

Application for a Research Permit for The BNP

Permit 2007-008

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Title of Research: Developmental and mortality effects of copper and light exposure in the Wood Frog (*Rana sylvatica*)

Briefly describe the research to be conducted at the BNP in non-technical language (this paragraph will be used in describing your research to the public).

Toxicology of temporary ponds is a major factor in amphibian conservation. Ponds provide essential habitat in which amphibians congregate in the spring to mate. Although resultant embryos remain in the pond surrounded by a protective gelatinous membrane, they are still vulnerable to any toxins in the pond. Disturbance of natural habitats also often leads to a decrease in vegetation surrounding ponds. This disruption allows more sunlight to reach the surface of breeding ponds. An increase in sunlight combined with nutrient-rich aquatic environments is favorable for the growth of algae. A commonly used algaecide to address this problem in ponds is copper sulfate. The toxicity of this algaecide may be influenced by the level of solar radiation is present. A number of studies have revealed correlations between delayed development and mortality in embryos and hatchlings with metals, and ultraviolet radiation. However, research is lacking in ponds with levels of pH as high as those generally found in northeastern Ohio. Furthermore, interaction between the effects of metals and harmful ultraviolet (UV-B, 280-315 nm) radiation found in direct sunlight may exacerbate the effects of each individual factor.

This study will investigate the combined influence of copper sulfate (a common algaecide) and sunlight on development and survival of Wood Frog (*Rana sylvatica*) embryos and larvae. The altered levels will reflect common anthropogenic changes found in natural systems. This study will be conducted in an array of small cattle tanks. Utilizing four replicate tanks for each treatment, a fully factorial experiment with two levels of ultraviolet radiation, and two copper treatments will allow us to test the way in which these factors interact to influence the development and mortality of Wood Frogs.

Briefly describe the goals of the proposed research.

The goal of this work is to determine the degree to which accepted dosages (for algal control) of Copper Sulfate influence Wood Frog developmental rate and survivorship

under realistic light regimes. Amphibian declines, and the utility of amphibians as an indicator species, provides the impetus for this research. Previous studies have been conducted to investigate the use and safety of copper sulfate with regard to fish, birds, livestock, and plants; however, not much is known of its affect on amphibians especially in the high pH systems. Results from this research will be beneficial in understanding the extent to which the use of copper sulfate combined with defoliation contribute to amphibian decline.

On which research areas of the preserve will your research occur? Martin Field Center Grounds

What are the GPS coordinates or locations of your proposed research? Cattle tank array located on grassy knoll east of Martin Field Center Grounds

Does your proposal involve areas that are not part of the designated research areas? No

(if yes, this permit will require approval from the Bath Trustees – this can only be sought four times a year, and so approval of your permit may take up to several months). Talk with the BNP Committee for more details.

What is the expected duration of your proposed research? March - May 2007

What is the Web address of your research outline?
http://_____

Briefly outline of methods to be used. Be sure to include outlines of the equipment to be used (if any) in the research.

Sixteen 380-liter (300 l to fill height) cattle tanks placed into holes in the ground will represent pond mesocosms. The tank array was built for use by Dr.s Moore and Bagatto in conjunction with Misty Ayres. The upper lip of the cattle tanks will be level with the ground. A screen cover on each cattle tank will allow airflow, sunlight, and rainwater in and keep predators out.

Beginning in March of 2007 (exact date determined by breeding of local populations) freshly laid Wood Frog egg masses (<24 hrs) will be pooled together in pond water in cattle tanks. 100 eggs will be placed into each cattle tank 15 cm below the surface of the water in each pond.

A fully factorial design of two levels (ambient and 50% shade) solar radiation and two levels of copper (ambient and 0.51 mg/l [Cu]). This represents a normal treatment as recommended by the manufacturer, and total copper treatment concentration will be well below federal safe drinking water standards of < 1.0 mg/l. Treatment placement will be randomized across the array of tanks, with four replicates of each treatment combination. Developmental stage and larval survival will be assessed 28 days after transplantation of eggs, before metamorphosis. Stage of embryonic development will be determined using Gosner's method (1960). This will allow assessment of the individual and combined influence of the three treatment variables.

Outline the use of markers/cages/fences/etc. for your research. Note: in receiving a research permit, you must agree to remove all such research tools at the completion of your study.

The cattle tanks in which these experiments will take place will be renovated by Dr. Moore under a previously approved proposal immediately upon completion of this study.

What is the potential impact of your research on nature preserve?

Impact should be minimal as infrastructure is already in place. Approximately 1200 gallons of water from Bath pond will be used to fill the cattle tanks.

At 0.51 mg/l of copper the treatment levels of Copper sulfate are low enough that if tanks were intentionally or unintentionally drained the effluent would fall below the EPA maximum limits for safe drinking water (1.3 mg/l). At the end of the experiment we plan on draining the tanks in the field surrounding the Tank Farm. While most or all of the copper in the treatment tank will have been bound into inaccessible forms by the end of the experiment even at the initial treatment levels the water is within EPA normal safe drinking water standards (1.0 mg/l).

The small size of mesocosms and application of Copper at recommended application levels ensures that even if the tanks were mistakenly emptied into Bath Pond maximum levels of copper lost to Bath pond would be well below .2 parts per billion. US Public Health Service Maximum contaminant level for drinking water is 1.0 part per million, five thousand fold higher than the maximum possible contamination. Typical recommended copper applications to ponds range between .5 and 2.5 PPM and treatment levels in the ponds will be at recommended levels. Given the clay soils in the area of the tanks the copper should bind to the clay and become biologically inaccessible. We therefore feel there is no risk of damage to the watershed.

Have you looked at the listings and web sites of the research being conducted at BNP?

<http://www3.uakron.edu/biology/bath/active.html> Yes No

Are there any potential conflicts of your research with others at BNP? Yes No

Explain: _____

To be granted a research permit for work at the BNP, you must agree to the following terms:

- Researchers are responsible for obtaining the appropriate state or federal permits for the conduct of their research on the BNP (e.g., when working with regulated species).
- Researchers are responsible for removing all markers, etc. from their research plots when the research is completed.
- Researchers must build a web site (immediately after being granted a permit) outlining their research at the BNP so that other researchers can avoid the proposed research site(s). Therefore, the web site must clearly outline, using one or more maps, the exact location(s) of the proposed research.
- Researchers will file an annual (due in yearly increments based on the date of the permit) and final report. Such reports will include: user days on the BNP, a summary of results of the project(s), a list of data generated and contact information for those interested in the data, and a list of publications resulting from the project(s).
- Any publications resulting from research conducted at the BNP must acknowledge the use of the preserve by referencing the BNP permit number. A copy of any such publication should be filed with the BNP committee.
- To abide by the rules and regulations of the BNP in any and all conduct of research at the BNP.

By signing the request for a BNP research permit below, I agree to the above terms and state that all of the above information is correct to the best of my knowledge. I also agree to amend my above permit request if my research plans change such that they are no longer well represented in the information supplied in this permit request. If I fail to notify the BNP oversight committee of significant changes in my research, or if I do not follow the rules of the BNP, I realize that the BNP oversight committee can revoke my research permit, and disallow any further work by me, research or otherwise, at the BNP.

Signature: _____

Date: 02/22/2007

Print Name: Colleen C.R. Sharp

Signature: _____

Date: 02/22/2007

Print Name: Francisco B.-G. Moore