

## Golf Course Maintenance and Amphibian Conservation

### Principal Investigator:

Dr. James H. Howard-Frostburg State University

### Cooperating Investigator:

Dr. David Morton-Frostburg State University

### Graduate Students:

James Julian and Shannon Julian

**Goals:***Laboratory Studies-1) To test the relative toxicity of the most commonly used pesticides (insecticides, fungicides and herbicides) with three diverse taxa of amphibians. 2) To develop a more complete and biologically realistic testing protocol including: a) multiple species; b) short term acute and long term chronic tests; c) multiple life history stages; d) multiple indicators of biological impact; and e) an environment that provides the opportunity to detoxify or potentiate chemicals in a more biologically realistic way.*

*Field Studies-1) To access the feasibility of "stocking" wetlands in order to establish breeding populations of desired amphibian species. 2) To evaluate the relative success of small temporary wetlands versus larger permanent bodies of water more typical of golf course design.*

### Progress to date-Laboratory studies

We determined the LC50s (dose lethal to 50% of the population) for three insecticides; Merit (Imidacloprid), Dursban (Chlorpyrifos) and Sevin (Carbaryl) on wild caught Wood Frog tadpoles (*Rana sylvatica*) and commercially available Gray Treefrog tadpoles (*Hyla versicolor*) and Bullfrog tadpoles (*Rana catesbeiana*). The LC50s for all three species are listed in Table 1.

**Table 1. LC50s for Carbaryl, Chlorpyrifos and Imidacloprid (all values expressed in parts/billion)**

Species	Pesticides		
	Imidacloprid	Carbaryl	Chlorpyrifos
Wood Frogs	248,250	20,523	494
Gray Tree Frogs	32,100	1664	39
Bullfrogs	175,500	45,294	273

The first egg hatching trials also demonstrate differences in toxicity among pesticides. These trials were conducted on Leopard Frog eggs. The egg hatching trial (unlike the LC50 determinations) uses sediment in the bottom of aquaria to absorb pesticides and produce a more realistic test simulation. Data obtained on water samples suggest that concentrations of pesticides drop dramatically in our protocol. For example, Carbaryl concentrations decline from over 800 ppb in our highest concentration to an average of 175 ppb over the first 24 hrs and

*Table 1 reflects the large differences among insecticides in their short-term acute toxicity to amphibians. For example, amphibians were 50-500 times more sensitive to Chlorpyrifos than the other agents tested. It also suggests substantial differences among frogs in terms of their sensitivity, however, the tadpoles tested were not of similar ages and ongoing studies will address the question of age-specific sensitivities to these insecticides.*

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### Personnel

#### Principal Investigator:

Dr. James H. Howard-Frostburg State University-20% of full time duties

#### Graduate Students:

James Julian -50% of full time duties

Shannon Julian - 50% of full time duties

### Budget Expenditures

### Spent or Encumbered

Salary	
P.I. (one month summer salary)	\$5,300
Graduate Students (Stipend) \$7,000@ X 2	\$14,000
Field Assistants \$6.00/hr.	\$2,750
Fringe Benefits (Approximately 8% of salaries)	\$1,721
Travel	\$726
Equipment and Supplies	\$3674
Consulting (American Land Concepts)	\$1000
Subcontract for chemical testing (TIWET)	\$2829
Indirect Costs	\$3,367
Total	\$35667
Balance	\$1369*

\*Most of this will be spent on travel and supplies before the end of January.

after one week the compound is not detectable. The preliminary results show no appreciable toxic effect on egg hatching except at the highest exposure levels of Carbaryl (approaching the LC50s for Bullfrogs).

In addition, long term chronic exposures of young Gray Treefrog tadpoles were conducted for six weeks to evaluate our protocol and develop behavioral as well as teratological indicators of toxicity. At two week intervals, beginning 24 hr. after initial exposure, water samples were collected and shipped to The Institute for Wildlife and Environmental Toxicology (TIWET) for chemical analyses. TIWET is currently reviewing procedures to evaluate sediment samples in order to estimate how much pesticide remains in the sediment and be bioavailable through an ingestion pathway. The concentrations of pesticide in the water and sediment will be critical to extrapolate our laboratory data to field conditions.

#### **Field studies**

We constructed six ponds to initiate studies on stocking to establish breeding populations of amphibians. These ponds were constructed with the technical input of mitigation specialists at American Land Concepts. Our study design consists of three pairs of ponds of approximately the same surface area. Four of these ponds are located in mitigated areas and two are in an adjacent existing wetland. We graded one of each of the pairs of ponds to a depth of 30-50cm and one to a depth of 70-100cm. The later pond in each pair is expected to hold water year round. In order to accelerate the suitability of these sites for the establishment of breeding populations we planted one shoreline of each pond with emergent aquatic vegetation (*Carex stricta*, *Carex crinita*, and *Glyceria stricta*). All ponds were completely enclosed with drift fencing and pitfall traps so all animals entering or exiting the ponds can be captured, examined and marked if necessary. We collected juvenile amphibians (northern cricket frogs and Jefferson salamanders) from nearby populations and individually marked animals were subsequently released at each of the six ponds. In addition, our mitigation sites and the larger bodies of water associated with the playing surface of the Rocky Gap Golf Resort were monitored throughout the summer and fall for natural colonization.

**Future work-Laboratory studies**-We will complete our first larval experiment in February 1998. At the same time we will also be able to gather recently deposited eggs of one local species (probably *Ambystoma jeffersonianum*) for our second egg hatching trial. By the end of summer 1998 we should have data on the effects of the three insecticides under investigation for at least three species of amphibians. We will have data on egg hatching success and the relative sensitivity of life history stages as well as data on larval growth, survivorship, behavior, and developmental abnormalities. After these trials are complete we will begin a new set of experiments on the three herbicides. In addition, Dr. Morton will direct a new graduate student to conduct a pathology evaluation. They are scheduled to begin those investigations in Madison, WI at the USFWS Wildlife Toxicology Laboratories.

**Field Studies**-Beginning in February, 1998 drift fences and pitfall traps will be examined every other day to capture amphibians migrating to the ponds to breed. Once per week other bodies of water in the golf course development outside the mitigation areas will be monitored by visual searches and auditory sampling. By late spring egg masses from target species will be located in nearby natural populations and these masses will be translocated to the six study ponds to evaluate egg hatching success, larval development and survival to metamorphosis in the constructed ponds.