K. Emerson. 1977. Aqueous ammonia equilibrium calculations. Technical Report No. 74-1. Bozeman, MT.

U.S. Environmental Protection Agency. 1986. Quality criteria for water 1986. EPA 440/5-86-001. Technical Report. Washington, DC.

U.S. Environmental Protection Agency. 1998. 1998 update of ambient water quality criteria for ammonia. EPA 822-R-98-008. Technical Report. Washington, DC.

U.S. Fish and Wildlife Service. 1996. Standard operating procedures for environmental contaminant operations. Division of Environmental Contaminants Quality Assurance Task Force. Washington D.C.

Wederkinch, E. 1988. Population size, migration barriers and other features on *Rana dalmatina* populations near Køge, Zealand, Denmark. Memo from Societe of Fauna Flora Fenn 64:101-103.

Xu, Q., and R. S. Oldham. 1997. Lethal and sublethal effects of nitrogen fertilizer ammonium nitrate in common toad (*Bufo bufo*) tadpoles. Archives of Environmental Contamination and Toxicology 32:298-303.

Appendices

Analytical Results

Appendix A1. Water quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado for pre-irrigation (5/12/99). Conductivity measured in μmhos/cm, pH unitless, remainder of analytes are mg/L.

Sample id	Site	pH	Conductivity	Ca	Mg	Na	K	B	CO_3	HCO_3	SO_4
NTMLWT01	Mortenson Lake	8.5	735	46.6	40.0	58.0	3.3	0.12	<0.1	265.8	151.6
NTMLWT02	Mortenson Lake	8.3	729	47.2	40.6	59.7	3.2	0.12	<0.1	264.8	154.5
NTLGWT01	Lake George	8.1	1430	195.9	70.7	57.8	5.2	0.09	<0.1	222.7	729.5
NTLGWT02	Lake George	7.9	1479	193.8	70.1	56.4	4.9	0.08	<0.1	226.4	729.5
NT7MWT01	Laramie River	8.0	302	32.0	11.0	12.3	1.0	<0.01	<0.1	85.9	68.5
NT7MWT02	Laramie River	7.9	305	31.5	10.7	12.1	0.9	<0.01	<0.1	84.4	59.5

Sample id	Cl	Hardness as $CaCO_3$	Alkalinity as $CaCO_3$	Total Dissolved Solids	NO_3	NO_3-N	NO_2	NO_2-N	NH_4	NH_4-N
NTMLWT01	16.2	281	218	582	<0.01	<0.01	0.13	0.03	<0.01	<0.01
NTMLWT02	15.5	285	217	586	<0.01	<0.01	0.22	0.05	<0.01	<0.01
NTLGWT01	13.2	779	183	1295	<0.01	<0.01	0.18	0.04	<0.01	<0.01
NTLGWT02	20.6	772	186	1302	<0.01	<0.01	0.22	0.05	<0.01	<0.01
NT7MWT01	2.5	125	70	213	<0.01	<0.01	0.27	0.06	<0.01	<0.01
NT7MWT02	2.7	122	69	202	<0.01	<0.01	0.27	0.06	<0.01	<0.01

Appendix A2. Water quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado beginning irrigation (6/03/99). Conductivity measured in μmhos/cm, pH unitless, remainder of analytes are mg/L.

Sample id	Site	pH	Conductivity	Ca	Mg	Na	K	B	CO_3	HCO_3	SO_4
NTMLWT03	Mortenson Lake	7.8	709	81.7	34.7	24.9	0.3	0.09	<0.1	358.5	72.8
NTMLWT04	Mortenson Lake	7.8	704	81.1	34.3	23.6	1.0	0.08	<0.1	348.3	74.5
NTLGWT03	Lake George	7.5	1510	194.0	71.2	59.6	5.3	0.12	<0.1	265.8	760.7
NTLGWT04	Lake George	7.9	1510	196.0	72.1	59.5	5.3	0.12	<0.1	264.8	781.9
NT7MWT03	Laramie River	7.7	261	26.8	8.2	11.0	1.2	<0.01	<0.1	85.9	51.9
NT7MWT04	Laramie River	7.6	259	27.0	8.3	11.3	1.2	<0.01	<0.1	84.4	52.0

Sample id	Cl	Hardness as $CaCO_3$	Alkalinity as $CaCO_3$	Total Dissolved Solids	NO_3	NO_3-N	NO_2	NO_2-N	NH_4	NH_4-N
NTMLWT03	11.5	346	294	585	2.5	0.6	0.49	0.11	<0.01	<0.01
NTMLWT04	13.3	343	285	577	2.6	0.6	0.04	0.01	<0.01	<0.01
NTLGWT03	13.4	777	218	1370	2.1	0.5	0.22	0.05	0.06	0.05
NTLGWT04	13.2	787	217	1394	2.5	0.6	0.31	0.07	0.31	0.24
NT7MWT03	2.0	100	70	187	2.4	0.6	0.18	0.04	0.32	0.25
NT7MWT04	2.0	101	69	187	2.7	0.6	0.35	0.08	0.13	0.10

Appendix A3. Water quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado mid-irrigation (7/08/99). Conductivity measured in μmhos/cm, pH unitless, remainder of analytes are mg/L.

Sample id	Site	pH	Conductivity	Ca	Mg	Na	K	B	CO_3	HCO_3	SO_4
NTMLWT05	Mortenson Lake	7.7	374	57.7	14.8	4.4	2.1	0.10	<0.1	220.7	30.7
NTMLWT06	Mortenson Lake	7.8	380	57.0	14.6	3.8	2.4	0.10	<0.1	218.0	29.3
NTLGWT05	Lake George	7.9	1590	187.6	81.3	61.5	6.8	0.18	<0.1	105.2	840.0
NTLGWT06	Lake George	7.7	1530	186.0	84.5	58.1	6.7	0.18	<0.1	107.6	820.0
NT7MWT05	Laramie River	7.9	650	58.1	29.0	32.3	3.3	0.11	<0.1	134.8	237.0
NT7MWT06	Laramie River	7.9	621	56.5	28.1	29.7	3.4	0.12	<0.1	130.8	232.0

Sample id	Cl	Hardness as $CaCO_3$	Alkalinity as $CaCO_3$	Total Dissolved Solids	NO_3	NO_3-N	NO_2	NO_2-N	NH_4	NH_4-N
NTMLWT05	19.6	205	181	351	NR*	0.6	<0.1	<0.01	<0.01	<0.01
NTMLWT06	20.6	202	179	346	NR	0.6	0.07	0.02	<0.01	<0.01
NTLGWT05	37.8	802	86	1321	NR	0.6	<0.1	<0.01	<0.01	<0.01
NTLGWT06	32.8	811	88	1296	NR	0.7	<0.1	<0.01	<0.01	<0.01
NT7MWT05	20.9	264	110	516	NR	0.7	<0.1	<0.01	<0.01	<0.01
NT7MWT06	19.5	257	107	501	NR	0.6	<0.1	<0.01	<0.01	<0.01

*Value not reported by laboratory

Appendix A4. Water quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado for post-irrigation (8/25/99). Conductivity measured in µmhos/cm, pH unitless, remainder of analytes are mg/L.

Sample id	Site	pH	Conductivity	Ca	Mg	Na	K	B	CO_3	HCO_3	SO_4
NTMLWT07	Mortenson Lake	7.9	820	92.7	42.6	28.1	0.0	0.06	<0.1	388.1	143.0
NTMLWT08	Mortenson Lake	7.9	817	93.6	43.4	31.3	0.0	0.06	<0.1	388.4	132.0
NTLGWT07	Lake George	8.3	1740	201.2	93.8	76.1	5.4	0.07	<0.1	57.0	1017.2
NTLGWT08	Lake George	9.0	1740	205.5	95.8	75.4	6.0	0.07	35.3	45.3	1026.4
NT7MWT07	Laramie River	8.2	1040	89.8	47.8	65.4	2.7	0.06	<0.1	163.9	395.3
NT7MWT08	Laramie River	7.8	1020	89.7	48.0	65.6	2.2	0.06	<0.1	163.1	378.1

Sample id	Cl	Hardness as $CaCO_3$	Alkalinity as $CaCO_3$	Total Dissolved Solids	NO_3	NO_3-N	NO_2	NO_2-N	NH_4	NH_4-N
NTMLWT07	35.4	406	318	731	1.0	0.23	0.03	0.01	<0.01	<0.01
NTMLWT08	34.0	412	318	724	1.0	0.22	0.07	0.02	<0.01	<0.01
NTLGWT07	31.3	888	47	1483	0.9	0.20	0.03	0.01	<0.01	<0.01
NTLGWT08	33.1	907	37	1524	1.0	0.22	0.03	0.01	<0.01	<0.01
NT7MWT07	9.5	421	134	776	0.9	0.24	0.03	0.01	<0.01	<0.01
NT7MWT08	39.2	421	134	787	1.0	0.21	<0.01	<0.01	<0.01	<0.01

Appendix B1. Sediment quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado for pre-irrigation (5/12/99). Units are mg/kg dry weight.

Sample id	Site	NH_4-N	NO_3-N	NO_2-N
NTMLSD01	Mortenson Lake	5.39	3.36	0.60
NTMLSD02	Mortenson Lake	7.22	5.11	0.75
NTLGSD01	Lake George	1.44	4.18	0.70
NTLGSD02	Lake George	1.87	3.27	0.47
NT7MSD01	Laramie River	3.22	4.18	0.62
NT7MSD02	Laramie River	1.91	4.95	0.55

Appendix B2. Sediment quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado beginning irrigation (6/03/99). Units are mg/kg dry weight.

Sample id	Site	NH_4-N	NO_3-N	NO_2-N
NTMLSD03	Mortenson Lake	1.5	1.4	0.02
NTMLSD04	Mortenson Lake	1.7	1.4	0.02
NTLGSD03	Lake George	0.7	1.4	<0.01
NTLGSD04	Lake George	2.8	1.2	0.02
NT7MSD03	Laramie River	2.9	1.4	0.02
NT7MSD04	Laramie River	1.5	1.3	<0.01

Appendix B3. Sediment quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado mid-irrigation (7/08/99). Units are mg/kg dry weight

Sample id	**Site**	**NH_4-N**	**NO_3-N**	**NO_2-N**
NTMLSD05	Mortenson Lake	10.0	1.4	<0.01
NTMLSD06	Mortenson Lake	9.7	1.8	<0.01
NTLGSD05	Lake George	12.8	1.5	<0.01
NTLGSD06	Lake George	11.7	1.6	<0.01
NT7MSD05	Laramie River	4.8	1.4	<0.01
NT7MSD06	Laramie River	4.4	2.1	<0.01

Appendix B4. Sediment quality results from Colorado State University Soil, Water, and Plant Testing Laboratory, Fort Collins, Colorado for post-irrigation (8/25/99). Units are mg/kg dry weight.

Sample id	**Site**	**NH_4-N**	**NO_3-N**	**NO_2-N**
NTMLSD07	Mortenson Lake	8.5	1.2	<0.1
NTMLSD08	Mortenson Lake	8.5	1.5	0.1
NTLGSD07	Lake George	9.7	1.0	<0.1
NTLGSD08	Lake George	10.9	0.9	0.1
NT7MSD07	Laramie River	25.0	1.3	<0.1
NT7MSD08	Laramie River	20.8	1.3	<0.1