Request for Research Funding ($500.00*)

Date of Request 2-22-09

Student's Name _____

Local Address

Anticipated Graduation Date (month/year) May 2010

Faculty Research Sponsor's Name Dr. Rod Williams

Department Forestry and Natural Resources

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Research Title Assessing the effects of Eutrophication on the Prevalence of Ribeiroia Ondatra Infection in Planorbid Snails Within Forested vs Agriculture Landscapes

Please attach research proposal and letter of recommendation from research sponsor.

✓ Proposal

✓ Letter of Recommendation

* NOTE: The **Agriculture** student makes this request on behalf of the professor. The research funds will be allocated to the **faculty research sponsor** to help defray costs associated with the student's research.

Name:
Research Advisor: Rod Williams

ASSESSING THE EFFECTS OF EUTROPHICATION ON THE PREVALENCE OF *RIBEIROIA ONDATRA* INFECTION IN PLANORBID SNAILS WITHIN FORESTED VS AGRICULTURAL LANDSCAPES

Introduction

Eutrophication of aquatic ecosystems due to increased levels of nutrients (particularly nitrogen and phosphorous) has been experimentally proven to serve as a source of emerging disease affecting both human and wildlife populations (Johnson et al. 2007; Patz et al. 2004). Anthropogenic alterations to the environment such as row crop agriculture, livestock production, erosion, sewage waste effluent, and surface water run-off undoubtedly affects water quality, but also can affect host-parasite interactions (Johnson et al. 2007). Parasites are an integral part of the trophic food web, as they are found in most species and can have detrimental impacts on host behavior, growth, abundance, and population dynamics (Johnson et al. 2004). Environmental perturbations (such as increased aquatic nutrient loads) that disrupt the natural balance of parasite-host interactions can have devastating effects on host populations. Recent increases in observed rates of amphibian malformations have been linked to infection of larvae by the trematode *Ribeiroia ondatrae*, while aquatic snail hosts (*Planorbella spp.*) have been found to be significant predictors of the presence and abundance of the parasite (Johnson et al. 2002; 2007).

Ribeiroia ondatrae is a digean trematode with a complex multiple host lifecycle commonly found in ephemeral breeding ponds used by amphibians (Johnson et al. 1999). Wetlands supporting *Ribeiroia* have shown an increased prevalence of amphibian malformations at a rate greater than 20%. Eggs of the trematode are deposited into the aquatic ecosystems through wetland bird or mammal defecation following growth and reproduction within the gastrointestinal tracks of these host organisms. After hatching, the parasite, in the form of free swimming miracidia, infects an intermediate host (typically pond snails within the genus *Planorbella*). Within the snails, the miracidia asexually reproduce to form free-living cercariae which are then released from the snail and seek larval amphibian hosts. Within the larval host, the trematode transforms and encysts as metacercaria around the limbs, often causing malformations. The trematode's life cycle completes when the malformed amphibians (presumably experiencing higher rates of predation in comparison to non-malformed individuals) are consumed by avian or mammalian predators (Schorrhoefer et al. 2003).

A recent experimental study by Johnson et al. (2007) found that eutrophication promotes increased parasite infection rates and amphibian disease risk via two methods: (1) by increasing primary production and thus, increasing population growth of herbivorous *Planorbella* snails, the intermediate host of the parasite *Ribeiroia ondatrae* and (2) by increasing resource availability for pond snails, individual fitness increased and translated into higher levels of parasite infection and subsequent per-snail *Ribeiroia* production and transmission. The results of this experimental study clearly demonstrated a strong positive relationship between increased nutrient loads, snail abundance, parasite densities, and amphibian malformations within small mesocosm enclosures. The relationship between eutrophication and parasite infection in planorbid snails, however, has not been tested in a natural setting.

This study will assess the relationship between water quality, planorbid snail abundance, and parasite (*Ribeiroia ondatrae)* prevalence among eight naturally occurring wetlands (four adjacent to agriculture and four wetlands buffered by forests) across central and southern Indiana. I predict that an increase in

nutrient load and corresponding increase in snail infection densities will be found in wetlands adjacent to agriculture. The results of this study will be crucial to assessing the impacts of anthropogenic affects on *Ribeiroia* densities throughout the state and possible implications for the future survival of amphibian populations.

Goals

The goal of this project is to investigate the interaction between levels of eutrophication on the densities of snail (*Planorbella)* populations and rates of infection of the trematode *Ribeiroia ondatrae*. This will be achieved by:

1. Collecting and analyzing weekly water samples from eight naturally occurring wetlands for levels of nitrate, total phosphate, and chlorophyll *a*.
2. Sampling *Planorbella* snails from each site in order to estimate population densities.
3. Analyzing collected snails for the presence of the trematode cercariae.

Methods

Study Sites. — The study sites for this project will be located in Morgan and Tippecanoe Counties in Indiana. Morgan County was chosen based on large tracts of contiguous forests and numerous natural wetlands located along ridge tops. Within this landscape, delineating wetlands associated with agriculture and ridge tops is relatively straightforward. Tippecanoe County was chosen based on geographic proximity to campus and its history of intensive agriculture. Given that Tippecanoe County is highly fragmented and predominantly agriculture, the delineation of wetlands associated with non-agriculture requires the application of wetland buffer zones. A wetland buffer (e.g., forest, hayfield, etc.) of 159-290 m (Semlitsch and Bodie 2003) will be required for forested ridge top simulation in Tippecanoe County. A total of four sites will be selected in Morgan County with two representing agricultural adjacent wetlands and two representing forested ridge tops. A total of four sites will be selected in Tippecanoe County with two representing agriculturally adjacent wetlands and two representing sites with adequate wetland forested buffers to simulate ridge tops. Sites will be separated from each other by at least 300m.

Water Quality Sampling. — Water samples will be collected weekly from March 7 through May 30. At each site, one 250mL clear polyethylene bottle and one 250mL dark polyethylene bottle will be filled with water for analysis of nitrogen (i.e., ammonia, nitrite, and nitrate), orthophosphate, and chlorophyll *a* using the Hach Dr/2400 spectrophotometer (Johnson 2002). Samples will be filtered before analysis to remove particulates.

Snail Sampling. — Snails will be collected from study sites weekly using a quadrat method adapted from Hairston et al. (1958). Samples will be taken at 10m intervals around the circumference of the wetland at one meter and two meters perpendicular to the shoreline, for a total of 16-24 samples per site . A cylindrical apparatus with an area covering .14m^2will be placed at a depth of 15cm into the substrate at each selected sample point. The area within the circle will be sieved through and hand-picked thoroughly. All snails collected from each sample will be placed in a plastic bottle and covered with a lid on site. After collection is complete, snails will be identified to genus (*Planorbella)* and classified into four size categories (1-5mm, 5.1-10mm, 10.1-15mm, and >15mm; Johnson et al. 2007). To estimate snail infection prevalence, a random sub-sample of 20-30 *Planorbella* will be selected per site. Each snail will be placed in 5mL of distilled water in a 20mL centrifuge tube on site and brought to the lab for analysis.

Analysis of trematode cercariae.— A minimum of 20 snails will be screened from each site to calculate infection prevalence. Snails will be suspended in 5mL of water, placed in individual 20mL centrifuge tubes, and then exposed to intense fluorescent light for 24 hours which induces snails to shed cercariae. The water from each tube will then be removed and examined under a dissecting scope for the presence or absence of cercariae (Kiesecker 2002). A sample of infected snails will be crushed and *Ribeiroia ondatrae* tissue will be isolated and collected as rediae and cercariae following the protocol of Wilson et al. (2005) for another project involved with examining the genetics of the parasite in this system.

Expectations

I expect that increased eutrophication of aquatic systems will increase primary production, thereby increasing population growth and vitality of *Planorbella* snails, the intermediate host of the trematode *Ribeiroia ondatrae*. As a result of increasing population density, *Planorbella* will correspondingly experience higher rates of infection. I expect that study sites in both central and southern Indiana adjacent to agriculture will experience the highest nutrient loads and thus will contain the highest prevalence of infection. Study sites adjacent to forested buffers will experience significantly lower nutrient loads and correspondingly lower rates of infection.

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