DEPARTMENT OF
ECOLOGY
State of Washington

Quality Assurance Project Plan

New Zealand Mud Snail Potassium Chloride and Magnesium Chloride Bioassay

April 2009

Publication No. 09-03-109

Publication Information

This plan is available on the Department of Ecology's website at www.ecy.wa.gov/biblio/0903109.html.

Ecology's Project Tracker Code for this study is 09-532.

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FWTCT – Freshwater Technical Coordination Team
EAP - Environmental Assessment Program
EOS – Eastern Operations Section
WOS – Western Operations Section
SCS – Statewide Coordination Section

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Abstract

Each study conducted by the Washington State Department of Ecology (Ecology) must have an approved Quality Assurance (QA) Project Plan. The plan describes the objectives of the study and the procedures to be followed to achieve those objectives. After completion of the study, a final report describing the study results will be posted to the Internet.

The following QA Project Plan focuses on laboratory bioassay testing of the chemical compounds, potassium and magnesium chloride, on the aquatic invasive species New Zealand Mud Snail *Potamopyrgus antipodarum* (NZMS). NZMS specimens will be collected from the Long Beach area and brought to the laboratory, where they will be positively identified and exposed to varying concentrations of potassium and magnesium chloride for set durations. After a recovery period, the number of snails killed by each exposure will be determined, including a control group exposed to unaltered source water.

The bioassay will be performed in two stages: a preliminary screening followed by a final replicate test. Results from the screening will determine concentrations to be used in the replicate test. For potassium chloride, a single additive may be included to increase effectiveness: either a degreaser (Simple Green®) or a relaxant (low concentration magnesium chloride).

The replicate test will determine whether either potassium chloride or magnesium chloride offers a viable decontamination option for field equipment potentially contaminated by the NZMS. Recommendations will be made about optimal concentration, exposure time, and choice of additive (if any).

Background

The New Zealand Mud Snail *Potamopyrgus antipodarum* (NZMS) is a highly invasive aquatic nuisance species known to inhabit a number of natural waterbodies in the Pacific Northwest, including some in Washington State. It can be spread through activities such as recreational fishing, boating, firefighting, and research. Because it is a clonal reproducer, only a single NZMS specimen needs to be transported to infect a new waterbody. Once this species is established within a waterbody, it is impossible to control or eradicate.

Due to high population density (up to 500,000 per square meter) it can dominate a river system's ecology, to the detriment of native species. Juvenile salmon and other fish are known to feed on NZMS but are starved of nutrition due to the mud snail's capacity to pass through the fish's intestinal tract alive.

To prevent Ecology personnel from inadvertently spreading NZMS as a result of field work, efforts have been made to both increase staff awareness and improve field decontamination procedures. Potential field decontamination methods to remove NZMS include visual inspection and removal, physical treatment, and chemical immersion. Visual inspection and removal is insufficient for all but the simplest equipment, due to the minute size of the NZMS (often < 2 mm). Physical treatment (freezing or heating) is not feasible during much field work due to required time and equipment. Therefore, decontamination by chemical immersion is an attractive option for many field situations.

Of the chemical immersion methods previously investigated by others for destroying the NZMS, few are 100% effective. This is because of the ability of this creature to close its operculum (a shell plate which closes when the snail retracts) and survive the treatment. For example, the NZMS is reported to survive exposure to some bleach solutions. Currently, one of the most practical and effective chemical immersions uses quaternary ammonia compounds (QACs) which have been demonstrated to result in 100% mortality to NZMS using reasonable concentrations and exposure times. Unfortunately, these QAC compounds sometimes pose a risk for sample contamination, negating their use in certain situations.

Potassium Chloride and Magnesium Chloride

To efficiently kill NZMS without risking sample contamination by QACs, potassium chloride and magnesium chloride are being evaluated as potential decontamination options. We will determine if at least one of these compounds can be demonstrated 100% effective at killing the NZMS using concentrations and exposure times reasonable for field work. Both compounds are salts which, with proper handling, are economical as well as safe for personnel, equipment, and the environment.

Previous research into the lethality of potassium chloride against NZMS has been performed by the State of California Department of Fish and Game; however, their exposure times (4 and 8 hours) are excessively long for field decontamination. Their 4-hour test reported 100% mortality against NZMS using concentrations of 24.5 g/L potassium chloride and higher (Aquatic

Toxicology Lab Report P-2454, 2007). Temperature was maintained at 13°C during their testing. We will investigate whether or not higher concentrations of potassium chloride can achieve 100% mortality against NZMS using shorter exposure times (30 minutes or less). To investigate improving the effectiveness of potassium chloride solutions, we will test two additives during screening: low concentration magnesium chloride as a relaxant and Simple Green® as a degreaser. Based on screening results, one of these additives may be included in subsequent replicate testing.

The decision to test low concentration magnesium chloride as a relaxant is based on a report that a solution of 30 g/L was found to be an effective relaxant for the sea snail, Queen Conch. Within 20 minutes, the solution resulted in an extended mantle, relaxed foot, and slow response to physical manipulation (Acosta-Salmon and Davis, 2007). If a similar relaxation response is experienced by the NZMS, it will greatly enhance lethality of the potassium chloride solution by allowing the solution to penetrate the snail's operculum. Magnesium chloride is commonly used as a deicer on roads.

Additives

We will also test four additional relaxants to determine if they relax the NZMS individually: MS-222, clove oil, nicotine, and magnesium sulfate (Epsom salt). MS-222 and clove oil are used as fish relaxants, and nicotine is known to relax leeches. Epsom salt will provide magnesium ions that may provoke a response similar to magnesium chloride, with the advantage that Epsom salts are cheap and easily obtained.

The decision to test Simple Green® is based on a previous study (Schisler et al., 2008) which speculates that a degreaser may loosen the NZMS's operculum, increasing exposure to lethal compounds. According to the manufacturer's MSDS[1], the only ingredient in Simple Green® with known exposure limits is 2-butoxyethanol (CAS 111-76-2). The NIOSH[2] guide gives the chemical formula for this substance as $C_4H_9OCH_2CH_2OH$, which does not appear to pose a sample contamination issue for most projects. However, because the MSDS lists Simple Green® as having < 1% nitrogen by weight, further evaluation may be needed to ensure that it poses no threat of sample contamination.

No data were found regarding acute toxicity of magnesium chloride on NZMS or other mollusks, but such data are available for other aquatic species. By comparing acute toxicity data from the online Pesticide Action Network (PAN) pesticide database for aquatic species tested with both potassium and magnesium chloride, we conclude that magnesium chloride may be toxic to NZMS at concentrations similar to potassium chloride. The PAN database also lists magnesium chloride as a molluscicide.

[1] MSDS = Material Safety Data Sheet
[2] NIOSH = National Institute for Occupational Safety and Health

Project Description

We will collect New Zealand Mud Snails in the field and test them in the laboratory by exposing them to known concentrations of potassium chloride and magnesium chloride (with and without additives) for set exposure times. After a recovery period has passed, we will record the number of snails killed within each exposure group.

We know that 24.5 g/L potassium chloride will kill 100% of NZMS in 4 hours; it is not known whether magnesium chloride will kill NZMS. Our hypothesis is that using higher concentrations of potassium chloride will result in shorter exposure times, and that similar concentrations of magnesium chloride may also kill NZMS. During the preliminary screening, we will identify those concentration ranges that reduce exposure time to 30 minutes or less. The replicate test will then determine optimal concentrations and exposure times.

Potassium chloride's solubility limit is listed as 340 g/L at 20°C. However, we found the practical solubility of the K-Life® water softener salt (99% potassium chloride) used to create the solution is much lower, approximately 260 g/L. Preliminary screening will use potassium chloride solutions at 100%, 75%, 50%, and 25% of the practical solubility limit (260, 195, 130, and 65 g/L, respectively). We will concurrently test these same concentrations using the two additives. Magnesium chloride's solubility limit is listed as 543 g/L at 20°C; preliminary screening will test the following concentrations: 340, 255, 170, and 85 g/L. Magnesium chloride will not be tested using any additives during the preliminary screening.

For the final replicate test, we will expose NZMS to two concentrations of either potassium chloride or magnesium chloride, using 20 and 30 minute exposure times, and possibly a single additive (either relaxant or degreaser). Modifications to this scheme may be made based on screening results.

To create the relaxant additive, we will mix magnesium chloride to a concentration of 30 g/L, similar to Acosta-Salmon and Davis (2007). To create the degreaser additive, we will mix Simple Green® as a 1:10 ratio, per manufacturer's specifications. All solutions will be pre-mixed using de-chlorinated tap water prior to collecting snails. We will record the mass and volume of salt used to create all solutions.

We will collect NZMS specimens from the Long Beach area of Washington State and perform laboratory testing at the Washington Department of Fish and Wildlife (WDFW) Nahcotta field station. After testing is concluded, we will dispose of specimens and contact water in a manner that ensures the species does not spread as a result of this project.

The goal of this project is to identify a concentration of potassium chloride or magnesium chloride that will kill 100% of exposed NZMS in 20 or 30 minutes. An additional objective is to determine whether toxicity is increased by adding a relaxant or a degreaser.

Organization and Schedule

The following people are involved in this project. All are employees of the Washington State Department of Ecology.

Table 1. Organization of project staff and responsibilities.

Staff (all are EAP except client)	Title	Responsibilities
Dave Hallock Freshwater Monitoring Unit WOS Phone: (360) 407-6681	Project Manager	Oversees the project, coordinates with management, and assists the principal investigator.
Evan Newell Central Regional Office EOS Phone: (509) 575-2825	Principal Investigator	Writes the QAPP, conducts field sampling lab tests, conducts QA review of data, analyzes and interprets data, and writes the draft report and final report.
George Onwumere Directed Studies Unit WOS Phone: (509) 457-7136	Unit Supervisor for the Project Manager	Provides internal review of the QAPP, approves the budget, and approves the final QAPP.
Gary Arnold Central Regional Office EOS Phone: (509) 454-4244	Section Manager for the Principal Investigator	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, and approves the final QAPP.
Bob Cusimano WOS Phone: (360) 407-6596	Section Manager	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, and approves the final QAPP.
Will Kendra SCS Phone: (360) 407-6698	Section Manager	Approves the final QAPP.
William R. Kammin Phone: (360) 407-6964	Ecology Quality Assurance Officer	Reviews the draft QAPP and approves the final QAPP.
Stuart Magoon Manchester Environmental Laboratory Phone: (360) 871-8801	Director	Approves the final QAPP.

EAP – Environmental Assessment Program.
QAPP – Quality Assurance Project Plan.
WOS – Western Operations Section.
EOS – Eastern Operations Section.
SCS – Statewide Coordination Section.

Table 2. Proposed schedule for completing field and laboratory work, data entry into EIM, and reports.

Field and laboratory work	
Field work completed	March 25, 2009
Laboratory analyses completed	April 1, 2009
Environmental Information System (EIM) system	
EIM data engineer	NA
EIM user study ID	NA
EIM study name	NA
Data due in EIM	NA
Final report	
Author lead	Evan Newell
Schedule	
Draft due to supervisor	April 15, 2009
Draft due to client/peer reviewer	May 1, 2009
Draft due to external reviewer(s)	N/A
Final report due on web	June 30, 2009

Project Costs

The budget for the project is about $1500, excluding staff time (Table 3). Laboratory supplies and space are being donated by WDFW.

Table 3. Expenses.

Item	Cost
Per diem and lodging (8 days, 6 nights)*	$724
Travel (two round trips, 1000 miles)*	<$550
Chemicals (potassium/magnesium chloride, Simple Green®)	$100
Lab analyses (2 samples, hardness and alkalinity)**	$78
Total	<$1,452

*For the principal investigator.
**Costs include a 50% discount for Manchester Laboratory.

Quality Objectives

Bias will be assessed on the basis of NZMS control group survival. Precision will be ensured through the use of replicate groups. A successful outcome is defined as 100% mortality in all replicates of a particular treatment with less than 10% mortality in each of the control replicates.

Sampling Process Design (Experimental Design)

We will perform a 20-minute pre-test of magnesium chloride to determine if it relaxes the NZMS both as a single solute and in combination with the four screening potassium chloride concentrations. Based on this pre-test result, we will decide whether or not to separate the potassium/magnesium chloride combination by exposing snails first to magnesium chloride for a few minutes (T1) and subsequently exposing them to potassium chloride for a few more minutes (T2), choosing the two exposure times T1 and T2 based on observed relaxation times (T1+T2<30 min).

The following additional relaxants will be tested individually: MS-222, clove oil, nicotine, and magnesium sulfate. Each relaxant will be tested on a group of 10 snails, alongside a control group.

During the preliminary screening, each tested group will consist of 10 snails, including two control groups. The two control groups will be exposed to unaltered source water. The total number of snails needed: 18 groups x 10 snails per group = 180 snails. Exposure duration will be 30 minutes.

In addition to two control groups, the following concentrations and additive combinations of potassium and magnesium chloride will be tested (Table 4).

Table 4. Chemicals to be tested (g/L).

KCl Only 30 minutes	KCl (+ $MgCl_2$) 30 minutes	KCl (+ Degreaser) 30 minutes	$MgCl_2$ Only 30 minutes
65	65	65	85
130	130	130	170
195	195	195	255
260	260	260	340

KCl = potassium chloride.
$MgCl_2$ = magnesium chloride.

Final replicate testing will consist of the approximate design shown below. NZMS will be exposed to both 230 g/L potassium chloride and 340 g/L magnesium chloride using three exposure times of 10, 20, and 30 minutes (Table 5). Each treatment will include four replicates of 10 snails. The control will consist of 4 replicate groups of 10 snails each exposed to unaltered source water.

Snails will be chosen randomly by selecting the snail closest to a random grid node. Only snails which are observed to be feeding will be selected (to avoid testing compromised specimens).

Table 5. Approximate bioassay design for final test.

Chemical	Concentration (g/L)	Minutes		
		10	20	30
(Control)	0	-----	-----	X
Potassium chloride	230	X	X	X
Magnesium chloride	340	X	X	X

Tested chemicals were obtained from the following sources:

- Potassium chloride is a K-Life® brand water softener salt, which is similar to the salts expected to be obtained by field crews for use in decontamination. Percentage composition is listed on the package as potassium chloride (98.9), sodium chloride (0.9), calcium (0.02), magnesium (0.01), bromium (0.04), sulfate (0.04), water insolubles (0.01), and moisture (0.10).
- Magnesium chloride is lab grade $MgCl_2 \cdot 6H_2O$ obtained through Fisher Scientific Supply.
- Simple Green® is a concentrated brand name and will be mixed with dechlorinated tap water.
- Epsom salt is Kroger® brand magnesium sulfate ($MgSO_4 \cdot 7H_2O$).
- Nicotine solution was prepared by heating tobacco from a single menthol cigarette in 1000 ml of dechlorinated tap water to form a “tea”.
- MS-222 was obtained from PHARMAQ and mixed as 15 g/L.
- Clove oil is Humco brand essential oil.

Sampling Procedures

We will collect NZMS from the Long Beach, Washington area. Snails will be collected twice, once for the screening and again for the replicate testing. We will use field meters at the time of collection to record water temperature, pH, and conductivity during both collection events. During the second collection event, we will obtain water samples for hardness and alkalinity of the source water, to be analyzed by Manchester Laboratory.

We expect to find snails on the bottom of shallow water areas. If possible, snails will be collected from the shore using macroinvertebrate nets. Alternatively, chest waders will be worn if it is necessary to enter the water.

We will immediately place snails in a 1-mm sieve to remove sediment and smaller specimens, then place approximately 100 snails at a time into a clear 1-liter, wide-mouth jar filled halfway with source water, to estimate the total number of snails collected. For preliminary testing, three jars will be filled; for subsequent testing, four jars will be filled. Jars will be securely lidded and protected from breakage using bubble wrap and rubber bands, then placed in an unrefrigerated cooler on shore.

Additionally, we will collect five gallons of source water from the waterbody where the snails were collected, for laboratory storage of the snails. Prior to leaving the collection site, we will use a scrub brush as needed to remove mud and debris from waders and sampling equipment. We will decontaminate waders and tools used during NZMS collection by either drying at low humidity for several days or freezing overnight prior to subsequent use.

Measurement Procedures

Storage and exposure testing of NZMS will be performed in an unheated room at the WDFW Nahcotta field station, where the temperature is expected to be reasonably similar to the water from which specimens were collected. Temperature in the room will be recorded and is expected to be approximately 10-13°C.

This testing water temperature should provide reasonable values for lethality, since a previous report indicates that exposures made at low temperature (5 °C) are more lethal than those performed at higher temperature (15 °C), due to slower operculum closure (Hosea and Finlayson, 2005).

Upon arrival at the WDFW laboratory, relaxation pre-tests will be performed on a subset of snails, while the remaining snails will be left in a cooler to gradually acclimate to room temperature, reducing stress on the snail population prior to testing.

For exposure, we will decant NZMS onto a wire strainer, collecting all storage water for later use as rinse water. We will place 10 specimens in each prepared test vessel (600-ml beaker filled with 250-ml test solution) maintained at the same temperature as the storage water. We expect snail opercula to be closed at time of exposure, resulting in conservative estimates for lethality. The test vessel will be briefly swirled to ensure contact with solution, and then allowed to rest for the allotted contact time of either 10, 20, or 30 minutes. We will observe snails to ensure that they remain within the solution.

After the allotted contact time has passed, we will again decant the snails using the wire strainer, and collect the test solution for proper disposal. Previously collected storage water will be poured over the snails while gently shaking them to ensure a proper rinse, again collecting this water for disposal.

We will then return snails to labeled storage vessels filled with 500 ml of fresh source water, for observation. Storage jars will remain un-lidded to allow oxygen exposure and will be clearly labeled for identification. Screens will be used as necessary to prevent escape. To provide snails with a food source, snails will be exposed to 10 hours of fluorescent lighting per day to maintain algae within the source water. We will assess death at least 48 hours after exposure using the following criteria:

1. Operculum is loose, missing, or easily rotated, and rotting tissue is observed.
2. Snail is unresponsive to being pulled out of shell and remains without movement for a 5-minute observation period.

Manchester Environmental Laboratory will determine hardness and alkalinity using EPA Methods 200.7 and 310.2, respectively.

Quality Control Procedures

Field

Specimen species will be identified by emailing photographs of the collected snails to Robyn Draheim, a researcher at Portland State University, who is familiar with the species. Live, healthy specimens will be collected to the extent possible.

Laboratory

For preliminary screening, we will include two control groups of 10 snails, which will be exposed to source water using the same procedures as snails being exposed to test solutions.

For the subsequent replicate testing, we will perform each test on four replicate groups of 10 snails each; we will also maintain four replicate control groups of 10 snails. We will expose the control groups to source water using the same technique as snails exposed to test solutions.

Manchester Laboratory will follow their usual quality control procedures. We will not collect field quality control samples for hardness or alkalinity.

Data Management Procedures

We will record in laboratory notebooks the solution composition, exposure time, and the number of snails killed within each group.

Audits and Reports

We will prepare a final report describing the procedures used and the percentage of snails killed by the various potassium chloride concentrations with and without degreaser/relaxants. The report will include recommendations regarding concentration, additive, and exposure time.

References

Acosta-Salmon, H. and M. Davis, 2007. Inducing relaxation in the queen conch *Strombus gigas* (L.) for cultured pearl production. Aquaculture, volume 262, issue 1, pp. 73-77.

Aquatic Toxicology Lab Report P-2454, 2007. California Department of Fish and Game. March 12, 2007.

Hosea and Finlayson, 2005. Controlling the Spread of New Zealand Mud Snails on Wading Gear. California Department of Fish and Game. Administrative Report 2005-02.

Schisler, G.J., N.K.M. Vieira, and P.G. Walker, 2008. Application of Household Disinfectants to Control New Zealand Mudsnails. North American Journal of Fisheries Management, 28:1172-1176.

Appendix. Acronyms and Abbreviations

Ecology	Washington State Department of Ecology
EPA	U.S. Environmental Protection Agency
g/L	Grams per liter
NZMS	New Zealand Mud Snail
PAN	Quaternary ammonia compounds
QAC	Quaternary ammonia compounds
WDFW	Washington Department of Fish and Wildlife