

A Water Quality Bibliography for Amphibians

R. Andrew Odum

Arthur, J. and J. Eaton (1971). "CHLORAMINE TOXICITY TO THE AMPHIPOD GAMMARUS PSEUDOLIMNAEUS AND THE FATHEAD MINNOW(PIMEPHALES PROMELAS)."

amphipods (gammarus pseudolimnaeus) were subjected to 96 hr and 15 week exposures, and fathead minnows (pimephales promelas) to a 21 week exposure of various chloramine concentrations under continuous flow conditions. The most marked sublethal effects were reductions in the number of young produced by the amphipod and of eggs produced by the minnow. The 96 hr median tolerance limit (tlm-50) for the amphipod was 0.220 mg/l total chloramine. Fathead minnows in the long term study were all killed at the highest concentration, 0.154 mg/l total chloramine, within three days. The lowest measured total chloramine concentration in the long term tests having no significant effect was less than 0.0034 mg/l for the amphipod and 0.0165 mg/l for the fathead minnow. (legore-washington)

Bai, H. and A. C. Yeh (1997). "Removal of CO₂ Greenhouse Gas by Ammonia Scrubbing." Industrial & Engineering Chemistry Research **36**(6): 2490-2493.

This paper provides preliminary results on the novel study of ammonia scrubbing for the removal of carbon dioxide from flue gas. Experimental results indicated the potential of CO₂ reduction by NH₃ scrubbing is very promising. The overall CO₂ removal efficiencies could be above 95% under proper operation conditions. The absorption capacity of NH₃ was around 0.9 kg of CO₂/kg of NH₃ reagent being used. This should be higher than that by a MEA solution. The reaction products were analyzed and determined by X-ray diffraction analysis, SEM picture, and pH measurements. All the measurements indicated that an ammonium bicarbonate solution and its crystalline solids are the major products of reaction.

Ball, I. (1967). "The relative susceptibilities of some species of fresh-water fish to poisons--I. Ammonia." Water Research **1**(11-12): 767-775.

The acute toxicity of ammonia in a hard, well-aerated water to bream, perch, roach, rudd, and rainbow trout was determined. While trout responded more quickly than the coarse fish so that they were more sensitive over a short period of exposure (1 day), their mortality rate subsequently dropped. The coarse fish, however, continued to die and extension of the test period to the time when the toxicity curves had become asymptotic with the time axis showed that all the species were equally sensitive. The importance of the slope of the concentration response curve in assessing concentrations permissible in the field is discussed.

Birge, J., A. Black, et al. (1980). Effects of organic compounds on amphibian reproduction. Available from the as PB 80-147523, Price codes: A 03 in paper copy, A 01 in microfiche. Research Report, National Technical Information Service, Springfield VA 22161.

Aquatic toxicity tests were conducted with atrazine, carbon tetrachloride, chloroform, methylene chloride, trisodium nitrilotriacetic acid (NTA), and phenol. Each compound was administered to developmental stages of three to five amphibian species. Exposure was initiated at fertilization and maintained through 4 days post-hatching. Test responses included lethality and teratogenesis. Different amphibian species exhibited varying degrees of tolerance to the selected compounds. Greatest tolerance usually was observed for the more broadly adapted semi-aquatic and terrestrial species (e.g., *Bufo americanus*, *Bufo fowleri*). The more sensitive amphibians usually included those species which normally are restricted to aquatic or moist habitats (e.g., *Rana catesbeiana*, *Rana pipiens*). Median lethal concentrations (mg/l) determined at 4 days posthatching ranged from 0.41 to 48 for atrazine, 0.90 to 2.83 for carbon tetrachloride, 0.27 to 35.14 for chloroform, 17.78 to >32 for methylene chloride, 39.3 to 252.3 for NTA, and 0.04 to >0.89 for phenol. The most toxic compounds always included phenol, carbon tetrachloride, and atrazine, and the least toxic consistently were NTA and methylene chloride. For three chlorinated alkanes, including methylene chloride, chloroform, and carbon tetrachloride, toxicity increased with chlorination. Toxicity of the different compounds was further characterized by calculating concentrations which produced embryo-larval lethality or teratogenesis at frequencies of 10% and 1%.

BLAUSTEIN, A., A. HATCH, et al. (2001). "Influence of abiotic and biotic factors on amphibians in ephemeral ponds with special reference to long-toed salamanders (*Ambystoma macrodactylum*)."
Israel Journal of Zoology **47**(4): 333-346.

Animals living in ephemeral habitats are subjected to various abiotic and biotic selection pressures that may not be present to the same degree in permanent habitats. For example, pond drying can lead to increased predation and competition as resources become limited and temperature and water quality undergo drastic fluctuations. Amphibians provide an excellent model for studying factors associated with survival in temporary habitats. Aquatic amphibians developing in ephemeral habitats must find food, avoid predators, and cope with potentially great fluctuations in abiotic parameters under increasingly harsh conditions. In Oregon (U.S.A.), larval long-toed salamanders (*Ambystoma macrodactylum*) often inhabit temporary ponds that gradually dry during the summer. We have been studying several factors that may influence long-toed salamander behavior, growth, and survival in ephemeral montane ponds in the Cascade Range. These include biotic interactions focusing on cannibalism and predation, and abiotic factors, including ultraviolet (UV) radiation, an agent that can harm developing amphibians. Long-toed salamanders living in ephemeral ponds, like some other salamander species, exhibit trophic polymorphism, with some individuals having disproportionately broader and longer heads and enlarged vomerine teeth. The ingestion of different types of prey contributes to plasticity in head shape but other cues are essential to induce extreme trophic polymorphism. During periods of food limitation, individuals capable of using alternative food sources, including conspecifics, may experience a competitive advantage over their conspecifics. Morphological plasticity may therefore play an important role in resource partitioning and in alleviating intraspecific competition. The results of field experiments suggest that long-toed salamander embryos are highly susceptible to ambient levels of UV-B radiation. UV radiation reduces hatching success and increases deformities. Laboratory tests suggest that larvae from populations from high elevations may be more resistant to UV-B radiation than larvae from low-elevation populations. Like other amphibian species that may inhabit temporary ponds, long-toed salamanders are potentially vulnerable to a wide array of pollutants, including those associated with nitrate and nitrite fertilizers. Synergistic interactions with a number of agents, including UV radiation and various toxic substances, may be especially harmful to long-toed salamanders.

Boga, A., S. Binokay, et al. (2009). "The toxicity and teratogenicity of gibberellic acid (GA3) based on the frog embryo teratogenesis assay-Xenopus (FETAX)."
Turk J Biol **33**: 181-188.

This study investigated the developmental toxicity of a plant growth regulator (a type of pesticide) using the frog embryo teratogenesis assay-Xenopus (FETAX). *Xenopus laevis* embryos were exposed to 11 different concentrations of gibberellic acid (GA3), from stage 8 to 11, for 96 h under static renewal test conditions. The median lethal concentration (LC50), malformation (EC50), non-observed adverse effect concentration (NOAEC), and lowest observed adverse effect concentration (LOAEC) were calculated. The corresponding LC50 and EC50 values determined for GA3 exposure were 1117.5 mg/L and 658 mg/L, respectively. The TI (LC50/EC50) value calculated for GA3 was 1.69. Different anomalies occurred in the embryos, depending on the GA3 concentration. Based on these results, we conclude that GA3 is toxic and teratogenic to *Xenopus laevis* embryos. Moreover, our results confirm that the FETAX assay can be a useful pretest for integrated biological hazard assessment of chemical agents used in agriculture.

Boyer, R. and C. Grue (1995). "The need for water quality criteria for frogs." Environmental health perspectives **103**(4): 352-357.

Boyer, R. and C. Grue (1995). "The need for water quality criteria for frogs." Environmental health perspectives **103**(4): 352.

Amphibians are considered reliable indicators of environmental quality. In the western United States, a general decline of frog populations parallels an apparent worldwide decline. The factors thought to be contributing to declines in frog populations include habitat loss, introduction of exotic species, overexploitation, disease, climate change, and decreasing water quality. With respect to water quality, agroecosystems use 80-90% of the

water resources in the western United States, frequently resulting in highly eutrophic conditions. Recent investigations suggest that these eutrophic conditions (elevated pH, water temperature, and un-ionized ammonia) may be associated with frog embryo mortality or malformations. However, water quality criteria for frogs and other amphibians do not currently exist. Here, we briefly review data that support the need to develop water quality parameters for frogs in agroecosystems and other habitats. Key words:

Broomhall, S. (2002). "The effects of endosulfan and variable water temperature on survivorship and subsequent vulnerability to predation in *Litoria citropa* tadpoles." *Aquatic Toxicology* **61**(3-4): 243-250.

The effects of short-term exposure of stage 25 *Litoria citropa* tadpoles to sublethal concentrations of endosulfan in combination with either a stable or a variable temperature cycle (20 ± 2 vs. 21 ± 7.5 °C) were investigated. Both exposure to 0.8 µg/l endosulfan and the wider temperature range over 96 h had significant adverse effects on survivorship. In addition, variable water temperatures at the time of endosulfan exposure increased tadpoles' vulnerability to predation by odonates 24 days later, but only if they had also been exposed to endosulfan. This effect occurred despite maintenance of all tadpoles at 19 ± 1 °C during the intervening 24 days. Correlates of fitness such as this represent a move towards more biologically relevant experimental endpoints. This is an important step if we are to gain an understanding of how exposure to agricultural chemicals may affect frog populations in the natural environment. The results indicate that a short, pulsed exposure to a sublethal concentration of endosulfan and extremes in temperature may then have long-term impacts on fitness.

Browne, R., R. Odum, et al. (2007). "Facility design and associated services for the study of amphibians." *ILAR journal* **48**(3): 188.

The role of facilities and associated services for amphibians has recently undergone diversification. Amphibians traditionally used as research models adjust well to captivity and thrive with established husbandry techniques. However, it is now necessary to maintain hundreds of novel amphibian species in captive breeding, conservation research, and biomedical research programs. These diverse species have a very wide range of husbandry requirements, and in many cases the ultimate survival of threatened species will depend on captive populations. Two critical factors have emerged in the maintenance of amphibians, stringent quarantine and high-quality water. Because exotic diseases such as chytridiomycosis have devastated both natural and captive populations of amphibians, facilities must provide stringent quarantine. The provision of high-quality water is also essential to maintain amphibian health and condition due to the intimate physiological relationship of amphibians to their aquatic environment. Fortunately, novel technologies backed by recent advances in the scientific knowledge of amphibian biology and disease management are available to overcome these challenges. For example, automation can increase the reliability of quarantine and maintain water quality, with a corresponding decrease in handling and the associated disease-transfer risk. It is essential to build facilities with appropriate nontoxic waterproof materials and to provide quarantined amphibian rooms for each population. Other spaces and services include live feed rooms, quarantine stations, isolation rooms, laboratory space, technical support systems, reliable energy and water supplies, high-quality feed, and security. Good husbandry techniques must include reliable and species-specific management by trained staff members who receive support from the administration. It is possible to improve husbandry techniques for many species by sharing knowledge through common information systems. Overall, good facility design corresponds to the efficient use of space, personnel, energy, materials, and other resources.

Burgett, A., C. Wright, et al. (2007). "Impact of ammonium nitrate on wood frog (*Rana sylvatica*) tadpoles: effects on survivorship and behavior." *Herpetological Conservation and Biology* **2**(1): 29-34.

Nitrogenous based fertilizers, such as ammonium nitrate, are commonly used in agriculture, entering aquatic ecosystems through runoff. Ammonium nitrate has been shown to affect the survivorship and behavior of anurans. We conducted an experiment to examine the potential toxic effects of ammonium nitrate on Wood Frog (*Rana sylvatica*) tadpoles. We also examined whether ammonium nitrate might interact with predator cues to affect tadpole behavior. Ammonium nitrate decreased survivorship of Wood Frog tadpoles (> 50 mg L⁻¹ NH₄NO₃). Activity level of Wood Frog tadpoles decreased when exposed to ammonium nitrate, as well as in the presence of predator (*Mosquitofish*, *Gambusia affinis*) chemical cues. Our results suggest that ammonium

nitrate can have significant effects on anuran larvae, both through direct toxicological effects on survivorship, but also through behavioral effects.

Burrell, P., C. Phalen, et al. (2001). "Identification of Bacteria Responsible for Ammonia Oxidation in Freshwater Aquaria." Applied and environmental Microbiology **67**(12): 5791–5800.

Culture enrichments and culture-independent molecular methods were employed to identify and confirm the presence of novel ammonia-oxidizing bacteria (AOB) in nitrifying freshwater aquaria. Reactors were seeded with biomass from freshwater nitrifying systems and enriched for AOB under various conditions of ammonia concentration. Surveys of cloned rRNA genes from the enrichments revealed four major strains of AOB which were phylogenetically related to the *Nitrosomonas marina* cluster, the *Nitrosospira* cluster, or the *Nitrosomonas europaea*-*Nitrosococcus mobilis* cluster of the α subdivision of the class Proteobacteria. Ammonia concentration in the reactors determined which AOB strain dominated in an enrichment. Oligonucleotide probes and PCR primer sets specific for the four AOB strains were developed and used to confirm the presence of the AOB strains in the enrichments. Enrichments of the AOB strains were added to newly established aquaria to determine their ability to accelerate the establishment of ammonia oxidation. Enrichments containing the *Nitrosomonas marina*-like AOB strain were most efficient at accelerating ammonia oxidation in newly established aquaria. Furthermore, if the *Nitrosomonas marina*-like AOB strain was present in the original enrichment, even one with other AOB, only the *Nitrosomonas marina*-like AOB strain was present in aquaria after nitrification was established. *Nitrosomonas marina*-like AOB were 2% or less of the cells detected by fluorescence in situ hybridization analysis in aquaria in which nitrification was well established.

Byrne, P. and J. Roberts (1999). "Simultaneous mating with multiple males reduces fertilization success in the myobatrachid frog *Crinia georgiana*." Proceedings of the Royal Society B: Biological Sciences **266**(1420): 717-721.

Approximately 50% of matings in the frog *Crinia georgiana* involve two or more males. We report reduced fertilization success as a major cost of mating with multiple males. For single-male matings, fertilization success was consistently high averaging 96%. Only 68% of eggs were fertilized when females were amplexed by two males and this dropped to 64% when females were amplexed by three to five males. Multiple regression analysis revealed the reduction in fertilization success was significantly related to the number of amplexant males but not to clutch size or three measures of water quality (depth, temperature and oxygen concentration) at the site of oviposition. The most likely cause of reduced fertilization success is struggles amongst males which interfere with ejective sperm transfer.

Collins, S. and R. Russell (2009). "Toxicity of road salt to Nova Scotia amphibians." Environmental Pollution **157**(1): 320-324.

The deposition of chemical pollutants into roadside wetlands from runoff is a current environmental concern. In northern latitudes, a major pollutant in runoff water is salt (NaCl), used as de-icing agents. In this study, 26 roadside ponds were surveyed for amphibian species richness and chloride concentration. Acute toxicity tests (LC50) were performed on five locally common amphibian species using a range of environmentally significant NaCl concentrations. Field surveys indicated that spotted salamanders (*Ambystoma maculatum*) and wood frogs (*Rana sylvatica*) did not occupy high chloride ponds. American toads (*Bufo americanus*) showed no pond preference based on chloride concentration. Acute toxicity tests showed spotted salamanders and wood frogs were most sensitive to chloride, and American toads were the least. Spring peepers (*Pseudacris crucifer*) and green frogs (*Rana clamitans*) showed intermediate sensitivities. We concluded that chloride concentrations in ponds due to application of de-icing salts, influenced community structure by excluding salt intolerant species.

Cummins, C. (1989). "Interaction between the effects of pH and density on growth and development in *Rana temporaria* L. tadpoles." Functional Ecology **3**(1): 45-52.

The common frog (*Rana temporaria* L.) occurs in areas where waters are naturally acid and where breeding sites are susceptible to acidification from anthropogenic sources. Acid conditions have been shown to cause embryo mortality. They can also delay metamorphosis and reduce size at metamorphosis among individual tadpoles. Time of metamorphosis and size at metamorphosis may also be influenced by crowding effects. It is conceivable

that reductions in tadpole density arising from embryo mortality will offset depressant effects of acid conditions on individual tadpoles. Furthermore, interactions among tadpoles may be modified by individuals' responses to acid conditions. To investigate such possible effects, common frog tadpoles were raised to metamorphosis in the laboratory at three densities (4, 8 and 16 tadpoles litre⁻¹) and two levels of pH (nominally pH 4 and pH 7) on a limiting ration. Overall, the effects of density on size at metamorphosis and time of metamorphosis far outweighed those of low pH. The depressant effect of low pH on mean development rate and mean size at metamorphosis decreased with increasing tadpole density. Low pH accentuated hierarchical effects within density replicates, and dominant tadpoles suffered little or no net retardation of growth or development due to low pH. Tadpoles which grew and developed rapidly at low pH suffered debilitating limb deformities. The implications of these interactive effects of density and pH are discussed in the context of freshwater acidification and its possible effects on frog populations. The response of individuals in a group is likely to be more important than the mean response of the group.

Emerson, K., R. Russo, et al. (1975). "Aqueous ammonia equilibrium calculations: effects of pH and temperature." Journal of the Fisheries Research Board of Canada **32**: 2379-2383.

The toxicity of ammonia to fishes has been attributed to the un-ionized ammonia chemical species present in aqueous solution. Because the percent of total ammonia present as un-ionized ammonia (NH₃) is dependent on pH and temperature, an exact understanding of the aqueous ammonia equilibrium is important for toxicity studies. A critical evaluation of the literature data on the ammonia-water equilibrium system has been carried out. Results of calculations of values of pK_a at different temperatures and of percent of NH₃ in aqueous ammonia solutions of zero salinity as a function of pH and temperature are presented. (Katz)

EPA (2009). "Risks of Prometryn Use to Federally Threatened California Red-legged Frog."

The purpose of this assessment is to evaluate potential direct and indirect effects on the California red-legged frog (*Rana aurora draytonii*) (CRLF) arising from Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) regulatory actions regarding use of prometryn on agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of the species' designated critical habitat. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (U.S. FWS) and National Marine Fisheries Service (NMFS) Endangered Species Consultation Handbook (U.S. FWS/NMFS 1998) and procedures outlined in the Agency's Overview Document (U.S. EPA 2004). The CRLF was listed as a threatened species by U.S. FWS in 1996. The species is endemic to California and Baja California (Mexico) and inhabits both coastal and interior mountain ranges. A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (U.S. FWS 1996) in California. Prometryn is a symmetric triazine (s-triazine) herbicide. Formulation types registered include liquids and wettable granules; therefore, all formulations are designed for liquid application. Currently, labeled uses of prometryn include uses on celery, fennel, dill, parsley, and cotton. These uses are considered as part of the federal action evaluated in this assessment. Prometryn is stable to abiotic hydrolysis and direct photolysis in water and soil. Biotransformation has been identified as a major dissipation process, albeit slow. There are no data on the persistence, transformation, or partitioning in water-sediment systems. It is very mobile to mobile in soils (average K_{oc} = 244 L/kg-OC). Based on the value of the vapor pressure and Henry's Law Constant it is not expected to volatilize from soil or water. The low n-octanol-water partition coefficient (Log K_{ow} < 4) of prometryn suggests low bioaccumulation potential. The major biotransformation product is a hydroxy-triazine that is potentially a biotransformation product in common with hydroxyl degradates of other s-triazine herbicides. Based on the persistence and mobility of prometryn, major routes of transport are likely to be runoff to surface water bodies or to adjacent fields and leaching to ground water. Prometryn could also reach non-target sites by drift and/or wind erosion. Water monitoring data from California has identified that the concentrations of prometryn in surface water range from 0.0054 to 0.621 µg/L. Although prometryn has been detected in rain and wet deposition, the vapor pressure suggests low potential for volatility. Since CRLFs exist within aquatic and terrestrial habitats, exposure of the CRLF, its prey, and its habitats to prometryn are assessed separately for the two habitats. The PRZM/EXAMS aquatic exposure model was used to estimate high-end exposures of prometryn in aquatic habitats resulting from runoff and spray drift from different uses. The

1-in-10-year peak model-estimated environmental concentrations (EECs) for selected CA scenarios range from 37.6 to 377.3 µg/L. This residue accumulation is the result of high persistence of prometryn in conjunction with the static hydrology (no flow) of the standard pond. These estimates are supplemented with analysis of available California surface water monitoring data from U. S. Geological Survey's National Water Quality Assessment (NAWQA) program and the California Department of Pesticide Regulation. The maximum concentration of prometryn reported by NAWQA for California surface waters with agricultural watersheds is 0.621µg/L. This value is approximately 607 times less than the maximum model-estimated environmental concentration. The T-REX model is used to estimate prometryn exposures to the terrestrial-phase CRLF and its potential prey resulting from uses involving prometryn applications. The AgDRIFT model is also used to estimate deposition of prometryn on terrestrial and aquatic habitats from spray drift. The TerrPlant model is used to estimate prometryn exposures to terrestrial-phase CRLF habitat, including plants inhabiting semi-aquatic and dry areas, resulting from uses involving foliar prometryn applications. The effects determination assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF itself, as well as indirect effects, such as reduction of the prey base or modification of its habitat. Direct effects to the CRLF in the aquatic habitat are based on toxicity information for freshwater fish, which are generally used as a surrogate for aquatic-phase amphibians. In the terrestrial habitat, direct effects are based on toxicity information for birds, which are used as a surrogate for terrestrial-phase amphibians. Given that the CRLF's prey items and designated critical habitat requirements in the aquatic habitat are dependant on the availability of freshwater aquatic invertebrates and aquatic plants, toxicity information for these taxonomic groups is also discussed. In the terrestrial habitat, indirect effects due to depletion of prey are assessed by considering effects to terrestrial insects, small terrestrial mammals, and frogs. Indirect effects due to effects to the terrestrial habitat are characterized by available data for terrestrial monocots and dicots. Degradation products of prometryn in soil metabolism studies are 2,4-bis(isopropylamino)-6-hydroxy-s-triazine (CS-11526) at 27% of the applied radioactivity after one year post-treatment and 2-amino-4-isopropylamino-6-methylthio-s-triazine (GS-11354) at less than 10% after one year post-treatment. There are no available ecotoxicity data and limited environmental fate data for these degradation products. Based on EPA's human health assessment conducted to support the prometryn RED (Wassell 1998), the only residue of concern is prometryn. Therefore, the degradation products of prometryn are not considered in this assessment. Risk quotients (RQs) are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the Agency's levels of concern (LOCs) to identify instances where prometryn use within the action area has the potential to adversely affect the CRLF and its designated critical habitat via direct effects or indirectly via direct effects to its food supply (i.e., freshwater invertebrates, algae, fish, frogs, terrestrial invertebrates, and mammals) or habitat (i.e., aquatic plants and terrestrial upland and riparian vegetation). When RQs for each particular type of effect are below LOCs, the pesticide is determined to have "no effect" on the CRLF. Where RQs exceed LOCs, a potential to cause adverse effects is identified, leading to a conclusion of "may affect." If a determination is made that use of prometryn use within the action area "may affect" the CRLF or its designated critical habitat, additional information is considered to refine the potential for exposure and effects, and the best available information is used to distinguish those actions that "may affect, but are not likely to adversely affect" (NLAA) from those actions that are "likely to adversely affect" (LAA) the CRLF. Similarly for critical habitat, additional information is considered to refine the potential for exposure and effects to distinguish those actions that do or do not result in effects to its critical habitat. Based on the best available information, the Agency makes a Likely to Adversely Affect determination for the CRLF from the use of prometryn. Additionally, the Agency has determined that there is a potential for effects to the CRLF designated critical habitat from the use of the chemical. A summary of the risk conclusions and effects

Erickson, R. (1985). "An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms." *Water Research* **19**(8): 1047-1058.

Available data on the pH and temperature dependence of ammonia toxicity to aquatic organisms were examined and their agreement with various models was evaluated. A model which considers alteration of the relative concentration of un-ionized ammonia at the gill surface failed to adequately describe either pH or temperature dependence. A model that assumes that un-ionized ammonia and ammonium ion are jointly toxic

was strongly supported by the data on pH dependence, but could not explain observed temperature dependence. Temperature dependence can be described empirically by a simple log-linear model. The effects of pH and temperature were generally found to be qualitatively and quantitatively similar among fish species.

Fellers, G., D. Bradford, et al. (2007). "Demise of repatriated populations of mountain yellow-legged frogs (*Rana muscosa*) in the Sierra Nevada of California." Herpetological Conservation and Biology **2**: 5-21.

Abstract.—In the late 1970s, *Rana muscosa* was common in the Tableland area of Sequoia National Park, CA. Surveys in 1993- 1995 demonstrated that this frog had disappeared from this and other areas, even though the species was still common 30 km to the northeast. To evaluate potential causes of the extirpation, we repatriated *R. muscosa* eggs, tadpoles, subadults, and adults to four previously occupied sites in the Tableland area in 1994 and 1995. We surveyed the release sites every few days in the summers of 1994 and 1995, and once a month in 1996-1997. During the first week after release, survival of all life history stages was high at each of the release sites. At the end of the first summer there were metamorphosing tadpoles, and adult frogs were still present. However, we detected no evidence of reproduction at three sites and nearly all life history stages disappeared within 12 months. At the fourth site, there was limited reproduction, but it was not sufficient to maintain a population. It appears either that the causal factors for the demise of *R. muscosa* in the 1970s were still operating in the 1990s, or that a new limiting factor has developed. Dispersal, weather, water quality, and predation do not appear to be causative agents; because fish have never been present in the portions of the watershed where we were working, they were not a factor. Observations and data are consistent with the hypotheses that chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis*, and/or exposure to airborne pesticides caused both declines. However, at the time of our study, chytridiomycosis had not been described and the potentially significant role of contaminants was largely undocumented.

Fellers, G., D. Bradford, et al. (2008). Experimental Repatriation of Mountain Yellow-legged Frogs (*Rana muscosa*) in the Sierra Nevada of California, USGS.

In the late 1970s, *Rana muscosa* (mountain yellow-legged frog) was common in the Tableland area of Sequoia National Park, California where it was possible to find hundreds of tadpoles and adults around many of the ponds and lakes. Surveys in 1993-1995 demonstrated that *R. muscosa* was absent from more than half of all suitable habitat within the park, including the Tableland area. At that same time, *R. muscosa* was still common at Sixty Lake Basin, Kings Canyon National Park, 30 km to the northeast. To evaluate the potential causes for the extirpation, we repatriated *R. muscosa* eggs, tadpoles, subadults, and adult frogs from Sixty Lake Basin to four sites in the Tableland area in 1994 and 1995. We subsequently surveyed each release site and the surrounding area 2 - 3 times per week in 1994-1995, and intermittently in 1996-1997, to monitor the survival of all life history stages, and to detect dispersal of adults and subadults. We also monitored predation, water quality, weather, and water temperature. Our techniques for capturing, holding, transporting, and releasing *R. muscosa* were refined during the study, and during 1995 resulted in high initial survival rates of all life history stages. Adult frogs were anaesthetized, weighed, measured, tagged, and held in plastic boxes with wet paper towels. Tadpoles were collected and held in fiberglass screen cages set in the water at the edge of a pond. This resulted in relatively natural conditions with less crowding and good water circulation. Frogs, tadpoles, and eggs were placed in Ziploc bags for transport to the Tableland by helicopter. Short-term survival of tadpoles, subadults, and adults was high at all four release sites, tadpoles reached metamorphosis, and adult frogs were still present. However, we detected no evidence of reproduction at three sites (e.g., no new eggs or small tadpoles) and nearly all life history stages disappeared within 12 months. At the fourth site, there was limited reproduction, but it was insufficient to maintain a population.

It appears that the causal factors for the demise of *R. muscosa* in the Tableland during the 1970s were still operating in the 1990s or that a new limiting factor has developed. Dispersal, weather, water quality, and predation do not appear to be causative agents; since fish have never been present in the portions of the watershed where we were working, they were not a factor. Observations and data are consistent with the hypotheses that chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis*, and/or exposure to airborne pesticides caused both declines. However, at the time of our study, chytridiomycosis had not been described and the potentially significant role of contaminants was largely undocumented.

Flynn, R. (2009). Fertilizer exposure affects *Hyla versicolor* (gray tree frog) larval growth and development. Amphibian populations worldwide are experiencing mass extinctions, the causes of which have yet to be fully determined. Portions of this have been linked to addition of mineral nitrogen to water bodies in which amphibians breed. This study set out to determine whether two types of nitrogen fertilizer addition to the water column—that is spills and run-off—differentially affect larval growth, development, activity, and survivorship. Concentrations of 10 mg/L of granular urea were added as pulses—simulating one-time large spills—and presses—simulating run-off—to pools containing *Hyla versicolor* tadpoles. Survivorship and activity did not differ among treatments. However, growth and development were both affected by the fertilizer: growth rate was higher in fertilizer treatments than in control, and the stage of development was higher in fertilizer treatments than in control ones. Except for one day, there was no statistically significant difference between press and pulse treatments. These results indicate that previous studies' results can be applied to both fertilizer spill and run-off situations. In addition, it shows that fertilizer may not always be harmful to tadpole growth and development if added to the water column during the larval stage, which may have agricultural and wildlife management implications.

Gauthier, L., Y. Levi, et al. (1989). "Evaluation of the clastogenicity of water treated with sodium hypochlorite or monochloramine using a micronucleus test in newt larvae (*Pleurodeles waltl*)." *Mutagenesis* **4**(3): 170-173.

We have previously described a micronucleus test using erythrocytes from larvae of the urodele amphibian *Pleurodeles waltl* (pleurodele). The test is based on a comparison of the levels of micronucleated erythrocytes in blood smears from larvae reared in water containing a clastogen, with the levels from larvae reared in purified water. We have employed this test to evaluate mutagenic activity of chlorinated or monochloraminated water devoid of all organic matter. (i) The level of micronuclei in erythrocytes was compared between a group of larvae reared for 12 days in chlorinated reconstituted ultrapure water treated with sodium hypochlorite, and a control group reared in just the reconstituted water. Sodium hypochlorite was added when both the food and medium were changed each day. Chlorine levels of 0.125 and 0.25 p.p.m. led to significant elevations of micronuclei. (ii) The possibility of indirect effects of chlorine through chemical interactions with the food were also investigated, using the following scheme: larvae were left for 3 h in chlorinated reconstituted ultrapure water and then placed in non-chlorinated water. Food was only introduced when they were transferred to the non-chlorinated water. This procedure was repeated for 12 consecutive days. Control larvae were reared in non-chlorinated water throughout this period. In this case results were also positive when the larvae were exposed for only 3 h to the chlorine (0.2 p.p.m. for 12 days) in the absence of food. (iii) This was the same as experiment 1 except that the water was chlorinated with monochloramine instead of sodium hypochlorite. The level of micronuclei increased with increasing concentration of monochloramine (0.05, 0.1 and 0.15 p.p.m.) although only the 0.15 p.p.m. concentration gave a statistically significant response. The results indicate that free chlorine and monochloramine are responsible for the clastogen effect in newt larvae.

Glos, J., T. Ulmar Grafe, et al. (2003). "Geographic Variation in pH Tolerance of Two Populations of the European Common Frog, *Rana temporaria*." *Copeia* **2003**(3): 650-656.

In a laboratory experiment, we investigated the effects of low pH environment, a key parameter of acidity, on mortality, growth, and development in two populations of *Rana temporaria* that differ in the pH of their breeding ponds. In a population with pH neutral breeding sites, low pH treatment caused a prolongation in embryogenesis and an increased embryonic mortality, a higher proportion of deformed hatchlings and an increased larval time. Embryos and larvae from a population that was exposed naturally to low pond pH were more pH tolerant, as the only effect of low pH was increased larval time.

Griffis-Kyle, K. (2005). "Ontogenic delays in effects of nitrite exposure on tiger salamanders (*Ambystoma tigrinum tigrinum*) and wood frogs (*Rana sylvatica*)." *Environmental Toxicology and Chemistry* **24**(6): 1523-1527.

Under certain conditions, nitrite can be present in freshwater systems in quantities that are toxic to the fauna. I exposed wood frog (*Rana sylvatica*) and eastern tiger salamander (*Ambystoma tigrinum tigrinum*) embryos and young tadpoles and larvae to elevated concentrations of nitrite in chronic toxicity tests: 0, 0.3, 0.6, 1.2, 2.1, 4.6,

and 6.1 mg/L NO₂-N, exposing individuals as both embryos and larvae. Nitrite caused significant declines in wood frog hatching success (3.4 mg/L NO₂-N, wood frog), and lower concentrations caused significant mortality during the early larval stages (4.6 mg/L NO₂-N, salamander; 0.5 mg/L NO₂-N, wood frog). Later tests exposing individuals to nitrite only after hatching showed that both wood frog and tiger salamander vulnerability to nitrite declined shortly after hatching. Hence, examining a single life-history stage, especially later in development, may miss critical toxic effects on organisms, causing the researcher potentially to underestimate seriously the ecological consequences of nitrite exposure.

Griffis-Kyle, K. (2007). "Sublethal effects of nitrite on eastern tiger salamander (*Ambystoma tigrinum tigrinum*) and wood frog (*Rana sylvatica*) embryos and larvae: implications for field populations." *Aquatic Ecology* **41**(1): 119-127.

Ephemeral pools, which can have high animal biomass and low dissolved oxygen, may be prone to nitrite accumulation. As such, it is important to understand how exposure to nitrite might affect development and growth of amphibians that breed in these ephemeral pools. Woodfrog (*Rana sylvatica*) and eastern tiger salamander (*Ambystoma tigrinum tigrinum*) embryos and tadpoles and young larvae were exposed to elevated concentrations of nitrite derived from sodium nitrite: 0, 0.3, 0.6, 1.2, 2.1, 4.6, and 6.1 mg l⁻¹ NO₂-N. Increasing nitrite exposure slowed embryonic and larval development in both the eastern tiger salamander and the wood frog, reduced growth in tiger salamander embryos and larvae, and delayed metamorphosis in the wood frog. At concentrations less than 2 mg l⁻¹ NO₂-N nitrite delayed hatching, and at concentrations above 2 mg l⁻¹ time to hatching decreased causing more individuals to hatch at less developed stages. Nitrite also increased asynchrony in tiger salamander hatching. The sublethal effects of nitrite on amphibian development, growth and hatching could have serious repercussions on amphibian fitness in ephemeral environments. Potential increases in mortality on field populations caused by sublethal effects of nitrite are discussed.

Hannas, B. (2009). Mechanistic evaluation of the reproductive and developmental toxicity of nitrate and nitrite. Raleigh, North Carolina, North Carolina State University. **Ph.D.**

The purpose of this research was to investigate the mechanism for reproductive and developmental toxicity associated with the ubiquitous aquatic contaminants nitrate and nitrite, in the crustacean *Daphnia magna*. Two hypotheses were tested: 1) Nitrate and nitrite are converted to the signaling molecule NO, resulting in disruption of endocrine-related processes; and 2) NO interferes with endocrine signaling by lowering steroid hormone levels or by binding a heme-containing nuclear receptor involved in steroid signaling. In the first study, the toxicity of nitrate and nitrite to daphnids was evaluated. Both compounds increased incidence of developmental abnormalities and reduced fecundity in a concentration-dependent manner. Toxicity was consistent with toxicity elicited by the NO donor sodium nitroprusside. Developmental toxicity of nitrite and NO was ameliorated by the NO-scavenger β -carotene. Since toxicity of nitrate and nitrite mimicked that of NO, experiments were performed to determine if arthropod cells could convert nitrate and nitrite to NO. *Drosophila* S2 cells converted both nitrate and nitrite to NO in a substrate and cell concentration-dependent manner. Together, these results are consistent with nitrate and nitrite eliciting toxicity via their intracellular conversion to NO. Although the observed toxicity was indicative of an anti-ecdysteroid mechanism of action, we were unable to detect significant, consistent decreases in ecdysteroid levels in daphnids exposed to the NO donor. These results suggest that an alternative mechanism was responsible for the observed NO-induced toxicity. The next study focused on characterizing a potential target of NO toxicity in the ecdysteroid signaling pathway. In this study, the nuclear receptors E75 (group NR1D) and HR3 (NR1F) were cloned and sequenced from *Daphnia magna*. Both receptors shared identity with the insect and human orthologs. E75 possessed conserved histidine and cysteine amino acid residues in the ligand binding domain that likely bind heme. NO potentially binds E75-heme as a ligand. HR3 was significantly induced by 20-hydroxyecdysone, whereas E75 was minimally responsive to the hormone. The results of this study implicate both E75 and HR3 of daphnids in the ecdysteroid signaling pathway as potential targets of the action of exogenous NO. Finally, E75 and HR3 were functionally characterized in regards to regulating gene transcription, to determine if NO alters this regulatory activity. HR3 cloned from *Daphnia pulex* activated transcription of a retinoid orphan receptor element (RORE)-driven luciferase reporter. E75 did not activate the reporter, but served to repress HR3 activation. Experiments revealed no evidence that NO interferes with E75 repression of HR3. Therefore, the mechanism by which nitrate

and nitrite-derived NO elicits developmental and reproductive toxicity remains unknown. Overall, this research highlights the potential threat posed to the reproductive and developmental success of aquatic organisms exposed to nitrate and nitrite. Additionally, this work advances understanding of crustacean endocrinology, while demonstrating the need for further information to identify the mechanism of action for interfering environmental contaminants.

Henrikson, L. and M. Olsson (1988). "Effects of pH and aluminium on embryonic and early larval stages of Swedish brown frogs *Rana arvalis*, *R. temporaria* and *R. dalmatina*." *Holarctic Ecology* **11**(2): 127-135.

The effects of pH and aluminium on embryonic and early larval stages of Swedish brown frogs *Rana arvalis*, *R. temporaria* and *R. dalmatina* were tested in laboratory bioassays. In all three species egg mortality and time needed for embryonic development to hatching increased when pH declined, but no significant effects were found on embryonic development when aluminium level was elevated. In *R. arvalis* and *R. temporaria* larval mortality was affected by both pH and aluminium. In both species the frequency of occurrence of larval deformities increased in acid water, and there was a synergistic effect of pH and aluminium. In *R. arvalis* swimming behaviour was disturbed by high levels of aluminium at pH 5. In all three species the frequency of stressed larvae increased when pH was depressed and aluminium concentration elevated, and there was a synergistic effect when both were combined. The three species differed significantly in egg mortality, time needed for embryonic development, larval mortality, larval deformities and larval stress at low pH and high aluminium levels. *R. arvalis* showed the highest acid tolerance and *R. dalmatina* was the most sensitive to low pH.

Hofman, T. and H. Lees (1953). "The biochemistry of the nitrifying organisms. 4. The respiration and intermediary metabolism of *Nitrosomonas*." *Biochemical Journal* **54**(4): 579.

Hostettler, S. (2002). "Tropical montane cloud forests: a challenge for conservation." *Bois et forêts des tropiques* **274**: 4. Tropical montane cloud forests (TMCF) have been identified in 736 sites in 59 countries. The important role of TMCF in sustaining the livelihoods of local populations by protecting watersheds and sustaining unpolluted freshwater sources has been generally recognised. Cloud forests are important sources of nontimber forest products and are an essential habitat for many endemic and threatened plant and animal species. During the past 20 years, cloud forests worldwide have been disappearing rapidly. They are facing considerable localised threats from clearance and further fragmentation, as a result of population pressure, unsustainable harvesting and poor management practices. External pressures such as mining, road building, air pollution and global warming further exacerbate the problem. Converting TMCF to other uses almost invariably affects water quality, and may significantly reduce water availability further downstream. Sustainable management and conservation of cloud forests faces many challenges including population pressure, poor awareness of their value and the lack of reliable information, political will and donor assistance. However, various successful conservation and sustainable use projects illustrate the potential of a range of approaches to cloud forest conservation. Furthermore, networks and initiatives are promoting cloud forest conservation at local, national, regional and global levels. Much hope is being placed in the International Year of the Mountain and Rio +10 to raise awareness and encourage donors, governments, businesses, NGO's and local communities to conserve the cloud forests that still remain.

Hovanec, T., L. Taylor, et al. (1998). "Nitrospira-Like Bacteria Associated with Nitrite Oxidation in Freshwater Aquaria." Oxidation of nitrite to nitrate in aquaria is typically attributed to bacteria belonging to the genus *Nitrobacter* which are members of the a subdivision of the class Proteobacteria. In order to identify bacteria responsible for nitrite oxidation in aquaria, clone libraries of rRNA genes were developed from biofilms of several freshwater aquaria. Analysis of the rDNA libraries, along with results from denaturing gradient gel electrophoresis (DGGE) on frequently sampled biofilms, indicated the presence of putative nitrite-oxidizing bacteria closely related to other members of the genus *Nitrospira*. Nucleic acid hybridization experiments with rRNA from biofilms of freshwater aquaria demonstrated that *Nitrospira*-like rRNA comprised nearly 5% of the rRNA extracted from the biofilms during the establishment of nitrification. Nitrite-oxidizing bacteria belonging to the a subdivision of the

class Proteobacteria (e.g., *Nitrobacter* spp.) were not detected in these samples. Aquaria which received a commercial preparation containing *Nitrobacter* species did not show evidence of *Nitrobacter* growth and development but did develop substantial populations of *Nitrospira*-like species. Time series analysis of rDNA phylotypes on aquaria biofilms by DGGE, combined with nitrite and nitrate analysis, showed a correspondence between the appearance of *Nitrospira*-like bacterial ribosomal DNA and the initiation of nitrite oxidation. In total, the data suggest that *Nitrobacter winogradskyi* and close relatives were not the dominant nitrite-oxidizing bacteria in freshwater aquaria. Instead, nitrite oxidation in freshwater aquaria appeared to be mediated by bacteria closely related to *Nitrospira moscoviensis* and *Nitrospira marina*.

Huey, D. and T. Beitinger (1980). "Toxicity of nitrite to larvae of the salamander *Ambystoma texanum*." Bulletin of Environmental Contamination and Toxicology **25**(1): 909-912.

Larvae of the salamander, *Ambystoma texanum*, from a Texas pond were highly sensitive to nitrite in low chloride (5.0 mg per liter) water. The 96 hour LC50 was 1.09 mg per liter nitrite. Nearly 100% mortality was seen at concentrations of 2.5 mg per liter nitrite and above. In water containing 300 mg per liter chloride and 10 mg per liter nitrite, no mortality was observed in 96 hours, probably as a result of lower nitrite uptake rates. (Cassar-FRC)

Jofre, M. and W. Karasov (1999). "Direct effect of ammonia on three species of North American anuran amphibians." Environmental Toxicology and Chemistry **18**(8): 1806-1812.

Leopard frog (*Rana pipiens*), green frog (*Rana clamitans*), and American toad (*Bufo americanus*) embryos were exposed to different un-ionized ammonia (NH sub(3)) levels over an ecologically relevant range (0-2 mg NH sub(3)/L H sub(2)O). Hatching success and prevalence of deformities were recorded after acute exposures (3-5 d duration) at 23 degree C and pH 8.7. Green frog tadpoles were exposed to different NH sub(3) levels in a subchronic experiment (114 d), and growth, survival, and metamorphosis were monitored. Survival declined, the prevalence of deformities increased, and growth and development were slow in anuran embryos and tadpoles exposed to NH sub(3) concentrations in excess of 0.6 mg/L (green frogs) or 1.5 mg/L (leopard frogs). No effects were observed in American toads up to a concentration of 0.9 mg/L NH sub(3). It appears from the few data available that anurans may not be particularly sensitive to NH sub(3) when compared with many fish species and that water quality criteria determined using data collected on fish species will be protective for many anuran amphibians. The NH sub(3) concentrations that caused negative effects in these experiments are higher than measured values for water in the Fox River-Green Bay ecosystem (WI, USA) but lower than for pore sediment water. In this ecosystem, anuran amphibians are potentially exposed to hazardous levels of NH sub(3) when they hibernate on the bottom or buried in sediments or during episodic releases of NH sub(3) from sediments.

Jofre, M., M. Rosenshield, et al. (2000). "Effects of PCB 126 and ammonia, alone and in combination, on green frog (*Rana clamitans*) and leopard frog (*R. pipiens*) hatching success, development, and metamorphosis." Journal of the Iowa Academy of Science **107**(3): 113-122.

The Green Bay watershed in Wisconsin is polluted with polychlorinated biphenyls (PCBs), dioxin, heavy metals, ammonia, and over 100 organic contaminants. In this study we exposed embryos and larvae of two ranid species commonly occurring in the Green Bay ecosystem, the green frog (*Rana clamitans*) and the leopard frog (*R. pipiens*), to PCB 126 (3,3', 4,4', 5-Pentachlorobiphenyl, nominal concentrations 0-50 µg/l, two control treatments: water plus 0.08% acetone as carrier for the PCB, water alone), unionized ammonia (0-2 mg/l), and mixtures of both contaminants. Exposure to PCB 126 did not cause significant mortality of embryos before hatching. However, exposure to unionized ammonia (NH sub(3)) concentrations in excess of 0.6 mg/l (green frogs) or 1.5 mg/l (leopard frogs) caused a decline in hatching success and an increase in prevalence of deformities. PCB 126 and NH sub(3) in combination had a significant negative effect on hatching success. Survival of larvae was significantly reduced at the highest PCB concentration (50 mg/l) for both species. Few skeletal deformities were observed in tadpoles at this concentration, but the incidence of edema was significantly increased. A slowing of growth was also observed in anuran tadpoles exposed to PCB 126. NH sub(3) exposure caused a decrease in the survival and growth of green frog tadpoles. When exposed to mixtures of both chemicals, green frog tadpoles showed a decrease in survival. However, growth was not affected. Fewer

tadpoles metamorphosed with increasing PCB 126 and NH sub(3) concentrations. In tadpoles exposed to PCB 126, tissue concentrations of PCB 126 at the end of the experiment increased with increasing nominal concentrations, ranging from 1.2-9600 ng/g wet weight. Our data indicate that anurans may not be particularly sensitive to NH sub(3) as compared to many fish species, and that water quality criteria determined using data collected on fish species will be protective for many anuran amphibians. At high concentrations, PCB 126 and unionized NH sub(3) affected both ranid species. However, no sublethal effects were apparent at water concentrations that occur in the Green Bay ecosystem.

Kinne, O. (1976). Cultivation of marine organisms: water quality management and technology. Marine ecology: a comprehensive, integrated treatise on life in oceans and coastal waters: 3. Cultivation. O. Kinne. London, Wiley. **3**: 19-300.

Landé, S. and S. Guttman (1973). "The effects of copper sulfate on the growth and mortality rate of *Rana pipiens* tadpoles." Herpetologica **29**(1): 22-27.

Rana pipiens eggs and tadpoles were each reared in water treated with copper sulfate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ at concentrations of 0.04, 0.05, 0.06, 0.16, 0.31, 0.62, or 1.56 mg/liter of copper (Cu). Eggs were not affected by the copper salt. Newly-hatched tadpoles were killed by the three highest concentrations. The LD-50 (72) was 0.15 mg/liter Cu. Weights of tadpoles grown in the 0.06 and 0.16 mg/liter concentrations were lower than controls and those grown in 0.04 and 0.05 mg/liter Cu. Tadpoles of varying weights and age classes were put into copper salt solutions ranging from 0.05 to 1.56 mg/liter Cu. Survival time was correlated to the weight of the tadpoles ($r = +0.74$, $P = 0.05$), i.e., the greater the weight of the tadpole, the longer was its survival time.

Lees, H. (1952). "The biochemistry of the nitrifying organisms. 1. The ammonia-oxidizing systems of *Nitrosomonas*." Biochemical Journal **52**(1): 134.

Lees, H. and J. Simpson (1957). "The biochemistry of the nitrifying organisms. 5. Nitrite oxidation by *Nitrobacter*." Biochemical Journal **65**(2): 297.

Little, E., R. Calfee, et al. (2002). Determination of impacts on the endangered Wyoming toad (*Bufo baxteri*) at Mortenson National Wildlife Refuge from ammonium nitrate concentrations. Contaminant Report Number: R6/719C/02. US Geological Survey, Columbia, Missouri, and US Fish and Wildlife Service, Cheyenne, Wyoming. Che, F&WS USFWS - Region 6 24.

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 $\text{NO}_3\text{-N}$ (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. USFWS - Region 6 - Environmental Contaminants Report - R6/719C/02 iii 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 effects 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.

Marco, A. and A. Blaustein (1999). "The effects of nitrite on behavior and metamorphosis in Cascades frogs (*Rana cascadae*)."
Environmental Toxicology and Chemistry **18**(5): 946-949.

Amphibian metamorphosis is a period of drastic morphologic reorganization, during which larvae experience a decrease in locomotor ability and are more vulnerable to predation. Our results indicate that exposure to sublethal concentrations of nitrite in the water induces behavioral and morphologic changes in the Cascades frog (*Rana cascadae*). Tadpoles exposed to a nitrite concentration of N-NO₂ at 3.5 mg/L transformed more slowly than control tadpoles exposed to dechlorinated tap water. No difference was found in time at emergence and snout-vent length at emergence between experimental and control tadpoles, but development was retarded in tadpoles exposed to nitrite and they emerged at an earlier developmental stage. Also, tadpoles exposed to nitrite occupied shallow water more frequently than did control tadpoles. Keywords—Amphibian Fertilizers Metamorphosis Nitrite *Rana cascadae*

Massal, L., J. Snodgrass, et al. (2007). "Nitrogen pollution of stormwater ponds: Potential for toxic effects on amphibian embryos and larvae." Applied Herpetology **4**(1): 19-29.

Runoff from impervious surfaces associated with areas of residential, commercial and industrial development is commonly managed through the construction of stormwater ponds that are designed to slow runoff and reduce pollutant inputs to streams. It has been suggested that stormwater ponds may also provide habitat for wildlife. However, wildlife attracted to ponds may be exposed to pollutants entering ponds in runoff. To assess the potential toxicity of nitrogen pollution of stormwater ponds to pond-breeding amphibians we monitored nitrogen levels in waters of eleven ponds in Baltimore County, Maryland. Levels of NH₃, NO₂ and NO₃ exceeded lowest sublethal effects concentrations reported in the literature in <2% of the water samples collected, and when relatively high concentrations did occur they were restricted to only a portion of the pond. Water sampling during and following rain events also indicated little input of nitrogen to stormwater ponds through runoff. While the number of amphibians recorded at ponds varied from three to six species, there was no relationship between nitrogen levels and amphibian occurrence at ponds. Overall, nitrogen pollution of stormwater ponds in our study area appears to represent little or no direct risk to developing embryos and larvae of pond-breeding amphibians, although indirect effects and interaction of inorganic nitrogen with other pollutants warrant further investigation.

May, T. W., Missouri. Dept. of Conservation., et al. (2007). Concentration of elements in hellbender blood and fish fillets

from the 2006 Missouri Department of Conservation Monitoring Programs. Reston, Va., U.S. Geological Survey: iv, 18 p.

McTammany, M. (2004). Recovery of southern Appalachian streams from historical agriculture.

Stream ecosystems are influenced by the surrounding landscape, and agriculture within their catchments has changed many characteristics of streams. Agriculture has been a prominent land use activity in the southern Appalachian Mountains of the eastern United States for over 500 years. However, recent socioeconomic changes in the region have caused many farmers to abandon agriculture leading to widespread reforestation of historical farmland. I investigated the influence of agriculture on the physical, chemical, and biological structure and ecosystem processes of streams in the southern Appalachians. In addition, I studied streams in watersheds previously agricultural but currently reforested to determine how historic agriculture generates long-term effects on streams. Stream draining agricultural catchments (i.e., agricultural streams) had higher temperatures, light inputs, nutrients, and suspended sediments than forested streams and contained smaller substrate, dominated by sand and silt. Temperature and light regimes recovered in streams of reforested catchments, but the other aspects of stream physicochemistry remained elevated or changed due to historical agriculture. I expected biological community structure and ecosystem processes to reflect these altered conditions in streams with current and historical agriculture. Higher chlorophyll, lower macroinvertebrate biodiversity, fewer shredder/detritivore invertebrates, and more pollution-tolerant organisms characterized agricultural streams compared to forested streams, but each of these biological features was similar in long-term forested streams and streams with reforested catchments but with agricultural histories. Agricultural streams had higher rates of gross primary production (GPP) and GPP to respiration (P/R) ratios than forested streams, indicating that agriculture enhances autotrophic metabolism in streams. Agriculture did not have a significant effect on wood breakdown or microbial biofilm development on wood substrates. Together, these data suggest that agriculture causes many different changes in stream physical and chemical properties and that many of these properties do not recover following reforestation of catchments over the past 50 years. However, biological community structure and ecosystem processes appear to respond to physical aspects of streams that do recover from historic agriculture including light, temperature, and organic matter supply and type.

ODUM, R. A. and K. C. ZIPPEL (2008). Amphibian water quality: approaches to an essential environmental parameter. International Zoo Yearbook. **42**: 40-52.

Appropriate water quality is essential for maintaining and breeding amphibians in captivity. Aquatic systems that maintain water quality have been employed for many years in the aquaculture and aquarium industries. These techniques are now more commonly being utilized for amphibians. Using information from the work of the authors and published literature on amphibians and fish, benchmarks are provided for common water-quality parameters for amphibians.

Pessier, A. (2002). An overview of amphibian skin disease, Elsevier.

The skin of amphibians has unique structural properties and physiologic functions that make amphibians particularly sensitive to environmental perturbations and cutaneous injury. This inherent sensitivity makes the skin a critically important site of evaluation in both clinical and postmortem examination of the amphibian patient. Many of the described amphibian skin diseases can be traced back to factors related to captive husbandry. Cutaneous injuries, alterations in water quality (such as pH, ammonia), and exposure to chemical irritants, are all potential hazards of the captive environment that can lead to skin irritation. Furthermore, many infectious diseases of amphibian skin such as "red leg" syndrome, mycobacteriosis, and saprolegniasis are often secondary to environmental factors and require correction of underlying lapses in husbandry for control. Other amphibian skin diseases, such as chytridiomycosis and systemic iridovirus infections are not yet associated with specific environmental cofactors but have been associated with significant mortalities in captive and/or wild amphibians. Amphibian skin diseases often have a similar gross appearance with cutaneous hyperemia and discoloration, cutaneous papules and nodules, and ulceration being among the most common presentations. This review emphasizes conditions most often encountered in amphibian dermatology as well as recently recognized diseases such as chytridiomycosis.

Punzo, F. and S. Law (2006). "Effect of nitrate-related compounds on growth, survival and hematological responses in tadpoles of the Cuban tree frog, *Osteopilus septentrionalis*(Boulenger)." Journal of Environmental Biology **27**(2): 187-190.

Experiments were conducted to assess the effects of nitrate-related compounds on survival, growth, and hematological responses in tadpoles of the Cuban treefrog, *Osteopilus septentrionalis*. Stage-25 tadpoles were exposed to a nitrate dilution series and exposed to distilled water (controls), 40 ppm nitrate, or 100 ppm nitrate. Survivorship was significantly higher for control animals as compared to those exposed to 40 and 100 ppm nitrate. Total blood Hb concentrations were not significantly altered by exposure to sodium nitrite, and a significant positive correlation was found between methemoglobinemia and nitrite concentration over the test range of 1.0 to 50.0 mg/l. Percentage Hb was significantly correlated with nitrite concentration. Percentage MHB for all treatment groups was significantly higher (18.4 to 45.3 %) than that of controls (5.4%).

Romansic, J., K. Diez, et al. (2006). "Effects of nitrate and the pathogenic water mold *Saprolegnia* on survival of amphibian larvae." Diseases of aquatic organisms **68**(3): 235-243.

We tested for a synergism between nitrate and *Saprolegnia*, a pathogenic water mold, using larvae of 3 amphibian species: *Ambystoma gracile* (northwestern salamander), *Hyla regilla* (Pacific treefrog) and *Rana aurora* (red-legged frog). Each species was tested separately, using a 3 × 2 fully factorial experiment with 3 nitrate treatments (none, low and high) and 2 *Saprolegnia* treatments (*Saprolegnia* and control). Survival of *H. regilla* was not affected significantly by either experimental factor. In contrast, survival of *R. aurora* was affected by a less-than-additive interaction between *Saprolegnia* and nitrate. Survival of *R. aurora* was significantly lower in the *Saprolegnia* compared to the control treatment when nitrate was not added, but there was no significant difference in survival between *Saprolegnia* and control treatments in the low and high nitrate treatments, consistent with increased nitrate preventing *Saprolegnia* from causing mortality of *R. aurora*. Survival of *A. gracile* followed a similar pattern, but the difference between *Saprolegnia* and control treatments when nitrate was not added was not significant, nor was the nitrate × *Saprolegnia* interaction. Our study suggests that *Saprolegnia* can cause mortality in amphibian larvae, that there are interspecific differences in susceptibility and that the effects of *Saprolegnia* on amphibians are context-dependent.

Rouse, J., C. Bishop, et al. (1999). "Nitrogen pollution: an assessment of its threat to amphibian survival." Environmental health perspectives **107**(10): 799.

The potential for nitrate to affect amphibian survival was evaluated by the areas in North America where concentrations of nitrate in water occur above amphibian toxicity thresholds. Nitrogen pollution from anthropogenic sources enters bodies of water through agricultural runoff or percolation associated with nitrogen fertilizer loss, and effluents from industrial and human wastes. Environmental concentrations of nitrate in watersheds throughout North America range from < 1 to > 100 mg/L. Of the 8,545 water quality samples collected from states and provinces bordering the Great Lakes, 19.8% contained nitrate concentrations exceeding those which can cause sublethal effects in amphibians. In the laboratory lethal and sublethal effects in amphibians are detected at nitrate concentrations between 2.5 and 100 mg/L. Furthermore, amphibian prey such as insects and predators of amphibians such as fish are also sensitive to these elevated levels of nitrate. From this we conclude that nitrate concentrations in some watersheds in North America are high enough to cause death and developmental anomalies in amphibians and impact other animals in aquatic ecosystems. In some situations, the use of vegetated buffer strips adjacent to water courses can reduce nitrogen contamination of surface waters. Ultimately, there is a need to reduce runoff, sewage effluent discharge, and the use of fertilizers, and to establish and enforce water quality standards for nitrate for the protection of aquatic organisms. Key words: amphibians, nitrate, toxicity, water quality.

Russo, R., C. Smith, et al. (1974). "Acute toxicity of nitrite to rainbow trout(*Salmo gairdneri*)." J. Fish. Res. Board Can. **31**(10): 1653-1655.

Flow-through bioassays on the acute toxicity of nitrite to *S. gairdneri* of 4 different sizes (2-235 g) showed median lethal concentration (LC50) values for 4 days ranging from 0.19 to 0.39 mg/l NO₂-N. For 12-g rainbow trout the asymptotic LC50 was 0.14-0.15 mg/l NO₂-N after 8 days.

Schmuck, R., W. Geise, et al. (1994). "Life cycle strategies and physiological adjustments of reedfrog tadpoles (Amphibia, Anura, Hyperoliidae) in relation to environmental conditions." Copeia **1994**(4): 996-1007.

The relationship between different degrees of intraspecific crowding of reedfrog tadpoles and their physiological responses to a deterioration of the natal pond water quality was examined under laboratory conditions. Tadpoles that were reared at a lower density metamorphosed significantly earlier than those raised at a higher density. As density increases, the average body length at metamorphosis decreases. However, at low tadpole density, a significantly higher diversity of body size classes among freshly metamorphosed froglets was observed than under more crowded conditions. Mortality increased during metamorphic climax and was inversely correlated with the tadpole density. In ephemeral ponds, an accumulation of nitrogenous wastes from metabolic processes and/or a concentration by evaporation in prolonged rainless periods can pose a considerable chemical stress to reedfrog tadpoles. *Hyperolius viridiflavus ommatostictus* responded to an increasing ammonia concentration with an activity increase of the ornithine cycle (intensified urea synthesis). In contrast, *Hyperolius marmoratus taeniatus* exhibited a strong tolerance against high ammonia levels. A deterioration of the natal pond water quality caused *H. v. ommatostictus* and *H. v. nitidulus* tadpoles to adjust to harsher climatic conditions at the time of metamorphosis. This physiological preadjustment enabled the froglets to start feeding and growing immediately after metamorphosis even at low air humidity and rare precipitation events. In contrast, froglets that were raised in daily refreshed water exhibited high mortality rates if subjected to identical conditions. As one possible indicator of the actual climatic conditions prevailing in the surrounding terrestrial habitat, fluctuations in the water ammonia level are discussed.

SCHMUTZER, A., M. GRAY, et al. (2008). "Impacts of cattle on amphibian larvae and the aquatic environment." Freshwater Biology **53**(12): 2613-2625.

1. Agricultural practices such as cattle farming may have direct or indirect negative effects on larval amphibians by decreasing water quality through deposition of nitrogenous waste, causing eutrophication, and grazing shoreline vegetation that contributes to detrital cover and food. 2. We sampled amphibian larvae on the Cumberland Plateau, Tennessee, U.S.A., twice per week, water quality twice per month and algal and detrital biomass once per month at seven wetlands (three cattle-access and four non-access) from March to August 2005 and 2006. 3. In general, species richness and diversity of amphibian larvae were greater in wetlands without cattle. Mean relative abundance of green frog (*Rana clamitans*) and American bullfrog (*Rana catesbeiana*) tadpoles was greater in non-access wetlands. Body size of some ranid larvae was larger in cattle-access wetlands but this trend did not exist for juveniles or adults. Dissolved oxygen was lower, while specific conductivity and turbidity were higher in cattle-access wetlands. Mean biomass of detritus was lower in cattle-access wetlands compared to non-access wetlands; no differences were detected in algal biomass. 4. Given the negative impacts of cattle on water quality, detrital biomass, larval amphibian species richness and relative abundance of some amphibian species, we recommend that farmers consider excluding these livestock from aquatic environments.

Schuytema, G. and A. Nebeker (1999). "Comparative toxicity of ammonium and nitrate compounds to Pacific treefrog and African clawed frog tadpoles." Environmental Toxicology and Chemistry **18**(10): 2251-2257.

The effects of ammonium nitrate, ammonium chloride, ammonium sulfate, and sodium nitrate on survival and growth of Pacific treefrog (*Pseudacris regilla* [Baird and Girard]) and African clawed frog (*Xenopus laevis* [Daudin]) tadpoles were determined in static-renewal tests. The 10-d ammonium nitrate and ammonium sulfate LC50s for *P. regilla* were 55.2 and 89.7 mg/L NH₄-N, respectively. The 10-d LC50s for *X. laevis* for the three ammonium compounds ranged from 45 to 64 mg/L NH₄-N. The 10-d sodium nitrate LC50s were 266.2 mg/L NO₃-N for *P. regilla* and 1,236.2 mg/L NO₃-N for *X. laevis*. The lowest observed adverse effect level (LOAEL) of ammonium compound based on reduced length or weight was 24.6 mg/L NH₄-N for *P. regilla* and 99.5 mg/L NH₄-N for *X. laevis*. The lowest sodium nitrate LOAELs based on reduced length or weight were <30.1 mg/L NO₃-N for *P. regilla* and 126.3 mg/L NO₃-N for *X. laevis*. Calculated un-ionized NH₃ comprised 0.3 to 1.0% of measured NH₄-N concentrations. Potential harm to amphibians could occur if sensitive life stages were impacted by NH₄-N and NO₃-N in agricultural runoff or drainage for a sufficiently long period.

Shinn, C., A. Marco, et al. (2008). "Inter-and intra-specific variation on sensitivity of larval amphibians to nitrite." Chemosphere **71**(3): 507-514.

Several authors have suggested that nitrogen-based fertilizers may be contributing to the global amphibian decline. We have studied the impact of sodium nitrite on early aquatic stages of *Epidalea calamita*, *Pelophylax perezi* and *Hyla meridionalis* larvae from Doñana National Park (coastal wetland) and *P. perezi* from Gredos Mountain (high mountain ponds), exposed during 10 to 16 days. After 8 days of exposure all *P. perezi* larvae from Doñana presented 100% mortality at 5 mg l(-1)N-NO2(-) while *E. calamita* larvae mortality rates were significantly lower at that concentration after 15 days. However, for *H. meridionalis* at day 15 no deaths were registered at 5 mg l(-1)N-NO2(-) and at 20 mg l(-1)N-NO2(-) presented intermediate mortality rates. In Doñana the 10 d LC50 of older *H. meridionalis* larvae was between 20 and 30 mg l(-1)N-NO2(-) whilst for *P. perezi* it was below 5 mg l(-1)N-NO2(-). These results indicate inter-specific variation of the sensitivity of larval amphibians to nitrite. Gredos Mountain *P. perezi* larvae exposed since the egg stage were highly sensitive to nitrite, with a 16 d LC50 below 0.5 mg l(-1)N-NO2(-). The same species in Doñana had a 15 d LC50 between 5 and mg l(-1)N-NO2(-). These results suggest that there is also intra-specific variation in sensitivity of amphibian larvae to nitrite: mountain amphibian populations appear to be more sensitive to polluted environments than coastal populations. Geographic and genetic variation and evolutionary adaptation of tolerance may also be the keys to variation amongst populations of the same species.

Siefert, R. and W. Spoor (1973). "Effects of reduced oxygen on embryos and larvae of the white sucker, coho salmon, brook trout and walleye."

Embryos and resulting larvae of 4 fish spp were subjected to reduced O Sub(2) concs at optimal temps and a flow of 60 ml/min (velocity {approx} 3.3 cm/min) until all fish were feeding. White suckers (*Catostomus commersoni* (Lacepede)) and walleyes (*Stizostedion vitreum vitreum* (Mitchell)), were not harmed at 50% saturation of dissolved O Sub(2). White sucker development was delayed at 25% saturation and walleye survival dropped at 35%. Developmental delay and mortality of coho salmon (*Oncorhynchus kisutch* (Walbaum)) increased progressively with each reduced O Sub(2) conc tested, including the highest reduced tension, 50% saturation. Good survival and slight delays in the development of brook trout (*Salvelinus fontinalis* (Mitchell)) occurred at all reduced concs down to 20% saturation, where poor survival occurred. All spp died at 12.5% saturation.

Smith, G., K. Temple, et al. (2005). "Effects of Nitrate on the Tadpoles of Two Ranids (*Rana catesbeiana* and *R. clamitans*)." Archives of Environmental Contamination and Toxicology **49**(4): 559-562.

Nitrate is a potential stressor of amphibian larvae. Previous research has shown variation in the effects of nitrate among species and even populations of amphibians. However, relatively few species and populations of amphibians have been examined for nitrate tolerance, especially from populations in the agricultural regions of the United States. Using a 15-day laboratory experiment, we investigated the effects of nitrate on the larvae of two species of anurans (*Rana catesbeiana* and *R. clamitans*) that are common throughout the agricultural Midwest. Survival of *R. catesbeiana* and *R. clamitans* tadpoles was negatively affected by higher concentrations of nitrate (20 mg L⁻¹), with *R. clamitans* being more affected than *R. catesbeiana*. The final mass of *R. catesbeiana* tadpoles was affected by nitrate concentration, with tadpoles exposed to intermediate concentrations of nitrate (5 mg L⁻¹) being the heaviest. The final mass of *R. clamitans* tadpoles was not affected by nitrate concentration. These experiments suggest that these two species differ in their responses to the toxic effects of nitrate and, along with previous results on Ranids, suggest there is a great deal of variation in nitrate tolerance in this family.

Smith, G., D. Vaala, et al. (2004). "Effects of nitrite on bullfrog (*Rana catesbeiana*) tadpoles from central Ohio, USA." Bulletin of Environmental Contamination and Toxicology **72**(5): 1012-1016.

The effect of nitrite on the growth, survival and behaviour of bullfrog (*Rana catesbeiana*) tadpoles from an area with a history of agriculture (central Ohio, USA) was studied. Nominal nitrite concentrations of 0, 0.375, 0.75, 1.5, 3.0 and 6.0 mg N-NO2- litre⁻¹ were used in this study. Tadpole final mass was marginally affected by nitrite

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

Theron, J., L. Hoffman, et al. (1992). "Aspects of the chemical control of *Xenopus laevis* tadpoles under laboratory conditions." *SOUTH AFRICAN JOURNAL OF WILDLIFE RESEARCH* **22**: 110-110.

The chemical control of *Xenopus laevis* tadpoles, under laboratory conditions, was tested using different concentrations of chlorine. Simultaneously, the toxicity of chlorine to *Clarias gariepinus* juveniles was investigated. *X. laevis* was more sensitive to chlorine concentrations, with 100% mortality occurring at 0,5 mg/l (tap water) and 12,0 mg/l (pond water and acidified pond water), whilst for *C. gariepinus* juveniles, this only occurred at 5,0 mg/l (tap water) and 12,0 mg/l (pond water and acidified pond water), respectively. The residual toxicity of chlorine sharply decreased after 72 h.

Wall, A. (1999). CORRELATION BETWEEN FROG MALFORMITIES AND HEAVY METALS IN WARD MARSH, WEST HAVEN, VT, AND MUD CREEK, ALBURG, VT, Middlebury College.

Studies performed by the Vermont Agency of Natural Resources (VTANR) and Jim Andrews of Middlebury College have found frog malformations in a number sites across the Lake Champlain Basin. The surveys documented rates of morphological abnormalities in the Northern Leopard frog (*Rana pipiens*) ranging from 2% to 45% depending on the site (VTANR, 1998). Further laboratory research by VTANR, using the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX), showed that frog embryos grown in water from Ward Marsh WMA, in West Haven, VT, had an 89% malformity rate with a 6.6% mortality rate. In the assays using sediment from Ward Marsh, there was a 100% malformity rate and a 38.3% mortality rate (VTANR, 1997). This evidence indicates that the sediment is a potential source of teratogens. Mud Creek, in Alburg, VT, which was used as a control in this study, has very little evidence of malformities in the field and laboratory FETAX assays. In other studies, frogs exposed to Cu²⁺, Zn²⁺, Co²⁺, Ni²⁺, Cd²⁺ in FETAX assays have shown these metals to be teratogens, causing malformities and mortality. The malformities of frogs exposed to these metals include retinal depigmentation and pelvic and hind limb malformities (Luo et al, 1993; Plowman et al., 1991,1994). These types of malformities have been found in Ward Marsh by VTANR, which leads us to suspect that the frog malformities may be related to high concentrations of metals in the sediment. EPA method 200.2 for total recoverable analytes was used to prepare sediment samples from Ward Marsh and Mud Creek for ICAP analysis. Samples at Ward Marsh and Mud Creek were collected in transects ranging from subaerially exposed soils adjacent to the marsh to subaqueous sediment in the marsh, including all levels in between. Concentrations of Cu²⁺, Zn²⁺, Co²⁺, Ni²⁺, Cr²⁺ in the sediment at the two sites were determined using the ICP, with replicate runs. Sediment samples from Ward Marsh contain statistically higher concentrations of Zn²⁺ (mean=103 + 34 mg/kg), Co²⁺ (mean=18.3 + 3 mg/kg), Ni²⁺ (mean=39.4 + 7.7 mg/kg), and Cr²⁺ (mean=43.4 + 11.8 mg/kg), than Mud Creek. Cu²⁺ concentrations were similar between the two sites. Furthermore, metal concentrations in Ward Marsh indicate a trend ranging from low concentrations in soils farther from East Bay to higher concentrations in subaqueous sediment closer to the bay. This relationship implies that East Bay is a potential source of heavy metals, which are accumulating in the sediment and that elevated metals concentrations could be responsible for frog malformities observed in the field at Ward Marsh.

Warner, S., W. Dunson, et al. (1991). "Interaction of pH, density, and priority effects on the survivorship and growth of two species of hylid tadpoles." *Oecologia* **88**(3): 331-339.

We examined the interactions of an abiotic factor (pH) and a biotic factor (density) on the survival and growth of two species of anuran larvae (*Hyla gratiosa* and *Hyla femoralis*) in outdoor tanks. Three levels of pH (4.3, 4.6, or 6.0) and three levels of density (0, 30 or 60 embryos) were arranged in a blocked design and replicated three times for *Hyla gratiosa*. At the end of this experiment the effects of pH (4.3, 4.6, or 6.0), density of *H. femoralis* (30 or 60), and prior use by *H. gratiosa* (at 0, 30, or 60 larvae per tank) on the survival and growth of *H. femoralis*, were examined. Higher density increased larval period and decreased size at metamorphosis of *H. gratiosa*. Lower pH decreased survival rate and also decreased size at metamorphosis. Body sodium concentrations were lowest at the low pH values. Lower pH increased the susceptibility of *H. gratiosa* tadpoles to the adverse effects of higher densities. For *H. femoralis* higher density decreased survival, increased larval

period and decreased size at metamorphosis. *Hyla femoralis* also had lower survivorship at low pH and exhibited decreased size at metamorphosis. However, unlike the results with *Hyla gratiosa*, there were no interactive effects between pH and density for any of the life-history traits studied. The effect of previous colonization by *H. gratiosa* on *H. femoralis* survival was facilitative. Body sodium concentrations of *H. femoralis* were lowest at the highest pH value. Metamorphs of the same size had much lower levels of sodium in *H. femoralis* than *H. gratiosa*. In general, *H. femoralis* was less affected by pH variation than *H. gratiosa*. These results demonstrate that abiotic factors can interact strongly with biotic effects such as density and they suggest that interspecific interactions can be strongly modulated by the background abiotic environment.

- Westin, D. T. (1974). "Nitrate and Nitrite Toxicity to Salmonoid Fishes." *The Progressive Fish-Culturist* 36(2): 86-89. The median tolerance limit (TLM) concentration of nitrates and nitrites were determined for chinook salmon and rainbow trout. The effect of varying salinity on rainbow trout after exposure to nitrate and the effect of exposure time on mortality were examined. The relative activity of nitrite to Chinook fingerlings in freshwater was 2,000, 1,480, and 2,700 times that of nitrate in freshwater, at 96-hour, 7 and 10-day TLM, respectively. All of the trout subjected to nitrate concentrations showed acute signs after 2 days exposure, while these signs were not observed in the lower nitrate concentrations until after 5-8 days. Guidelines for hatchery water quality may be set from the data. Best results of growth and good health would indicate nitrate concentrations of 25-35 ppm and 0.12 ppm nitrite. Mode of action of nitrate as a toxicant must still be determined. (Katz)
- Wurts, W. and R. Durborow (1992). "Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds." Water quality in fish ponds is affected by the interactions of several chemical components. Carbon dioxide, pH, alkalinity and hardness are interrelated and can have profound effects on pond productivity, the level of stress and fish health, oxygen availability and the toxicity of ammonia as well as that of certain metals. Most features of water quality are not constant. Carbon dioxide and pH concentrations fluctuate or cycle daily. Alkalinity and hardness are relatively stable but can change over time, usually weeks to months, depending on the pH or mineral content of watershed and bottom soils.