Mercury Speciation and Bioaccumulation in Amphibian Populations

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Mercury contamination is well-documented and continues to be a public-health issue of great concern for certain sectors of the global human population. Mercury contamination of wild amphibians has received little attention, however, despite reports of worldwide population declines. Documentation of the pervasiveness of this contaminant is a first step toward understanding the potential environmental health and ecological implications of mercury pollution. Identifying broad scale distribution patterns of mercury bioaccumulation can convey to regulators that certain ecosystems may be degraded and require development of policies and regulations that may reduce mercury emissions, and ultimately, improve air and water quality. A more synthesized, holistic, perspective on the mechanisms related to aquatic and terrestrial biogeochemistry linkages of fate, transport, and bioavailability of mercury in aquatic ecosystems will result from long term, multi-ecosystem monitoring programs coupled with process-oriented research questions. Here I present total and methyl mercury amphibian data from freshwater and terrestrial ecosystems in the conterminous United States, including sites where amphibian disease and die-offs have been documented. We evaluate variation in mercury bioaccumulation and distribution in these ecosystems across a broad gradient of physical, climatic, biotic, and ecosystem settings to identify the species, environmental conditions and ecosystem types that are most sensitive to mercury pollution. The role of disturbance (i.e., fire, acidification, eutrophication, high mercury deposition, and land use) mechanisms and abiotic and biotic factors governing mercury distribution, bioaccumulation and its potential ecotoxicological effects, in amphibians inhabiting the different ecosystem types will also be discussed. **S-7**

Unravelling the Mechanism(s) of Ranavirus Transmission

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Ranaviruses are double-stranded DNA virus of fish, reptiles, and amphibians. They have been associated with mass mortality events in both aquaculture and wild populations. The tiger salamander ranavirus, Ambystoma tigrinum virus (ATV), for instance, causes recurrent epidemics in ponds throughout North America that can kill an entire year class. ATV is also being moved in the live animal trade, in the form of infected "waterdogs." In order to better understand how these viruses "get around," we conducted several experiments to explore the form, routes, and timing of ATV transmission among tiger salamanders. Our data suggest that ATV is efficiently transmitted by direct interactions between live animals (bumping, biting, and cannibalism) as well as by necrophagy and indirectly via water and fomites. Determining which form of transmission is most important in nature is essential for understanding transmission at the population level. Our experiments also revealed an important temporal aspect to infectiousness: larval salamanders become infectious soon after exposure to ATV and their propensity to infect others increases with time. These results begin to clarify the mechanisms and dynamics of ATV transmission, and lead to key questions that need to be addressed in future research. **S-7**

Toxicity of Nitrogenous Fertilizers and Pesticides to Snapping Turtles Eggs (*Chelydra Serpentina*)

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Many reptiles oviposit in soil, including agricultural landscapes. We evaluated the toxicity of nitrogenous fertilizers, atrazine, and a complex mixture of pesticides and fertilizers simulating those associated with corn production in Ontario, to snapping turtle (*Chelydra serpentina*) eggs. Eggs were also exposed in vegetable gardens to simulate realistic exposures, and in the laboratory in covered bins so as to minimize loss of volatile compounds. Compounds or mixtures were applied at typical field application rates, and at 10 times these rates. Hatching success, deformities and body size were evaluated for all exposures, whereas for the atrazine exposure gonadal development was also evaluated. Neither urea nor ammonium nitrate had any impact upon hatching success or development in the exposed vegetable garden, despite overt toxicity of ammonium nitrate to endogenous plants. Both laboratory exposures resulted in reduced hatching success, lower body mass at hatching, and reduced post-hatching survival

compared to controls at the highest concnetrtaions. A complex mixture of pesticides (atrazine, glyphosate, dimethamid, tefluthrin) and ammonia did not affect turtle development at typical application rates, although at higher rates caused 100% mortality in both laboratory and field exposures. For the atrazine only exposure, some males with testicular oocytes and females were produced in the atrazine-treated groups (3.3–3.7%) but not in the control group, although no statistical differences were found among treatments. Future work may focus on the accumulation of pesticides in eggs from soil exposures. **S-7**

Herpetofauna Population Changes in a Highly-Polluted Urban Ecosystem: Onondaga Lake, NY

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Onondaga Lake (Syracuse, NY) and its surrounding terrestrial and wetland habitats have been greatly disturbed by human activities for over 150 years. The ecosystem has received wastes from major adjacent industries and from the sewage treatment system for the city of Syracuse; these multifaceted disturbances affected all flora and fauna. Over the last 25 years, some of the major sources of pollutants at the lake have changed in important ways. We have studied the amphibian and reptile populations at the site since 1994, making comparisons with herpetofauna communities in other parts of the state. At the start of the study, the species composition and population densities in the Onondaga Lake ecosystem were dramatically less than comparable sites elsewhere in the region. From 1994 through 2001, no amphibian species were using the lake or the connected wetlands for breeding, although a few species bred successfully in surrounding, but hydrologically-separated wetlands. However, since 2001, some amphibian species have begun to reinvade parts of the lake and connected wetlands possibly due to upgrades in the sewage treatment systems feeding the lake. The species that have been successful here are similar to those found in some other urban areas in NY. **S-7**

Emerging Infectious Amphibian Diseases and Potential Stressors in Acadia National Park Wetlands

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Amphibian mortality events in protected and relatively pristine settings have increased dramatically over the past two decades. We investigated amphibian larval die-off events in Acadia National Park (ANP), Maine, USA, to determine disease ecology and incidence on two scales: within a single breeding pond and at the individual level. Within each breeding pond, we measured biological, chemical, and physical stressors to identify stressors associated with disease incidence. To approach individual amphibian responses, we used three years of comprehensive disease screenings of free-living amphibians combined with in-field health screenings in 26 wetlands to determine potential amphibian reservoir hosts, vectors, and amplifying hosts. We confirmed five major amphibian pathogens within ANP: *Ranavirus*, *Batrachochytrium dendrobatidis (Bd)*, *Ichthyophonus*, *Saprolegnia*, and a *Perkinsus*-like organism, but did not confirm suspected occurrences of *Riberoia*. *Ichthyophonus*, *Bd*, and *Saprolegnia* were relatively benign in our study, and may have natural controls in ANP. Few sublethal stressors were significantly associated with ranavirus or the *Perkinsus*-like organism. We suggest that for *Ranavirus*, the *Perkinsus*-like organism, and *Ichthyophonus*, disease ecology is more important than environmental conditions, with the potential exception of aluminum and temperature stressors. However, for *Bd* and *Saprolegnia*, environmental conditions may be important in controlling and instigating outbreaks. Although some amphibian populations in ANP experienced extensive die-off events caused by *Ranavirus* and the *Perkinsus*-like organism, disease events do not seem to exacerbate natural population fluctuations. **S-7**

Potential Effects of Endocrine Disrupting Chemicals on Diamondback Terrapins (*Malaclemys terrapin*)

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Millions of gallons of anthropogenic chemicals, including endocrine disrupting chemicals (EDCs), are discarded into waterways everyday and little is known about their effects on wildlife. The waters of Jamaica Bay, NY contain known endocrine disrupting chemicals (EDCs) as well as New York's largest

population of diamondback terrapins (*Malaclemys terrapin*). The purpose of this study was to determine possible effects of EDCs on terrapins by comparing egg and hatchling growth, locomotor performance, shell abnormalities, and aromatase levels of hatchlings from four locations. Terrapin clutches from Jamaica Bay and three comparison sites (Cape May Peninsula, NJ, Peconic Bay, NY, and Barrington River, RI) were collected from naturally laid nests. Eggs were incubated at a constant temperature and massed periodically. Body size was measured routinely for each hatchling. Shell abnormalities were noted for each turtle. Righting response was tested to determine locomotor performance. Eggs and hatchlings from Jamaica Bay were larger than the other sites. Cape May Peninsula turtles had the lowest percentage of shell abnormalities. Locomotor performance of turtles from all sites markedly increased with age. Although Jamaica Bay is probably the most heavily polluted site from which turtles were obtained, the preliminary results did not indicate that Jamaica Bay turtles were adversely affected. Major effects from EDCs on terrapins may occur over a more extended period of time in nature. The aromatase levels of hatchlings will be determined and presented, as they may provide further insight to the effects of EDCs on diamondback terrapin hatchlings. **S-7**

A Review of Diagnostic Methods for Reptile Infectious Diseases

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Reptiles may be infected with bacterial, viral, fungal, and parasitic agents. Identifying an infectious agent in an ill or dead reptile can be accomplished by several methods, each with limitations. Microbiological cultures and fecal parasite tests are best interpreted in combination with histopathology. Histopathology samples must be collected within 48 hours of death. Bodies should be refrigerated, not frozen, prior to necropsy. Autolysis will occur rapidly under warm conditions and will limit histopathologic interpretation. At necropsy, tissues should be fixed in neutral buffered formalin, and a second set of tissues should be frozen. Frozen tissues may be used for later microbiology or molecular diagnostics. While histopathology may be very informative, it may not demonstrate the specific pathogen. Electron microscopy may be useful for demonstrating the presence of viral particles or for further characterizing agents seen histologically. If electron microscopy is planned, it is best to save a set of necropsy tissues in a fixative such as gluteraldehyde. Molecular diagnostic tests such as polymerase chain reaction (PCR) may be useful for demonstrating the presence of specific pathogen genetic material, and can often be run on frozen tissues. Several laboratories offer PCR tests for reptilian infectious agents. Viral isolation may be successful in some cases but may require reptile-specific cell cultures. Immunologic techniques such as in situ hybridization, serology, and immunohistochemistry may also be useful, but generally require the production of monoclonal antibodies. Serologic tests are limited by difficulty in differentiating past exposure from current infection. **S-7**

Is Exposure to Agricultural Runoff Leading to Sexual Disruption in *Rana Pipiens*?

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Intensive row crop agriculture featuring corn and soybean production, is predominant in southwestern Ontario where the two crops account for over 50% of the total acreage of crops grown. The corn herbicides atrazine and metolachlor, used in row crop agriculture, are two of the most heavily applied pesticides in Ontario and are routinely detected in tributaries draining agricultural watersheds. We measured circulating sex steroids, vitellogenin expression, and gonad histology in wild northern leopard frogs (*Rana pipiens*) from agricultural and non-agricultural sites in southwestern Ontario. Agricultural sites, particularly those in Chatham had a significantly higher percentage of males with ova-testes (42%), as compared to non-agricultural sites (7%). To determine if this gonadal abnormality was linked to exposure to water borne chemicals from agricultural activity, we took eggs from a non-agricultural reference site where no intersex individuals had been detected, and raised them in four agricultural sites to determine if the gonadal abnormalities persisted and for comparison in two non-agricultural sites. While effects were seen at earlier life stages there was no consistent difference between agricultural and non-agricultural sites in terms of survivorship to metamorphic transformation, body size, sex ratio or deformity rates. Testicular oocytes were seen in males raised in both agricultural and reference sites, but rates were significantly higher at some agricultural sites. We have also begun to compare sperm viability in male leopard frogs from populations with and without testicular oocytes. **S-7**

Evidence of Increasing Incidence of Disease in Native Turtles and Research Needs

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In recent years the number of turtles being brought in from the wild for rehabilitation due to illness rather than injury has increased greatly. These illnesses include respiratory infections, aural abscesses, eye infections, dermal lesions, opportunistic infections and documented cases of iridovirus in several states including a semi-aquatic turtle in New York State. Several snakes have also been treated for eye and skin infections. Previously, illnesses treated were predominantly limited to eastern box turtles with respiratory infections and aural abscesses. The reason for the increased number of illnesses is not clear. There is speculation that environmental factors may play a role as well as introduction of disease to isolated populations. Current research on box turtles has shown a possible correlation between levels of organochlorine compounds, which are disruptors of vitamin A metabolism, and aural abscesses. In one study site alone, a researcher this year located five spotted turtles with various illnesses. Several cases will be discussed. There are tremendous research needs in this area including water quality issues, environmental contaminants, infectious diseases, disease vectors and immune suppressants. Are environmental stresses such as diminished habitat and climate change also factors? These issues present many challenging research opportunities. **S-7**

Amphibian Abnormalities and Parasite Incidence on National Wildlife Refuges, Regional/National Perspectives

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The US Fish and Wildlife Service has been studying the prevalence of abnormal frogs on National Wildlife Refuges (NWRs) since 2000. The primary objectives have been to 1) determine if refuges have sites with a high frequency of abnormal frogs, 2) evaluate whether abnormality frequencies are consistent within seasons and among years, 3) investigate possible causes of the abnormalities. Standard operating procedures were developed to insure consistency and replicability nationwide. From 2000–2006, 137 refuges in 46 states were monitored. In the Northeast, 24 refuges have been surveyed with over 200 site-specific collections. Surveys include field exams and subsamples for parasitology and x-ray examination. In the Northeast, the trematode *Ribeiroia*, was detected on six refuges: Aroostook (ME), Erie (PA), Great Bay (NH), Great Swamp (NJ), Iroquois (NY), and Missisquoi (VT). We discuss the relationship between abnormalities and *Ribeiroia* intensity in *Rana clamitans*, *R. sphenocephala*, and *R. pipiens*, and summarize the types of abnormalities across species, refuges, and years. **S-7**

Environment Canada Research on Disease and Contaminant Effects in Reptiles and Amphibians

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The Wildlife Toxicology and Disease Program of Environment Canada funds studies on the effects of contaminants on wildlife, including reptiles and amphibians, and on the incidence and effects of wildlife disease. Research is also being conducted to establish standard rearing, breeding, and toxicological test methods for contaminant screening and risk assessment with amphibians. Environment Canada's contaminant research studies with reptiles and amphibians have typically examined pesticides and priority industrial contaminants, with particular studies focussed on the effects on amphibians of priority in-use pesticides as well as industrial solvents and brominated flame retardants. For example, research conducted on the Roundup® formulation of glyphosate with native amphibians showed that components of the formulation were responsible for the toxicity, and a series of research projects with the herbicide atrazine, from laboratory exposures of individual animals, to outdoor mesocosm exposures, to field work in corn growing areas, has revealed that atrazine exposure alters amphibian development and physiological processes. Both of these herbicides have also shown an ability to alter amphibian gonadal

development; possible mechanisms of atrazine's observed effects are currently being studied. The current focus of the amphibian disease research program in Environment Canada is to determine the incidence, distribution and pathogenicity of disease in Canadian amphibians. Studies are also being conducted on the influence of exposure to pesticides on the rate and severity of infection. These studies are complemented by studies that relate surrounding land-use patterns to measures of amphibian health in the field. **S-7**