Experimental use of Zequanox® to control dreissenid mussels adhering to native mussels in Keyes Lake.

Project scope/overview

There is an immediate need for safe and effective control measures to reduce the impact of dreissenid mussels (zebra *Dreissena polymorpha* and quagga mussels *D. rostriformis bugensis*) whose attachment and feeding behavior disrupts aquatic food webs, fouls spawning habitats, and threatens native aquatic species. This project will evaluate the efficacy of Zequanox® to control zebra mussels adhering to native mussels in Keyes Lake and the long-term survival of native mussels following exposure to Zequanox.

Problem/Project Objectives

Dreissenid mussels continue to expand their range within Wisconsin lakes and rivers, while management agencies lack access to effective tools to control their populations. One potential tool for limited open-water control of dreissenid mussels is the commercially formulated product, Zequanox, which contains killed cells of the common soil bacterium *Pseudomonas fluorescens* (strain CL145A). Zequanox is produced by Marrone Bio Innovations (Davis, CA) and is registered by the U.S. Environmental Protection Agency for control of dreissenid mussels in defined discharges (e.g. in cooling and service water systems for industrial facilities). Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the United States government. A 3-year, multiagency (U.S. Geological Survey, U.S. Fish and Wildlife Service, and New York State Museum) research project was recently completed to assess the potential impacts of Zequanox on native fish and unionid mussel species during open water applications (see http://cida.usgs.gov/glri/projects/invasive_species/zm_control.html). As part of this research effort, an initial field trial was completed to assess the efficacy of Zequanox to control zebra mussels (ZM) adhering to native mussels in Lake Darling, located near Alexandria, MN. Preliminary data indicates an approximately 50% mortality rate of ZM adhering to native mussels. Unfortunately, this initial field trial had to be terminated prematurely due to a heavy filamentous algae bloom in Lake Darling. The early study termination and filamentous algae bloom may have compromised the results of this trial. In addition, during the post-exposure observation period, mortality of some of the native mussels was observed. However, there appeared to be no difference in native mussel mortality between the control and treatment groups. This suggests that the native mussels were initially in poor condition, likely as a result from heavy ZM colonization prior to the Zequanox treatment.

The objectives of this study are to (1) assess the efficacy of Zequanox to control ZM adhering to native mussels in Keyes Lake, located near Florence, WI; (2) assess the impacts of Zequanox exposure on sub-lethal measures of native mussel health (i.e. glycogen concentration) and (3) assess the survival of native mussels with varying levels of adhered ZM following exposure to Zequanox.

Methods

Experimental design – Two groups of native mussels with adhering ZM (group 1) and without or very low densities of adhering ZM (group 2) will be collected from Keyes Lake or other WI waters by wading or SCUBA (collection conducted by WI-DNR). A total of 270 mussels (of a single species) will be collected for each group. Native mussels from the two groups will be indiscriminately assigned to assessment cohorts and tagged with individually numbered and color coded Hallprint® tags. The assessment cohorts will include (1) an initial ZM colonization density cohort (N = 45/group), (2) a mussel

health cohort (i.e., glycogen content in foot tissue [N = 45/group]), (3) a cleaned cohort (assessed for survival and zebra mussel colonization (i.e. cleaned of adhering ZM) at 28-d, [N = 90/group]), and (4) an uncleaned cohort (assessed for survival only at 28-d and immediately returned to lake without removing ZM [N = 90/group]). All assessment cohorts will be assessed for survival and ZM colonization/ recolonization at 9-12 month post-exposure. Native mussels from all assessment cohorts will be comingled during the treatment and post-exposure observation periods and the color coded tags will be used to facilitate the field identification of assessment cohorts.

A pre-study survey will be conducted by the WI-DNR and if multiple species are present in sufficient numbers for testing, a sampling design (utilizing fewer animals of each species) that meets project goals will be developed in consultation with WI-DNR.

The initial wet weight of all native mussels (regardless of assessment cohort) will be determined and recorded (i.e., adhered ZM will not be removed) prior to Zequanox treatment. This data will be used later to calculate the difference between the initial wet weight and the cleaned wet weight of each individual native mussel to better characterize the initial ZM colonization prior to treatment.

After all the native mussels are tagged (color coded per assessment cohort) and their initial wet weights are recorded, additional data and samples will be collected for two of the assessment cohorts: the initial ZM colonization density cohort and the mussel health cohort. For the ZM colonization cohort, the ZM adhering to these native mussels will be removed, categorized as alive or dead, and enumerated to estimate the number of live ZM per unit measure (g) adhering to native mussels prior to Zequanox exposure. For the mussel health cohort, an initial sample of native mussel foot tissue (10-mg plug) will be collected, placed into individually labeled cryovials, and stored at -80 C until glycogen analysis. Then all the mussels assigned to all assessment cohorts will be randomly allocated to one of nine replicated enclosure areas (~9 m^2 area that will be enclosed during the treatment by an impermeable membrane barrier) and placed into a retention barrier (Figure 1a).

Figure 1. Example retention barrier (a, left) and enclosure (b, right).

Zequanox treatment levels (control + 2 Zequanox treatments [levels to be determined in consultation with WI-DNR and Marrone Bio Innovations]) will be randomly assigned to each replicate. An impermeable

membrane (i.e. enclosures; Figure 1b) will be placed around each enclosure area replicate to contain the Zequanox during the exposure period. At the end of the Zequanox exposure period, the membrane enclosures will be removed and the mussels with associated adhered ZM will remain within the retention barrier to aid in recovery during the post-treatment assessments. A more detailed sampling design is shown in Figure 2.

Native mussels assigned to the 28-d cleaned assessment cohort will be assessed for survival and characterized for the number of live ZM adhering to surviving native mussels before Zequanox application using pre- and 28-d post-exposure (i.e. cleaned) wet weights. The number of live ZM adhering to native mussels after Zequanox application will be determined by removing, categorizing (live or dead), and enumerating all ZM (> 5 mm length) adhering to each native mussel at 28-d post exposure. After assessment, the surviving, cleaned native mussels will be returned to the appropriate treatment level replicate for a subsequent (9-12 month post-exposure) survival and ZM re-colonization assessment.

Native mussels assigned to the 9-12 month not cleaned assessment cohort will be assessed only for survival (i.e., no ZM will be removed) at 28-d and then placed directly back into their respective treatment level replicate retention barrier or into larger holding corrals (the use of larger holding corrals for overwintering will be determined from the results of the WI-DNR spring survey). At 9-12 months post-exposure, native mussels assigned to this assessment cohort will be assessed for survival and characterized for the number of live ZM adhering to surviving native mussels before Zequanox application using pre- and 9-12 month post-exposure (i.e. cleaned) wet weights using the methods previously described for the 28-d cleaned assessment cohort.

Native mussels assigned to the mussel health and initial ZM colonization assessment cohorts will be allocated to and retained within a retention barrier replicate throughout the study duration. Native mussels from these cohorts will be assessed for (1) survival and glycogen content (mussel health cohort) at the 28-d post exposure assessment and (2) survival, glycogen content (mussel health cohort), and ZM colonization at the 9-12 month post-exposure assessment.

Results from survival, glycogen content, and zebra mussel colonization densities will be compared between treatment levels and mussels groups (with and without adhering ZM) prior to Zequanox treatment and at 28-d and 9-12 month post-exposure. Native mussels assigned to the mussel health, initial ZM colonization assessment and cleaned cohorts will be treated as covariate groups to account for the additional stressors.

Project timeline

Event	Who	Completion Date
Pre-study survey	WI-DNR	5/30/14
Mussel collection	WI-DNR	7/28/14
Treatment application	USGS	8/1/14
28-d assessment	USGS	8/29/14
9-12 month assessment	USGS	8/29/15
Final report	USGS	1/30/16

Estimated Costs

Item	Cost
Salary	$51,000
Materials	$13,000
Travel	$7,500
Subtotal	$71,500
Indirect costs	$38,687
Total project cost	**$110,187**