Frontline EXPRESS

Canadian Forest Service, Great Lakes Forestry Centre

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MULTIPLE STRESSOR EFFECTS ON NATIVE AMPHIBIANS

INTRODUCTION

Several individual factors, or stressors, may be contributing to declining amphibian populations that have been observed worldwide. A combination of stressors may induce even greater impacts on amphibian populations. The goal of this study was to determine the importance of multiple stressors, particularly simultaneous stress from herbicide and acid contamination, as possible factors affecting native amphibian populations in forested ecosystems of northeastern North America.

A green frog, *Rana clamitans.*

This project incorporated four component studies:
1) Laboratory toxicity testing on different amphibian species;
2) Laboratory studies on multiple stressor interactions;
3) Studies in two natural wetlands, where experiments were conducted in enclosures made in the wetlands;
4) Chemical and biological monitoring of operational aerial herbicide programs.

The first study provided toxicological data specific to three native amphibians (leopard frog, green frog and American toad), as well as the standard test species, the African clawed frog (*Xenopus laevis*). The second study examined the interaction mechanisms among multiple stressors in laboratory test systems. This study enabled us to identify potential additive or synergistic effects among stressors such as acidity, food availability and herbicides. Additive effects occur when stress from one factor is experienced on top of stress from another factor. Synergistic effects occur when two or more stressors interact to yield a greater impact than would be experienced from adding the effects from the two individual stressors.

The third study used field enclosures to examine herbicide impacts under two different natural forest wetlands to validate predictions from both laboratory tests and models. Finally, the fourth study provided data required to determine the range in herbicide exposure levels and direct acute effects that may be experienced by sensitive native amphibian larvae during typical aerial herbicide applications.

The main herbicides being investigated in this study were glyphosate (brand name Vision), the most widely used herbicide in Canadian forestry, and triclopyr (brand name Release), the herbicide most recently registered for use in Canadian forestry.

LOCATION/SITE

This was an international collaborative study with laboratory experiments being conducted at the University of Guelph, Guelph, Ontario and at Dartmouth College, New Hampshire. Field studies were carried out in the Turkey Lakes and Icewater Creek Watersheds north of Sault Ste. Marie, Ontario.

Operational monitoring studies were conducted at various locations across northern Ontario in collaboration with Bowater Inc., Tembec Inc., and Domtar Inc.

A helicopter applies herbicide to a plantation. (Photo by D. Thompson).

RESULTS

Results of this project confirm that there is a significant interaction between acidity and herbicide stressors for both amphibian and zooplankton species. In zooplankton studies, significant interaction with food availability was also observed. All results support the postulate of multiple stress interactions occurring in forest wetland ecosystems. Results from laboratory toxicity testing indicate that late stage amphibian larvae are more sensitive to herbicide stress than embryos. Among the three amphibian species tested, no single species was consistently most sensitive, and the African clawed frog appeared to be a reasonable stand-in for native species that are more difficult to rear in a laboratory. In laboratory experiments, a

less acidic environment resulted in greater toxicity for Vision herbicide; whereas a highly acidic environment resulted in greater toxicity for Release. This result suggests that the two herbicides do not interact in the same way with acidity. Further studies on mechanisms of interaction are currently under investigation.

Enclosure studies in natural wetlands demonstrated that toxicity to native amphibian larvae also occurs under typical field conditions, but generally only at concentrations of herbicide that are in excess of theoretical "worse-case" expected environmental concentration (EEC) scenarios. However, there is an exception with Release, which is toxic at or below worst-case EEC levels under acidic conditions. Results of the operational monitoring studies with Vision showed that wetlands protected by vegetated buffer zones reduced herbicide exposures to essentially nil. Operational monitoring also indicated that exposures typical of direct overspray or adjacent wetland scenarios are insufficient to cause significant acute mortality in most sensitive life stages of native amphibians.

MANAGEMENT INTERPRETATIONS

This four-part study shows that late larval stages of native amphibians are very sensitive to the effects of both Vision and Release herbicides, and that toxicity may be enhanced by simultaneous exposure to other stressors such as pH. The interaction between acidity and herbicides varies with different herbicides, which is particularly relevant to wetlands of northeastern forest ecosystems where a wide range in pH levels has been documented. These findings indicate that potential effects on amphibians should be considered by pesticide manufacturers, and in risk assessments conducted by federal and provincial regulators. Further, interactive effects of other simultaneous stressors that may occur in the environment should also be considered as part of the evaluation process.

In the case of the herbicide Vision, field studies in two natural wetlands and an extensive operational monitoring program demonstrated that applications of this herbicide, as typically conducted in northern Ontario, do not pose a significant risk of acute effects to the most sensitive aquatic life stages of native amphibians in forest wetland environments.

In contrast, field studies with the herbicide Release showed substantial acute toxicity at exposure levels similar to those in lab studies and at or below estimated worst-case environmental exposure concentrations. Although these results indicate a relatively greater risk for harmful effects on native amphibians, the fact that Release herbicide is often applied using ground-based directed application techniques reduces the potential for contamination of wetlands.

Field enclosures were used to examine herbicide impacts under natural forest wetland conditions.

Pending the completion of further chemical and biological monitoring studies to provide data for risk assessment for the herbicide Release, we suggest that forest managers and herbicide applicators take precautions when making broadcast applications to avoid contamination of wetland areas comprising important breeding habitat for native amphibian species. In this regard, results of the operational monitoring study with Vision, confirmed the value of standing timber buffers in reducing herbicide inputs to sensitive aquatic ecosystems and thus significantly diminishing potential effects on resident biota.

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