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Plenary Session

Kentucky Water Resources Research Institute, University of Kentucky

EVALUATING THE IMPACT OF HEMLOCK WOOLLY ADELGID INVASION ON HEADWATER STREAMS

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Invasions by non-native species are widely considered among the most important causes of the loss of native biota. A serious example of a devastating exotic forest pest recently established in Kentucky is the hemlock woolly adelgid (HWA: *Adelges tsugae*, Hemiptera: Adelgidae). HWA is a xylem feeding insect native to Asia that feeds on all ages, sizes, and species of hemlock (*Tsuga* spp). While certain hemlock species exhibit some measure of resistance, HWA infestations usually lead to mortality of eastern hemlock (*T. canadensis*). Furthermore, native predators appear unable to regulate HWA populations in eastern North America (Wallace and Hain 2000). HWA has the capacity to functionally eliminate eastern hemlock from the landscape.

Eastern hemlock is considered a foundation species that dictates microclimatic conditions and strongly influences ecological processes and interactions (Ellison et al. 2005). It is also an irreplaceable component of riparian zones, particularly within headwater streams. The loss of eastern hemlock may create a cascade of changes from stand to ecosystem level, including increased nutrient exports (Jenkins et al. 1999), altered transpiration rates (Ford and Vose 2007), changes in stream macroinvertebrate diversity (Snyder et al. 2002), loss of unique microclimates (Ellison et al. 2005), and community shifts to deciduous species (Spaulding and Rieske, in review). Benthic macroinvertebrate community composition is correlated with surrounding forest composition (Snyder et al. 2002) and many species are sensitive to slight changes in stream characteristics (Karr 1999). Thus the loss of eastern hemlock from riparian zones has the potential to significantly alter benthic community abundance and functional feeding guild composition (Snyder et al. 2002). This can have far reaching ecosystem-level consequences, since benthic macroinvertebrates form a vital part of the aquatic food chain as both immatures and adults (Merritt and Cummins 1996).

The overall goal of this project is to characterize the composition and structure of benthic macroinvertebrate communities associated with eastern hemlock dominated headwater streams, which will be compared with those from deciduous dominated headwater streams. We are also characterizing chemical and physical characteristics within these streams, which will enable us to correlate stream characteristics with the benthic macroinvertebrate community to evaluate the extent to which HWA-induced eastern hemlock mortality impacts stream health. Research sites are located in eastern Kentucky at Kentucky Ridge State Forest, Natural Bridge State Nature Preserve/Red River Gorge, and Robinson Forest. At each site three hemlock dominated headwater streams have been paired with three deciduous dominated streams with similar physical characteristics for a total of eighteen study streams. A 30 m segment ("reach") was established in each

study stream ~150 m above the confluence. Within each reach, transects were established across three riffles. Benthic macroinvertebrates are sampled from each riffle at 30 d intervals using a standard kick-net and a 0.1 m^2 Surber sampler. Artificial substrates (2.5 × 5 cm five plate Hester-Dendy samplers) provide a means of monitoring long-term colonization of benthic macroinvertebrates; one sampler is deployed at each end of the designated 30 m reach and monitored at 30 d intervals. Benthic macroinvertebrates are being identified to the highest resolution possible and categorized into functional feeding guilds (Merritt and Cummins 1996) in order to elucidate functional differences within the benthic communities of hemlock and non-hemlock headwater streams.

Stream chemistry is evaluated by collecting two 500 ml water samples concurrent with benthic macroinvertebrate sampling. These samples are taken ~2 m downstream from each 30 m reach, placed on ice and returned to the laboratory. Nitrate, ammonia, phosphorus, sulfate, and hardness are evaluated using colorimetric methods (Hach Corporation, Loveland, CO). Total carbon and total organic carbon are being measured using an automated total organic carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Chlorophyll is measured using standard spectroscopic methods. Stream velocity is measured using a flow meter, and width and depth measurements are taken to calculate total flow. Dissolved oxygen, pH, temperature, and specific conductance are measured using a Multi-Probe System (YSI Inc., Yellow Springs, OH). Daily maximum, minimum, and mean water temperature is being monitored using waterproofed iButtons (Maxim Integrated Products, Sunnyvale, CA) anchored in-stream.

Preliminary results indicate significant differences between eastern hemlock-dominated and deciduous-dominated streams in water chemistry characteristics as well as benthic macroinvertebrate function feeding groups. On average, concentrations of sulfate, total carbon, total organic carbon, as well as conductivity are lower in streams draining eastern hemlock. Further, benthic macroinvertebrate shredders and collectors are more abundant in eastern hemlock dominated streams. These results suggest that loss of eastern hemlock from headwater riparian zones due to hemlock woolly adelgid-induced mortality will likely lead to functional changes in headwater stream communities.

Works Cited

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EFFECTS OF ROUNDUP® EXPOSURE ON BEHAVIOR AND REPRODUCTIVE FUNCTION IN A POND-BREEDING SALAMANDER

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Project/Research Objectives

Contamination of water resources by pesticides can pose serious risks to humans and wildlife. More research is needed on sublethal and low-dose effects of exposure so that action can be taken before large-scale, irreversible damage occurs. Endocrinological effects are of particular interest, as hormones drive key processes related to development, reproduction, and relative fitness. In amphibians, biocide exposure may affect reproduction and behavior by interfering with production, delivery, and/or receptor binding of hormones relevant to these processes. We are utilizing a pond-breeding salamander as a model to examine the acute effects of a common herbicide, Roundup®, on courtship behavior, feeding response, and related plasma levels of sex steroids.

Methodology

Adult spotted salamanders (*Ambystoma maculatum*) were collected from two field sites in Calloway County and semi-randomly assigned to one of four nominal exposure concentrations: Negative Control (0.00 mg AI/L), Low (0.05 mg AI/L), Medium (0.50 mg AI/L), and High (5.00 mg AI/L). The Low and Medium treatments were below the maximum glyphosate concentration of 2.6 mg AI/L documented in natural habitats. All treatments were more dilute than the manufacturer's maximum recommended concentration for application. Stock solutions were prepared using Roundup® Ready-to-

[1] The views expressed are those of the authors and do not necessarily reflect the position of the U.S. Environmental Protection Agency.

Use Weed and Grass Killer and stored in airtight polypropylene containers in a dark room. Because of the proprietary nature of the surfactant in Roundup®, no surfactant control was included.

Prior to exposure, specimens were maintained in well water and housed in individual containers in an environmental chamber (6°C ±2). At test initiation, treatment animals were individually submersed in solution for 72 hours. Control animals were newly submersed in untreated well water for an equivalent duration.

Snout-vent length, mass, and reproductive status were recorded for each specimen prior to exposure. Courtship behavior was videotaped for eight hours following treatment and was evaluated using methods from previous studies. Individual feeding response was documented as the number of mealworms eaten (maximum=10) in a 24-hour period immediately following the courtship trials. Plasma was obtained from individual blood samples at the conclusion of behavioral trials and was stored at -80°C ±3 prior to hormone extraction.

Preliminary Findings and Significance

Unexpectedly, 100% mortality was noted in the High treatment within 24 hours of application. An LC_{50} could not be determined because no mortality was noted at either of the other treatment levels. However, the results suggest that the LC_{50} for adult *Ambystoma maculatum* acutely exposed to Roundup® is considerably less than the LC_{50} for amphibians exposed to the active ingredient, glyphosate, in other formulations (*eg.*, Rodeo®) and in non-formulated applications. This suggests that the surfactant and/or other "inert" ingredients in Roundup® (1) are directly toxic to the salamander and/or (2) enhance the toxicity of the active ingredient to the salamander. Alternatively, reproductive status may affect the vulnerability of adult salamanders to glyphosate exposure.

For the Low and Medium treatments, no significant differences in courtship behavior were noted. However, preliminary analysis of log transformed data suggests that Roundup® exposure significantly decreased feeding response in male salamanders ($F_{2,29}=12.15$, $p<0.001$), when controlling for mass. Both treatment levels were significantly different from the Control ($p<0.05$), but not from one another. Decreased foraging activity and/or effectiveness following Roundup® exposure may inhibit the salamander's ability to re-establish energy reserves that are depleted during the breeding season.

Ongoing Research

To determine whether endocrine endpoints related to courtship and feeding response are affected by Roundup® exposure, steroid hormone extraction and enzyme immunoassay (EIA) are being performed on plasma samples as the necessary antisera (C. Munro, U.C. Davis) become available.