LARRATT AQUATIC

# Limiting the Spread of Aquatic Invasive Species into the Okanagan

This report prepared for:
Okanagan Basin Water Board and
Glenmore-Ellison Improvement District

Larratt Aquatic Consulting, 3025 Ensign Lane, West Kelowna, B.C. V4T 2Z4

Phone: 250.769.5444 Fax: 250.769.3233 Email: heather@larratt.net

## Executive Summary

This project was designed to help slow the advance of aquatic invasive species (AIS) into the Okanagan by studying the best decontamination methods, establishing monitoring points, and by creating educational materials. All of the AIS covered by this project can be accidentally transported to new lakes in life stages invisible to the naked eye. It is illegal to transport invasive mussels, live or dead, in British Columbia as of December 2012.

### Decontamination Experiments

LAC tested the most commonly recommended decontamination methods for their effectiveness against *Didymosphenia geminata* (Didymo or rock snot) and zooplankton. Zooplankton are analogous to the larval stages of mussels and mud snails, as well as invasive zooplankton species such as the spiny water flea. We defined successful decontamination when a treatment achieved both a >95% mortality in Didymo and 100% decontamination for zooplankton, in a reasonable time frame.

The most effective decontamination methods for Didymo were drying in the sun and soaking in 1% salt solution. Other slightly less effective options included 50% pine oil cleaner and 1% bleach, but these products can damage rubber equipment such as waders. Drying indoors in the dark was not effective. Very hot water (>60°C) was surprisingly ineffective against Didymo. Our experiments probably over-estimated treatment survival because we used intact chloroplasts as the sole determinant of viability. Overall results indicated that Didymo was hardy and resistant to decontamination efforts.

In the zooplankton experiments, most decontamination options proved very effective. Hot water (>60°C) was completely effective with contact time of only one minute, or two minutes for >45°C. Pine oil cleaner and salt water were both effective in less than five minutes of contact, while vinegar and tri-sodium phosphate were effective in less than ten minutes. These options are not ideal in all situations because they can harm equipment or damage the environment. Overall our results confirm the “Clean, Drain, Dry” approach, provided sufficient contact times are observed.

### Okanagan Veliger Monitoring

Samples were collected at 5 boat launches and 7 intakes throughout the Okanagan for zebra/quagga mussel veligers. No mussel veligers were detected anywhere in the Okanagan during 2012.

## Education

This report includes a sample poster lay-out, a Power Point Presentation, and background information on the AIS that currently threaten the Okanagan. These materials present one detailed decontamination protocol that is effective against a wide range of AIS. This report also reviews methods for invasive mussel control for water supplies in Appendix 7. Chlorination at the intake mouth is the recommended option.

These photos were taken during the course of the 2012 research project on AIS. The photo on the left shows a dock piling at a boat launch monitoring site. The photo below shows one example of a Didymo algae control, compared to a Didymo treatment in 2% bleach

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## Table of Figures & Tables

**Suggested Reference**

Self, J., Larratt, H. 2013. Limiting the Spread of Aquatic Invasive Species into the Okanagan. Prepared for the Okanagan Basin Water Board and the Glenmore-Ellison Improvement District.

Authors: Jamie Self H.B.Sc.; Heather Larratt H.B.Sc., R.P.Bio.

# 1.0 Goals of Project

This research focused on developing a simple, low-cost decontamination protocol effective against the microscopic and adult life stages of the most serious AIS that are advancing on the Okanagan. Its purpose is to alert Okanagan residents, municipalities and water suppliers to the imminent environmental and economic damage that will be done by aquatic invasive species when they arrive, and what we can do to protect our Okanagan lakes, streams, and domestic water infrastructure. In this report, emphasis is placed on the zebra and quagga mussels because they pose the greatest threat to the Okanagan.

This project was divided into three major areas:

## 1.1 Best Decontamination Practices

- Conduct literature search to find all recommended techniques for cleaning equipment contaminated with AIS
- Experimentally test techniques on actual AIS or AIS analogues and use statistical analysis to determine effectiveness during the Okanagan boating season
- Determine best practices based on: contact time, cost, potential damage to equipment, and safety for users
- Develop a simple, low-cost decontamination protocol effective against the microscopic larval and adult life stages of the most serious AIS that are advancing on the Okanagan

## 1.2 Monitoring Okanagan Lake for AIS

- Collect plankton hauls for mussel larvae (veligers) in Okanagan lakes during routine monitoring (mainstem, upland reservoir lakes, and mainstem creeks) during the 2012 field season, focusing on boat launches; also watch for adult invasive mussel infestations on likely surfaces
- Provide GPS locations for monitoring sites for AIS monitoring (zebra/quagga mussels, *Didymo* and NZ mud snails)
- With the assistance of RDCO, provide GIS mapping of vulnerable locations (boat launches, marinas, and ideal habitats)

## 1.3 Create Educational Materials

- Based on results from experiments, help develop educational materials to convey to the public the best practices for cleaning equipment and preventing the spread of AIS
- Recommend changes to boat launches/valet services to make decontamination simple and available; consider use of car washes
- In conjunction with RDCO, OBWB, MoE, SOSIPS, and IPCBC, provide the how-to for spotting, sampling and documenting a suspected infestation in the Okanagan, including contact numbers for reporting a suspected infestation

- Develop a web site link that includes space to accumulate a list of consultants, researchers, municipalities, businesses, community groups, and more who will commit to voluntary decontamination when they move a boat from one lake to another
- Assist with preparation materials for an Okanagan information blitz with feedback from OBWB and RDCO staff, and BC societies, to include a laminated card lay-out for boaters, a web site link, a PP presentation that can be given to interested parties, a sign lay-out, and a press release(s)
- Provide a detailed summary of the best available methods for limiting the damage of zebra/quagga mussels on water intakes, pumps and distribution systems (Appendix 7)

## 1.4 Terminology and Abbreviations

The following terms are defined as they are used in this report.

**ALGAE BLOOM: A superabundant growth of algae. Many species are capable of colouring the water or covering the surface of a lake.**

**ANOVA (Analysis of Variance): A set of statistical tests that determines if the observed difference in the means of two or more variables is statistically significant.**

**AQUATIC INVASIVE SPECIES (AIS): Any aquatic organism that will readily invade a new habitat and cause damage to local biology and/or economy.**

**DIATOMS: The family of algae containing chlorophyll as the primary photosynthetic pigment and having hard, silica-based "shells" (frustules). Diatoms affect filtration and produce a range of taste and odors. Some species can form nuisance blooms.**

**EUTROPHIC: Refers to a nutrient-rich, biologically productive water body where concentrations of mineral and organic nutrients have reduced dissolved oxygen, producing environments that frequently favor plant over animal life.**

**GENERA: The usual major subdivision of a family or subfamily in the classification of organisms, usually consisting of more than one species.**

**LARVA: Juvenile form of organism, not reproductively mature.**

**LIMITED, NUTRIENT LIMITATION: In any environment, a nutrient or other growth requirement will limit or restrict the potential growth of organisms. For example, phosphorus usually limits algae production in lakes; if there is an increase in all of the other nutrients, no increase in algae growth will result because phosphorus is the "bottleneck". Conversely, even a small increase in the phosphorus supply will result in increased algae growth.**

**LIMNOLOGY: The study of freshwater; physical and chemical considerations such as lake thermal behavior, nutrient cycling, basin morphology, sediment structure, etc.**

**LITTORAL: Of or pertaining to the bio-geographic zone between the high and low water marks, usually the most productive area of a lake that supports rooted aquatic plants.**

**MACRONUTRIENT: Macronutrients are the major constituents of cellular protoplasm and usually limit biological production. (They include nitrogen, phosphorus, carbon, hydrogen sulphur.)**

**MEAN: A statistical term that describes the middle of a sampling distribution by dividing the sum of the distribution by the number of data points.**

**MEDIAN: A statistical term that describes the middle of a sampling distribution by measuring the middle value (i.e. the 3$^{rd}$ number in a distribution of 5 values).**

**MESOTROPHIC: Refers to a lake or pond, etc., having a moderate amount of plant growth: the mesotrophic stage is intermediate between the oligotrophic and eutrophic stages.**

**METALIMNION: The water layer containing the thermocline that is between the surface epilimnion and the bottom hypolimnion.**

**MICRONUTRIENT: Relatively minute amounts of a micronutrient are required to maintain plant growth within its environmental constraints. These include; Mn, Fe, Co, Zn, Cu, Mo etc.**

**MODE: A Statistical term that describes the middle of a sampling distribution by measuring the most common value in the distribution**

**OLIGOTROPHIC: Designating or of a lake, pond, etc. poor in plant nutrient minerals and organisms and usually rich in oxygen at all depths.**

**PARTHENOGENESIS: The asexual reproductive process whereby females are born or hatch already impregnated with developing embryos and do not need to find males.**

**PEDIVELIGER: Non-planktonic larval stage of zebra and quagga mussels.**

**PHOTIC ZONE: The zone in a water body that receives sufficient sunlight for photosynthesis.**

**PHYTOPLANKTON: Algae that float, drift or swim in standing water.**

**PLANKTON: Organisms that float or swim in water. Phytoplankton refers to plants; zooplankton to animals.**

**RIPARIAN: A riparian zone or riparian area is the interface between land and a stream or lake. Plant communities along the river margins are called riparian.**

**SAPONIFICATION: The chemical process where fats and oils are converted to soaps in the presence of a highly alkaline chemical. It is commonly used by cleaning products.**

**TRISODIUM PHOSPHATE (TSP): Is a basic chemical that can be added to detergents to increase effectiveness. It works by saponifying fats and oils into soaps that can be washed clean with water.**

**t-TEST: A statistical test that determines if the observed difference between the means of two variables is statistically significant**

**VELIGER: Planktonic larval stage of zebra and quagga mussels.**

**ZOOPLANKTON: Minute animals that graze algae, bacteria and detritus.**

**Report Abbreviations:**

Entities

*BCLSS = BC Lake Stewardship Society*
*CAISN = Canadian Aquatic Invasive Species Network*
*DFO = Department of Fisheries and Oceans*
*DSF = David Suzuki Foundation*
*GEID = Glenmore Ellison Improvement District*
*IHA = Interior Health Authority*
*LAC = Larratt Aquatic Consulting;*
*MFLNRO = Ministry of Forests, Lands, and Natural Resources*
*MoE = Ministry of Environment*
*MoTI = Ministry of Transportation and Infrastructure*
*MPI = New Zealand Ministry of Primary Industries*
*OASISS = Okanagan and Similkameen Invasive Species Society*
*OBWB = Okanagan Basin Water Board*
*PFBC = Pennsylvania Fish & Boat Commission*
*RDCO = Regional District of Central Okanagan*
*SIDWT = Southern Interior Drinking Water Team*
*TOTA = Thompson Okanagan Tourism Association*
*USACE = United States Army Corps of Engineers*
*USDoA = United States Department of Agriculture*

**Technical Phrases, Regulations**

*AIS = Aquatic Invasive Species*
*BCERMS =British Columbia Emergency Response Management Systems*
*BCWQ = BC Water Quality*
*BMP = Best Management Practices*
*FIM = Foreshore Inventory mapping*
*GCDWQ = Guidelines for Canadian Drinking Water Quality*
*GUDI = Groundwater Under Direct Influence (of surface water)*
*IPZ =Intake Protection Zone*
*NZMS = New Zealand Mud Snail*
*OKBS =Okanagan Basin Study*
*ROV = Remotely Operated Vehicle*
*SCADA =Supervisory Control And Data Acquisition (system)*
*SHIM = Sensitive Habitat Inventory Mapping*
*WTP = Water Treatment Plant*

# 2.0 Background on Aquatic Invasive Species

Aquatic invasive species (AIS) introductions are one of the most important threats to endangered plants and animals in BC and the freshwater ecosystems of southern BC are deemed to be particularly vulnerable (Biodiversity Branch, 2004). Those of greatest concern have the following characteristics:

- Wide environmental tolerance and capacity for rapid adaptation
- Rapid reproduction
- Capable of being transported on boats and gear

Most Okanagan lakes are at high risk to a very dangerous invader, the Zebra and Quagga mussels (Mackie, 2010), as well as New Zealand mud snails, *Didymo*, and the spiny water flea (Biodiversity Branch, 2004). These species pose an imminent economic and environmental threat to the Okanagan Region (Warwick-Sears, 2011; McCamman, 2010). The invasive mussels will cost the Okanagan region millions in damaged infrastructure and lost revenue from tourism. Currently, there is no practical management for controlling an alien mussel infestation of a stream, river, large lake or reservoir after it arrives (Mass. Gov., 2011).

Although transport by wildlife is theoretically possible, aquatic invaders are invariably carried by boats and sporting gear from infected systems into uninfected systems (Hoodle, 2011). Unlike invasive aquatic plants, all of the alien species to be covered by this research report can be transported to new lakes in life stages invisible to the naked eye.

Preventing and detecting the invasion of mussels starts with education of the public, especially boaters and fishermen (CAISN, 2011). BC Lake Stewardship Society, the Invasive Species Council of BC, the South Okanagan-Similkameen Invasive Plant Society, and the Okanagan Basin Water Board have all launched educational programs to convince boaters that they need to clean, drain, and dry their boats and gear to prevent the spread of aquatic invasive species.

## 2.1 Zebra and Quagga Mussels

### 2.1.1 Origin and Current Distribution

Zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena rostiformis bugensis*) are native to southern Russia (Hoddle 2, 2011). After their spread through Europe, they were inadvertently introduced to the Great Lakes in ballast water. Zebra mussels were first discovered in Lake Erie in 1986 (Figure 2.1). Within four years they had spread to all of the Great Lakes and by 1991 zebra mussels had infested the Mississippi system. In 2007, quagga mussels were detected in the southwestern United States for the first time. Every year the mussels spread further on contaminated boats and equipment.

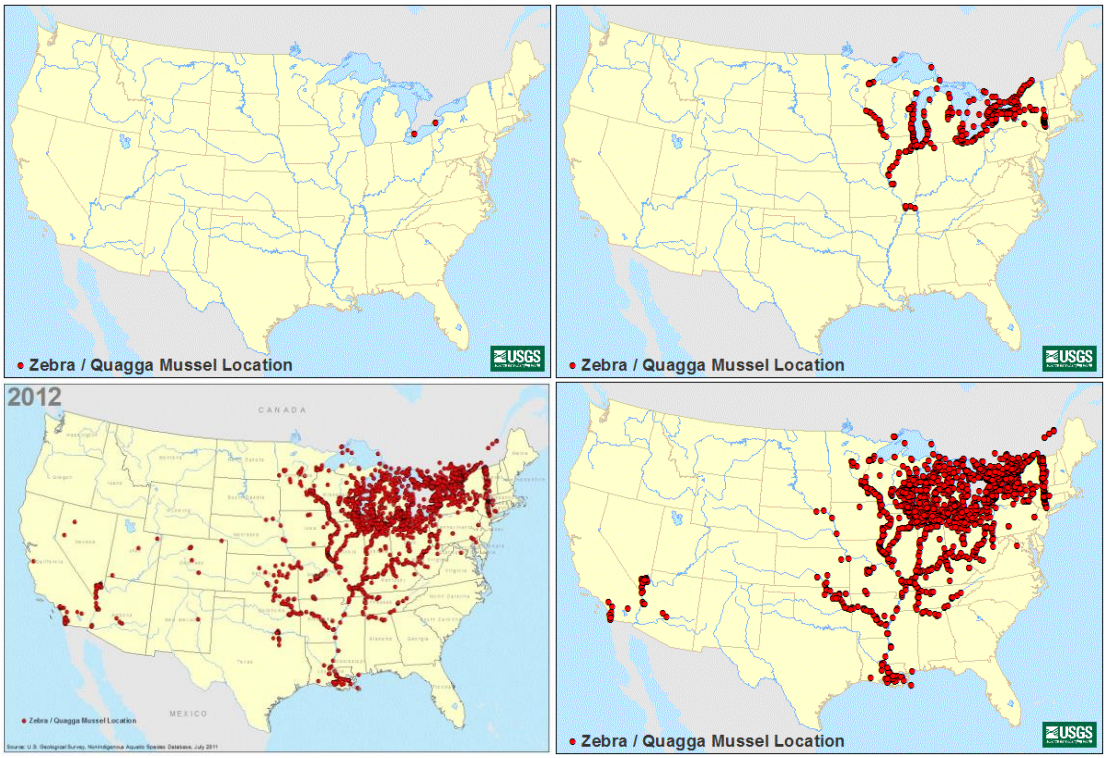


Figure 2.1: Spread of dreissenid mussels (clockwise from top left: 1986, 1991, 2007, and 2012) (USGS, 2012)

### 2.1.2 Impact to Okanagan

Okanagan lakes have low acidity and abundant dissolved calcium to grow mussel shells, making it an ideal environment for invasive mussels (Mackie, 2010). Most lakes in the Southern Interior are at high risk of an infestation (Mackie, 2010). Invasive mussels will have a huge impact on the Okanagan lake ecosystem by altering food webs, concentrating pollutants in their wastes, and inducing bird and fish kills. They also pose a significant threat to the Okanagan economy by clogging water intakes, damaging pumps, and clogging water distribution systems, as well as fouling structures, boats, and beaches. A more detailed economic impact analysis is presented in Section 5 of this report.

From 1989-2004, invasive mussels cost Great Lakes' water utilities an average of over $30,000 per year per facility in additional regular maintenance expenses (Connelly et al, 2007). For the City of Kelowna that has four active intakes in Okanagan Lake, costs could exceed $100,000 per year in additional maintenance expenses. This does not include the cost of retrofitting older facilities which can also exceed $100,000 per facility (National Atlas, 2013) (Appendix 7). The costs will not be restricted to large utilities; any intake into Okanagan Lake will be affected. Once invasive mussels become established in a water body, they are impossible to eradicate with any technology currently available.

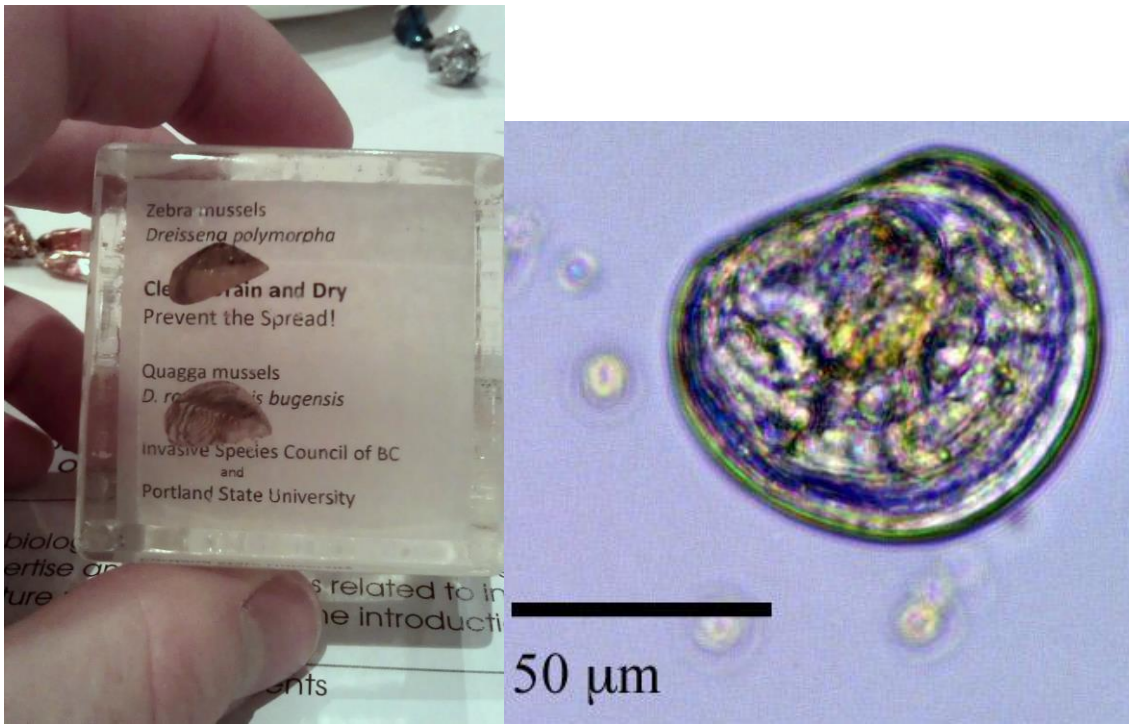


Figure 2.2: Adult dreissenid mussels (left) and microscopic mussel veliger (right) (DeLeon et al., 2009)

Unlike native North American freshwater mussels, dreissenids have a holdfast allowing them to grow on virtually any submerged surface (Benson et al. 1, 2012). Zebra mussels prefer to grow on hard surfaces like rocks and metal. Quagga mussels will grow on hard and soft surfaces enabling them to colonize significantly more of a water system than zebra mussels. Overall, quagga mussels are more flexible and therefore more invasive. In some parts of the Great Lakes quagga mussels are overtaking zebra mussels (Grigorovich, 2003).

### 2.1.3 Life Cycle and Vulnerability to Decontamination

Each adult invasive mussel will live about three years, with a maximum lifespan of nine years (Benson et al. 1, 2012). An adult zebra mussel will reach sexual maturity within 6-7 weeks of attaching onto a surface (Borcherding, 1991). Each individual will undergo several reproductive cycles every year, releasing up to 40,000 eggs each time (Benson et al. 1, 2012). Each female mussel can produce a million offspring in a single year. Eggs will develop into a planktonic free floating larval form called a veliger. The larvae of all other native North American freshwater mussels must attach to fish and are not free to disperse anywhere the water moves them (Sprecher & Getsinger, 2000). It is this trait, perhaps more than any other that makes dreissenid mussels so invasive. Equipment contaminated with viable veligers that are invisible to the naked eye can easily be introduced into another system if precautions are not taken.

Table 2.1: Comparison of zebra and quagga mussels physical appearance and habitat tolerances (many sources)

| | Zebra Mussels | Quagga Mussels |
|---|---|---|
| **Shell** | Triangular shape, byssal (ventral) side flat. Obvious ridge between side and bottom | Rounder sides, byssal side rounded. ridge lacking |
| **Colour** | Variable colours and patterns, usually dark | Pale near hinge, dark concentric rings on the shell |
| **Byssal** | Large groove in middle of flat side; allows tight hold on rocks | Small byssal groove near the hinge |
| **Depth in lake** | 1-30 m, rarely found below 15 m | 1-130 m, commonly found down to 30 m |
| **Temperature tolerance** | 0° to 30°C | -2° to 30°C |
| **Temperature for growth** | 12° to 20°C preferred | 4° to 20°C preferred |
| **Reproductive Temperature** | Young present at 14° to 20°C | Young present as low as 8°C |
| **Substrate** | Hard substrates only | Soft or hard substrates muddy or sandy bottom |

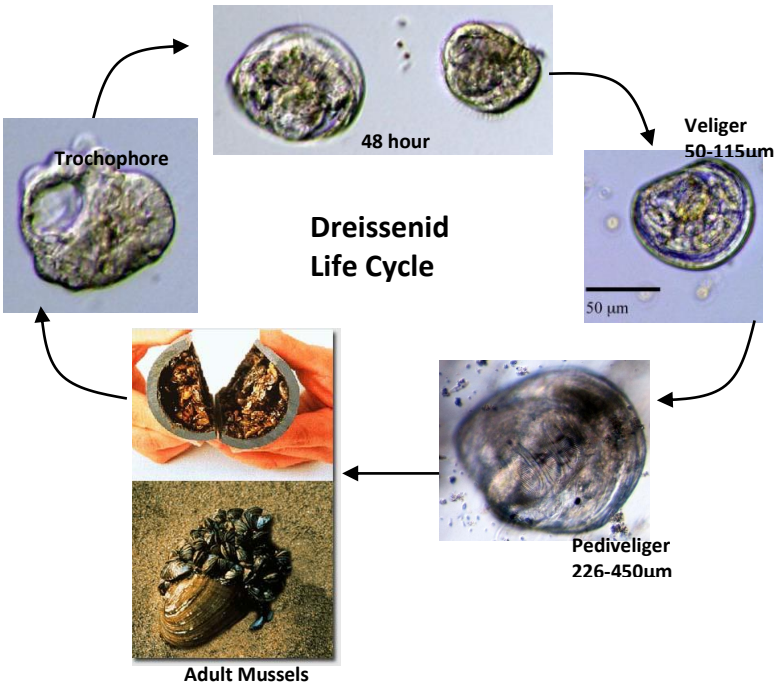


Figure 2.3: Dreissenid life cycle (DeLeon et al., 2009 & VDGIF, 2012)

The veliger and, to a lesser extent, pediveliger (a larger growth stage that has attached to a substrate but is not yet reproductively mature) are the most vulnerable stages in the mussel life cycle. Adult mussels will close their shells as soon as they detect any harmful compounds in their environment and can remain closed for several days (Sprecher & Getsinger, 2000). Veligers do not yet have a shell and cannot protect themselves from

chemical exposure or desiccation in the same way that adult mussels can. For adult mussels crushing and thermal treatment are most effective. Section 3 of this report focuses on the most effective methods for killing mussel veligers on equipment.

### 2.1.4 Close Call in Shuswap

A BC-bound boat with adult quagga mussels was detected in an Idaho boat check and they notified the BC authorities, but Idaho had no authority to detain the vessel. The boat had come directly from Lake Mead, Nevada – a quagga mussel hotspot. It was launched into Shuswap Lake for five days before BC Government staff removed and cleaned the boat. All evidence indicated that the mussels were dead before the boat went into Shuswap Lake but additional monitoring is now ongoing at the marina where the boat was launched. In 2011 and 2012, Idaho stopped more than six boats per year bound for British Columbia that were infested with mussels (Scott, pers. comm, 2012).

## 2.2 New Zealand Mud Snails (*Potamopyrgus antipodarum*)

### 2.2.1 Origin and Current Spread

New Zealand Mud Snails (NZMS) are native to New Zealand and have been invading new habitats since Europeans first colonized New Zealand (Hoddle 1, 2011). NZMS have currently colonized most western US states and the Great Lakes and unfortunately, have been detected on Vancouver Island.

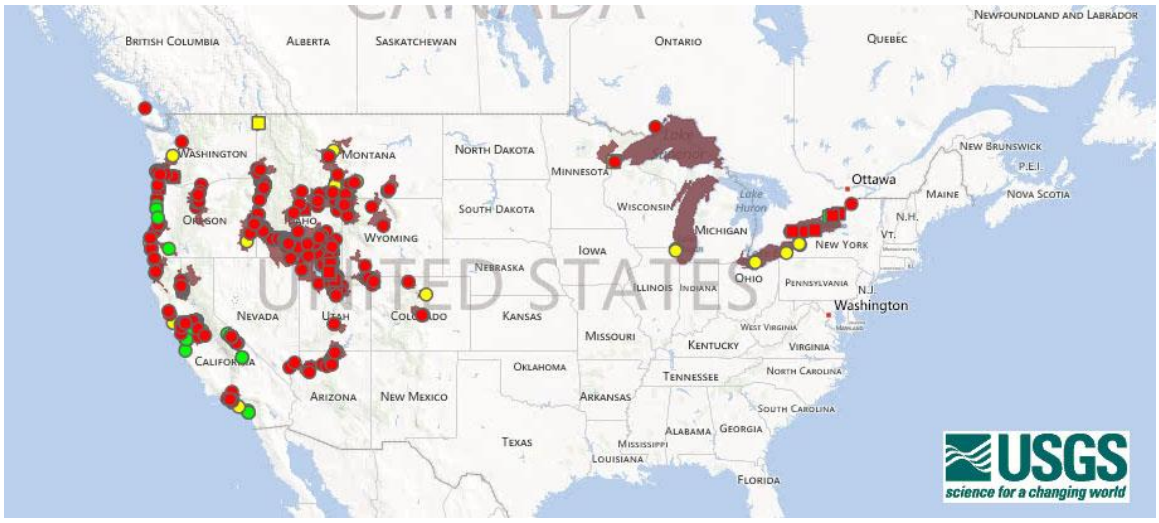


Figure 2.4: Spread of NZMS as of 2012 (Benson et al. 3, 2012)

### 2.2.2 Impact to the Okanagan

NZ mud snails (NZMS) are tiny freshwater snails that are rarely more than 5mm in length. NZMS can easily get into folds and nooks of equipment without being noticed. Felt soled waders are particularly notorious for transporting NZMS. These tiny snails can coat substrates much like the invasive mussels. At high densities, the NZMS can drastically alter aquatic food web structure. NZMS compete with native macro-invertebrate fauna for food and habitat, and are thought to be a poor food source for fish because they provide little energy and can pass through the gut of fish undigested.


Figure 2.5: NZMS next to a dime for scale (Hoddle 1, 2011)

### 2.2.3 Life Cycle and Vulnerability to Decontamination

Through a process known as parthenogenesis, female NZMS hatch pregnant and can reproduce rapidly once they reach a new habitat (Hoddle 1, 2011). As with dreissenid mussels, adult NZMS are hard to kill with chemicals because they can close their shells. Physical treatments such as heating, freezing, and crushing are more effective. Unlike adult dreissenid mussels, adult NZMS are mobile and can get onto personal equipment such as boots or waders. For this reason different approaches to decontamination may be appropriate. For example, it is not practical to freeze NZMS on a boat but it may be suitable for contaminated boots. Larval NZMS are more vulnerable to control treatments and would respond similarly to dreissenid veligers and other zooplankton.

## 2.3 Didymo (*Didymosphenia geminata*)

### 2.3.1 Geographic Origin and Spread

*Didymosphenia geminata* (Didymo) is a prolific freshwater diatom native to British Columbia but not to the Okanagan region. It grows attached to hard surfaces using polysaccharide tubes that accumulate as a coating on rocks resembling wet paper towels in look and feel. Occasionally, its growth explodes and Didymo mats cover every hard surface in a thick layer of growth (Sea Grant, 2012). These problematic blooms are only known to occur in moving water (Sea Grant, 2012).

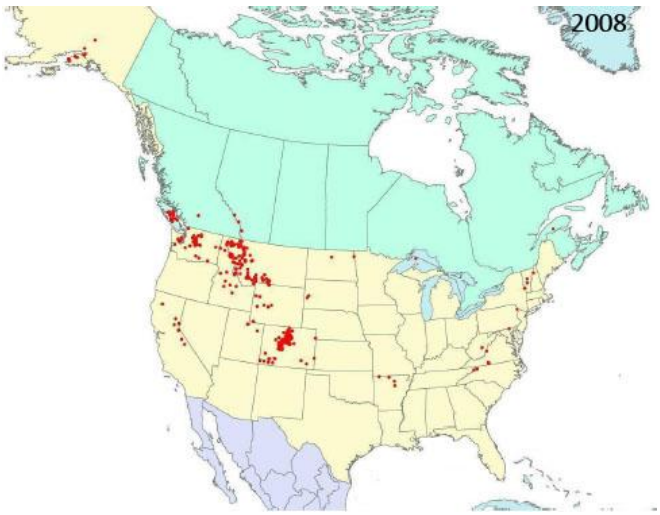


Figure 2.6: Spread of *Didymosphenia geminata* in North America in 2008 (USDoA, 2012)

### 2.3.2 Impact to the Okanagan

Didymo mats routinely coat substrates in clean, low nutrient creeks and shorelines. At low densities, the algae mats are not a concern, however, a bloom of this algae smothers the substrates in unsightly masses that disrupt the entire aquatic ecosystem.

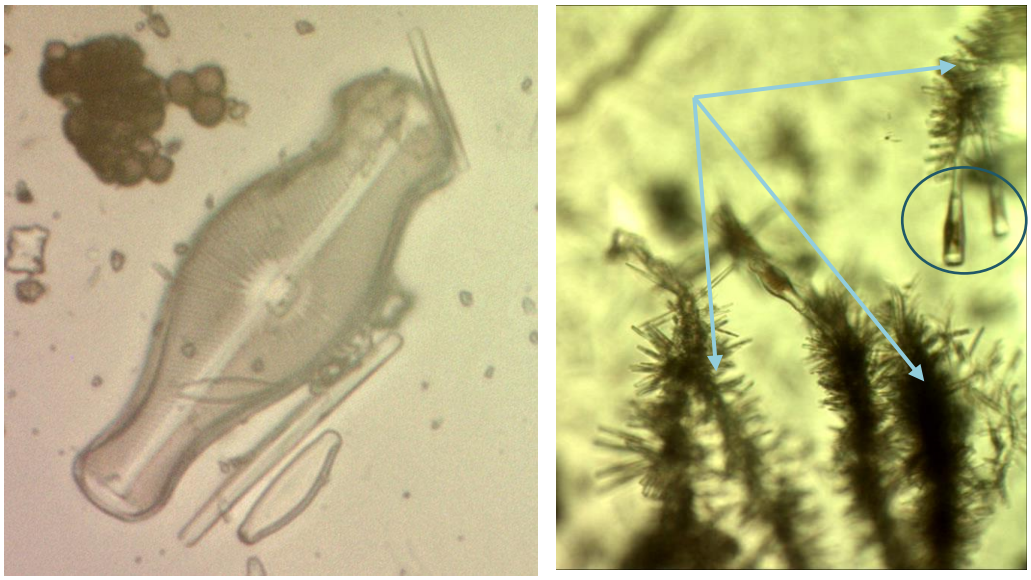

Figure 2.7: Left: Cleaned (dead) Didymo cell under microscope next to two other smaller diatoms; Right: Live Didymo cells (circled, larger) attached to stalks (arrows) that are hosting smaller diatoms

### 2.3.3 Life Cycle and Vulnerability to Decontamination

Didymo reproduces asexually and can spread rapidly in new systems (MPI, 2012). Didymo prefers cold, clear, low nutrient waterways because it is able to out-compete other algae for what nutrients are available. New research has shown that the fibrous mat also serves as habitat for symbiotic bacteria that can provide nutrients for the Didymo cells (NSF, 2012). During Didymo blooms, these mats can cover every surface in a waterway up to 20 cm thick (Sea Grant, 2012).

Figure 2.8: Handful of Didymo mat during a bloom (left) and Didymo in a normally pristine New Zealand river (right) (Sea Grant, 2012 & WRP, 2007).

These thick mats make it difficult to kill Didymo. The cells within the mat are protected from exposure to chemicals. Any chemical that is used to kill the Didymo cells must saturate the mass of growth. This will increase the amount of time equipment must be exposed to a decontaminating agent to become clean, possibly damaging it.

The mat also retains water, allowing the cells to avoid desiccation for long periods on absorbent surfaces such as felt-soled waders. In section 3 of this report we attempt to experimentally determine level of exposure is required to completely kill Didymo on equipment.

## 2.4 Spiny Water Flea (*Bythotrephes longimanus*)

### 2.4.1 Origin and Current Spread

The spiny water flea is a type of zooplankton that is native to Northern Europe and Asia. It was accidentally introduced into the Great Lakes in the 1980s and has since taken over that system, spreading to many other lakes in the region (Liebig et al, 2012).

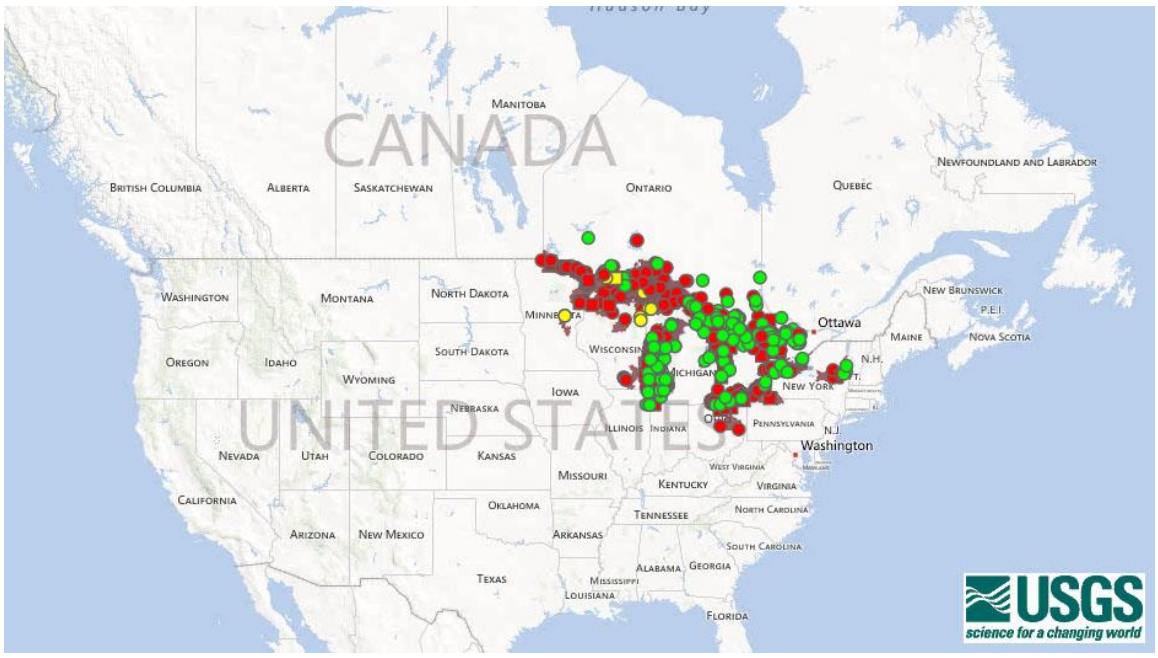

Figure 2.9: Current distribution of *Bythotrephes* in North America (Liebig et al, 2012).

### 2.4.2 Impact to the Okanagan

Spiny water fleas are a species of predatory zooplankton that feeds on other smaller zooplankton and can grow to over 1cm (Liebig et al, 2012). As their common name suggests, they have a long barbed tail that serves to protect from predation (Figure 2.10). The spiny water flea can therefore out-compete native zooplankton and fail to provide food to small fish. Like the deliberately introduced mysid shrimp, there may be other unforeseen impacts as well. The barbs on their spiny tails will readily entangle in fishing gear, fouling gear and possibly helping spread them to new lakes.

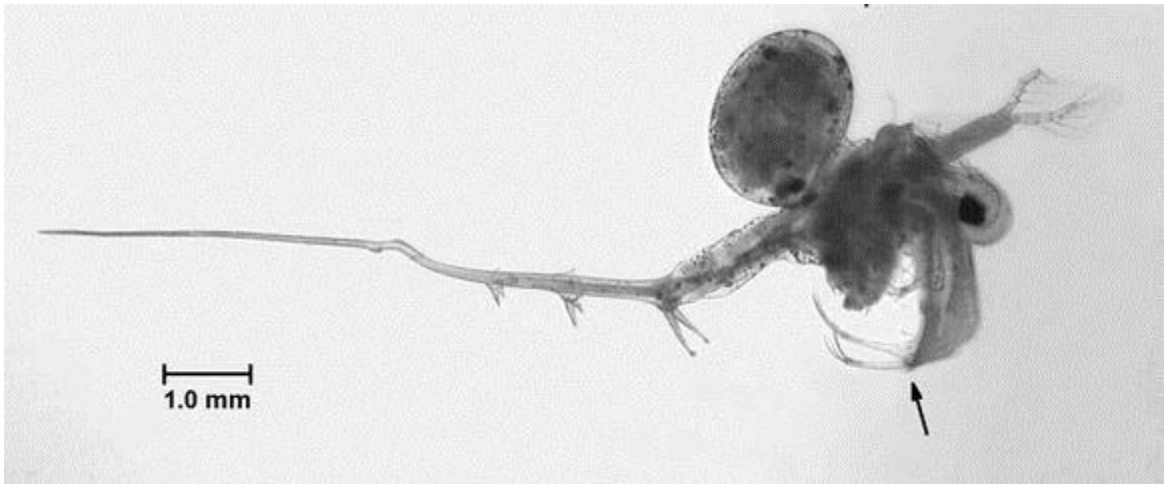


Figure 2.10: Spiny water flea (Kate Feil, 2012)

### 2.4.3 Life Cycle and Vulnerability to Decontamination

Spiny water fleas are able to reproduce asexually and can spread rapidly. They prefer cool low salinity lakes like Okanagan Lake (Liebig et al, 2012). They are a type of zooplankton and will to respond very similarly to decontamination agents as local zooplankton. That is to say, chemical exposure may be quite effective against the spiny water flea. The decontamination experiments in section 3 of this report use zooplankton as analogues for the potential invasive species and compare the effectiveness of various chemicals and physical treatments.

## 2.5 Vectors of Aquatic Invasive Species Transport

Aquatic invasive species are almost always introduced by people moving equipment from infected lakes to clean lakes. Anything that is in contact with a contaminated body of water can be a vector for the transport of aquatic invasive species. Soft materials that can remain wet long after being taken out of the water are particularly notorious for spreading aquatic invasive species. Felt-soled waders and carpeted boat trailers are the most commonly implicated soft surfaces. Parts of equipment that remain wet most of the time, such as boat bilges, are also problem areas.

Potential vectors of AIS into the Okanagan include but are not limited to:

- Boats of all varieties: fishing, power, canoes, kayaks, inflatables etc.
- Boat equipment: ropes, cables, chains, anchors, buckets etc.
- Boat trailers: carpeted runners, nooks and crannies, tires, etc.
- Float planes: In and on the pontoons
- Fishing equipment: waders, bait buckets, rods and line, boots, etc.
- Recreational equipment: life jackets, wet suits, etc.

The most notorious vectors for AIS transport are bilge and ballast water in large power boats, absorbent surfaces including carpet and felt-soled waders, and wads of aquatic plants or mud.

# 3.0 Results of Decontamination Research

Our experimental results are given in detail in Appendix 2. They illustrate that aquatic invasive species (AIS) are differently vulnerable to the methods commonly used to destroy them. We defined a success as achieving greater than 95% decontamination for Didymo and 100% decontamination for invertebrates. When ranking the different decontamination protocols, we gave greater weight to those that took less time. That is to say, if two protocols achieved similar results then the one that did so in less time was ranked higher (Table 3.3). A poor result was one that did not achieve 95% decontamination or that took longer than was recommended in the literature. We selected a high threshold for success because attempting to decontaminate equipment in the real world is more difficult than in a simplified lab environment and because of the risks posed by AIS are very high.

## 3.1 Didymo Decontamination Experiments

Didymosphenia geminate took longer to decontaminate than the small aquatic invertebrates.

The protocols that achieved a better than 95% Didymo decontamination rate include:

- 50% Pine oil cleaner for 5 minutes exposure
- 50mg/L salt solution for 30 minutes
- 1% solution of household bleach for 15 minutes
- Drying in the sun for >12 hours

The decontamination protocols tested that gave poor results against Didymo include:

- Freezing
- Hot (>60°C) water for 5 minutes or less
- 5% solution of dish soap
- Drying indoors for less than 2 weeks
- 5% trisodium phosphate solution
- Vinegar at any concentration for 5 minutes
- 5% TSP for 5 minutes

Overall, Didymo is a difficult invasive species to effectively remove from gear and additional decontamination must be taken if equipment has been exposed to Didymo contaminated waters. Didymo was significantly more resistant to all of the tested decontamination protocols than the zooplankton (Table 3.1). This is because Didymo is a simple single-celled algae that lives inside a protective fiber mat.

The literature consistently cites absorbent surfaces as taking longer to decontaminate than hard surfaces because they stay wet longer and provide places for microscopic organisms to hide (MPI, 2012). In our results, both bleach and freezing were less effective on carpet than on a hard plastic surface. The variation in the drying results was too great

to draw conclusions on effective drying times. We therefore recommend that absorbent and non-corrodible surfaces (e.g. felt-soled wader boots) be soaked in 1% salt solution for at least 60 minutes and then dried in the sun for one week for Didymo decontamination.

Table 3.1: Effectiveness of decontamination protocols on Didymo

| Method | Variable | Substrate | Maximum Effectiveness | Time to Reach Max. (mins) | Recommended Time in Lit. |
|---|---|---|---|---|---|
| Bleach | 2% | Plastic | 75.5% | 1 | 1 min |
| Bleach | 2% | Carpet | 96.4% | 30 | |
| Bleach | 1% | Plastic | 96.9% | 15 | - |
| Detergent | 5% | Plastic | 71.7% | 30 | 5% for 1 min |
| Detergent | 5% | Carpet | 91.2% | 30 | - |
| Freezing solid | | Plastic | 91.5% | 4 days | 4 hours |
| Hot Water | 45°C | Plastic | 49.8% | 5 | >20 mins |
| Hot Water | >60°C | Plastic | 75.0% | 5 | 1 min |
| NaCl | 100mg/L | Plastic | 94.3% | 30 | 2% for 1 min |
| NaCl | 50mg/L | Plastic | 99.0% | 60 | - |
| Pine oil cleaner | 50% | Plastic | 97.5% | 5 | 5 mins |
| TSP | 5% | Plastic | 89.9% | 5 | - |
| Vinegar | 100% | Plastic | 86.2% | 5 | 20 mins |
| Drying Indoors | | Plastic | 96.4% | 6 days | 5 days |
| Drying Indoors | | Carpet | 100.0% | 12 days | 5 days |
| Drying Outdoors in Sun | | Plastic | 100.0% | 2 days | 5 days |
| Freezing solid | | Plastic | 91.5% | 4 days | 4 hours |
| Freezing solid | | Carpet | 99.02% | 2 months | 4 hours |
| | | | | (Many cited references) | |

## 3.2 Invertebrate Decontamination Experiments

For the zooplankton surrogate for dreissenid mussels, NZ Mud Snails, and invasive zooplankton there were numerous effective options. The tests were directed at the microscopic larval forms of these species. Tests were only performed on hard plastic and not carpet because the zooplankton became entangled in the carpet fibres and it was impossible to determine viability. Adult mussels and NZ Mud Snails are very small but still visible with the naked eye and are best destroyed mechanically (crushed) or through >2 minute contact with very hot (>60°C) water.

The best option we found for killing the microscope veliger life stage was using very hot water. Very hot water was effective with contact time of only 1 minute. Hot water (45°C) was also effective with only 2 minutes of contact time. Hot water is therefore reasonable for cleaning large equipment such as boats or trailers that have been exposed to AIS. Commercial car washes typically operate near 50°C which could reduce the potential infrastructure costs of using hot water decontamination. Pure vinegar and concentrated salt solutions (100 mg/L) were also effective against zooplankton with contact times of only 5 minutes. Use of these solutions is advised for soaking smaller equipment or areas of a boat that are difficult to access like bilges. Salt water is

corrosive to metals parts and should be used with care and flushed thoroughly after use (consult equipment's warrantee prior to use). The boat owner could choose any of the successful protocols listed below for the cleaning stage of decontamination.

The successful protocols for invertebrate decontamination include:

- Hot water at 45°C for 2 minutes or >60°C for 1 minute
- 5% bleach for >60 minutes
- 1% Salt solution >50 mg/L for 5 minutes
- 50% Pine oil cleaner for 5 minutes
- 5% trisodium phosphate for 10 minutes
- 100% vinegar in 20 minutes
- Freezing solid for >4 hours

The decontamination protocols tested that gave poorer results against aquatic invasive invertebrates include:

- Dish washing detergent
- Pine oil cleaner at <50% concentration

Table 3.2: Effectiveness of agents on invasive invertebrate analogues

| Method | Variable | Maximum Effectiveness | Time to Reach Maximum (minutes) | Recommended Time in Lit. |
|---|---|---|---|---|
| Bleach | 5% | 100.0% | 60 | > 60 mins |
| Detergent | 10% | 96.9% | 30 | - |
| Detergent | 5% | 94.3% | 30 | 5% for 1 min |
| Freezing solid | | 100.0% | 240 | 240 |
| Hot Water | 45°C | 100.0% | 2 | - |
| Hot Water | >60°C | 100.0% | 1 | 1 min |
| NaCl | 100mg/L | 100.0% | 5 | 1% for 24 hrs |
| NaCl | 50mg/L | 100.0% | 5 | 1% for 24 hrs |
| Pine oil cleaner | 50% | 100.0% | 5 | 5 mins |
| Pine oil cleaner | 25% | 98.7% | 10 | - |
| TSP | 5% | 100.0% | 10 | - |
| Vinegar | 100% | 100.0% | 20 | 20 mins |
| Vinegar | 50% | 100.0% | 10 | - |
| Vinegar | 25% | 100.0% | 20 | - |
| | | | | (Many cited references) |

## 3.2 Summary of Effectiveness of AIS Decontamination Treatments

Table 3.3 summarizes and ranks our decontamination experiment findings and compares them to the suitability of those treatments on materials commonly found in boats, equipment, and recreation gear. In some cases, the treatment may be effective at decontaminating AIS but is likely to damage equipment.

The rankings are based on the time and level of decontamination achieved by each protocol. The individual scores for Didymo and invertebrate AIS were averaged for each protocol and given a net score. This score was used to sort the protocols in Table 3.3. This technique ranked the protocols based on effectiveness over a broad range of AIS but masked specific results. For example, vinegar was highly effective against invertebrates but relatively ineffective against Didymo and is therefore ranked low in Table 3.3.

All treatment options were more effective than plain water (Appendix 2).

Table 3.3: Summary of effectiveness and impacts of *Didymo* and invertebrate AIS decontamination protocols on boats and gear

| Protocol \ Material | Fiberglass/Plastic | Metal | Fabric | Rubber & Neoprene | Carpet |
|---|---|---|---|---|---|
| **1% Table Salt** | -Safe for use on this substrate | -Not recommended for use on metals | -Safe for use on this substrate | -Will reduce life expectancy of equipment but generally safe if flushed after sufficient contact time | -Will reduce life expectancy of equipment but generally safe if flushed after sufficient contact time |
| **50% Pine oil cleaner** | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Known to degrade rubber: not recommended for use on this substrate | -May potentially degrade bonding agents but is unlikely to damage carpet itself. |
| **Very Hot Water (>60°C)** | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate |
| **Hot Water (45°C)** | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate |
| **5% Bleach** | -Safe for use on this substrate | -Safe for use on this substrate | -Will remove colour and may potentially damage some fabrics | -Known to degrade rubber: not recommended for use on this substrate | -May remove colour and damage carpet fibres |
| **5% TSP** | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate (formerly used as a fabric detergent additive) | -Safe for use on this substrate | -Safe for use on this substrate (formerly used as a fabric detergent additive) |
| **Drying Outdoors** | -Safe for use on this substrate | -Safe for use on this substrate | -Sunlight will bleach and degrade fabrics over time reducing life expectancy | -Sunlight will bleach and degrade rubber equipment over time reducing life expectancy | -Sunlight will bleach and degrade carpet over time reducing life expectancy |
| **100% Vinegar** | -Safe for use on this substrate | -Safe on most metals & particularly effective on stainless steel but will damage aluminum. | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate |
| **5% Detergent** | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate |
| **Freezing** | -Safe for use on this substrate | -Safe for use on this substrate | -Safe for use on this substrate | -Will reduce flexibility of material that may lead to reduced life expectancy | -Safe for use on this substrate |
| **Drying Indoors** | -Safe for use on this substrate | -Safe for use on this substrate but may result in rust formation over time due to moisture | -Safe for use on this substrate but extended moisture may lead to aesthetic concerns | -Safe for use on this substrate but extended moisture may lead to aesthetic concerns | -Safe for use on this substrate but extended moisture may lead to aesthetic concerns |

Recommended ⇔ Not Recommended

## 3.3 Recommended Decontamination Protocol for AIS

This program has attempted to identify a simple, inexpensive decontamination protocol effective against all of the aquatic invasive species currently threatening the Okanagan system. In the event that you discover or suspect mussels on your boat, please do not launch and contact Matthias Herborg (BC Ministry of Environment AIS Coordinator) immediately (250-356-7683). A detailed start-to-finish guide for cleaning boats, equipment, and gear, based on this research project is provided below:

**CLEAN**

1. **Park the boat away from waterways** or stormwater drainage for vessel inspection and cleaning.
2. **Remove *all* plants and mud from boat**, trailer, and all equipment. Dispose of all material in the trash.
3. **Thoroughly inspect all exposed surfaces on the vessel and trailer**. If any adult mussels are found, scrape them off and kill them by crushing them. Dispose of the remains in a sealed bag the trash. Alert Matthias Herborg (BC MoE) @ 250-356-7683 *immediately*. If you can, please take a picture with your cell phone of the suspected mussels. PLEASE do not launch until your entire boat has been decontaminanted.
4. **Carefully feel the boat's hull for any rough or gritty spots** - these may be young mussels.
5. **Wash the boat**'s hull, trailer, equipment, bilge, and any other exposed surfaces with high-pressure, hot water. Collect all wastewater and dispose of away from waterways and stormwater drainage systems. The hot water (>60°C) should be in contact with all areas of the boat for at least 1 minute to kill mussels (>2 minutes for 45°C water, available at car washes). Flush engine cooling system and bilge system with hot water (>60°C for >1 minute) or salt water (>100 mg/L for >5 minutes) if the engine is marine-certified. *Complex engine systems may require a professional mechanic*.
6. **Clean *all* items that have been in the water** make sure that all items that have been in the water, including anchors, ropes, life jackets, etc., are inspected, cleaned and dried. Soak in >100 mg/L salt water for >1 hour, rinse and dry for 1 week in the sun. Thoroughly clean all fishing and recreational equipment using hot water (>60°C for 1 minute), salt water (>100 mg/L for >1 hour), or pine oil cleaner (50% >5 minutes).

**DRAIN**

7. **Drain all water from the boat** (pull all plugs), including the motor, motor cooling system, live wells, ballast tanks, bladders, bilges, and lower outboard units. Rinse as outlined above.

**DRY**

8. **Empty and dry all** buckets and dispose of all bait in trash receptacles. Please do not take bait home, leave it on the ground or dump it in any waterway.

9. **Dry outdoors** - Dry boats and gear outside or in dry, well ventilated area for at least a week (more in mild, wet weather, about 18 days) Watch absorbent surfaces – if they stay damp they can keep AIS alive.
10. **Clean and dry** personal belongings, clothing, and footwear that have come in contact with the water.
11. **Wash, dry and brush pets** that have been in the water.

**Precautions during decontamination:**
1. Waste wash water should always be collected, treated, and disposed of properly and NOT be allowed to enter waterways or storm water drainage systems.
2. Please observe all manufacturers precautions found on the labels of cleaning products and equipment.
3. Water above 45°C can scald and appropriate precautions should be observed.

Table 3.4: Examples of Appropriate Decontamination Solutions for the Highest Probability

| Gear | Best Decontamination Solution |
|---|---|
| Big Boats / yachts | Flush bilge, ballast, water systems with 5% bleach solution then rinse with clean water (consult with manufacturer)<br>Power wash entire hull with 45-60oC water for 5 minutes |
| Small power boats | Wash boat down inside and out with 50% pine-oil or 5% TSP cleaner and rinse and dry outdoors; drench carpeted trailer runners with cleaner and make sure they dry |
| Non-motorized boats | Wash boat down inside and out with 50% pine-oil or 5% TSP cleaner and rinse and dry outdoors |
| Felt Soled Waders | Soak boots in 1% salt solution for at least 60 minutes, rinse and dry in the sun for one week |

Invasive mussels can permanently wreck a boat's engine and steering systems. Done properly, CLEANING, DRAINING and DRYING boats and gear will improve their longevity and performance. Choose the cleaning solution best suited to the material, and consult the manufacturer when in doubt.

# 4.0 Okanagan Veliger Sampling 2012

## 4.1 Locations

In addition to the experimental decontamination component, this project also had a veliger monitoring component that involved sampling vulnerable locations throughout the Okanagan for zebra/quagga mussel veligers. Sampling locations are provided in Table 4.1 for the purpose of establishing long-term veliger monitoring sites. Sampling was broken into two types: primary and secondary (Figure 4.1). Primary sampling involved going out to specific sites in Okanagan Lake and doing veliger-specific plankton tows and inspecting structures for attached mussels. This sampling was done at boat launches because they are the most likely first site of colonization. Boat launches in the south, central, and north basins of the lake were sampled once a month from April to August 2012. Additional secondary sites were monitored in the course of regular LAC sampling. Throughout summer 2012, no veligers were detected at any primary or secondary sites in the Okanagan. Detailed results can be found in Appendix 3 of this report.

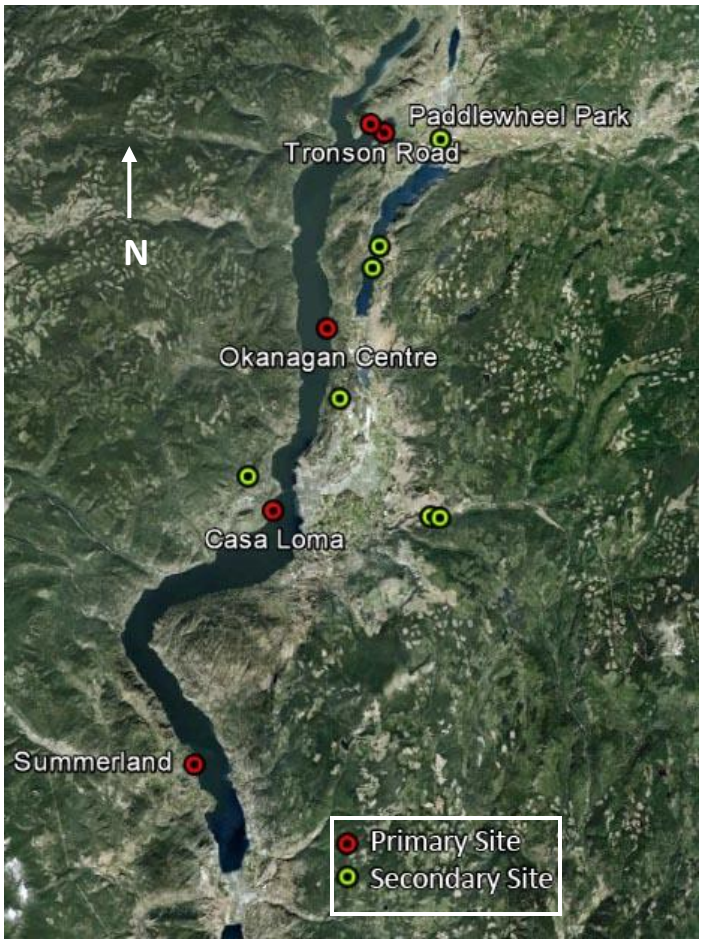


Figure 4.1: Veliger sampling locations. (Google Earth)

Table 4.1 Veliger sampling locations

| Veliger sampling Locations | Latitude | Longitude |
|---|---|---|
| Summerland Boat Launch | 49°36'36.52"N | 119°39'7.41"W |
| Casa Loma Boat Launch | 49°51'37.27"N | 119°31'58.30"W |
| Okanagan Centre Boat Launch | 50° 2'25.43"N | 119°27'0.79"W |
| Paddlewheel Park Boat Launch | 50°14'5.43"N | 119°21'41.84"W |
| Tronson Road Boat Launch | 50°14'34.88"N | 119°22'57.47"W |
| Rose Valley Reservoir | 49°53'38.54"N | 119°34'15.94"W |
| Stevens Reservoir | 49°51'10.54"N | 119°16'41.23"W |
| Hadden Reservoir | 49°51'14.66"N | 119°17'32.27"W |
| McKinley Reservoir | 49°58'15.88"N | 119°25'50.22"W |
| Wood Lake | 50° 6'1.51"N | 119°22'48.26"W |
| Kalamalka Lake South End | 50° 7'18.20"N | 119°22'9.58"W |
| Kalamalka Lake Coldstream Arm | 50°13'40.47"N | 119°16'28.00"W |

# 5.0 Economic Costs

The economic costs of an invasion of the Okanagan by AIS, particularly zebra/quagga mussels, would be enormous. The effects would be felt at the commercial activity level, throughout the tourism sector, and at the ecological level (Table 5.1).

## 5.1 Commercial Activity

### 5.1.1 Drinking Water Intakes

One of the main uses of Okanagan Lake water is for drinking water. There are currently hundreds of intakes in Okanagan Lake alone. Most municipalities on the Okanagan mainstem lakes have major intakes and many operate reservoirs that are also vulnerable to a mussel infestation. The hundreds of small, shallow private intakes would be especially vulnerable and difficult to protect.

Mussels will increase general maintenance costs by increasing wear/corrosion on pipes, pumps, clogged intakes screens, etc. Preventing mussels from clogging intakes will require expensive periodic cleaning by professional divers. Mussel infestations in eastern North America increased maintenance costs to water utilities by an average of $30,000 per year per intake (Connelly et al., 2007).

The best control method currently available involves retrofitting intakes with chlorine ejection technology. This creates a plume of chlorine in the lake around the end of the intake. Chlorine is not particularly effective at killing adult mussels but continuous concentrations of 1-3 mg/L at the end of the intake are effective at preventing mussels from attaching and growing on and in the intake (Rajagopal et al., 2002). Okanagan Lake raw water chlorine consumption is approximately 1 mg/L which means chlorine pumps will need to supply >2 mg/L to the lake in order to maintain a residual of at least 1 mg/L around the pipe (Hrasko, 2013). Most chlorine injector pumps currently in use in the Okanagan have a maximum capacity of 2 mg/L; higher concentrations would require specialized equipment. The cost of retrofitting an existing intake with chlorine ejection would cost in the between $25,000 to $100,000's depending on whether chlorine is already present at the pumphouse plus the cost of additional chlorine (Hrasko, 2013; Underwood, 2013; Phillips, 2005). Currently over $^2/_3$ of intakes into Okanagan lakes do not use chlorine. Chlorination also requires the installation of three-phase electricity. Although invasive mussels are capable of colonizing an entire water distribution system, these chlorine concentrations should prevent them from doing so.

### 5.1.2 Sewage Effluent Outfalls

The City of Kelowna and the District of West Kelowna operate treated sewage effluent outfalls at 60 m in the central basin of Okanagan Lake. These outflows are within the habitable range of quagga mussels and would certainly require periodic cleaning. At 60 m, specialized mixed-gas divers would be required to perform the maintenance. Mixed-gas diving to 60 m in Okanagan Lake would cost $25,000 per day per intake (All-Sea Enterprises Ltd., 2013 quote). Depending on the extent of cleaning required and the

layout of the outfall diffusers, it may be possible to use ROVs to clean the outfall. Given the depth and length of pipe (>1km to City of Kelowna's outflow), it may be impractical to attempt to retrofit the outfall with chlorine ejection technology.

### 5.1.3 Marinas and Boat Fouling

Fouling of boats and marina equipment is a serious concern for the Okanagan. Floating docks are particularly vulnerable to the added weight of mussels. Mussels create drag on boats, reducing fuel efficiency, and also can damage engine cooling systems. Marine antifouling paints are available to protect hulls, and depending on the type of paint used, applications can last up to 10 years and cost on average $1000-2500 per year per boat (Lydecker, 2013). Many antifouling paints are toxic to aquatic life and their use is restricted in many jurisdictions.

### 5.1.4 Bridge Fouling

The William R. Bennett (WRB) Bridge is the largest structure in Okanagan Lake. It has a surface area of over 3 hectares. In a mussel infestation, all submerged surfaces could be covered with up to 15cm of mussels. ProTrans WRB Bridge staff have expressed concern over the weight of mussels and the drag they would create on the bridge. ProTrans WRB Bridge currently spends $7000 per year inspecting the bridge pontoons with divers and $8500 every five years to use an ROV to inspect the bridge cables down to their anchors (Balogh, 2013). The increased costs to ProTrans WRB Bridge to deal with an invasion of mussels would be enormous and continuous. The only option, apart from physical mussel removal by divers, would be the use of antifouling coatings. Antifouling coatings cost $80-150 /m² and are generally only effective for 3-5 years (Skaja et al., 2012).

## 5.2 Ecological Costs

### 5.2.1 Drinking Water Quality

Okanagan Lake is the source of drinking water for much of the region's 300,000+ residents (Okanagan British Columbia, 2013). There are no alternate water sources large enough to supply the demands of the region's growing population. It is therefore important to protect the integrity and quality of the valley's lakes. Impaired water quality will result in higher treatment costs to water utilities.

### 5.2.2 Tourism

Tourism is a major industry in the Okanagan. 3.5 million visitors generated over $1.7 billion to the local economy and supported 15,000 jobs in 2010 (TOTA, 2012). Tourist activity is largely focused around the mainstem lakes in the summer months. Zebra/quagga mussels have the potential to damage tourism to the region. Invasive mussels would cover beaches with a decaying layer of razor-sharp shells. It is possible to clean beaches of shells but this is another added expense. Mussels also remove beneficial algae from the water column. This action encourages harmful cyanobacteria blooms ("green scum") that can compromise water quality and aesthetics. Clearer water would also encourage further expansion of invasive Eurasian water milfoil and

filamentous algae growth. Invasive mussels would make the Okanagan a less enjoyable summer destination and reduce waterfront property values.

### 5.2.3 Fisheries and Angling

Okanagan mainstem lakes and reservoir lakes support many recreational fishing options. Kokanee salmon are particularly popular sport fish. Researchers agree that there is the potential for significant damage to fish populations when zebra/quagga mussels arrive because the mussels strip out the algae and zooplankton that support the fishery food chains. Potential losses to the Okanagan region could exceed $15 million annually (TOTA, 2012; Cannings & Durance, 1998).

### 5.2.4 Total Costs

Based on the lowest prices listed in Table 5.1, we conservatively estimate a possible cost to the Okanagan of $42 million per year for at least the first few years of a mussel infestation. This includes direct costs and lost revenues. This figure is in line with research in other jurisdictions. For example, the US Army Corps of Engineers estimated that Lake Tahoe, a tourist-centric lake region in California, would suffer over $22 million per year in financial impacts from zebra/quagga mussels (USACE, 2009).

Information Sources for Table 5.1

1 Connelly, N., O'Niel, C.R., Knuth, B., Brown, T. 2007. Economic Impacts of Zebra Mussels on Drinking Water Treatment and Electric Power Generation Facilities. Environmental Management. Vol 40, Issue 1. Pp 105-112.
2 http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/Potential%20Zebra%20Mussel%20Impacts%20Hydro%20CRB.pdf
3 Pers. Comm. Terry Underwood (TRUE Consulting, Kamloops)
4 All-Sea Enterprises
5 http://www.boatus.com/magazine/2012/february/copper.asp
6 http://el.erdc.usace.army.mil/zebra/zmis/zmishelp/antifouling_foul_release_and_thermal_spray_coatings.htm
7 http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/Potential%20Zebra%20Mussel%20Impacts%20Hydro%20CRB.pdf
8 Pers Comm. Susan Balogh, ProTrans WRB Bridge
9 www.obwb.ca/fileadmin/docs/state_of_fish_habitat_obwb.pdf
10 http://www.totabc.org/corporateSite/regional-strategy/
11 http://www.naturewatch.ca/eman/reports/publications/99_montane/humans/humans07.html
12 http://www.theglobeandmail.com/globe-investor/personal-finance/mortgages/vacation-properties-still-struggling-as-buyers-play-the-waiting-game/article4491756/?page=all
13 Pers Comm. Hrasko, Bob. 2013. P.Eng and Administrator of the Black Mountain Irrigation District

Table 5.1: Summary of economic impacts to Okanagan region from zebra/quagga mussel invasion

| Type of cost | Existing asset / value | Cost per year with mussels | Information Source |
|---|---|---|---|
| **Commercial Activity** | | | |
| Intake/distribution system fouling | $0.00 – $5000 per year inspection and trash grate cleaning | -$30,000/yr/intake in total additional maintenance<br>-$25,000 per facility to install chlorine ejection IF chlorine is already present at the pumphouse<br>- $100,000s per facility to install chlorine ejection if chlorine absent at pumphouse<br>-Annual operation expenses of Cl ejection = <$100,000 per large intake<br>***130,000 x 30 large intakes = 3,900,000*** | 1,2,3, 13 |
| Outfall fouling | ROV inspection 8500.00 per outfall every few years | -$25,000 / day / outfall for divers to inspect and clean outfall several times per year (60 m depth)<br>***75,000 x 5 large outfalls = 375,000*** | 4 |
| Marina / boat fouling | Variable | -Anti-foul paint/cleaning = $1000-2500/year/boat (based on 40' boat) x 1000 marina slips + 2700 private docks<br>***1000 x 3700 moored boats = 3,700,000*** | 5 |
| Equipment fouling and corrosion | Variable | -Anti-fouling paint = $80-150/m² life expectancy is only 3-5 years per application<br>-More rapid replacement of piping, pumps, motors<br>***Annual expense of about 1,000,000*** | 6,7 |
| Bridge fouling | $7000/year for diver inspections<br>$8500/5 years for ROV inspection of deep components | -Cost to pressure-wash bridge unknown (not permanent solution)<br>-Cost to apply antifouling surface treatment = >$3M with life expectancy of only 3-5 years per application<br>***Annual expense = 600,000*** | 7,8 |
| **Ecological Loss** | | | |
| Safety of drinking water | Invaluable | -Treatment cost increase<br>-There is no "plan B" water source for most supplies | |
| Collapse of fisheries | 16 M in 2000; net with spin-off benefit 41.6 M | -Potential to devastate all Okanagan fisheries.<br>***Annual losses estimated at 12-16 M – 41.6 M*** | 9,11 |
| Loss of lakeshore real estate value | 450k condo<br>800K Townhouse<br>700k – 1 M lot<br>…3M+ waterfront homes | Lost value to residents, lakeshore home-owners, recreators<br>***Annual loss of water front real estate value estimated at 10 M*** | 12 |
| Okanagan Tourism | -$1.7B in 2010<br>-3.5M visitors<br>-15,000 jobs | AIS could significantly drop summer tourism<br>***Annual loss estimated at 12 - 22 M*** | 10 |

# 6.0 Recommendations for Further Action

Immediate action on many fronts is needed to adequately deal with the issue of aquatic invasive species. For example, relying solely on informing the public is critically important, but most BC authorities believe that it will not be enough. Reacting after an invasion is far more costly and irreversible environmental damage will occur.

## 6.1 Government Actions

To effectively deal with the AIS issue, there must be action by all levels of government.

PROVINCIAL: Within British Columbia, the first priority was passing legislation that lists and prohibits transport of invasive species into and within British Columbia. The legislation should be flexible enough that new species can be added to the list without delay. The species of concern should be prioritized based on their environmental and economic consequences. This step was completed for invasive mussels in December 2012. The British Columbia government included aggressive fines in the new legislation. For example, transporting mussels (alive or dead) on a boat could result in a fine of up to $100,000 (BC Government, 2012; BCLSS, 2013).

BC could introduce a boat licensing system modeled after the one in Idaho to help fund the AIS prevention programs such as boat checks and washes.

FEDERAL At the federal level, similar legislation is still required. Canadian Border Services agents can only act on federal legislation. For example, even if they saw a boat covered in zebra mussels today, agents could not stop the boat because zebra mussels are not listed as an invasive species in any federal legislation. The Canadian Department of Fisheries and Oceans began the consultation phase of introducing new legislation on AIS in January 2013. It could be mandatory for all boats entering BC from the US be washed properly at the border at their owner's expense

Legislation is only effective if there are resources provided to enforce it. In addition to legislation prohibiting transport of invasive species into BC, resources in the form of staff, equipment, and training are needed to monitor border crossings and key traffic corridors throughout the province. One potential source of revenue that has been successful in other jurisdictions is boating user fees for all boats that wish to use BC lakes. The funds generated from this program would then go directly into protecting BC lakes from aquatic invasive species. Additionally, BC could consider mandatory boat-check stations at highway weigh scales, provincial borders, National Parks, or other locations where infrastructure already exists.

MUNICIPAL Municipal governments can also play a very important role in dealing with AIS. They are able to target their efforts to local lake amenities and vulnerabilities. Local government cannot create environmental legislation but it can carry out very effective

public information campaigns about new laws that affect the region. RDCO and other governance plan to raise their AIS concerns in a resolution at the 2013 UBCM

It is also important that federal, provincial, and municipal staff coordinate their efforts. Information sharing allows for rapid coordinated operations when potential contaminated equipment enters BC. For example, Canadian Border Services agents could identify contaminated boats coming up from the US and inform BC Conservation officers or local RCMP who could quarantine that boat and prevent it from entering a BC waterway until it has been properly decontaminated.

Finally, the OBWB continues to provide leadership on the AIS issue. They are currently corresponding with the Federal and Provincial Ministers of the Environment to encourage protective legislation. To date, they have funded research (including this study), public education campaigns, and are considering printing information cards to be distributed by Border Services to boats entering BC from the US.

Targeting major boat launches, such as the Hotel Eldorado boat launch in Kelowna with information and services could be an effective public outreach option. SOSIPS summer students found in 2012 that 100% of boaters using the valet service at this launch would be interested in paying $5.00 to have their boats cleaned properly. It may be possible to install coin operated hot boat washes at major boat launches.

## 6.2 Public Education

Most people are unaware of the risks associated with aquatic invasive species and take no actions to prevent their spread. Providing concise, concrete steps that people can follow to ensure that they are not spreading invasive species is very important. Section 3.3 of this report provides a recipe for decontaminating boats and gear effective against all of the AIS that currently threaten the Okanagan. Maintaining a consistent message is also important; the findings in this report agree with the “Don’t Move a Mussel” and “Clean, Drain, Dry” campaigns that are underway in numerous jurisdictions.

It is also important to inform people of the economic costs and environmental risks associated with invasive species. An economic analysis for the Okanagan Valley conservatively estimated that an invasive mussel invasion could cost the Okanagan region $42 million annually (Section 5.0). Managing invasive mussels has cost the Great Lakes region $5 billion in one decade (Hoddle 2, 2011). Prevention is far less expensive than managing an invasive species and the ecological damage they cause is usually irreparable. Finally, public education materials must explain that BC has severe fines for transporting or possession of zebra/quagga mussels.

Tourism literature could carry the message that the Okanagan is “invasive mussel-free” and encourage tourism while raising awareness

Providing information, training, and resources to interested associations, groups, and societies can increase the number of people out monitoring for AIS with minimal cost to government.

## 6.3 Private Sector Involvement

Given the current economic climate, the provincial government is unlikely to finance a comprehensive AIS control program. It is crucial to involve the private sector in a substantive way in order to achieve success in preventing the spread of AIS. For example, it may not be necessary to invest in and staff expensive hot water decontamination equipment if commercial car-washes can be encouraged to fulfill that role. The results of this report indicate that water temperatures employed by car washes are sufficient to achieve 100% mortality of invasive mussels in less than five minutes of contact time. Training car wash staff to effectively clean boats and equipment would reduce the cost burden to the government and increase local business opportunities.

## 6.4 Further Research

This report is part of the effort to develop information on prevention of accidental introduction of aquatic invasive species. Other areas of research needed on AIS threatening the Okanagan and BC include:

- Identify transit corridors into and within BC where contaminated equipment is likely to travel to more efficiently use limited resources
- Maintain a current database of effective techniques for decontaminating equipment as new methods emerge
- Maintain a current database of new technologies to prevent equipment from being fouled by aquatic invasive species (e.g. anti-fouling paint)
- Identify new invasive species that may threaten BC and evaluate the decontamination recipe developed in this research to ensure that it is effective against them

This Report Prepared By:

Jamie Self H.B.Sc. Heather Larratt H.B.Sc., R.P.Bio.

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**Personal Communications**

Balogh, Susan. 2013. ProTrans WRB BRidge

Hrasko, Bob. 2013. P.Eng and Administrator of the Black Mountain Irrigation District

Scott, Lisa 2012. Coordinator, South Okanagan-Similkameen Invasive Plant Society

Underwood, Terry. 2013. TRUE Consulting, Kamloops

# Appendices

## Appendix 1: Experimental Methodology

The experiments were divided by invasive species and then by protocol. Didymo and small aquatic invertebrates (analogues for mussels, NZMS, spiny water fleas, etc.) were tested separately. In every case, five replicates of each protocol were compared to a control. Statistical evaluation of the protocols included t-Tests, and ANOVA with a >95% confidence level (a=0.05). t-Tests are used to compare two variables while ANOVA can compare two or more variables. The results of both tests will tell if any apparent difference between the means of the two variables is statistically significant. This is important when working with experimental data because there is always a variance in the results. A difference that is found to be statistically significant indicates that the means of the two variables are actually different and it is not a results of sampling variance.

Viability of Didymo was tested on both hard plastic surfaces (Petri-dishes) and on absorbent indoor/outdoor carpet to simulate different types of surfaces that may be contaminated. A viable clump of Didymo collected from the Lower Columbia River was sub-sampled with a one gram clump transferred to a small Petri-dish containing 10 mL of filtered Columbia River water. The test agent was added to the five replicates for specified times before they and the corresponding control were promptly evaluated using via microscopy. Sufficient microscope fields were counted to provide an evaluation of 200 – 300 Didymo frustules. Didymo is a non-motile algae and there is no good way to completely confirm viability. For these tests, cells were microscopically examined for intra-cellular deformities. If the cells were intact and the chloroplasts appeared normal, then the cell was considered to be viable. These results, therefore, overestimate the resistance of Didymo and underestimate the effectiveness of the agents tested.

In the next set of trials, zooplankton were placed in small Petri-dishes. Decontamination agents were mixed with spring water to the desired concentrations. Pure spring water was added to the control samples. Spring water was used over distilled water because it contains dissolved minerals that both more accurately reflect natural water and are necessary to zooplankton. The number of living zooplankton was counted in each Petri-dish before being exposed to the test agent. For the purposes of this experiment “living” was defined as any zooplankton that was swimming or moving in the Petri-dish. Each dish was flooded with the agent and at set intervals, the number of living zooplankton were counted (Figure A1.1).

Significant difference between the decontamination trials and their corresponding controls was taken as an estimate of effectiveness. When organisms survived the decontamination trial, the decontamination protocol was deemed to be inadequate. We defined 100% mortality as the ideal for success of a protocol.

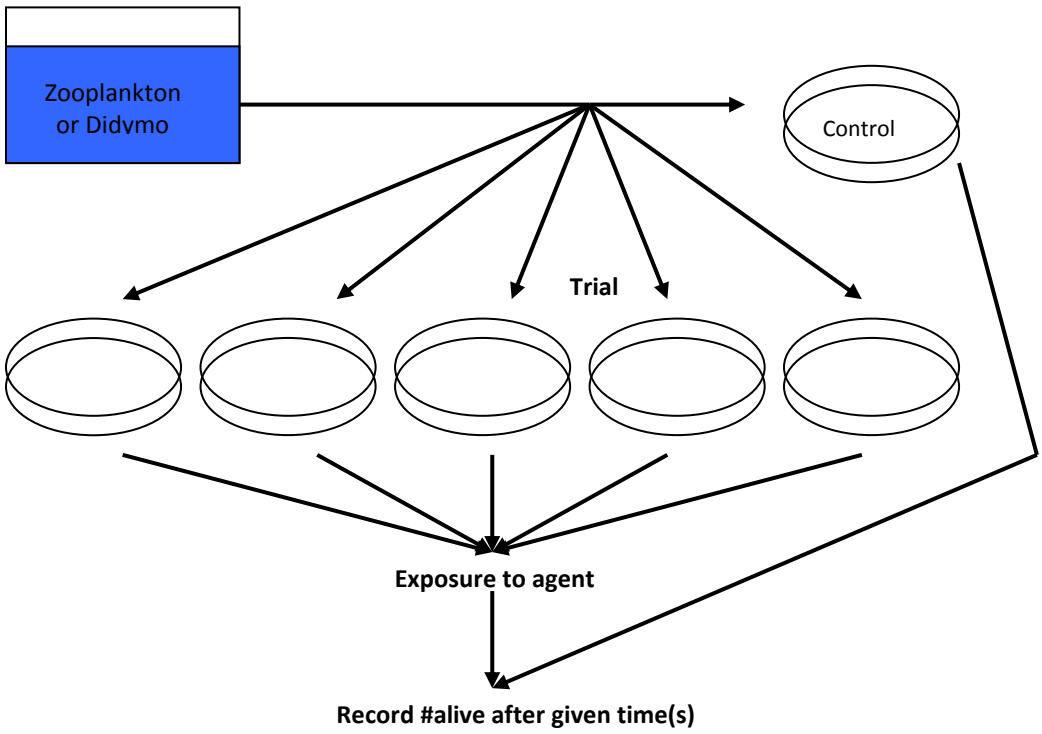



Figure A1.1: Methodology flow chart for decontamination experiments

We chose to trial readily available, inexpensive treatments, for periods that are feasible and therefore likely to be employed in real world situations. The decontamination agents tested in this research were collected from recommendations in published literature and are summarized in Table A1.1.

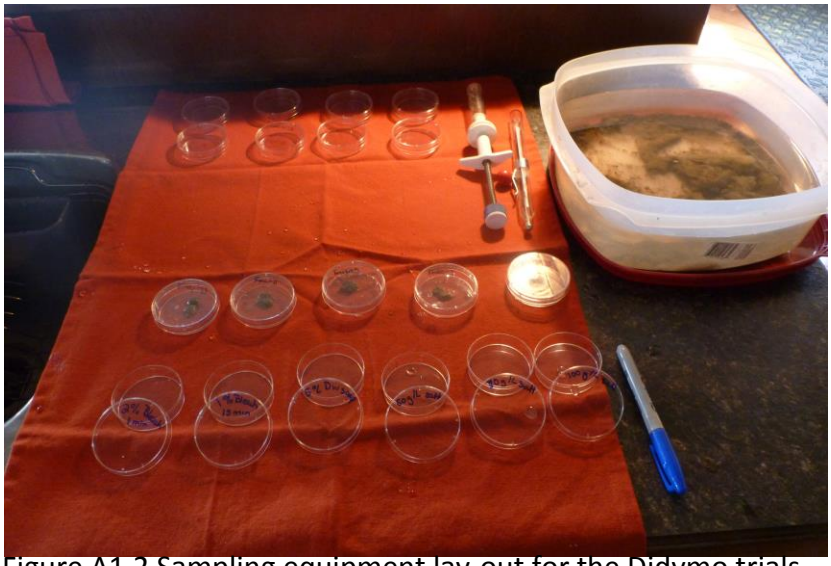


Figure A1.2 Sampling equipment lay-out for the Didymo trials

Figure A1.3 Didymo drying on carpet trials and controls

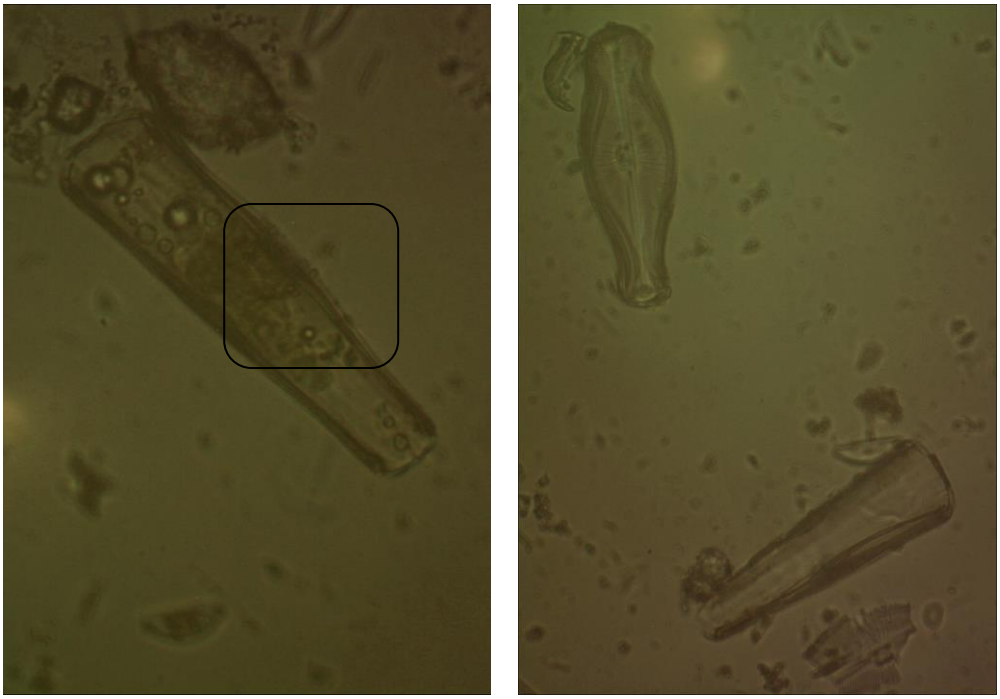


Figure A1.4 Didymo Treatments:
5% Dish detergent 30 minutes (Left image) did not kill the Didymo, chloroplast still visible in black box; but 1% salt solution for 30 minutes did, no chloroplast (Right image)

Table A1.1: Agents tested for effectiveness in decontaminating zooplankton and Didymo

| Agent | Advantages | Disadvantages |
|---|---|---|
| Bleach | inexpensive and readily obtainable | Poisonous, hard on fabrics, protective wear needed |
| Dishwashing detergent | Inexpensive, readily obtainable and safe to handle | None |
| Pine Oil cleaner | inexpensive and readily obtainable | Protective wear needed, hard on rubber |
| Vinegar | Inexpensive, readily obtainable, safe to handle, dissolves shells composed of CaCO3 (mussel, zoop), environmentally safe | None |
| TSP | inexpensive and available at hardware stores | Caustic, protective wear needed |
| Hot Water | Inexpensive, no chemicals involved, environmentally safe | Potential for scalding, difficult to deliver, requires expensive equipment to heat water to required temperatures |
| Salt Water | Inexpensive readily obtainable and safe to handle, less harmful to non-metal material than other chemicals | Corrosive to metals |
| Drying | Potentially zero cost, environmentally safe | Slower than chemical & thermal treatments |
| Freezing | Inexpensive | Not appropriate for large equipment or boats |

(PFBC, 2012(Hosea & Finlayson, 2005), (DSF, 2012) (DiVittoria et al, 2012). (DiVittoria et al, 2012) (MPI, 2012), (MSP, 2012).

# Appendix 2: Results of Decontamination Trials

## A2.1 - Decontamination Research on *Didymosphenia geminata*

The results of the trials are discussed in the following section and summarized in Tables A2.1 and A2.2.

### A2.1.1 Household Bleach

Bleach was tested at 2% (50:1 dilution from bottle) and 1% for between 1 and 30 minutes. Some literature cited 2% bleach for only 1 minute as being effective (Gil-Fox, 2008) but we found this to be inadequate. On hard plastic, after 1 minute in 2% bleach, 4.3±2.3% remained alive. After 15 minutes in 1% bleach only 0.36±0.35% remained alive. On carpet 2% bleach achieved a 96±0.8% elimination of Didymo after 30 minutes. All results are statistically different from the controls but there was no statistically significant difference between the results of the 1% and 2% concentrations. Bleach was not 100% effective in any of the tests. The viable cells were all found in the interior of the Didymo clumps. Our findings concur with the literature on this point – the mat can protect Didymo cells from chemical treatment.

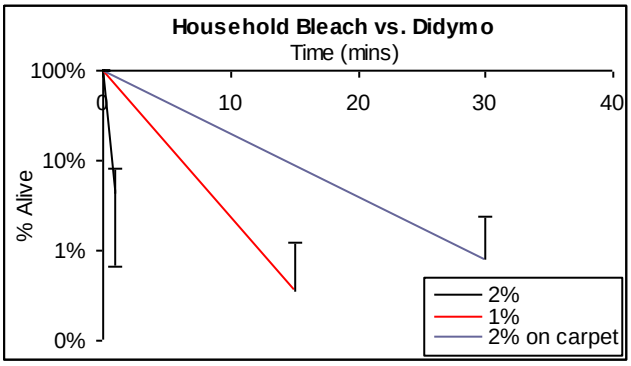



Figure A2.1: Effectiveness of household bleach in decontaminating Didymo from plastic and carpet

### A2.1.2 Dish Detergent

Dish detergent was tested at 5% for 30 and 60 minute exposures on clumps of Didymo in plastic Petri-dishes. After 30 minutes, a reduction in apparent viability of 72±0.81% occurred. After 60 minutes, the number of apparently viable Didymo cells decreased by only 3.2±2.0% compared to the control (Figure A2.2). The difference between t=30 & t=60 minutes was not statistically significant. When compared to the controls, the reduction in viability of Didymo after 30 minutes was statistically significant but not at 60 minutes. These anomalous results are probably due to the limitations of the method used to determine Didymo viability.

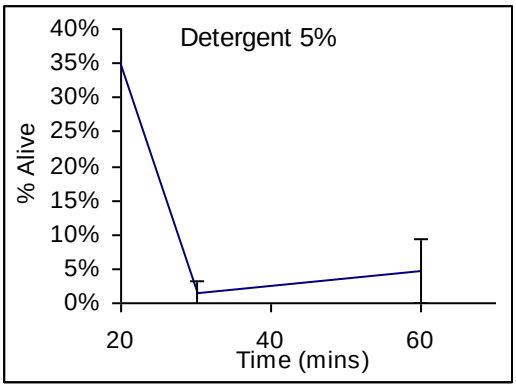



Figure A2.2: Effectiveness of detergent in decontaminating Didymo on plastic

Detergent was combined with hot water (45°C) and tested on Didymo on carpet. After 30 minutes the number of viable cells was reduced by 91±1.2%. There was a statistically significant reduction in viability compared to the carpeted control but not when compared with 30 minutes on plastic without hot water.

**A2.1.3 Pine Oil Cleaner (Pine-Sol)**

The product tested was Pine oil cleaner and it contained 8.7% pine oil by volume. A 1:2 dilution (50%) for 5 minutes was recommended as an appropriate concentration for cleaning equipment (Hosea & Finlayson, 2005). After 5 minutes of exposure, only one apparently viable cell was observed in the 5 replicates with a total reduction of 98±0.47%. According to a t-Test Assuming Unequal Variances, the pine oil cleaner was statistically more effective than the control. Pine oil cleaner was more effective than the other cleaning products trialed against Didymo.

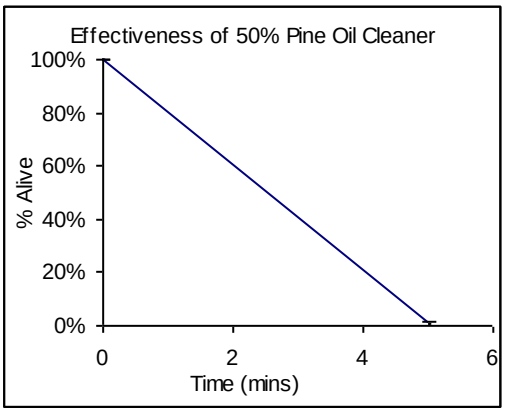



Figure A2.3: Effectiveness of 50% pine oil cleaner in decontaminating Didymo on plastic

**A2.1.4 Vinegar (Acetic Acid)**

Vinegar can dissolve calcium carbonate ($CaCO_3$). We trialed the recommended 100% vinegar (5% pure acetic acid) applied for 20 minutes. In our tests vinegar achieved a statistically significant reduction of 86±2.1% in Didymo viability after only 5 minutes.

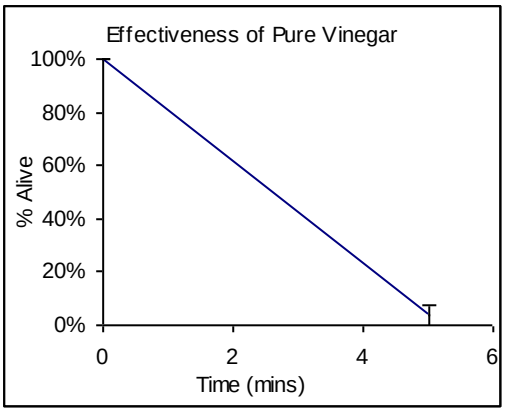



Figure A2.4: Effectiveness of pure vinegar in decontaminating Didymo on plastic

### A2.1.5 Trisodium Phosphate (TSP)

TSP was tested at 20:1 dilution (5%) for 5 minutes and achieved a 90±1.6% reduction in viable Didymo cells. According to a t-Test Assuming Unequal Variances, the reduction in Didymo viability by TSP was statistically significant.

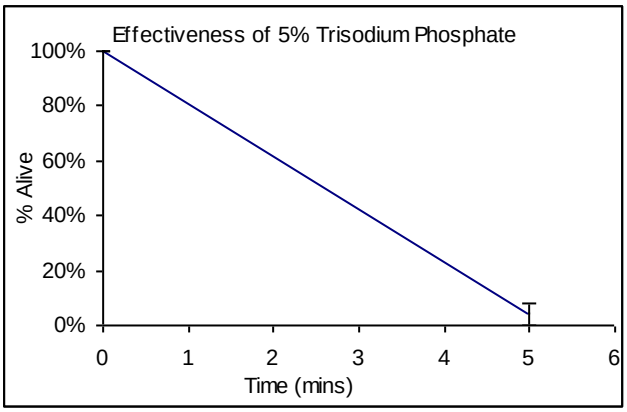



Figure A2.5: Effectiveness of 5% trisodium phosphate solution in decontaminating Didymo on plastic

### A2.1.6 Hot Water

Hot water is one of the most commonly used methods for cleaning equipment. We tested hot water (45°C) and very hot water (>60°C). After 5 minutes in 45°C, only 50±2.4% of Didymo cells were killed. In >60°C water, after 5 minutes, viable Didymo cells were reduced by 75±2.2%. The reductions were statistically significant when compared to the control but the difference between 45°C and 60°C was not significant. Didymo does not appear to be as vulnerable to hot water as the other treatments. Some literature recommends that only 1 minute of exposure to >60°C water is sufficient to guard against Didymo, but we found this to be inadequate (DiVittoria et al, 2012). 60°C water is hot enough to scald skin in only 5 seconds and precautions should be taken when working with it (PSEG, 2012).

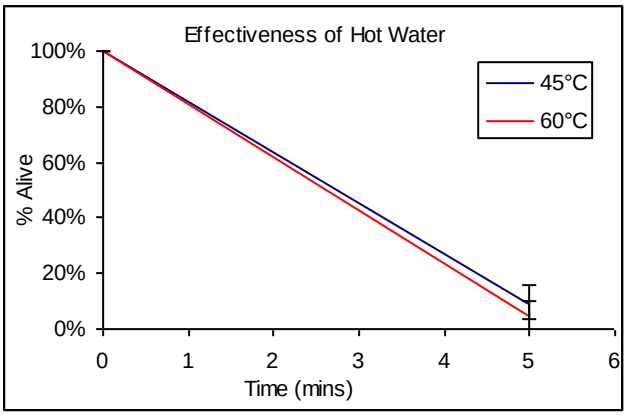



Figure A2.6: Effectiveness of 45°C and 60°C water in decontaminating Didymo on plastic

### A2.1.7 Salt Water

Didymo is a freshwater organism and can not survive in salt water. Salt water was tested at 50 mg/L and 100 mg/L concentrations for 30 and 60 minutes. After 30 minutes, there were total reductions of 98±0.81% in the 50 mg/L solution and 94±0.93% in the 100 mg/L solution. After 60 minutes in the 50 mg/L solution, the viable Didymo population was reduced by 99±0.54%. In the 100 mg/L solution, total reductions were only 91±1.2% (Figure A2.7). The overall reductions compared to the controls are statistically significant but there is no significant difference between the 50 and 100 mg/L concentrations. It is not clear why the higher concentration was not more effective, but the short-comings in ascertaining the viability of Didymo via microscopy were likely a factor.

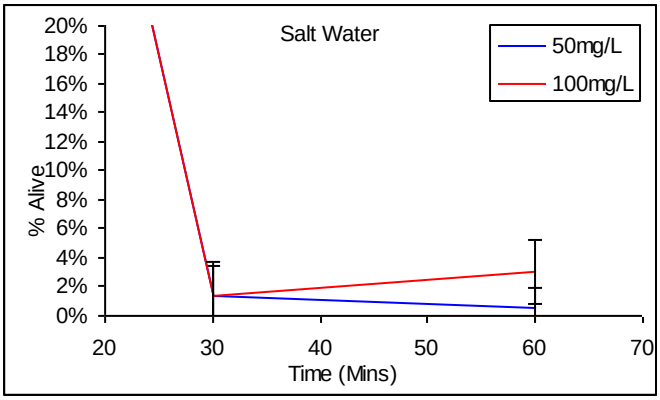



Figure A2.7: Effectiveness of salt water in decontaminating Didymo on plastic

### A2.1.8 Drying

Didymo is an aquatic organism and is vulnerable to drying out, however, the fibrous mats that Didymo grows retain water and protect the cells from desiccation. For this reason it takes days to fully kill Didymo by drying. In our tests it took 48 hours of drying outside with sun exposure to reach 100% mortality. In the trials dried indoors after 6 days, there was only 99±0.49% mortality (Figure A2.8). This reduction held up under

ANOVA statistical analysis and the difference between indoors and outdoors was also statistically significant.

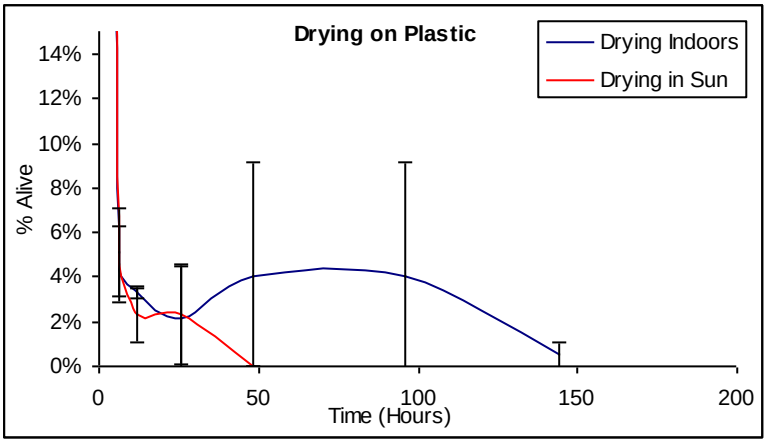



Figure A2.8: Effectiveness of decontaminating Didymo on plastic by drying

Drying Didymo on carpet took even longer to reach 100% mortality. The trials were dried inside away from the sun to replicate the worst case scenario of wet equipment left in a garage or basement. The results were highly variable and not statistically significant (Figure A2.9). This means attempting to dry absorbent equipment in a damp or poorly ventilated area is not an effective method of decontamination unless there are several weeks before the equipment is needed again. There are reports of Didymo surviving on felt-soled waders for up to one month (MPI, 2012). We checked the waders that were used in the Didymo collection for viable cells after one month of storage, and did not find any. The possibility of Didymo survival beyond what we found in this research cannot be discounted.

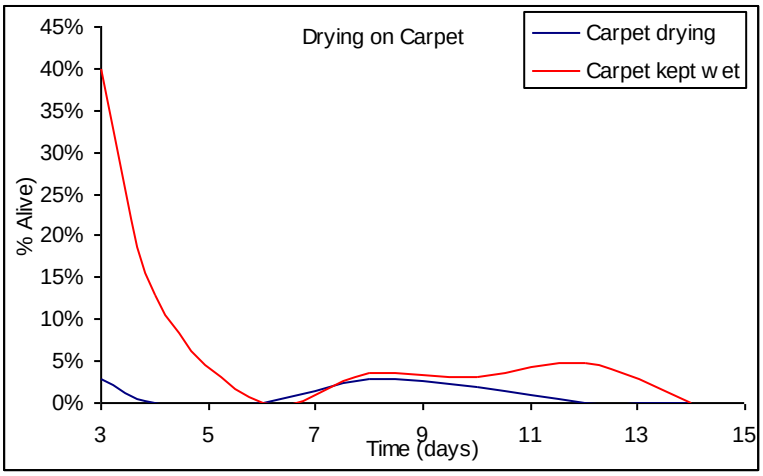



Figure A2.9: Effectiveness of decontaminating Didymo on carpet by drying

### A2.1.9 Freezing Solid

For smaller equipment such as waders it may be possible to freeze the equipment solid to kill organisms on it. The literature recommends being frozen for 4 hours (MSP, 2012). Based on our experiments freezing is an ineffective technique for dealing with Didymo. There was a statistically significant reduction in Didymo viability over time, but the freezing timeframes were on the order of days to weeks. No effect was detected in 4 hours and after 144 hours (6 days) of being frozen solid the mortality rate was 99±.81%. When Didymo was frozen on carpet, it survived even longer with some viable cells detected after 2 months in the freezer. The frozen samples showed far less bacterial activity than the corresponding controls. Freezing may prevent bacterial decomposition of the senescent Didymo cells.

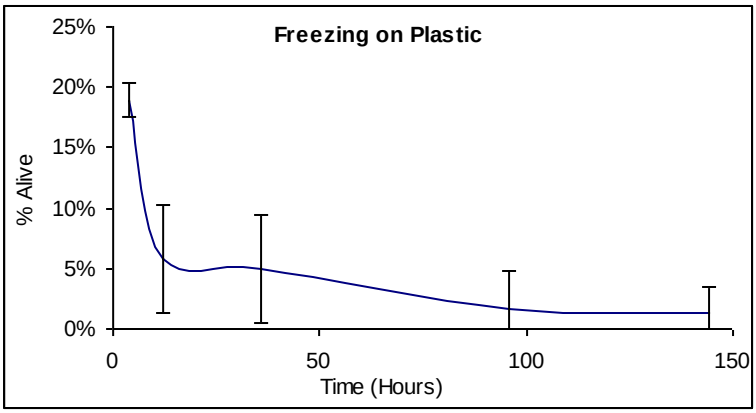



Figure A2.10: Effectiveness of decontaminating Didymo by freezing on plastic

### A2.1.10 Summary of Decontamination Results for Didymo

After testing several different recommended decontamination options, we found that drying in the sun for one week and soaking equipment with salt water was the most effective decontamination protocols.

Table A2.1: Viability of *Didymosphenia geminata* on carpet

| Treatment | Time | % Alive | StdDev | Ctrl Survival | % Reduction |
|---|---|---|---|---|---|
| Bleach 2% | 30 mins | 0.83% | 1.86% | 23.33% | ***96.43%** |
| Dish Soap 5% at 45°C | 30 mins | 1.92% | 2.82% | 21.88% | *91.24% |
| Drying | 3 days | 2.86% | | 20.0% | 97.14% |
| | 4 days | 0.00% | | 16% | 100.00% |
| | 6 days | 0.00% | | 13.8% | 100.00% |
| | 8 days | 2.94% | | 9% | 97.06% |
| | 10 days | 1.89% | | 3.85% | ǂ*50.94%* |
| | 12 days | 0.00% | | 23.08% | 100.00% |
| | 14 days | 0.00% | | 5.26% | 100.00% |
| | 1 month | 0.00% | | 3.26% | **100.00%** |
| Freezing | 2 weeks | 50.00% | | 5.26% | 50.00% |
| | 1 month | 11.11% | | 3.26% | 88.89% |
| | 2 months | 0.98% | 1.70% | Not available | 99.02% |

* Indicates statistically significant reduction compared to control
ǂ Aberration in data caused by poor control survival

Table A2.2: Viability of *Didymosphenia geminata* on plastic

| Treatment | Time (mins) | % Alive | StdDev | Ctrl Survival | % Reduction |
|---|---|---|---|---|---|
| Bleach 2% | 1 | 4.33% | 5.24% | 17.6% | *75.5% |
| Bleach 1% | 15 | 0.36% | 0.80% | 11.4% | ***96.9%** |
| Soap 5% | 30 | 1.35% | 1.86% | 4.8% | *71.7% |
| Soap 5% | 60 | 4.61% | 4.58% | 4.8% | ǂ*3.2%* |
| Pine oil cleaner 50% | 5 | 0.48% | 1.06% | 18.8% | ***97.5%** |
| Vinegar 100% | 5 | 3.44% | 4.76% | 25.0% | *86.2% |
| TSP Household 5% | 5 | 4.02% | 3.70% | 40.0% | *89.9% |
| Hot Water 45°C | 5 | 9.65% | 5.44% | 19.2% | *49.8% |
| Hot Water 60°C | 5 | 4.82% | 5.02% | 19.2% | *75.0% |
| Salt 50mg/L | 30 | 1.34% | 1.84% | 58.3% | ***97.7%** |
| | 60 | 0.56% | 1.24% | 58.3% | ***99.0%** |
| Salt 100mg/L | 30 | 1.43% | 2.13% | 25.0% | *94.3% |
| | 60 | 3.07% | 2.89% | 33.3% | *90.8% |
| Drying Indoors | 360 (6 hrs) | 4.55% | 1.54% | 37.5% | *87.9% |
| | 720 (12 hrs) | 3.32% | 0.25% | 58.3% | *94.3% |
| | 1560 (26 hrs) | 2.19% | 2.20% | 35.7% | *93.9% |
| | 2880 (2 days) | 4.05% | 4.61% | 28.0% | *85.5% |
| | 5760 (4 days) | 4.05% | 4.61% | 20.0% | *79.8% |
| | 8640 (6 days) | 0.50% | 0.86% | 13.8% | ***96.4%** |
| Drying in Sun | 360 (6 hrs) | 5.13% | 1.78% | 25.0% | *79.5% |
| | 720 (12 hrs) | 2.32% | 1.49% | 50.0% | ***95.4%** |
| | 1560 (26 hrs) | 2.33% | 2.24% | 72.2% | ***96.8%** |
| | 2880 (2 days) | 0.00% | 0.00% | 19.2% | ***100.0%** |
| Freezing Solid | 240 (4 hrs) | 18.93% | 8.65% | 17.6% | ǂ *-7.3%* |
| | 720 (12 hrs) | 5.84% | 4.31% | 25.0% | *76.6% |
| | 2160 (22 hrs) | 4.96% | 3.84% | 13.5% | *63.3% |
| | 5760 (4 days) | 1.71% | 2.35% | 20.0% | *91.5% |
| | 8640 (6 days) | 1.36% | 1.86% | 13.8% | *90.2% |

* Indicates statistically significant reduction compared to control
ǂ Aberration in data caused by poor control survival

## A2.2 Decontamination Research on Small Aquatic Invertebrates

It is illegal to bringing live dreissenid mussels into the Okanagan region for any purpose, even testing their viability in a lab setting. Three species of zooplankton were substituted as analogues to the invasive small aquatic invertebrates of interest (*Dreissena*, *Potamopyrgus*, *Bythotrephes*). The first and most numerous species tested was *Daphnia* (Figure A2.11a). *Daphnia* is a model organism in toxicity testing because it is easy to culture and work with. *Daphnia* is also representative of many other species in its sensitivity to chemicals (Gewin, 2005). Copepods are generally larger than *Daphnia* and were the next most numerous zooplankton type used in our decontamination trials (Figure A2.11b). Based on our experiments, copepods have similar sensitivity to chemicals to *Daphnia*. Finally, some *Hydracarina* (water mites) were used because anecdotal experience has shown that they are more resistant to chemical exposure. *Hydracarina* represented potentially more resistant invasive invertebrates (Figure A2.11c).

Figure A2.11: *Daphnia*, copepod, *Hydracarina* from left to right with all being approximately 1-2mm in size. Images not to scale (Gewin, 2005; Kils, 2008; Micrographia, 2012).

In addition to decontamination trials in plastic Petri-dishes, tests were also conducted on indoor/outdoor carpet because absorbent surfaces are the most commonly implicated transport vectors for aquatic invasive species. Unfortunately, the zooplankton became entangled in the carpet fibres and there was no way to properly inspect their viability. This test concluded that it takes longer for carpet to dry, and therefore organisms can stave off desiccation and potentially survive longer, indoors in the dark than outside in the sun. Some of the carpet remained damp outside even after 24 hours. Also, once absorbent equipment has been contaminated with small aquatic invertebrates it cannot effectively washed clean; full decontamination is required.

### A2.2.1 Household Bleach

The literature recommends using 5% household bleach (i.e. a 20:1 mixture of household bleach and water, not pure household bleach which is usually 5% hypochlorite) (Gil-Fox, 2008). The recommended time of exposure for 100% mortality was 60 minutes. Living zooplankton were counted after 1, 5, 20, and 60 minutes (Figure A2.12). After 20 minutes only 1 individual in 1 trial remained and by 60 minutes, 100% mortality had been

achieved in all trials. Using Single-Factor ANOVA analysis, the reduction in viability over time was statistically significant.

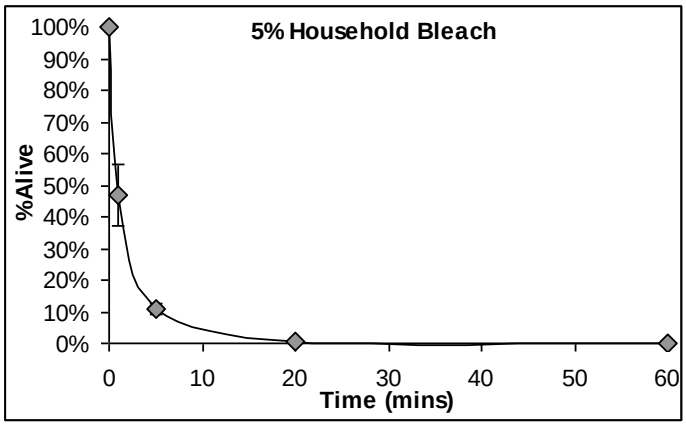



Figure A2.12: Effectiveness of 5% household bleach in decontaminating small aquatic invertebrates

### A2.2.2 Dish Detergent

The literature recommends 5% household detergent for 1 minute to achieve 100% mortality of Didymo (PFBC, 2012). We tested 5% and 10% and were unable to reach 100% mortality, with 2.6±1.3% surviving more than 30 minutes in the 10% solution (Figure A2.13). The overall reductions as well as the difference between the concentrations were statistically significant. The 5% detergent samples showed greater reductions within the first 10 minutes but less by 30 minutes. This trend was also statistically significant but the actual mechanism(s) involved is unclear.

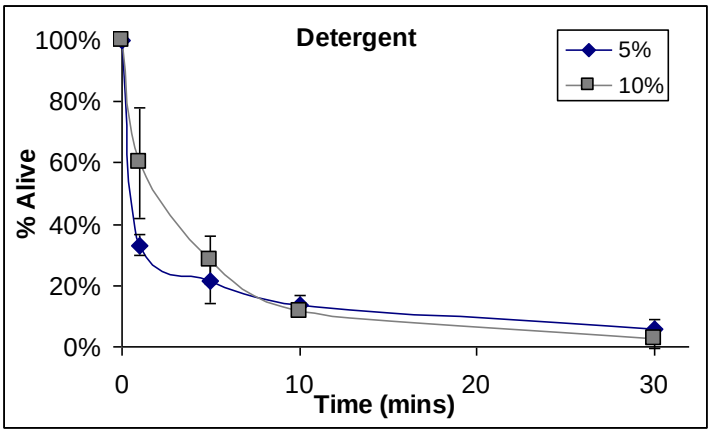



Figure A2.13: Effectiveness of household detergent in decontaminating small aquatic invertebrates

### A2.2.3 Freezing Solid

Zooplankton are complex multi-cellular organisms that are vulnerable to freezing. The literature recommends freezing for 4 hours (MPI, 2012). We interpreted this to mean that

the sample should be frozen for 4 hours and not just in a below 0°C environment for 4 hours. After 4 hours of being frozen solid, all 5 replications had 100% mortality.

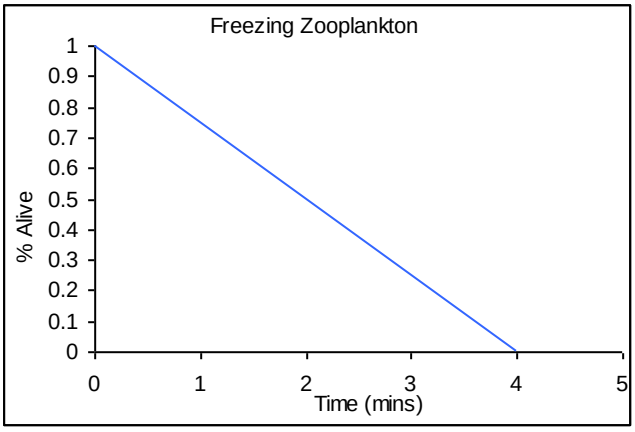



Figure A2.14: Effectiveness of freezing for decontaminating small aquatic invertebrates

### A2.2.4 Hot Water

Hot, high pressure water, is the recommended technique for cleaning boats (DiVittoria et al., 2012). The literature recommends >60°C for 1 minute of contact time. Our experiments found that >60°C was 100% effective in only 1 minute. We also tested 45°C and that was 100% effective after only 2 minutes. Hot water will cool rapidly in the environment so it is important that temperatures are maintained above the efficacy threshold for the necessary contact time. 60°C water can scald skin in only 5 seconds and precautions should be taken when working with it (PSEG, 2012).

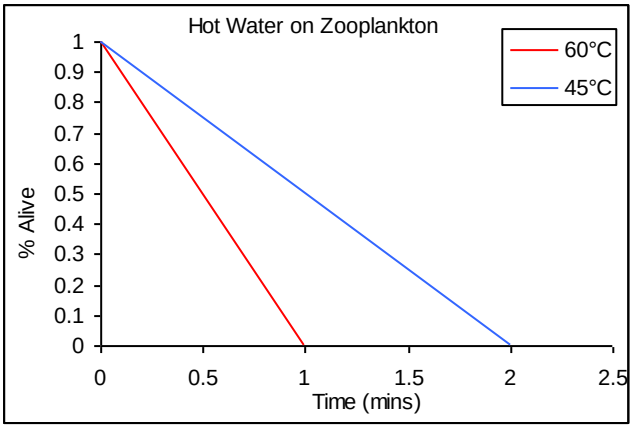



Figure A2.15: Effectiveness of hot water in decontaminating small aquatic invertebrates

### A2.2.5 Salt Water

We tested salt at 50 mg/L and 100mg/L. Literature recommends soaking in 1% salt for 24 hours (DiVittoria et al., 2012). Our results for both solutions were 100% effective on zooplankton after only 5 minutes (Figure A2.16). According to two-factor ANOVA the reductions over time were statistically significant but there was no statistically

significant difference between the effects of the concentrations. This may be because this test did not have the temporal resolution to differentiate between the two.

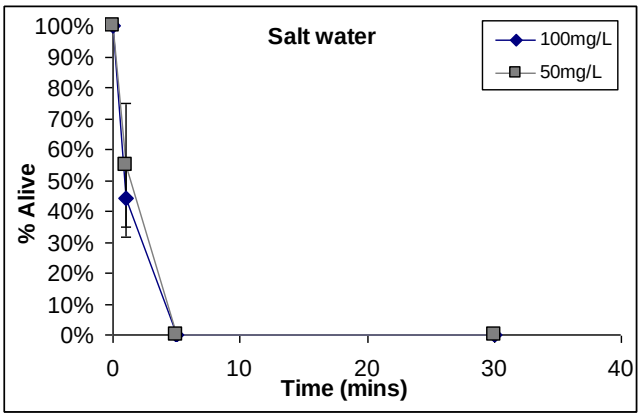



Figure A2.16: Effectiveness of salt water in decontaminating small aquatic invertebrates

### A2.2.6 Pine Oil Cleaner (e.g. Pine-Sol)

The literature recommends 50% pine oil cleaner for 5 minutes to be 100% effective (Hosea & Finlayson, 2005). After 5 minutes of exposure 50% pine oil cleaner achieved 100% mortality (Figure A2.17). Pine oil cleaner is alleged to be hard on rubber equipment so we also tested a 25% concentration and found it to be unreliably effective with 1.3±0.8% surviving over 10 minutes. Two-factor ANOVA revealed the reduction over time and the difference between the two concentrations are statistically significant.

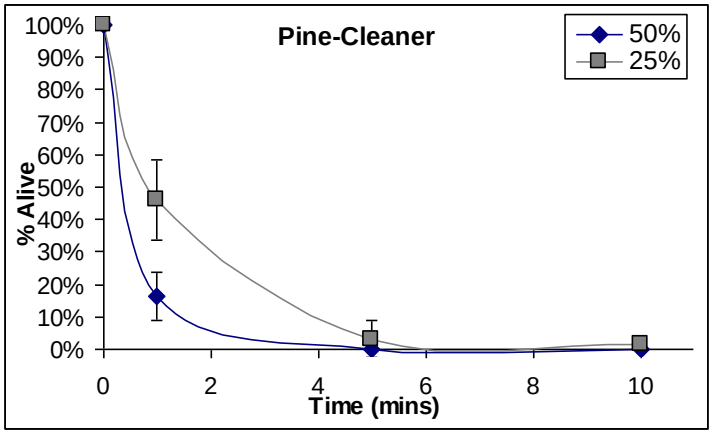



Figure A2.17: Effectiveness of pine oil cleaner in decontaminating small aquatic invertebrates

### A2.2.7 Trisodium Phosphate (TSP)

TSP is inexpensive and relatively safe for use on equipment so it was tested at 5%, the lowest concentration recommended on the packaging. TSP was highly effective on zooplankton, averaging over 99% effectiveness within 15 seconds of contact time. A single *Hydracarina* struggled on for over 5 minutes and 100% mortality was achieved by 10 minutes of contact with the 5% TSP solution (Figure A2.18). There was no statistically

significant decrease in effectiveness observed after 1 minute because virtually all zooplankton were dead by that point.

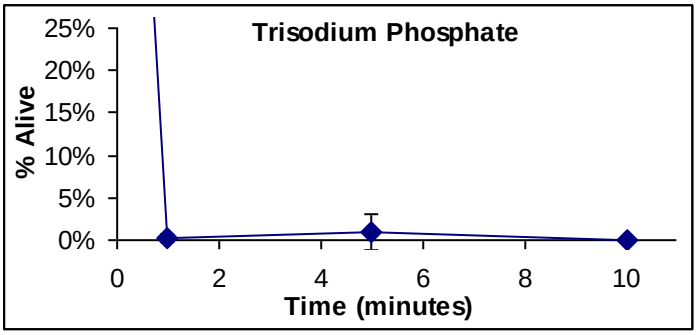



Figure A2.18: Effectiveness of trisodium phosphate in decontaminating small aquatic invertebrates

### A2.2.8 Vinegar (Acetic Acid)

The zooplankton tested have calcium carbonate ($CaCO_3$) shells that are vulnerable to acids such as vinegar. *Dreissena* and *Bythotrephes* also have $CaCO_3$ shells and would also be vulnerable to acid. Literature recommends soaking in pure vinegar for 20 minutes (DiVittoria et al, 2012). Trials were conducted at 100%, 50%, and 25% to confirm effectiveness in situations where potentially contaminated water is already present. Results showed that pure vinegar was very effective reaching 99±.034% mortality in less than 5 minutes. Reductions were all statistically significant and the weaker vinegar concentrations showed statistically slower effectiveness. All concentrations achieved 100% mortality by the recommended 20 minutes of exposure.

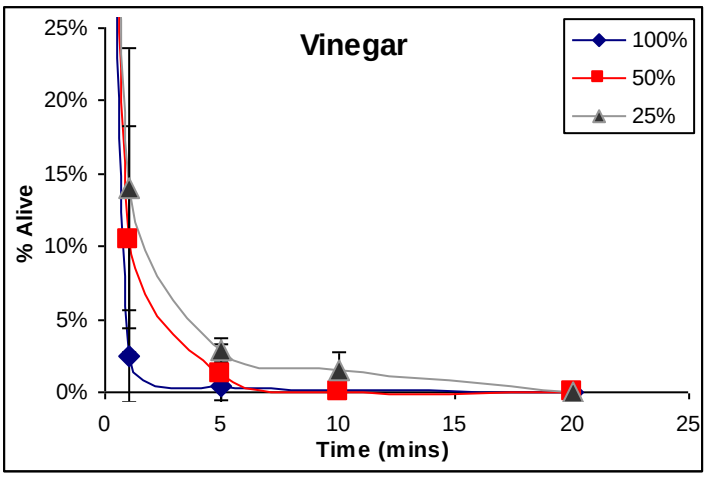



Figure A2.19: Effectiveness of household vinegar in decontaminating small aquatic invertebrates

### A2.2.9 Summary of Decontamination Results for Invertebrates

Of the decontamination protocols tested: bleach, salt water, pine oil cleaner, TSP, and vinegar all achieved 100% mortality within the recommended time frames. Hot water and freezing were also 100% effective within recommended timelines (Table 3.2).

Table A2.3: Summary decontamination results for small aquatic invertebrates

| Treatment | Time (mins) | % Alive | StdDev | % Reduction |
|---|---|---|---|---|
| Bleach 5% | 1 | 47.0% | 8.4% | 53.0% |
| | 5 | 11.0% | 4.8% | 89.0% |
| | 20 | 0.3% | 0.7% | 99.7% |
| | 60 | 0.0% | 0.0% | **100%** |
| 5% Detergent | 1 | 33.2% | 2.9% | 66.8% |
| | 5 | 21.4% | 7.2% | 78.6% |
| | 10 | 13.5% | 2.9% | 86.5% |
| | 30 | 5.7% | 4.5% | 94.3% |
| 10% Detergent | 1 | 60.0% | 21.0% | 40.0% |
| | 5 | 28.4% | 6.9% | 71.6% |
| | 10 | 11.6% | 2.7% | 88.4% |
| | 30 | 2.6% | 3.0% | 97.4% |
| Salt Water (50mg/L) | 1 | 44.2% | 11.0% | 55.8% |
| | 5 | 0.0% | 0.0% | **100%** |
| | 30 | 0.0% | 0.0% | **100%** |
| Salt Water (100mg/L) | 1 | 54.8% | 17.4% | 45.2% |
| | 5 | 0.0% | 0.0% | **100%** |
| | 30 | 0.0% | 0.0% | **100%** |
| 50% Pine oil cleaner | 1 | 16.3% | 7.7% | 83.7% |
| | 5 | 0.0% | 0.0% | **100%** |
| | 10 | 0.0% | 0.0% | **100%** |
| 25% Pine oil cleaner | 1 | 45.9% | 12.4% | 54.1% |
| | 5 | 3.2% | 5.1% | 96.8% |
| | 10 | 1.3% | 2.0% | 98.7% |
| 5% TSP | 1 | 0.2% | 0.5% | 99.8% |
| | 5 | 0.9% | 1.9% | 99.1% |
| | 10 | 0.0% | 0.0% | **100%** |
| 100% Vinegar | 1 | 2.5% | 3.0% | 97.5% |
| | 5 | 0.4% | 0.8% | 99.6% |
| | 20 | 0.0% | 0.0% | **100%** |
| 50% Vinegar | 1 | 10.5% | 7.8% | 89.5% |
| | 5 | 1.3% | 1.8% | 98.7% |
| | 10 | 0.0% | 0.0% | **100%** |
| | 20 | 0.0% | 0.0% | **100%** |
| 25% Vinegar | 1 | 14.0% | 10.8% | 86.0% |
| | 5 | 2.9% | 2.1% | 97.1% |
| | 10 | 1.5% | 2.2% | 98.5% |
| | 20 | 0.0% | 0.0% | **100%** |
| Hot Water (>60°C) | 1 | 0.0% | 0.0% | **100%** |
| Hot Water (45°C) | 2 | 0.0% | 0.0% | **100%** |
| Freezing | 4 hours | 0.0% | 0.0% | **100%** |

# Appendix 3: Veliger Sampling Methods and Results

At the primary sampling sites, veliger specific tows were taken (Figure 4.2). The veliger net has 63 µm pores and captures anything larger that is in the water column. At the base of the net, the contents are concentrated in the valve and then poured into a flask. The liquid in the flask contains all the plankton from several hundred litres of lake water concentrated to only a few dozen mL. For more detailed information on the process see Appendix 9. In the lab the tow was viewed under a microscope (Figure 4.3). Any algae or zooplankton present were identified and tabulated (Table 4.2). Selected samples were then preserved with elthyl alcohol and refrigerated for further taxonomic investigations. Throughout summer 2012, no veligers were detected at any primary or secondary sites in the Okanagan.

Figure A3.1: Veliger net (left) and veliger tow being performed (right)

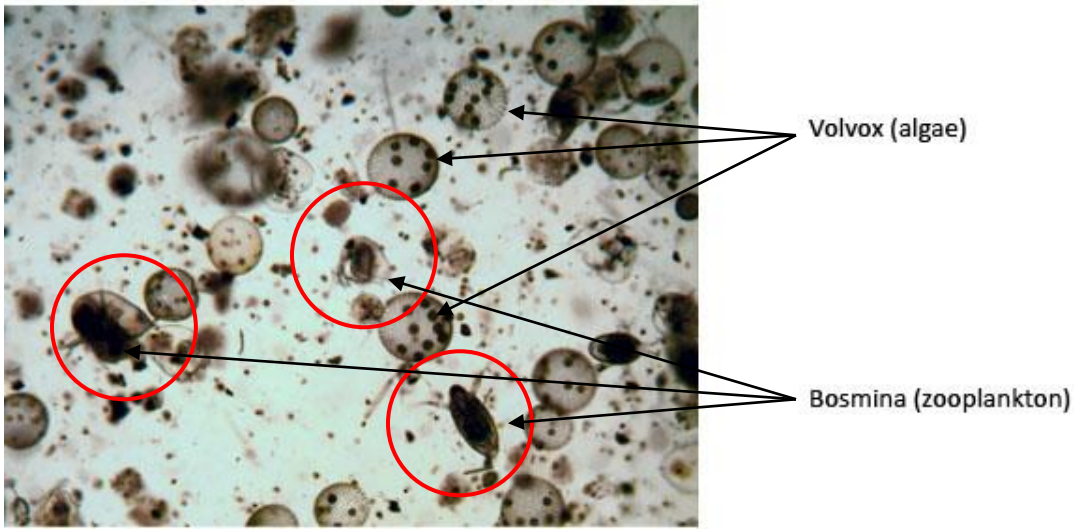



Figure A3.2: Example of plankton tow under microscope at 40x

Table A3.1: Results from veliger plankton haul sampling in 2012

| | | **DIATOMS** | Asterionella formosa | Cyclotella sp. | Cymbella spp. | Pinnularia | Gyrosigma | Gomphonema | Fragilaria | Navicula | Melosira italica | Rhopalodia | Stephanodiscus | Synedra acus | Synedra ulna | Tabellaria | **YELLOW-BROWN** | Cryptomonas ovata | Dinobryon spp. | **GREEN ALGAE** | Chlorella | Oocystis | Mougeotia | **BLUE-GREEN** | Anabaena circinalis | Anacystis cyanea | Gomphosphaeria | Aphanizomenon | Lyngbya | Planktothrix | **VELLIGERS** | No. of Velligers | **ZOOPLANKTON** | Chladocera | Copepods | Diptera | Kellicottia | Keratella | Polyarthra | Large Ciliates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| South Prairie Creek | 14-May | | | C | | | | | A | | C | | P | | C | C | | | C | | | | | | | | | | | A | | - | | | P | | | P | P | P |
| | 11-Jun | | A | C | | | | | A | | P | | | C | P | C | | | C | | | | | | | P | | | C | A | | - | | P | P | | P | P | | |
| | 16-Jul | | A | | P | P | | | A | | | | P | C | P | C | | | A | | | | P | | C | | | | | A | | - | | P | P | | P | P | | |
| | 13-Aug | | C | | P | P | | | A | | P | | P | P | P | C | | | | | | | | | C | P | | | | P | | - | | | C | | | | P | |
| Okanagan @ Kelowna | 14-May | | C | | | | | | A | | | | P | P | P | | | | A | | | | | | | | | | | A | | - | | P | P | | | | | P |
| | 11-Jun | | A | C | P | | | | A | | | | | C | P | C | | | C | | | | P | | | | | | C | A | | - | | | P | | P | P | | P |
| | 16-Jul | | A | | P | P | | | A | | | | | C | P | C | | | A | | | | P | | C | | | | C | C | | - | | P | P | | | P | | |
| | 13-Aug | | P | | | | | | C | | | | | P | | P | | | | | C | | P | | | C | P | | | | P | | - | | | P | | | P | P | |
| Okanagan Centre | 15-May | | A | C | P | P | | | A | | | | | | | C | | | A | | | | | | | P | | | | A | | - | | | P | | | | | P |
| | 11-Jun | | A | C | | | | | A | | | | | C | | | | | | | | | P | | | P | | | C | A | | - | | | P | | | P | | |
| | 16-Jul | | A | | P | P | | | A | | | | P | C | P | A | | | C | | | | | | C | | | | | C | A | | - | | | P | | P | P | | |
| | 13-Aug | | P | | P | P | | | C | | | | | | P | | | | | | C | | P | | C | P | | | A | | | - | | | P | | | | P | |
| Okanagan @ Vernon | 15-May | | A | P | | | | | A | | | | | | | C | | | A | | | | | | C | | A | | | A | | - | | | P | | | | | P |
| | 12-Jun | | A | C | | | | | A | | | | | C | | C | | | C | | | | P | | A | | | | | | | - | | | P | | | P | | |
| | 16-Jul | | A | | | | | | A | P | | | | C | C | C | | | C | | | | P | | C | P | | | | A | | - | | P | P | | | P | | |
| | 14-Aug | | C | | | | | | | | | | | | | P | | | | | A | | | | A | | | | P | | | - | | P | P | | P | | | P |
| | 17-Sep | | P | | P | P | | | A | P | | | | | P | | | | | | | | P | | C | C | | A | A | | | - | | P | C | | P | | A | |

P = Presnt in low numbers
C = Present in moderate numbers
A = abundant
D = Dominant

## Appendix 4:- Educational Materials

In the proposal for this OBWB grant, we committed to help develop educational materials to convey to the public the best practices for cleaning equipment and preventing the spread of AIS, and in conjunction with other agencies to provide the how-to for spotting, sampling and documenting a suspected infestation in the Okanagan, including contact numbers for reporting a suspected infestation. We have prepared several poster lay-outs for consideration and two Power-Points based on this research paper.


Figure A4.1: Sample signage created to alert boaters to threat of aquatic invasive species

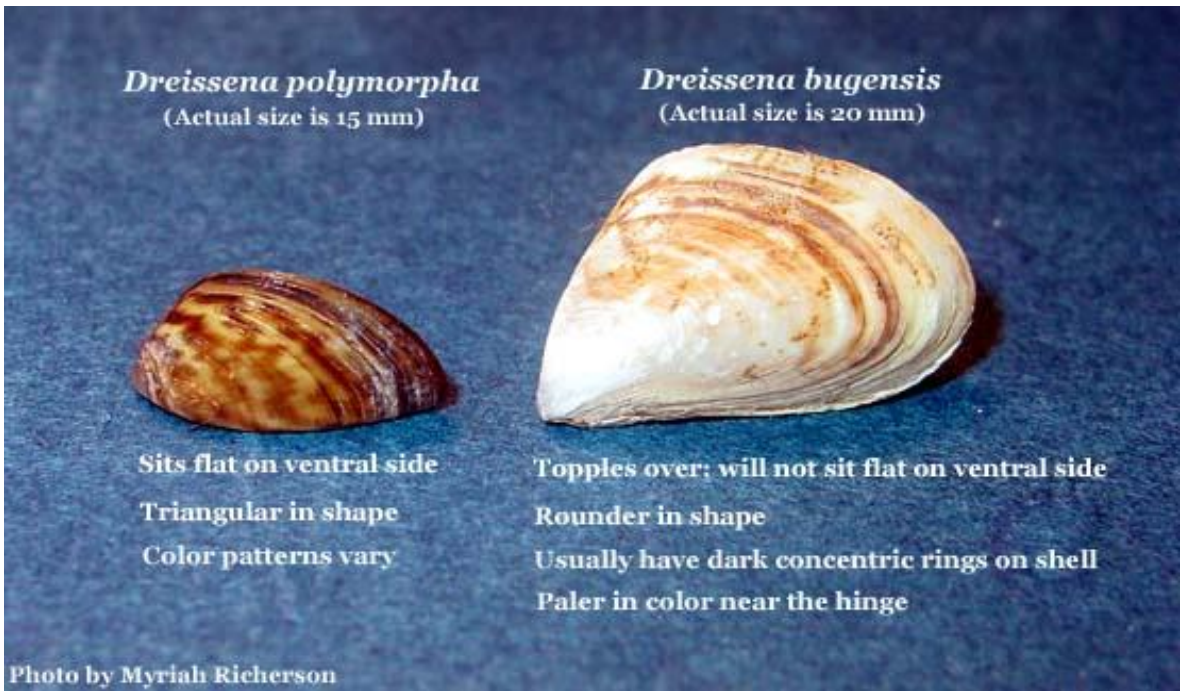
# Appendix 5: Additional information on Zebra and Quagga Mussels

**Why should we care about invasive mussels?** These mussels are heading towards us, carried by boats and fishing gear. Okanagan lakes have low acidity and abundant dissolved calcium to grow mussel shells, making it an ideal environment (Mackie, 2010). Most lakes in the Southern Interior are at high risk of an infestation (Mackie, 2010). Zebra mussels will also have a huge impact on the Okanagan lake ecosystem as a whole, and a huge impact on the economy. The base of the lake food chain is green algae, microscopic plants that feed larger plankton, and small invertebrates that in turn provide food for fish including kokanee and sockeye salmon (Warwick-Sears, 2011). Invasive mussels displace the zooplankton and shut down the food chain supplying fish. They also pose a significant threat to the Okanagan economy by clogging water intakes, damaging pumps and clogging water distribution systems, fouling structures, inducing fish-kills, fouling beaches, etc. After zebra mussels become established in a water body, they are impossible to eradicate with any technology currently available

**What are they?** Zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena rostriformis bugensis*) are two species of prolific, invasive freshwater mussels. These related species have rapidly spread throughout North America from the initial infestation in the Great Lakes during 1986-88. The microscopic mussel larvae or "veligers" are easily transported from infested waters through ballast water discharge and on or in boats, anchors, personal watercraft, dive gear, and bait buckets (Mills et al., 1996). They are notorious for their tendency to colonize water intake pipelines, boat hulls and docks in layers up to 15-60 cm thick (O'Neil, 1993).

Water intake structures provide an excellent habitat for zebra mussel colonies. The flow of water into the pipes carries with it a continuous source of food and oxygen for the mussels and carries away their wastes, while the structures themselves protect the mussels from predation and environmental conditions such as storm wave activity and scouring by ice. Zebra mussels can attach to intake pipes at water flow velocities of up to 2 m/second. Zebra mussels enter water intakes as veligers carried by the water flow, as juveniles when they crawl in using their clam-like foot and as adults when they break loose from colonies and travel on lake or river currents (O'Neil, 1993).

The invasive quagga mussel, *Dreissena rostriformis bugensis*, has out-competed the zebra mussel and taken its place in many North American waterways (Grigorovich et al., 2008; Mills et al., 1999). The resulting drastic changes to the ecology of infested lakes and rivers in North America is causing annual multimillion losses to the economy (USGS, 2002), estimated at 140 million per year in the Great Lakes region during 2007 (Pennsylvania Sea Grant).




## Comparison of Zebra and Quagga Mussels (Many cited sources)

| | Zebra Mussels | Quagga Mussels |
|---|---|---|
| **Shell** | Triangular shape, byssal (ventral) side flat. Obvious ridge between side and bottom | Rounder sides, byssal side rounded. ridge lacking |
| **Colour** | Variable colours and patterns, usually dark | Pale near hinge, dark concentric rings on the shell |
| **Byssal** | Large groove in middle of flat side; allows tight hold on rocks | Small byssal groove near the hinge |
| **Depth in lake** | 1-30 m, rarely found below 15 m | 1-130 m, commonly found down to 30 m |
| **Temperature tolerance** | 0° to 30°C | -2° to 30°C |
| **Temperature for growth** | 12° to 20°C preferred | 4° to 20°C preferred |
| **Reproductive Temperature** | Young present at 14° to 20°C | Young present as low as 8°C |
| **Substrate** | Hard substrates only | Soft or hard substrates muddy or sandy bottom |


Sizes of Zebra and Quagga Mussels

**Dreissena Mussel Requirements:**
Suitable environments for *Dreissena* growth are those with a pH between 7.4 and 9.4, 12°C to 24°C for optimal reproduction, within 2 – 70 m depth, with moderate to high plankton production and with a minimum calcium ion concentration of 12 -20 mg/L (Sprung 1993; Hincks and Mackie 1997; Whittier et a;., 2008). The table below gives the range of values tolerated by these invasive animals:

*General Dreissena* Environmental Tolerance (many cited sources)

| Parameter | High | Mod | Low | V Low |
|---|---|---|---|---|
| Diss oxygen mg/L | 8 - 10 | 6 - 8 | 8 - 4 | < 4 |
| Temperature °C | 18 - 25 | 16 - 18 | 9 - 16 | < 8 or > 30 |
| T Hardness mg/L | 90 - 125 | 45 - 90 | 25 - 45 | <25 |
| T-Calcium mg/L | 25 - 125 | 20 - 25 | 9 - 20 | < 9 |
| pH | 7.5 – 8.7 | 7.2 – 7.5 | 6.5 – 7.2 | <6.5 >9.0 |
| Conductivity us/cm | 83 - 110 | 37 – 82 | 22 - 36 | >22 |
| Secchi disk cm | 40 - 200 | 20 – 40 | | <10 >250 (2.5m) |
| Velocity m/sec | 0.1 – 1.0 | | | <0.075 >1.5-2 |

**Information on Life Cycle of Zebra Mussels**
Mussel spawning can take place when surface waters are approximately 10°C. Each female produces from 30,000 to 40,000 eggs two to three times a year. Zebra mussel eggs and sperm are released after water temperatures reach 12°C, with peak activity occurring when temperatures reach 15° to 17°C. Zebra mussel veligers are approximately 70 microns when they hatch from eggs (Benson et al., 2011).

Larval zebra mussels (veligers) remain planktonic for 2-4+ weeks (Claudi and Mackie 1994). During this stage, veligers are transported by water currents. When they reach the pediveliger stage (220-320 µm), they use byssal threads to settle and attach to hard substrates before metamorphosing into the adult form. Colder water temperatures can reduce growth rates and extend the period required for a mussel to reach a settleable size. Settlement and attachment usually occur in late June to late August and are

associated with a veliger size of around 175 to 200 microns. Successful mussel attachment to surfaces is more likely to occur in areas with a low water velocity of less than 1.5 m/second (Claudi and Mackie, 1994).

In river systems, larvae produced by a population can be transported far from the parent population before they reach settlement stages. River populations of zebra mussels remain dependent on new larvae from upstream sources, as these populations are rarely if ever self-sustaining, possibly through turbulence-induced mortality (Horvath et al 1996; Stoeckel et al. 2004; Rehman et al., 2003).

Zebra mussels grow rapidly, as much as 25 mm in their first year, but typically around 15 to 20 mm. They grow another 12 to 25 mm in their second year. Growth rates are dependent on water conditions, especially temperature. Zebra mussels can live four to six years, but generally survive only two years. An adult mussel can filter up to one liter of water per day, stripping it of plankton, bacteria and particulate organic matter (Ohio Sea Grant 1994).

**Information on Life Cycle of Quagga Mussels**. Quagga mussels are as prolific as zebra mussels; a single mature female mussel can produce more than 1 million eggs in a spawning season. Quagga mussels grow up to 20 mm in their first year and are more likely to grow in single layers and produce more patchy distributions than zebra mussels (Smythe 1996). Quagga mussels have thinner shells, put more energy into reproduction, can subsist on soft substrate, have a longer siphon, can spawn at lower temperatures, and have a higher assimilation efficiency than zebra mussels (Jude, 2010).

Adult mussels can crawl up to several meters per day (Maryland Sea Grant 1993), or move with currents after detachment. Relocation of adult mussels is more common in fall and winter (Claudi and Mackie 1994). Trailered boats and boating equipment are the most common vectors for mussel movement between water bodies. To a lesser extent, waterfowl and other aquatic organisms also assist in the dispersal of these mussels.

**Impact of Mussel Introduction - Enviromental:** Quagga and zebra mussels are a serious threat to aquatic environment and fisheries (McCamman, 2010). It only takes a few mussels to infest an entire waterway and destroy the ecosystem there (McCamman, 2010).

Quaggas are prodigious water filterers. By 2010 there were some 900 trillion quagga mussels in Lake Michigan and it is estimated that all the water in this massive lake can be filtered by these mussels in 9 to 12 days (Jude, 2010). By removing the phytoplankton, zebra and quagga mussels decrease the food source for zooplankton, and alter the food web. Their filtering impacts include:

- increases in water transparency (can stimulate aquatic plant or filamentous algae growth),
- decreases in mean chlorophyll-a concentrations,

- accumulation of pseudofeces (wastes) that can foul the environment (Claxton et al. 1998) and create taste and odor events (Pennsylvania Sea Grant).

Mussels accumulate organic pollutants within their tissues to levels more than 300,000 times greater than concentrations in the environment and these pollutants are found in their pseudofeces, which can be passed up the food chain, therefore increasing wildlife exposure to organic pollutants (Snyder et al. 1997). Nearly all fish species found in areas infiltrated by the Quagga have suffered due to the strain on the food chain. Quagga mussels have been found at depths up to 130 m in the Great Lakes (Mills et al. 1996, Claxton and Mackie 1998).

Zebra and quagga mussels can become carriers or attractants for dangerous bacteria such as *E. coli, or Clostridium botulinum* that can impact other aquatic species. For example, scientists believe a strange form of botulism known as type E was brought to the Great Lakes by foreign invaders such as zebra mussels, the round goby and quagga mussels. In the Great Lakes, the green filamentous alga *Cladophor*a and filamentous cyanobacteria (in Lake Erie) can be broken off the rocks during storms and accumulate in huge mats along the shoreline. Anoxic conditions develop in these mats, leading to optimal conditions for the development of Type E botulism, which is then filtered by mussels, eaten by round gobies, and then stricken round gobies are eaten by many bird and piscine predators, leading to their death (Jude, 2010). While this scenario may be unlikely in B.C., other related and unforeseen scenarios are possible.

Zebra and quagga mussels have been implicated in more cyanobacteria blooms in infested lakes. They eat favored algae, such as diatoms, leaving the undesirable algae to flourish. Many cyanobacteria are favored by this selective filtering in lakes. They produce cyanotoxins and taste and odor problems that are difficult to remove, even with water treatment plants (Jude, 2010).

**Impact of Mussel Introduction - Economic**

*Dreissena* species ability to rapidly colonize hard surfaces causes serious economic problems. Mussel populations on stationary structures can reach 750,000 adults per square meter. Colonies of this magnitude affect water intakes, pumping stations, bridges, cooling inlets, ballast intakes, locks, and other manmade structures. Their obstruction of valves, screens, impellers, and other moving mechanisms wreak havoc with irrigation, pumping and hydroelectric systems.

# Appendix 6: Additional Information on Didymo, New Zealand Mud Snails, and Spiny Water Flea

**Didymosphenia geminata**

Didymosphenia geminata, (AKA Didymo, or "rock snot,") a mat-forming species of freshwater diatom, has taken over low-nutrient rivers in North America and Europe, and it has also invaded water bodies in the Southern Hemisphere, including those in New Zealand and Chile. Because its blooms alter food webs and have the potential to impact fisheries, Didymo presents a threat to the ecosystem and economic health of these watercourses. Algae blooms are usually linked with the input of nutrients that fuel plant growth, so Didymo's ability to grow prolifically in waters where nutrients such as phosphorus are in short supply, puzzled scientists. A paper just published in the journal Geophysical Research Letters (Didymosphenia geminata: algal blooms in oligotrophic streams and rivers (P.V. Sundareshwar et al.) finds Didymo is able to colonize and dominate the bottoms of some of the world's cleanest waterways precisely because they are so clear. Didymo is able to concentrate phosphorus from the water with help from bacteria that live inside the algal mats and allow Didymo to make use of the sequestered nutrients.

The authors conducted their research in Rapid Creek, SD, an unpolluted mountain stream where Didymo was first observed in 2002. The creek regularly has Didymo blooms that cover 30 to 100% of the streambed over an area up to ten kilometers (6 miles) long. Didymo thrives in Rapid Creek because of biogeochemical processes in biofilms in the mats. Didymo cells adsorb both iron and phosphorus on their surfaces. Then bacterial processes in the algal mat interact with iron to increase the biological availability of phosphorus. As Didymo mats form, new stalks develop at the surface, and older stalks which have already bound phosphorus are displaced to the mats' inner regions. The process results in abundant phosphorus for cell division, resolving the paradox of Didymo blooms in oligotrophic streams and rivers. "This study solves the puzzle of how Didymo can produce such large blooms in low-nutrient rivers and streams," said Tim Kratz, program director in NSF's Division of Environmental Biology. "It has uncovered the fascinating mechanism by which Didymo 'scrubs' phosphorus from a stream or river, then creates a microenvironment that allows microbes to make this nutrient available for Didymo's growth". The results will help scientists and managers to identify water bodies susceptible to Didymo blooms, and have the potential to lead to discoveries that may stem this organism's prolific growth in rivers around the world.

(Excerpted from 'River Mystery Solved -- Scientists Discover how "Didymo" Blooms in Pristine Waters with Few Nutrients', NSF press release 11-109, June 2)

**New Zealand Mud Snails**

The New Zealand mudsnail *Potamopyrgus antipodarum*, is a small aquatic snail that is an invasive non-indigenous species in the Pacific Northwest. Since it was first discovered in the Snake River, Idaho in 1987, it has spread rapidly throughout the Western United States and British Columbia, and has reached population densities as high as 300,000 snails/$m^2$. *P. antipodarum* is now reported from North America: Canada (Port Alberni, British Columbia), all western states of United States, (with exception of New Mexico), and in Great Lakes in eastern part of US (http://rivrlab.msi.ucsb.edu/NZMS/maps.php). At high densities, the New Zealand mudsnail can drastically alter aquatic food web structure. Mudsnails compete with native macroinvertebrate fauna for food and habitat, and are thought to be a poor food source for fish because they provide little energy and can pass through the digestive tract of fish undigested. Their potential to alter the Columbia River Basin's benthic ecosystem is high due to their ability to spread through human activities, reproduce parthenogenetically, and rapidly colonize and occupy substrates at high densities. They are readily transported from infested waters inadvertently through gear, boats and other human activities. More information on the New Zealand mudsnail can be found here: http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=1008

In the Great Lakes Region, NZ mudsnails have been found at depths ranging from 4 to 45 meters (Zaranko et al. 1997; Levri et al. in prep.). They are very small (5-6mm in length) and may be found subtidally or intertidally on or under rocks and debris in fresh or brackish waters. Potamopyrgus antipodarum can be spread by people through the movement of gear such as waders, boots, angling equipment, and boats or by the translocation of aquaculture materials (live fish T.M. Davidson et al. 350 or eggs; Bowler 1991; Haynes et al. 1985; Hosea and Finlayson 2005). Secondary introductions may occur on birds that carry the snails among their feathers or by fish that consume but are unable to digest snails (Bondesen and Kaiser 1949; Haynes et al. 1985). In some invaded freshwater systems, P. antipodarum has become the most prevalent and numerically abundant species (Ponder 1988; Hall et al. 2006) reaching densities over 500,000 snails $m^2$ in vegetative and muddy substrates, and constituting between 65-92% of total invertebrate productivity (Hall et al. 2006).

These herbivorous and detritivorous snails can also dominate carbon and nitrogen fluxes (Hall et al. 2003). The high densities achieved by P. antipodarum in invaded systems suggest that it may compete with native species for resources (Brown et al. 2008). However, the field evidence for a negative competitive effect is mixed, with some negative (Kerans et al. 2005), non-significant (Cada, 2004), and positive (Schreiber, 2002) correlations between densities of P. antipodarum and native fauna. Potamopyrgus antipodarum may also reduce the colonization rate of some macroinvertebrates (Kerans et al. 2005) and affect the survivorship of fish that consume them (Vinson and Baker 2008). The interactions with different trophic levels coupled with the high densities observed in many systems may lead to substantial changes in trophic dynamics and nutrient cycling in aquatic ecosystems (Bronmark 1989; Hall et al. 2003; Hall et al. 2006).

There appear to be few (if any) feasible options in controlling P. antipodarum populations once they become established (New Zealand Mudsnail Management and Control Plan Working Group, 2007). Resource managers, however, are employing several options to prevent the future spread of P. antipodarum such as posting signs at boat ramps, distributing informational media (pamphlets, brochures, websites; pers. obs.), and by establishing permanent and mobile washing stations at boat ramps (New Zealand Mudsnail Management and Control Plan Working Group, 2007). Another option is to treat infected equipment. Richards et al. (2004) recommended two options to prevent the spread of P. antipodarum through infected equipment: 1) freezing for several hours or 2) drying infected equipment at 30ºC for at least 24 hours or at 40ºC for 2 hours. There are also several chemical options to decontaminate infected equipment including copper sulfate (252 mg/L Cu), Formula 409® Disinfectant (50% dilution), and benzethonium chloride compounds (1,940 mg/L)(Hosea and Finlayson, 2005). These types of chemical treatments only require five minutes of submergence to be effective and do not appear to damage neoprene and rubber wading gear, although care must be taken to dispose of those chemicals properly (Hosea and Finlayson 2005). While chemical options are effective in preventing the spread of P. antipodarum, knowledge of infested sites coupled with rigorous gear cleaning (scrubbing, draining, and drying) at these sites and elsewhere are cost effective means of limiting further transport of P. antipodarum and other aquatic invasive species. We urge resource managers to remain vigilant and aware of the threat P. antipodarum may hold for aquatic systems and to educate the public in order to prevent the further spread of P. antipodarum on the Pacific coast of North America.

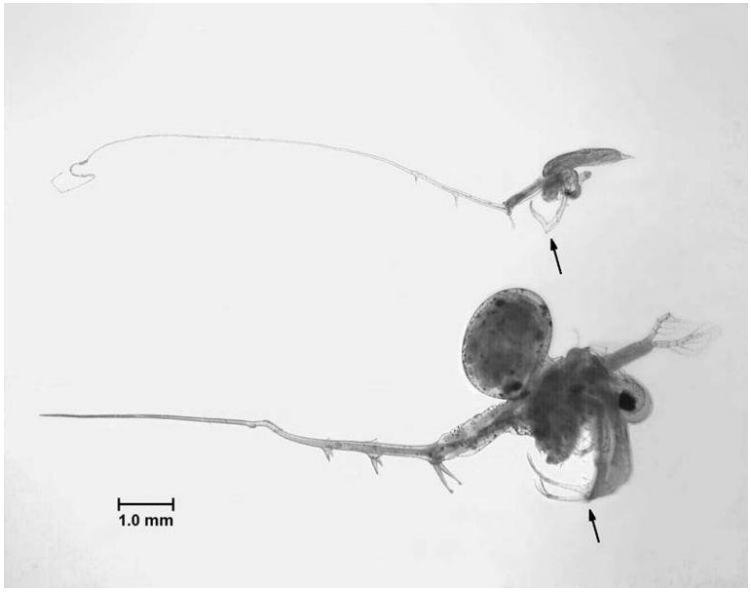
Sampling for NZMS involves the use of a standard heavy-duty D-shaped kick net with mesh size < 1 mm. The kick net is vigorously pushed through all available habitats, including vegetation, and also placed downstream of the biologist who vigorously kicks and agitates the substrate (cobbles, gravels, etc.) to collect what is kicked up with the net. Contents of the net are then placed in a large bucket of water; vegetation is washed in the bucket to remove snails and then safely discarded. Snails and other invertebrates are then poured into a < 1.0 mm mesh small, aquaria hand-net or suitable container. All that should remain in the bucket is heavier sand, gravel, or cobbles, which can be discarded. Contents of the small aquarium net are stored in 70-95% ethanol with

collection labels written in pencil or alcohol-proof pen placed both in the container and attached on the outside of container.

## Spiny Water Flea

Thus far, the spiny water flea has not been transported to western Canada or the western states, and as such, is not an imminent threat to the Okanagan. Its current range includes Wisconsin as of 2002, Minnesota, and Southern Ontario and near border with Manitoba. We include this tiny animal here as information of yet another aquatic invader that is transported in life stages invisible to the naked eye.

Discovered in 1984 in Lake Huron, scientists have hypothesized that the spiny water flea (Bythotrephes) came to North America in the water onboard freighters from European ports, especially the port of St. Petersburg, Russia. Spiny water fleas are crustaceans, a relative of the shrimp, lobster, and crayfish. They have a long, sharp, barbed tail spine. They are large zooplankton measuring about 1 centimeter in length and are active from late spring to late fall. Spiny water fleas can rapidly reproduce in summer because adult females can produce young without mating, when water temperatures are just right, at a rate of 10 young every two weeks. They live for several days up to two weeks. In fall, females mate and produce resting eggs which live through the winter.




Spiny water flea will do well in lakes with an abundance of edible prey, such as bosminids and small daphniids, and will multiply most rapidly in environments with a suitable temperature of around 22°C (typical mid-summer conditions in the Okanagan). Human mediated dispersal accounted for 99.75% of propagules to probability of Establishment. Management efforts controlling recreational boating traffic out of the largest lakes in the system will be the most effective way of slowing the spread of spiny

water flea in lakes within this system. In addition, Gertzen found that invasions were most likely to happen during the summer—a time when human-mediated introductions are at their peak. Boats are much more important than streams for spiny water flea spread, and that large lakes close to where people live have the highest invasion risk. CAISN (Canadian Aquatic Invasive Species Network) student Jennifer Petruniak demonstrated that the spiny water flea could concentrate in patches in lakes under certain weather conditions. When the wind blows in one direction for an extended period of time, large numbers of animals can build up at shorelines. Thus, if a boat launch is located in an infested area, and the lake is a popular destination for tourists, continued spread is inevitable. Finally, CAISN student Lifei Wang found that spiny water fleas seem to establish populations in larger, deeper lakes that are home to more sport fishes. While it is too late to eradicate the spiny water flea, CAISN has provided invasion researchers worldwide with a valuable case study of how species spread once they colonize a single system. These studies are also critical to management efforts to curtail future spread, as they have highlighted the role of human vectors in spread to inland lakes.

Bythotrephes is inedible to many fish because of the barbs on its tail. The spiny water flea's diet consists mostly of Daphnia zooplankton, leading to competition with small fish and fry, and also with native water flea species. Daphnia zooplankton populations have declined in recent years (in the Great Lakes) though there is no conclusive evidence as to the cause. Spiny water flea can actually eliminate zooplankton species. As zooplankton is the backbone of aquatic food chains, this tiny crustacean presents a serious risk to the ecosystem. The eggs survive even after being dried out or eaten by fish.

For More Information: http://dnr.wi.gov/org/caer/ce/eek/critter/insect/waterflea.htm

# Appendix 7: Methods of Invasive Mussel Control for Water Supplies

Physical Control

**Drawdown and exposure**: If the infestation is within an impoundment with water level control capability, drawdown may be a viable control technique. Removing all water from a lake or pond and allowing it to dry completely for a week in summer may eliminate the zebra mussel infestation; however, this technique involves many technical and biological issues. A drawdown of a reservoir or pond could result in the eradication of many desirable plant and fish species. An effort could be made to capture and relocate desirable species, but this would likely be an expensive and lengthy undertaking. The water pumped out of the impoundment would have to be filtered or otherwise treated to ensure no small eggs or larvae escaped to other water bodies. Alternatively, it may be possible to hold the water in a separate basin or to dispose of the water in a way that limits risk of zebra mussel transfer (e.g., ground water infiltration). However, drawdown and exposure will not be a viable option in most cases.

**Physical removal** Physical removal of the mussels using manual or mechanical scrapers and/or high pressure water jets can be used on a small, localized scale with success, but are not likely to be successful against large infestations. Physical removal causes minimal impact on native species, however it is unlikely to provide 100% eradication of all Dreissena life stages.

**Suffocation** Dreissena mussels need oxygen to survive. If the oxygen level drops below the lethal limit of mussels, they will die off. Lakes with anaerobic zones will not allow the mussels to infest the deeper water. Deliberately inducing anaerobic conditions is a technique that is usually confined to industrial applications.

**Thermal treatment** Hot water can kill zebra mussels, although many other aquatic organisms can also be harmed as well. Industrial and public utilities are experimenting with thermal controls for zebra mussels, and on a localized basis this approach may have merit. Generally, though, thermal treatments are best used to decontaminate boats.

Hot water can be used to keep intakes clear and is also becoming the treatment of choice for decontaminating boats. Hot water has a relatively low environmental impact in short duration treatment periods. It can be mitigated by rapid mixing with ambient water with an outfall diffuser. Hot water sprays at ≥60°C for 1 minute or 80°C for ≥5 seconds were 100% lethal to adult zebra mussels (Morse, 2009). Thus, presently recommended spray temperatures of 60°C may not be 100% effective unless the spray is applied for more than 10 seconds (Morse, 2009). In other work, adult quagga mussels were exposed to hot-water sprays at 20, 40, 50, 54, 60, 70, and 80°C for 1, 2, 5, 10, 20, 40, 80, and 160 seconds. In yet another recent work, Beyer et al., (2011) tested the acute upper thermal limits three aquatic invasive species; adult zebra mussels, quagga mussels, and spiny water fleas (Bythotrephes longimanus), employing temperatures

from 32 to 54°C and immersion times from 1 to 20 minutes. Immersion at 43°C for at least 5 minutes was required to ensure 100% mortality for all three species, but due to variability in the response by Bythotrephes, a 10 minute immersion was recommended. Overall there were no significant differences between the three species in acute upper thermal limits. Heated water can be an efficient, environmentally sound, and cost effective method of controlling aquatic invasive species potentially transferred by boats (Beyer et al., 2011).

**Electricity** Control of zebra mussel veligers in a river might be possible using an electric dispersal barrier. Plans are under way to eventually develop a barrier that will also be effective against various planktonic organisms such as zebra mussel veligers. If proven effective in the Illinois River, similar control tactics could feasibly be applied other rivers (Stoeckel et al., 2004; Hovarth et al., 1996).

**Biological Control**

Biological controls that are currently researched include selectively toxic microbes and parasites that may play a role in management of Dreissena populations (Molloy 1998). For example, Pseudomonas fluorescens, a common soil bacteria, is harmless to humans but toxic to zebra mussels. Other prospective biological approaches to controlling Dreissena populations may be to disrupt the reproductive process, by interfering with the synchronization of spawning by males and females in their release of gametes (Snyder et al. 1997). Another approach would be to inhibit the planktonic veliger from settling, since this is the most vulnerable stage in the life cycle (Kennedy. 2002). Biological control so far has not been effective in controlling *Dreissena* species.

Alternatively, augmenting or introducing natural predators may be considered, but is not likely to result in the eradication of the infestation. The change in ecosystem dynamics due to introductions of new organisms or the augmentation of present organisms may be detrimental to the overall health of the ecosystem in some cases, so extreme care must be taken with this approach. Predation by migrating diving ducks, fish species, and crayfish may reduce mussel abundance, though the effects can be short-lived (Bially and MacIsaac, 2000). An exception may be certain fish species, like freshwater drum, which prey upon zebra mussels effectively. As with most biological predator-prey interactions, cycles of abundance are typically set up and eradication is unlikely, but some measure of control can be achieved.

**Chemical Control**

There are no known chemical controls suitable for use against invasive mussels in an open environment. If the target area is small and water exchange can be controlled, it may be possible to apply some of the harsher chemicals with limited impacts to non-target populations in the lake, but great care must be taken and this approach has generally not been applied. The US Army Corps of Engineers has published a “Zebra Mussel Chemical Control Guide” that can be accessed at: http://el.erdc.usace.army.mil/zebra/pdf/trel00-1.pdf

Adult mussels can be especially challenging to control chemically since they may sense some chemicals in the water and close their shells for weeks, thus limiting their exposure. A summary of the most commonly used chemicals follows:

**Copper** Effective control of Zebra mussel larvae can be obtained within one day of exposure to a copper-containing algaecide at concentrations much lower than allowable dosage for treatment of algal blooms. The study found that an early life stage called the trochophore can be killed in the laboratory after just a few hours using copper exposures of 0.02 mg/L copper ion while killing adults with the algaecide was not possible after 24 hours exposure at 5 mg/L. Even after 96 hours of continuous exposure, it took almost 2 mg/L to kill most of the adults and that copper dose would likely have unintended ecological impacts. Such a strategy would need to be coordinated with spawning events and repeated seasonally for several years (the approximate life expectancy of adult mussels) to achieve effective control zebra mussel populations (Kennedy, 2002).

**Chlorine:** Pre-chlorination has been the most common treatment for control, but if this method is used to control both zebra and quagga mussels the amount of chlorine used may reach hazardous levels (Grime, 1995). Chlorine kills adult zebra mussels through asphyxiation and limited glycolysis over a prolonged period of exposure. Primary concerns with chlorine are its toxicity to non-target organisms and the production of carcinogenic trihalomethanes from dissolved organic materials.

Research has shown that mussels shut their valves as soon as the detect chlorine and open only after chlorine dosing is stopped. Under continuous chlorination mussels are constrained to keep the shell valves shut and they starve. Zebra mussels subjected to continuous chlorination at 1-3 mg/L showed 100% mortality after 25 days, while those subjected to intermittent chlorination at 1 mg/L showed very little or no mortality during the same periods (Rajagopal et al., 2003).

Mussel mortality also varies with water temperature. Mussels exposed to 0.25 mg/L chlorine residual took 45 days to reach 100% mortality whereas those exposed to 3 mg/L chlorine took 10.5 days. The effect of water temperature on D. polymorpha mortality in the presence of chlorine was significant. For example, it took 43 days to reach 95% mortality using 0.5 mg/L residual chlorine at 10°C, compared to only 19 days at the same 0.50 mg/L chlorine dose but at a warmer 25°C (Rajagopal et al., 2002).

**Potassium:** Potassium chlorate ($KClO_3$) or Potassium chloride (KCl) can be used to selectively kill invasive mussels, since toxicity data indicates that the target concentration is not lethal to non-target organisms other than freshwater mollusks (e.g., the threshold effect concentration for potassium is 272.6 ppm for Ceriodaphnia and 426.7 ppm for fathead minnows) (Aquatic Sciences, 1997). Elevated potassium levels in the range of 10-15 ppm have been reported as lethal to other freshwater mussel species over a few-week period. For example 1 to 4 applications of a 12% liquid potassium

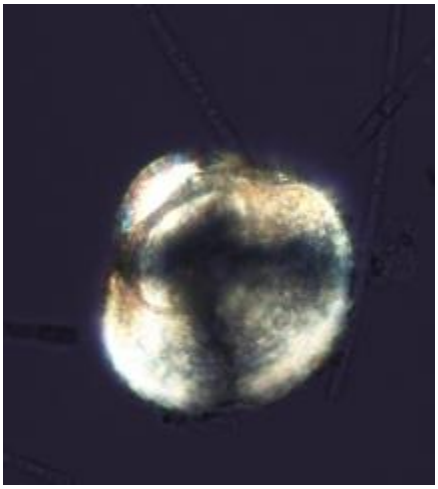
stock solution mixed from potassium chloride were proposed to kill a zebra mussel infestation in a flooded quarry. The proposed treatment would require 128,000 kg of active ingredient to treat 200,000,000 gallons of water (131,000 kg of dry muriate of potash) (USFWS, 2005). The magnitude of this application highlights the challenge of treating an infested water body.

Other potential methods of chemical control include: radiation, filtration, removable substrates, ozone, antifouling coatings, etc. A straining and ultraviolet (UV) light system was installed at Hoover Dam. The strainer removes large mussels followed by treatment with UV light to kill or disable veligers from settling (Willett, 2011).

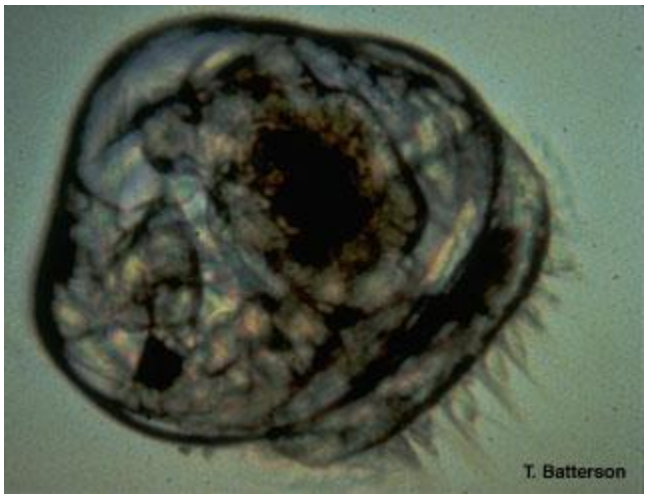
**Examples of Zebra and Quagga Mussel Infested Habitats**

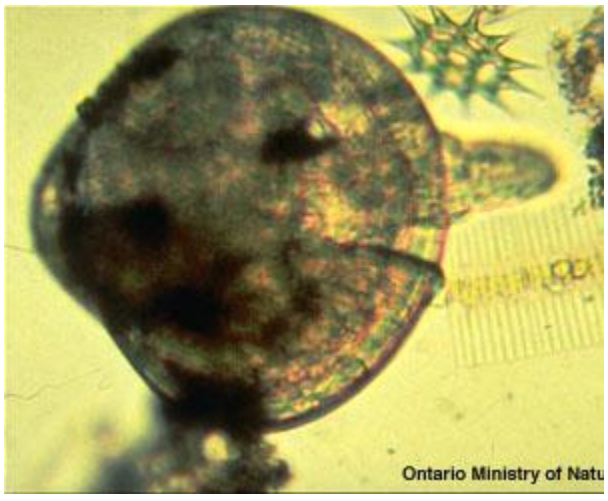
# Appendix 8: Mussel Veliger Identification Key

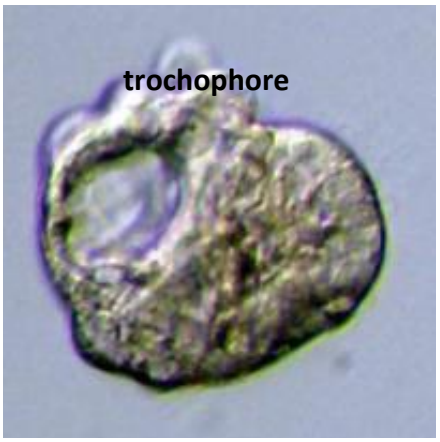
## Quagga Mussel Veligers

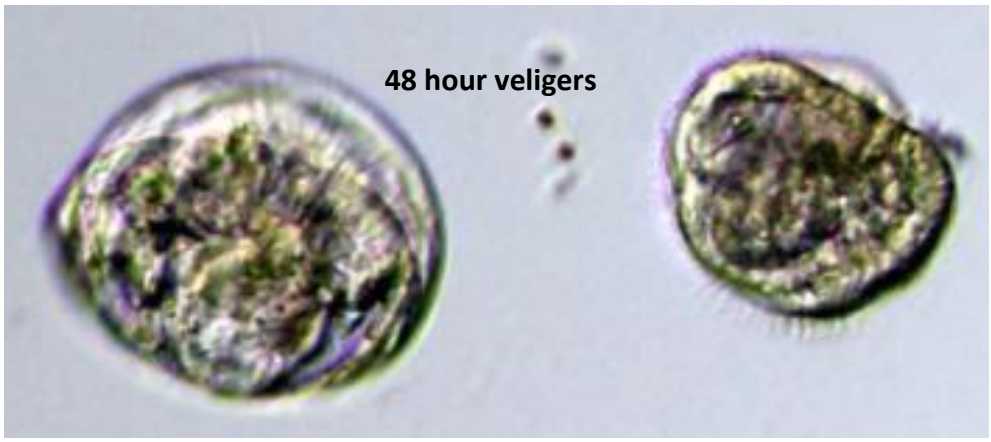


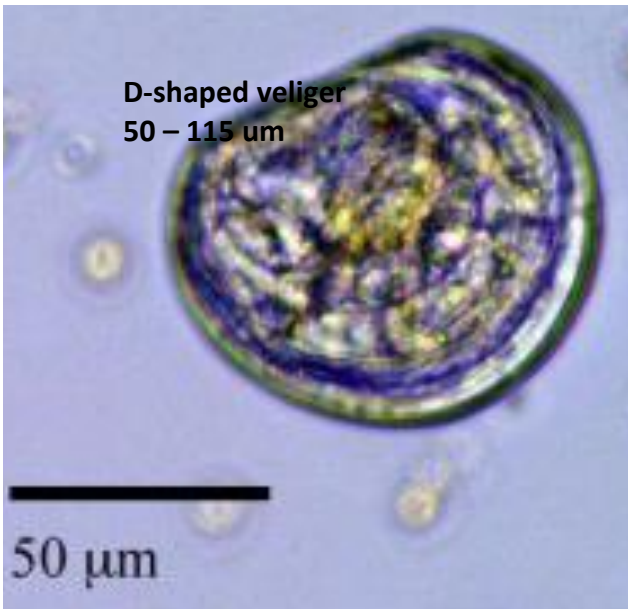
## Zebra Mussel veligers

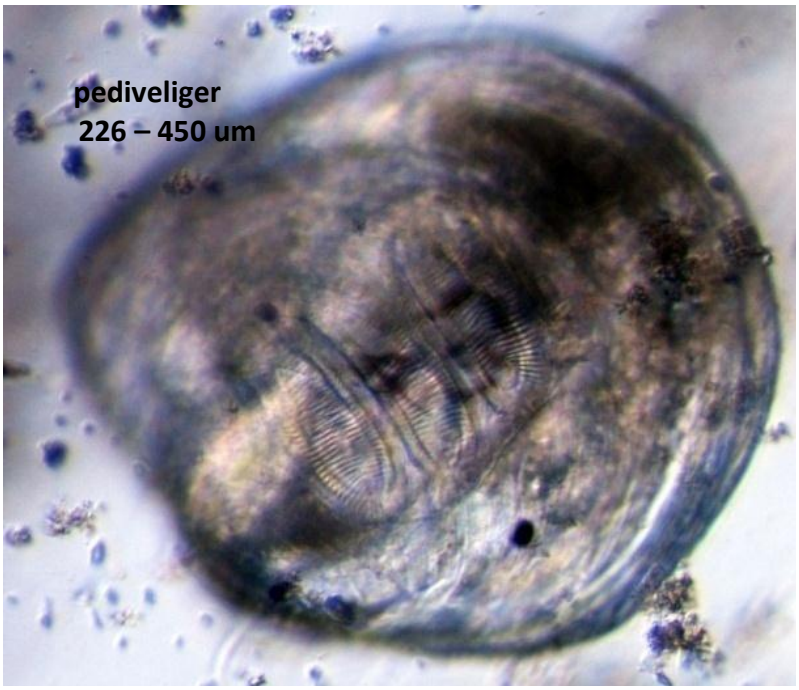





**Photos from De Leon, 2009**

# Appendix 9: Zebra / Quagga Mussel Monitoring and Detection Protocols

**Selection of Monitoring Sites**

1. Acquire a suitable map of the water body, preferably with water depth contours.
2. Concentrate the survey in areas with suitable hard substrates for attachment, especially those near boat launches.
3. Mark all sample sites in GPS and map on RDCO GIS layer
4. If zebra mussels are collected, mark the position with a GPS for future reference
5. Use an underwater video camera and camera to document samples sites

**Sample Collection for Detection of Initial Establishment of Zebra Mussel Populations**

**Qualitative Analysis of Veliger Samples:**

Since we anticipate very low or no veligers at present, researchers can use 2-3 one-minute plankton hauls in shallow water at each site to strain veligers and particles larger than 52/65 microns out of hundreds of liters. This should allow qualitative detection of very low concentrations of veligers. The net will be decontaminated.

The main goal of analysis for these samples is to identify any veligers that may be present. Site, date, replicate #, collection depth, water temperature will be recorded with the results of the analysis.

**Equipment:** Inverted or standard microscope at 100X, Sedgewick-Rafter counting chambers.

**Protocol:** Unpreserved samples will be examined within 48 hours of sample collection for swimming (live) veligers or dead veligers. (Large veligers nearing the settlement stage may move using their foot).

**Sample collection for Established Zebra Mussel Populations**

Should zebra mussels become established, we propose to shift to a quantitative method of veliger detection in which 10 L would be collected from mid-depth at three separate locations in the water body and batched to create a composite sample of 30 L.

**Quantitative Analyses of Veliger Samples:**

The ability of any sampling method to detect veligers is limited by the volume of water sampled, the number of subsamples examined, and the concentrated volume of the sample. The detection limit of any technique is calculated using the following formula (Marsden 1992):

$$\frac{(1 \text{ veliger/total subsample volume [mL]}) \times (\text{volume conc. sample [mL]})}{\text{volume water sampled [mL]}}$$

**Equipment:** Inverted phase contrast microscope at 100X, Sedgewick-Rafter counting chambers.

**Protocol:** Unpreserved samples will be examined within 48 hours of sample collection for swimming (live) veligers or dead veligers.

**Veliger stage assessment :** Veligers can be identified by developmental stage based on morphological differences (Farr and Alley 2003).
-Individuals (<100 µm) include those considered to be recently detectable veligers and will have a newly developed shell.
-Veligers 100-200 µm; these are detectable by cross-polarized lighting, but are not yet of sufficient size to settle.
-Veligers >200 µm are defined as "competent to settle".
-Pediveliger stage (220-320µm). Byssal threads will be used for attachment.

**Used sample disposal**
Used samples will be used to water a flower garden and will not be poured down drains.